UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

Dirleise Colle

AVALIAÇÃO DO POTENCIAL EFEITO PROTETOR DO PROBUCOL EM MODELOS EXPERIMENTAIS DA DOENÇA DE HUNTINGTON

Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal de Santa Catarina como requisito parcial para obtenção do título de Mestre em Bioquímica.
Orientadora: Profº. Dr. Marcelo Farina
Co-orientadora: Profº. Dr. Félix Alexandre Antunes Soares

Florianópolis
2012

Catalogação na fonte pela Biblioteca Universitária
da
Universidade Federal de Santa Catarina

C697a Colle, Dirleise
Avaliação do potencial efeito protetor do probucol em modelos experimentais da doença de Huntington [dissertação] / Dirleise Colle ; orientador, Marcelo Farina. - Florianópolis, SC, 2012.
161 p.: il., grafs.

Dissertação (mestrado) - Universidade Federal de Santa Catarina, Centro de Ciências Biológicas. Programa de Pós-Graduação em Bioquímica.

Inclui referências

1. Bioquímica. 2. Ácidos. 3. Estresse Oxidativo. I. Farina, Marcelo. II. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Bioquímica. III. Título.

CDU 577

"Avaliação do potencial efeito protetor do Probucol em modelos experimentais da Doença de Huntington"

por

Dirleise Colle

Dissertação julgada e aprovada em sua forma final pelos membros titulares da Banca Examinadora (Port. 08/PPGBQA/2012) do Programa de Pós-Graduação em Bioquímica - UFSC, composta pelos Professores Doutores:

Banca Examinadora:

Prof(a) Dr(a) Marcelo Farina (presidente/BQA/CCB/UFSC)

Prof(a) Dr(a) Juliano Ferreira (Química/CCNE/UFSM)

Prof(a) Dr(a) Carla Inês Tasca (BQA/CCB/UFSC)

Prof(a) Dr(a) Alexandra Susana Latini (BQA/CCB/UFSC)

Prof. Dr. Marcelo Farina
Coordenador do Programa de Pós-Graduação em Bioquímica

Florianópolis, 17 de fevereiro de 2012.

AGRADECIMENTOS

Primeiramente, agradeço a Deus por sempre me abençoar e iluminar o caminho que escolhi seguir. Aos meus pais, Geraldo e Dirte, por terem me ensinado a ser uma pessoa honesta e centrada nos meus objetivos, pela confiança, incentivo e carinho. Obrigada por tudo, sem o apoio de vocês, eu não seria ninguém.

Ao meu namorado, marido, amigo e companheiro Jaelson, por todo amor, carinho e por estar ao meu lado em todos os momentos bons ou ruins sempre me apoiando e me dando forças para eu conseguir conquistar meus objetivos.

Um agradecimento em especial ao meu orientador Prof. Dr. Marcelo Farina, por ter me recebido em seu laboratório, pela paciência, por todo empenho, incentivo e atenção com o meu trabalho durante o mestrado.

Agradeço ao meu co-orientador Prof.Dr. Félix Alexandre Antunes Soares, por todos os ensinamentos, apoio e amizade desde os tempos de iniciação científica na UFSM.

Um agradecimento especial a minha dupla dinâmica no lab. Danúbia, por todos os experimentos, apoio, troca de conhecimentos, conversas e principalmente pela nossa amizade.

Ao pessoal do lab., Mariana, Alessandra, Luciana, Renata, Eduardo, Aline, Carol e Juliana (minha IC) por toda amizade, companheirismo e auxílio durante a realização desse trabalho.

Aos meus colegas e amigos do laboratório do Prof Félix em Santa Maria nos meus tempos de IC, em especial á Daiana, por todos os ensinamentos no laboratório; aos demais colegas Priscila, Leticia, Jéssika, Nélson, Portella, Fernando, Guilherme, Rômulo, Thiago, Edovando, Gustavo, pelo apoio, conversas, rizadas, cervejas, jogatinas de truco... Sinto muita saudade de vocês, jamais esquecerei esses ótimos momentos.

Aos colegas do laboratório do Prof. Nélson, da Prof. Andreza e da Prof. Alexandra, pela amizade e convivência, Vicente, Daiane, Marcos, Jade, Bianca, Gianni, Aline, Roberta, Viviane, e demais colegas...

Aos técnicos do LAMEB Bibiana e Dênis, pela amizade, disponibilidade e auxílio na utilização dos aparelhos.

Aos colegas de corredor, pela amizade, brincadeiras e troca de experiências.

A CAPES, pela bolsa concedida, que me possibilitou o trabalho em tempo integral no laboratório durante este período.

Ao Programa de Pós-Graduação em Bioquímica e aos professores deste curso, pela dedicação e trabalho.

Um muito obrigado a todos os amigos e familiares, que direta ou indiretamente participaram e me apoiaram nesta etapa da minha vida.

RESUMO

A doença de Huntington (DH) é uma patologia neurodegenerativa, autossômica dominante caracterizada por sintomas atribuídos à morte de neurônios estriatais e corticais no cérebro. O mecanismo de neurodegeneração na DH parece estar relacionado com excitotoxicidade, disfunção mitocondrial e estresse oxidativo. O probucol (PB) é um composto fenólico antilipêmico, que apresenta propriedades anti-inflamatória e antioxidante em diferentes modelos experimentais de toxicidade/patologia. O objetivo deste estudo foi investigar o possível efeito protetor do PB sobre a neurotoxicidade e estresse oxidativo em modelos experimentais de DH *in vitro* e *in vivo*. Inicialmente, foi avaliada a relação entre prejuízo no metabolismo energético, excitotoxicidade e estresse oxidativo em fatias de estriado de ratos expostas ao ácido quinolínico (AQ), ácido 3-nitropropiônico (3-NP) e ao modelo combinado (AQ + 3-NP). Os dados sugerem que os modelos utilizados podem gerar um padrão complexo de dano, que envolve comprometimento metabólico, formação de espécies reativas de oxigênio (ERO) e estresse oxidativo. O PB preveniu o estresse oxidativo nas três condições experimentais e foi capaz de proteger contra disfunção mitocondrial induzida pelo AQ e AQ + 3-NP. Além disso, o potencial efeito protetor do probucol foi avaliado sobre a neurotoxicidade do 3-NP em ratos. O pré-tratamento com probucol (por 60 dias) aumentou a atividade da glutationa peroxidase (GPx) no estriado e no córtex e preveniu o prejuízo motor e o estresse oxidativo induzido pelo 3-NP em ratos. O efeito do PB sobre a GPx e suas propriedades antioxidantes estão provavelmente associados ao seu efeito benéfico neste modelo. Também foi verificado o possível efeito protetor do succinobucol, um análogo do PB, sobre a toxicidade induzida pelo 3-NP em preparações mitocondriais de cérebro de ratos *in vitro*. O probucol e o succinobucol preveniram o estresse oxidativo induzido pelo 3-NP, mas apenas o succinobucol foi capaz de prevenir a disfunção mitocondrial induzida pela toxina. Juntos este resultados sugerem um novo papel para o probucol e seu análogo succinobucol como potenciais agentes neuroprotetores em modelos de DH.

Palavras-chave: doença de Huntington, ácido-3-nitropropiônico, ácido quinolínico, probucol, succinobucol, estresse oxidativo.

ABSTRACT

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disorder characterized by symptoms attributable to the death of striatal and cortical neurons. The molecular mechanisms mediating neurodegeneration in HD seem to be related to excitotoxicity, mitochondrial dysfunction and oxidative stress. Probucol is a phenolic lipid-lowering agent with antiinflammatory and antioxidant properties, which plays protective effects in experimental models of toxicity/pathology. The objective of this study was to investigate the possible protective effects of probucol on neurotoxicity and oxidative stress in experimental models of HD *in vitro* and *in vivo*. Initially, we evaluated the potential relationship between energetic impairment, excitotoxicity and oxidative stress in rat striatal slices exposed to QA, 3-NP, as well as a combined model (AQ plus 3-NP). Our data suggest that the three studied toxic models can generate complex patterns of damage, which involve metabolic compromise, reactive oxygen species (ROS) formation, and oxidative stress. Probucol prevented oxidative stress in all used models, and was able to protect against the mitochondrial dysfunction induced by QA and QA plus 3-NP. Furthermore, we evaluate the potential protective effect of probucol against 3-NP neurotoxicity *in vivo*. Probucol pretreatment (60 days) increased glutathione peroxidase activity (GPx) in striatum and pre-frontal cortex and prevented 3-NP-induced motor impairment and oxidative stress in rats. The stimulatory effect of probucol toward GPx activity and its antioxidant properties are likely related to its beneficial effects in this model. The possible protective effect of succinobucol, a probucol analogue, against 3-NP-induced toxicity was also evaluated in mitochondrial brain preparations. Probucol and succinobucol prevented oxidative stress induced by 3-NP, but only succinobucol was able to prevent the mitochondrial dysfunction induced by the toxin. Taken together, the results suggest a new role for probucol and its analogue succinobucol as potential neuroprotective agents in HD models.

Keywords: Huntington's disease, 3-nitropropionic acid, quinolinic acid, probucol, succinobucol, oxidative stress.

LISTA DE FIGURAS

LISTA DE FIGURAS DA DISSERTAÇÃO

LISTA DE FIGURAS DO ARTIGO 1

LISTA DE FIGURAS DO MANUSCRITO 1

LISTA DE FIGURAS DO MANUSCRITO 2

LISTA DE ABREVIATURAS

Aβ	Peptídeo β-amilóide
AGI-1067	Succinobucol
AMPA	Ácido α-amino-3-hidróxi-5-metil-4-isoxazol propiônico
AQ	Ácido quinolínico
ATP	Trifosfato de adenosina
CAG	Citosina, adenina, guanina
Ca^{2+}	Íon cálcio
CoQ10	Coenzima Q10
Cu-Zn SOD	Cobre-zinco superóxido dismutase
DH	Doença de Huntington
DHJ	Doença de Huntington juvenil
DNA	Ácido desoxirribonucléico
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
GABA	Ácido gama-aminobutírico
GLT1	Transportador de glutamato 1
GPx	Glutationa peroxidase
GSH	Glutationa
HDL	Lipoproteína de alta densidade
H_2O_2	Peróxido de hidrogênio
iNOS	Óxido nítrico sintase induzível
InsP3	Inositol (1,4,5)-trifosfato
i.p.	Intraperitoneal
KA	Ácido caínico
Mg^{2+}	Íon magnésio
MK-801	Dizocilpina
Mn SOD	Manganês superóxido dismutase
Na^{2+}	Íon sódio
NAD^+	Nicotinamida adenina dinucleotídeo
Na^+/K^+ATPase	Sódio/potássio ATPase
NMDA	N-metil-D-aspartato
3-NP	Ácido 3-nitropropiônico
$^{\bullet}NO$	Óxido nítrico
PB	Probucol
PGC-1α	Co-ativador 1α do receptor ativado por proliferadores do peroxisoma γ

PSD-95	Proteína da densidade pós-sináptica-95
$O_2^{\bullet-}$	Radical ânion superóxido
$^{\bullet}OH$	Radical hidroxil
$ONOO^-$	Peroxinitrito
s.c.	Subcutânea
SDH	Succinato desidrogenase
SNC	Sistema nervoso central
SOD	Superóxido dismutase
TBARS	Substâncias reativas ao ácido tiobarbitúrico
TH	Tirosina hidroxilase
TNF-α	Fator de necrose tumoral-α

SUMÁRIO

1 INTRODUÇÃO

1.1 Doença de Huntington

A doença de Huntington (DH) é uma patologia neurodegenerativa caracterizada por alterações motoras progressivas, distúrbio emocional, demência e morte neuronal. Classicamente conhecida como coréia de Huntington (‘*khoreia*’ é a palavra grega para dança), foi primeiramente descrita pelo médico norte americano George Huntington, em 1872, que identificou as características clínicas da doença e o padrão de transmissão familiar (Bates, 2005). Contudo, foi apenas em 1983 que a mutação gênica causadora da DH foi localizada no cromosssomo 4 (Gusella et al., 1983) e, posteriormente, isolada em 1993 pelo *Huntington’s Disease Collaborative Research Group*. Este grupo identificou uma mutação na porção 5’ do gene IT15 ou “*Interesting Transcript 15*” no braço curto do cromossomo 4, que codifica a proteína Huntingtina. Tal mutação resulta numa expansão da seqüência de nucleotídeos citosina, adenina e guanina (CAG - que codifica o aminoácido glutamina), resultando em uma proteína mutante com uma sequência de poliglutaminas (poli-Q) no terminal amínico da proteína huntingtina (*Huntington’s Disease Collaborative Research Group*, 1993).

Geralmente, em indivíduos normais (que não apresentam a doença), a proteína huntingtina apresenta menos de 35 repetições. Já nos afetados pela desordem, a proteína apresenta acima de 36 resíduos de glutamina na porção N-terminal da cadeia polipeptídica (*Huntington’s Disease Collaborative Research Group, 1993*), sendo essa característica utilizada como parâmetro de diagnóstico para a doença (Perez-De La Cruz e Santamaria, 2007). Uma correlação inversa tem sido descrita entre o comprimento da sequência Poli-Q e a idade do desenvolvimento da doença, determinado pela primeira manifestação motora (Andrew et al., 1993; Roos, 2010). Quanto mais longa essa sequência, mais cedo ocorre o desenvolvimento da doença e mais severa é sua progressão (Vonsattel e DiFiglia, 1998). Quando a doença inicia antes dos 20 anos de idade, conhecida como Doença de Huntington Juvenil (DHJ), a sequencia poliglutamínica geralmente excede 55 repetições (Wheelock et al., 2003).

A DH possui herança autossômica dominante, onde o alelo normal transmite-se de geração em geração segundo as regras de hereditariedade Mendeliana. O alelo mutante é instável durante a meiose, alterando o seu comprimento na maioria das transmissões entre

as gerações, com um aumento de 1-4 unidades ou diminuição de 1-2 unidades do triplete CAG (Gil-Mohapel e Rego, 2011). Em casos raros podem ocorrer expansões maiores associadas à transmissão paterna, o que reflete uma maior taxa de mutação durante a espermatogênese (*Huntington's Disease Collaborative Research Group*, 1993).

A doença possui prevalência de 5-10 casos para cada 100.000 habitantes na Europa e América do Norte (Ho et al., 2001; Vonsattel e DiFiglia, 1998). A proteína huntingtina mutante é expressa durante toda a vida, porém, na maioria dos casos, o aparecimento dos primeiros sintomas surge apenas na idade adulta, entre os 35 e 50 anos de idade (Roos, 2010). A doença progride ao longo do tempo, tornando-se fatal entre 15 a 20 anos após o aparecimento dos primeiros sintomas (Ho et al., 2001; Ross e Tabrizi, 2011).

Clinicamente, a DH caracteriza-se por coréia progressiva, declínio cognitivo e distúrbios psiquiátricos (Roos, 2010). Os primeiros sinais da patologia são sutis e podem variar bastante entre cada indivíduo. Numa fase precoce, podem ser observadas alterações moderadas na execução dos movimentos, dificuldades na resolução de problemas, irritabilidade e depressão. As alterações motoras, associadas à perda de coordenação dos movimentos voluntários, progridem de forma lenta. Os movimentos involuntários dos músculos (especialmente membros superiores, inferiores e de face) tornam-se mais graves e os pacientes perdem gradualmente a capacidade de movimento, fala e deglutição em fases mais avançadas da doença (Garcia Ruiz et al., 2000; Roos, 2010; Sanchez-Pernaute et al., 2000; Thompson et al., 1988).

Além das alterações motoras, as funções cognitivas são também afetadas nos pacientes com DH (Gil-Mohapel e Rego, 2011). O declínio da capacidade intelectual e de memória são os principais sinais de déficit cognitivo nesses indivíduos (Roos, 2010) e, em alguns casos, podem ser detectados décadas antes do aparecimento dos sintomas motores (Gil-Mohapel e Rego, 2011). As alterações cognitivas tendem a piorar ao longo do tempo, onde os doentes em fase tardia podem apresentar demência severa (Folstein et al., 1983). Por outro lado, um comportamento maníaco-depressivo, alterações de personalidade, tais como irritabilidade, apatia e distúrbios sexuais e depressão fazem parte da síndrome psiquiátrica que caracteriza a DH (Dewhurst et al., 1970; van Duijn et al., 2007). A morte geralmente ocorre devido a complicações respiratórias infecciosas, cardiovasculares ou até mesmo por quedas, engasgos e suicídio (Roos, 2010).

Considerando as características acima descritas, os critérios usados para o diagnóstico da DH incluem: histórico familiar de DH, déficit motor progressivo associado à coréia ou rigidez, bem como alterações psiquiátricas com demência progressiva, sem outra causa definida (Ross e Tabrizi, 2011). Os indivíduos que apresentam estes sintomas são submetidos ao teste genético, de forma a avaliar a presença da mutação associada à DH e confirmar o diagnóstico (Gil-Mohapel e Rego, 2011).

1.2. Neuropatologia da Doença de Huntington

Neuropatologicamente, a DH é caracterizada por disfunção e degeneração de áreas específicas nos gânglios basais e no córtex cerebral (Reiner et al., 1988; Storey e Beal, 1993).

Os gânglios basais constituem um conjunto de estruturas cerebrais subcorticais envolvidos em diversos aspectos do controle motor e de cognição (Graybiel, 1990; Mitchell et al., 1999). Nesta região, o processo neurodegenerativo inicia no estriado (Vonsattel et al., 1985), uma região que recebe projeções (aferências) de diversas áreas corticais (Mitchell et al., 1999), onde a morte neuronal ocorre principalmente no núcleo caudado e no putamen (Reiner et al., 1988; Vonsattel e DiFiglia, 1998). A perda significativa de neurônios é também relatada no córtex cerebral de pacientes, incluindo as regiões frontal, parietal e temporal (Heinsen et al., 1994; Mann et al., 1993), apesar de essas alterações serem menos óbvias do que as observadas no estriado.

Uma escala de avaliação dos diferentes estágios patológicos da DH foi desenvolvida por Vonsattel e colaboradores em 1985 e permite determinar a severidade da degeneração na doença. Esta escala baseia-se nos padrões de degeneração estriatal observados em tecidos *pos mortem* e é classificada em 5 graus (0 a 4). O grau 0 é praticamente indistinguível de um cérebro normal, apesar de uma perda neuronal de 30 a 40% poder estar presente no núcleo caudado. No grau 1 são observadas algumas alterações como atrofia, diminuição neuronal e astrogliose no núcleo caudado. Já os graus 2 e 3 são caracterizados por progressiva e severa atrofia do estriado, e o grau 4 por sua vez, inclui o quadro mais grave de atrofia estriatal, com perda neuronal de até 95% (Vonsattel et al., 1985). Além disso, foi estabelecida uma correlação positiva entre o número de repetições CAG e a escala de Vonsattel, onde um maior número de repetições está associado a lesões mais

pronunciadas no estriado e a um grau mais elevado nesta escala (Vonsattel e DiFiglia, 1998).

Os neurônios mais afetados no estriado são os neurônios espinhosos médios, que correspondem a aproximadamente 95% do número total de neurônios estriatais. Esses neurônios são GABAérgicos e, portanto, utilizam o neurotransmissor inibitório ácido gama-aminobutírico (GABA). A perda do efeito inibitório causada pela morte desses neurônios tem sido diretamente associada aos movimentos involuntários característicos da doença (Han et al., 2010).

Estudos revelaram que diferentes graus de degeneração podem estar presentes em uma mesma população neuronal do estriado (Ferrante et al., 1987; Ferrante et al., 1997; Vonsattel, 2008). Esses estudos demonstraram que neurônios espinhosos médios GABAérgicos que se projetam para o globo pálido externo (via indireta) são os principais afetados na DH. Essas alterações na via indireta têm sido associadas com o desenvolvimento dos movimentos involuntários (Crossman, 1987; Crossman et al., 1988). Com a progressão da doença, os neurônios espinhosos médios que se projetam para o globo pálido interno (via direta) e neurônios piramidais corticais também são afetados. A degeneração tardia dos neurônios da via direta é responsável pelo desenvolvimento de bradicinesia e rigidez em estágios terminais da doença (Berardelli et al., 1999) (Figura 1).

Por outro lado, muitos interneurônios, tanto no estriado como no córtex, são “poupados” do dano no início da doença (Vonsattel, 2008). Porém, em fases mais avançadas, todas as projeções estriatais são afetadas, com atrofia estriatal extrema e perda considerável da população neuronal no grau 4, indicando que todos os tipos neuronais são vulneráveis nos estágios finais da doença (Zuccato et al., 2010). Dessa forma, nos graus 3 e 4, a neurodegeneração parece não ser restrita ao estriado, ocorrendo também em outras regiões como o córtex cerebral, *globus palidus*, tálamo, núcleo subtalâmico, substância *nigra*, cerebelo e hipocampo (Kassubek et al., 2004; Vonsattel e DiFiglia, 1998; Zuccato et al., 2010) são afetadas. Nessas condições, uma significativa atrofia com perda de massa encefálica pode ser observada, podendo ocorrer redução de até 40% da massa cerebral (Gil-Mohapel e Rego, 2011).

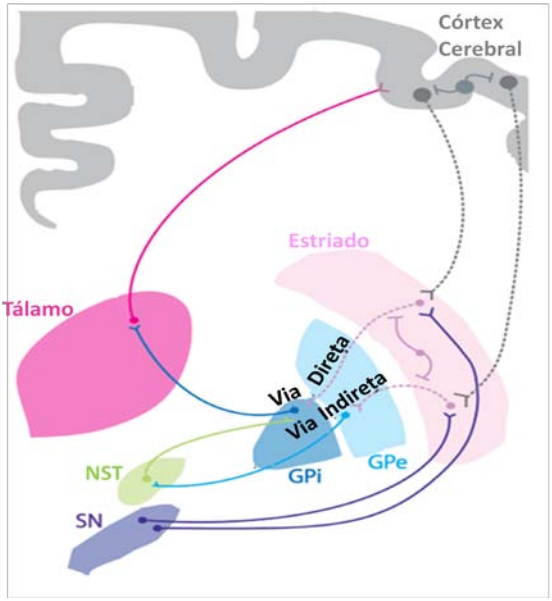

Figura 1: Principais projeções neuronais afetadas na DH. Os neurônios espinhosos médios GABAérgicos estriatais (linha tracejada rosa) e neurônios piramidais corticais (linha tracejada cinza) são as projeções neuronais mais vulneráveis na DH. A morte desses neurônios é responsável pelo desenvolvimento dos sintomas clínicos da doença. GPi, globo pálido interno; GPe, globo pálido externo; SN, núcleo subtalâmico; SN, substância *nigra* (Adaptado de: Han et al., 2010).

1.3. Proteína Huntingtina mecanismos de neurodegeneração na Doença de Huntington

Apesar de ter sido identificada há quase duas décadas, o exato papel da proteína huntingtina nas células ainda não está completamente elucidado. A huntingtina é uma proteína de 348 kDa expressa em humanos e roedores em níveis elevados no sistema nervoso central (SNC) (DiFiglia et al., 1997; Ferrante et al., 1997; Fusco et al., 1999), principalmente em neurônios piramidais corticais que se projetam para o estriado (Fusco et al., 1999). É encontrada principalmente no citoplasma, associada a organelas como mitocôndrias, complexo de Golgi, retículo endoplasmático, vesículas sinápticas e diversos componentes do citoesqueleto (Hoffner et al., 2002), mas pode também

estar presente, com menor frequência, dentro do núcleo das células (Landles e Bates, 2004).

Essa proteína parece exercer importante papel na sobrevivência celular por controlar vias de apoptose, regular o transporte intracelular, o tráfego e a secreção de vesículas, mediar processos de endocitose, promover a sinalização celular e regulação transcricional (Harjes e Wanker, 2003; Li e Li, 2004). Todas essas funções estão relacionadas com a sua capacidade de interação com outras proteínas nas células (Zuccato et al., 2010).

Na DH a mutação no gene da proteína huntingtina é responsável pela modificação conformacional da mesma. Essa proteína pode ser clivada por proteases, caspases (Goldberg et al., 1996; Kim et al., 2001; Sun et al., 2002) e calpaínas (Gafni e Ellerby, 2002; Kim et al., 2001; Sun et al., 2002) em fragmentos N-terminais, os quais podem formar agregados protéicos citoplasmáticos, bem como inclusões nucleares (DiFiglia et al., 1997; Gafni e Ellerby, 2002; Sun et al., 2002). A presença desses agregados em regiões como o estriado e o córtex cerebral é altamente tóxica (Kim et al., 2001) e é responsável por causar disfunção neuronal, a qual está diretamente envolvida nos sintomas clínicos da doença. Estudos demonstraram que a toxicidade estaria envolvida com o recrutamento de muitas proteínas celulares para dentro dos agregados, causando a perda de função dessas proteínas, bem como severa disfunção de vias de sinalização intracelulares (Zuccato et al., 2010).

Inúmeras linhas de evidência sugerem que a presença da mutação da proteína na DH confere uma nova função à huntingtina que torna-se tóxica para a célula (ganho de função) (Browne e Beal, 2006; Imarisio et al., 2008; Zuccato et al., 2010). Por outro lado, a perda de função da proteína normal (devido à diminuição de sua expressão ou por ser sequestrada para dentro de agregados da proteína mutante) também contribui para alterações na homeostase intracelular (Browne e Beal, 2006; Zuccato et al., 2010). Tanto o ganho de função da proteína mutante como a perda de função da proteína normal contribui para a disfunção e morte neuronal observadas na DH.

Neste contexto, diversos mecanismos patológicos parecem contribuir para a neurodegeneração, como desregulação transcricional, comprometimento do sistema proteolítico, prejuízo no transporte axonal, comprometimento metabólico e disfunção mitocondrial, alteração na homeostase do cálcio, excitotoxicidade, estresse oxidativo, ativação de caspases, resposta inflamatória, entre outras (Krobitsch e Kazantsev, 2011).

1.3.1. Excitotoxicidade e Doença de Huntington

O glutamato é o neurotransmissor excitatório mais abundante no SNC e é essencial para uma variedade de processos fisiológicos, como integridade da função cerebral e desenvolvimento neuronal (Ribeiro et al., 2011). Por outro lado, o glutamato está também relacionado com morte celular, desempenhando um importante papel na patogênese da DH (Calabresi et al., 1999; DiFiglia, 1990; Ferraguti et al., 2008).

Quando liberado nos terminais sinápticos, o glutamato ativa diferentes tipos de receptores em neurônios pós-sinápticos: (i) os metabotrópicos, que são receptores associados a segundos mensageiros intracelulares, geralmente acoplados à proteína G, e (ii) os receptores ionotrópicos, que são canais iônicos que permeiam cátions para dentro das células e desencadeiam respostas excitatórias, como os receptores N-metil-D-aspartato (NMDA), ácido -amino-3-hidróxi-5-metil-4-isoxazol propiônico (AMPA) e ácido caínico (KA) (Dhami e Ferguson, 2006; Dingledine et al., 1999; Olney, 1994; Ribeiro et al., 2010). A ativação de receptores não-NMDA induz influxo de íons sódio e subsequente despolarização da membrana plasmática, promovendo a extrusão do íon magnésio que normalmente bloqueia o canal do receptor NMDA. Uma vez o íon magnésio liberado, a interação do glutamato (e do seu co-agonista glicina) com seus respectivos sítios de ligação ativa o receptor NMDA, levando a um influxo de cálcio (Ca^{2+}) para o interior da célula (Edmonds et al., 1995). A ação do glutamato é finalizada através da sua captação pelos astrócitos ou pelos neurônios pré-sinápticos (Danbolt, 2001).

A homeostase do glutamato deve ser estritamente regulada, uma vez que um excesso deste neurotransmissor na fenda sináptica superestimula os receptores NMDA, causando aumento no influxo de Ca^{2+}. O influxo aumentado de Ca^{2+} leva à ativação de proteases, endonucleases e fosfolipases que culminam na degradação de diferentes componentes celulares e na ativação de vias de morte celular (Estrada Sanchez et al., 2008), num fenômeno conhecido como excitotoxicidade.

É importante salientar que o dano excitotóxico pode também ocorrer em circunstâncias onde os níveis extracelulares de glutamato são fisiológicos, mas há prejuízos no metabolismo energético, sendo conhecido como “excitotoxicidade secundária” (Beal et al., 1993). Em condições de déficit no metabolismo energético, a redução na produção de ATP pode alterar a regulação do gradiente iônico através das

membranas celulares mantida pela bomba Na^+/K^+-ATPase, causando inapropriada ou prolongada abertura de canais iônicos dependentes de voltagem e parcial despolarização da membrana (Browne e Beal, 2006). Dessa forma, mesmo concentrações extracelulares fisiológicas de glutamato podem ativar receptores NMDA e aumentar o influxo de Ca^{2+} para o interior das células.

A hipótese da excitotoxicidade na patogênese da DH iniciou com diferentes estudos mostrando que a administração de glutamato ou ácido caínico em ratos causava dano neuronal semelhante ao observado na doença (Beal et al., 1986; Coyle e Schwarcz, 1976). Essa hipótese foi confirmada posteriormente por achados que demonstraram que a excitotoxicidade glutamatérgica é aumentada em modelos animais transgênicos para a essa patologia (Hodgson et al., 1999; Levine et al., 1999). Esses resultados sugerem que a superestimulação de receptores NDMA pode desempenhar um papel significativo na patogênese da DH.

Alterações na remoção do glutamato na fenda sináptica também podem contribuir de maneira expressiva para aumentar o dano excitotóxico na DH. Estudos em modelos animais transgênicos da doença (R6/2 e R6/1) demonstraram comprometimento na regulação do GLT1, uma das proteínas responsáveis pela remoção do glutamato extracelular, o que está correlacionado com uma diminuição da captação de glutamato no estriado desses animais (Behrens et al., 2002; Lievens et al., 2001; Shin et al., 2005). Alterações na captação de glutamato também foram observadas em cérebros *pos mortem* de pacientes com DH (Arzberger et al., 1997; Hassel et al., 2008).

Além disso, outras evidências também mostraram o efeito direto da proteína mutante sobre os receptores NMDA, nos quais a presença dessa proteína inibiu a interação da proteína huntingtina normal com a proteína pós-sináptica PSD-95, (responsável por regular a atividade dos receptores NMDA), causando um aumento na sensibilidade desses receptores (Sun et al., 2001). Outros trabalhos também mostraram um aumento nos níveis de metabólitos das vias das quinureninas, como o ácido quinolínico, que é um agonista do receptor glutamatérgico NMDA (Coyle e Puttfarcken, 1993; Guidetti et al., 2004; Zucker et al., 2005) em cérebros *pos mortem* de pacientes com DH.

Dessa forma, a considerável inervação glutamatérgica no estriado, a partir do córtex cerebral, parece ser responsável por exacerbar o risco de dano excitotóxico nos neurônios estriatais (Browne e Beal, 2006). Porém, estudos em tecido *post mortem* de pacientes em estágios mais avançados da doença demonstraram que os receptores NMDA são seletivamente depletados no estriado, sugerindo uma grande

perda de neurônios que expressam esses receptores e que a excitotoxicidade poderia ser mais importante nas fases iniciais da doença (Young et al., 1988).

Além do aumento dos níveis intracelulares de cálcio através de receptores NMDA, a huntingtina mutante pode também aumentar a concentração de Ca^{2+} por desestabilizar a regulação mitocondrial deste íon (Choo et al., 2004) e por sensibilizar o receptor InsP3 (inositol (1,4,5)-trifosfato) no retículo endoplasmático, culminando com a liberação de Ca^{2+} dos estoques intracelulares (Tang et al., 2003). Juntos esses eventos contribuem com progressiva neurodegeneração do estriado (Figura 2).

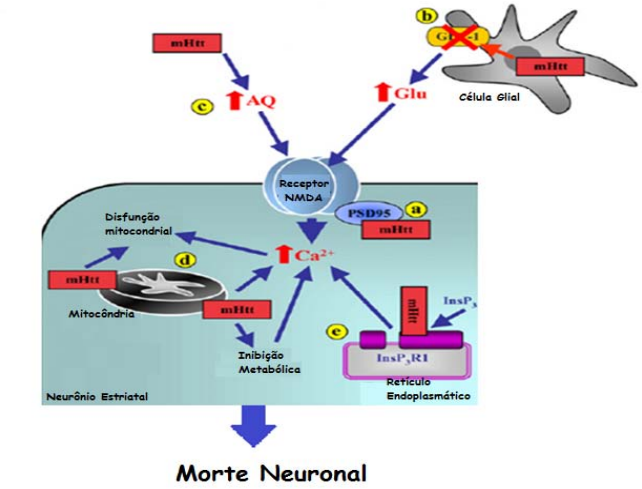

Figura 2: Mecanismo de excitotoxicidade na DH. A presença da proteína mutante huntingtina inibe a interação da proteína normal com a proteína pós-sináptica PSD-95 aumentando a sensibilidade dos receptores NMDA (a). Além disso, a proteína mutante diminui a atividade do GLT-1 reduzindo a captação de glutamato da fenda sináptica (b) e aumenta a produção de ácido quinolínico (c), um agonista de receptors NMDA, aumentando a ativação desses receptores (c). A superestimulação dos receptores NMDA causa aumento no influxo de cálcio nos neurônios estriatais. Esses eventos tóxicos são potencializados pela disfunção mitocondrial (d) e pela sensibilização dos receptores InsP3 no retículo endoplasmático (e), culminando com a liberação de Ca^{2+} dos estoques intracelulares. Todos esses eventos contribuem com a ativação de enzimas dependentes de cálcio como calpaínas, caspases e endonucleases culminando

com morte neuronal. QA, ácido quinolínico; Glu, glutamato; mHtt, huntingtina mutante (Adaptado de Popoli et al., 2007).

1.3.2. Déficit energético e disfunção mitocondrial na Doença de Huntington

As mitocôndrias são importantes integradoras da função celular e, portanto, afetam o estado de equilíbrio das células. Além do seu papel na produção de trifosfato de adenosina (ATP) através da fosforilação oxidativa, elas estão envolvidas em outros processos como o controle das concentrações de cálcio citosólicas, metabolismo de intermediários celulares e morte celular programada. As mitocôndrias são também importantes produtores de espécies reativas de oxigênio (ERO). Diversas patologias humanas, incluindo as doenças neurodegenerativas, estão associadas com disfunção mitocondrial e produção de ERO (de Moura et al., 2010).

As primeiras hipóteses de disfunção no metabolismo energético na DH surgiram a partir de observações da pronunciada e inexplicável perda de peso em pacientes, apesar do consumo calórico normal (Djousse et al., 2002; Sanberg et al., 1981). Posteriormente, estudos revelaram que o metabolismo da glicose nos gânglios basais e no córtex cerebral era significativamente reduzido em indivíduos sintomáticos (Andrews e Brooks, 1998; Grafton et al., 1992; Kuhl et al., 1985; Kuwert et al., 1990; Kuwert et al., 1993). Além disso, outras alterações metabólicas, tais como elevada produção de lactato (Browne, 2008; Jenkins et al., 1993; Jenkins et al., 1998; Koroshetz et al., 1997; Reynolds et al., 2005) e diminuição da síntese de ATP (Gines et al., 2003; Milakovic e Johnson, 2005; Seong et al., 2005), foram evidenciadas no estriado e no córtex de pacientes e em modelos animais da doença. Uma significativa redução na atividade de algumas enzimas do metabolismo oxidativo, como a aconitase, o complexo da piruvato desidrogenase e a α-cetoglutarato desidrogenase (Butterworth et al., 1985; Mochel e Haller, 2011; Sorolla et al., 2008; Tabrizi et al., 1999), foi observada em pacientes sintomáticos com atrofia no caudado e no putamen.

Por outro lado, alguns trabalhos apontam que, além do cérebro, outros tecidos também podem apresentam déficit energético (van der Burg et al., 2009). Esses estudos mostraram distúrbio energético no tecido muscular esquelético similar ao observado em neurônios estriatais de pacientes (Gizatullina et al., 2006; Strand et al., 2005), bem como diminuição nos níveis de fosfocreatina e ATP no músculo de indivíduos

sintomáticos e pré-sintomáticos (Lodi et al., 2000; Saft et al., 2005). Além disso, outras alterações foram encontradas, tais como diminuição na razão lactato/piruvato no fluido cérebro espinhal (Koroshetz et al., 1997), alterações na morfologia mitocondrial e diminuição do potencial de membrana em mitocôndrias isoladas de linfoblastos (Panov et al., 2002; Squitieri et al., 2006), bem como depleção do DNA mitocondrial em leucócitos de pacientes com DH (Liu et al., 2008).

Vários mecanismos parecem contribuir para o déficit energético no cérebro, incluindo alterações na atividade da cadeia transportadora de elétrons, prejuízos na fosforilação oxidativa (Milakovic e Johnson, 2005), estresse oxidativo (Tabrizi et al., 1999), prejuízos no tamponamento do cálcio pela mitocôndria (Lim et al., 2008; Oliveira et al., 2006), anormalidades no tráfego mitocondrial (Li et al., 2010), desregulação de fatores chaves na biogênese mitocondrial (Cui et al., 2006) e diminuição da glicólise (Powers et al., 2007).

Estudos em cérebro *pos morten* de pacientes com DH mostraram uma marcante redução da atividade principalmente dos complexos II, III e uma menor deficiência do complexo IV da cadeia transportadora de elétrons no caudado e no putamen, com atividades normais no córtex frontal e no cerebelo (Benchoua et al., 2006; Brennan et al., 1985; Browne et al., 1997; Gu et al., 1996; Stahl e Swanson, 1974). De particular importância, deficiências no complexo II têm sido foco de atenção na DH devido ao fato de que a inibição da enzima succinato desidrogenase (SDH) por compostos como malonato e ácido 3-nitropropiônico mimetizam diversas características clínicas e neuropatológicas da doença em modelos animais (Mochel e Haller, 2011). Esta enzima (SDH) é o principal componente do complexo II mitocondrial (Ackrell, 2000), o qual desempenha papel central na cadeia respiratória, no ciclo dos ácidos tricarboxílicos e no controle da produção de radicais livres (Ackrell, 2000; Damiano et al., 2010; Rustin et al., 2002). Benchoua e colaboradores (2006) relataram uma redução na expressão das subunidades Ip de 30 kDa e Fp de 70 kDa deste complexo no núcleo caudado e no putamen de pacientes com DH, que foi proporcional a morte neuronal, sugerindo que a depleção das subunidades do complexo II pode preceder a morte neuronal. Além disso, a disfunção do complexo II na doença parece estar diretamente associada com o estresse oxidativo e excitotoxicidade, que levam a morte celular (Benchoua et al., 2006; Browne e Beal, 2006; Mochel e Haller, 2011).

Aliado a disfunção na cadeia transportadora de elétrons e fosforilação oxidativa, alterações na homeostase do Ca^{2+} mitocondrial

parecem contribuir na patogênense da DH. As mitocôndrias, juntamente com o retículo endoplasmático, são reguladores centrais da concentração de Ca^{2+} nas células; aumentando sua captação quando as concentrações no citosol estão elevadas, ou liberando esse íon em caso de queda de seus níveis citoplasmáticos (Nicholls, 2009). No entanto, a capacidade da mitocôndria de captar íons Ca^{2+} do citosol é limitada, onde concentrações aumentadas desse íon podem levar à abertura de poros na membrana plasmática mitocondrial e conseqüente morte celular (Leung e Halestrap, 2008; Nicholls, 2009).

Neste contexto, estudos demonstraram alterações na capacidade da mitocôndria de regular as concentrações de Ca^{2+} no cérebro de animais em modelos experimentais da doença e em linhagens celulares (Lim et al., 2008; Oliveira et al., 2006), bem como em células sanguíneas (linfócitos e plaquetas) de pacientes com DH (Panov et al., 1999). Essas alterações foram relacionadas com uma interação da proteína mutante huntingtina com a membrana mitocondrial externa (Orr et al., 2008; Panov et al., 2002; Yu et al., 2003), aumentando a sensibilidade da mitocôndria à abertura de poros de transição permeáveis a íons Ca^{2+} ou a outros estímulos apoptóticos (Damiano et al., 2010).

Apesar de a disfunção mitocondrial estar diretamente relacionada com prejuízos no metabolismo energético, muitos estudos têm mostrado que alterações no tráfego mitocondrial neuronal também podem contribuir para a neurodegeneração na DH (Li et al., 2010; Mochel e Haller, 2011). O mecanismo pelo qual a proteína mutante afeta o tráfego intracelular de organelas como as mitocôndrias ainda não está completamente esclarecido, mas estudos sugerem que os agregados formados a partir da clivagem da proteína mutante huntingtina podem bloquear o movimento mitocondrial nos neurônios (Chang et al., 2006), além de um efeito indireto desses agregados em prejudicar o transporte axonal normal dependente da proteína huntingtina (Trushina et al., 2004). Estudos demonstram que essas alterações no tráfego mitocondrial precedem a disfunção mitocondrial e morte neuronal em modelos *in vitro* e *in vivo* da DH (Trushina et al., 2004).

Alterações na expressão do PGC-1α também estão relacionadas com a disfunção mitocondrial na DH (Weydt et al., 2006). O PGC-1α é um co-ativador transcricional e importante regulador metabólico envolvido com a regulação da função mitocondrial, como biogênese, respiração, detoxificação de ERO, metabolismo energético e termogênense (Puigserver e Spiegelman, 2003). A expressão do PGC-1α é reprimida no estriado em modelos *in vivo* e *in vitro*, bem como em

pacientes com DH, pela interação da proteína huntingtina mutante com fatores de transcrição que regulam sua expressão (Cui et al., 2006), acarretando disfunção mitocondrial possivelmente por prejuízos na fosforilação oxidativa (Cui et al., 2006). Todos os mecanismos descritos relacionados à disfunção mitocondrial são representados na figura 3.

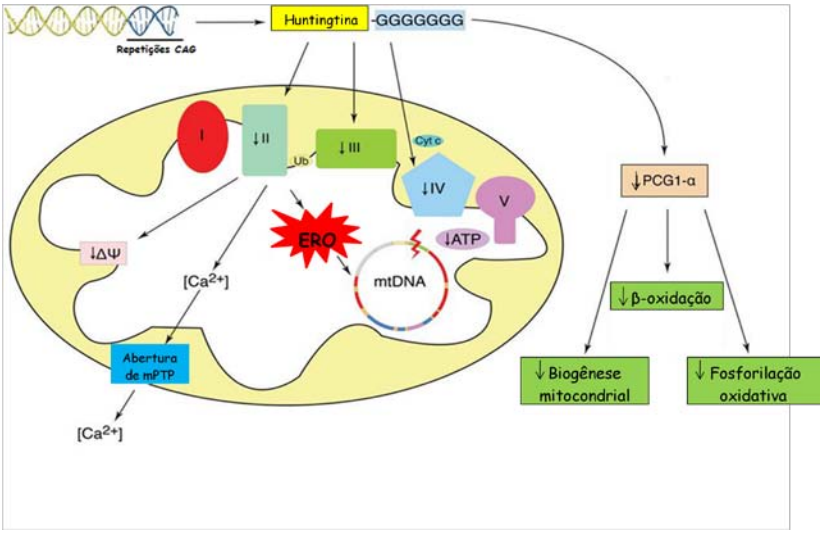

Figura 3: Mecanismos de disfunção mitocondrial na DH. A presença da proteína mutante huntingtina pode exercer um efeito direto sobre a função mitocondrial através de uma diminuição da atividade dos complexos II, III e IV da cadeia transportadora de elétrons. Alterações na atividade do complexo II podem diminuir o potencial de membrana mitocondrial, causando a abertura de poros de transição com liberação de íons cálcio. Além disso, alterações no complexo II podem também induzir a produção de ERO, que por sua vez, podem promover dano oxidativo ao DNA mitocondrial. A diminuição da expressão do PGC-1α pela proteína mutante contribui para a disfunção mitocondrial na DH (Adaptado de de Moura et al., 2010).

1.3.3. Estresse oxidativo e Doença de Huntington

O estresse oxidativo é um importante fator que tem sido relacionado com as doenças neurodegenerativas (Beal, 1995). O cérebro é altamente suscetível ao dano induzido por espécies reativas devido a sua alta utilização de oxigênio e à presença de concentrações relativamente baixas de enzimas antioxidantes e *scavengers* de radicais

livres (Shinomol e Muralidhara, 2008). A geração de ERO e o resultante estresse oxidativo desempenham relevante papel na neurodegeneração observada na DH (Mochel e Haller, 2011; Stack et al., 2008; Zuccato et al., 2010).

Evidências apontam para um aumento de produtos de peroxidação lipídica, como malondialdeído e 4-hidroxinonenal no tecido cerebral e no sangue de pacientes com a doença (Browne e Beal, 2006; Browne et al., 1997; Browne et al., 1999; Chen et al., 2007). Além disso, marcadores da oxidação de DNA (8-hidroxi-2-deoxiguanosina) e de proteínas (proteínas carboniladas) também foram detectados em cérebro e sangue de pacientes (Browne et al., 1997; Hersch et al., 2006). Outros indicadores de estresse oxidativo, como o aumento da atividade da enzima superóxido dismutase mitocondrial e citoplasmática (MnSOD e Cu-Zn SOD, respectivamente), têm sido observados em cérebros de pacientes (Sorolla et al., 2008) e em modelos animais da doença (Santamaria et al., 2001). Ainda, um aumento na atividade da glutationa peroxidase (GPx), uma importante enzima responsável pela detoxificação de peróxidos nas células (Lubos et al., 2011), foi observado no estriado e no córtex de pacientes com DH (Sorolla et al., 2008). Além disso, foi demonstrado que a agregação da proteína huntingtina mutante induz formação de peróxido de hidrogênio (H_2O_2) em modelos *in vivo* e *in vitro* da doença (Hands et al., 2011). Essas evidências apontam para o envolvimento da GPx e dos peróxidos no estresse oxidativo observado da DH.

Apesar de a origem da elevada geração de ERO na DH e em modelos animais dessa patologia não estar completamente esclarecida, a disfunção mitocondrial parece contribuir de maneira significativa para este aumento (Mochel e Haller, 2011). Além disso, alguns estudos sugerem que a produção dessas espécies reativas está envolvida com a repressão do PGC-1α na presença da proteína huntingtina mutante. O PGC-1α é necessário no processo de transcrição de enzimas antioxidantes como a SOD e a GPx (St-Pierre et al., 2006), sendo um importante protetor contra o dano oxidativo na DH.

1.4 Modelos animais da Doença de Huntington

Desde 1970, diferentes modelos animais da DH foram desenvolvidos. O primeiro foi um modelo de morte neuronal baseado na administração de ácido cainíco, um composto excitotóxico que causava morte neuronal (Coyle e Schwarcz, 1976). Outros modelos também foram estabelecidos como o modelo induzido pelo malonato, ácido

quinolínico (AQ) e ácido 3-nitropropiônico (3-NP). Mais recentemente, foram desenvolvidos os modelos transgênicos da DH em camunongos (Mangiarini et al., 1996), primatas não humanos e outras espécies (Marsh et al., 2003; Parker et al., 2001), os quais expressam o gene mutante da proteína huntingtina.

No entanto, os modelos experimentais não genéticos ainda são bastante utilizados no estudo dos processos neurodegenerativos na DH devido à fácil aquisição, controle e uso (Tunez et al., 2010). Nesses modelos, a morte celular é induzida po mecanismos excitotóxicos (ácido caínico e AQ) ou por alterações no metabolismo mitocondrial (malonato e 3-NP). Dessa forma, esses modelos, mesmo com algumas limitações, são importaantes para o melhor entendimento dos fenômenos relacionados com a doença, bem como na busca de novos alvos terapêuticos para o tratamento de tal patologia.

1.4.1 Ácido quinolínico e o modelo excitotóxico

Inicialmente, o ácido cainíco era utilizado como modelo excitotóxico da DH (Coyle e Schwarcz, 1976). Apesar de importantes estudos utilizando esse composto, anos mais tarde, pesquisadores começaram a testar outras toxinas excitotóxicas como o ácido ibotênico e o ácido quinolínico (Ramaswamy et al., 2007). Por diversas razões, detalhadas a seguir, o AQ tornou-se o composto excitotóxico preferido e passou a ser utilizado como modelo para estudar a DH.

O AQ ou ácido piridino-2,3-dicarboxílico é um metabólito endógeno da via das quinureninas nas células gliais. Essa via é a principal rota de catabolismo do triptofano, resultando na produção de NAD^+ e outros intermediários neuroativos como o ácido quinolínico (Ruddick et al., 2006).

Em concentrações fisiológicas, o AQ não causa danos, porém discretos aumentos em seus níveis podem gerar toxicidade. Schwarcz e colaboradores (1988) demonstraram aumentos na atividade de enzimas da via das quinurenias no estriado de pacientes com DH. Posteriomente, outros estudos também mostraram aumentos nas concentrações de intermediários da via das quinureninas no cérebro de pacientes afetados por esta doença (Connick et al., 1989; Pearson e Reynolds, 1992; Perez-De La Cruz e Santamaria, 2007; Schwarcz et al., 1988).

O AQ é incapaz de atravessar a barreira hematoencefálica e, portanto, experimentalmente é administrado diretamente no estriado (Foster et al., 2004). Shear e colaboradores (1998) demonstraram que a injeção intraestriatal de AQ é responsável por causar alterações motoras,

além de prejuízos cognitivos. A administração de AQ também causa neurodegeneração em ratos (Bordelon et al., 1997; Ribeiro et al., 2006), camundongos (McLin et al., 2006) e primatas (Emerich et al., 2006), a qual se assemelha àquela observada em humanos com DH (Kendall et al., 2000; Ramaswamy et al., 2007).

As lesões induzidas pelo AQ causam sintomas que frequentemente mimetizam os danos vistos em estágios iniciais da DH (Perez-De La Cruz e Santamaria, 2007; Ramaswamy et al., 2007), tais como a hiperatividade. Entretanto, a hipoatividade que ocorre em fases tardias da doença não é observada em nenhuma dose da toxina (Ramaswamy et al., 2007). Em roedores, a administração unilateral de AQ causa comportamento rotacional assimétrico resultante de um desbalanço nas vias dopaminérgicas entre o hemisfério lesionado e o intacto (Vazey et al., 2006). Além disso, as lesões induzidas pelo AQ em roedores produzem déficit cognitivo evidenciados em testes que avaliam funções de memória (Furtado e Mazurek, 1996; Isacson et al., 1984; Shear et al., 1998).

O AQ reproduz o padrão de perda de neurônios GABAérgicos similar ao que ocorre na DH, afetando especialmente neurônios espinhosos médios estriatais (Beal et al., 1986; Bordelon et al., 1997). Por isso, tornou-se um bom modelo experimental para estudar a DH, pois além das alterações comportamentais, esse composto também reproduz mecanismos de morte neuronal observados na doença. Como já mencionado, na DH a morte celular parece estar diretamente envolvida com excitotoxicidade mediada por receptores NMDA. O AQ atua como um agonista parcial de receptores NMDA (Stone e Perkins, 1981) e, portanto, sua administração no estriado, uma região rica em receptores NMDA causa superestimulação desses receptores com despolarização da membrana e consequente aumento no influxo de Ca^{2+} resultando em dano quando testado em concentrações tóxicas (Bordelon et al., 1997). Além disso, o AQ estimula a liberação sinaptossomal de glutamato e inibe sua captação pelos astrócitos o que contribui para o aumento da concentração extracelular de glutamato, levando a uma superestimulação do sistema glutamatérgico (Tavares et al., 2005; Tavares et al., 2002, 2005).

Por outro lado, evidências apontam para um efeito do AQ sobre o metabolismo energético no cérebro. Alterações metabólicas tais como diminuição da captação de glicose, redução do consumo de O_2 mitocondrial, diminuição nos níveis de ATP e NAD^+ e inibição do complexo II no tecido cerebral foram observados em animais tratados com AQ (Bordelon et al., 1997; During et al., 1989; Foster et al., 1983;

Kalonia et al., 2010; Santamaria e Rios, 1993; Schuck et al., 2007; Vezzani et al., 1987). Recentes estudos *in vivo* e *in vitro* mostraram que o AQ é capaz de causar inibição dos complexos mitocondriais I, II e IV da cadeia transportadora de elétrons, bem como estresse oxidativo (Kalonia e Kumar, 2011; Kalonia et al., 2010; Schuck et al., 2007). Evidências experimentais também sugerem que parte da toxicidade induzida pelo AQ envolve mecanismos diretos de geração de radicais livres, estresse oxidativo e peroxidação lipídica (Behan et al., 1999; Santamaria et al., 2001; Stone et al., 2000).

Considerando a potencial capacidade pró-oxidante do AQ, seus efeitos tóxicos têm sido associados à produção de radical hidroxil ($^{\bullet}OH$) no estriado em condições *in vivo*, num mecanismo que parece ser independente de receptores NMDA (Santamaria et al., 2001). Além disso, outros achados também apontam para o envolvimento do AQ na produção de $^{\bullet}NO$ e de $O_2^{-\bullet}$ mostrando sua potencial habilidade em produzir peroxinitrito ($ONOO^-$), uma espécie reativa de nitrogênio altamente tóxica para as células (Noack et al., 1998; Perez-De La Cruz et al., 2005; Ryu et al., 2004).

As alterações acima descritas podem ser atribuídas ao mecanismo primário do AQ sobre os receptores NMDA, mas um efeito secundário do mesmo sobre a produção de energia através da geração de ERO não pode ser descartado (Schuck et al., 2007). Assim, o estresse oxidativo parece ser uma importante alternativa para explicar, mesmo que parcialmente, os eventos neurotóxicos induzidos pelo AQ, já que o uso de antioxidantes tem se mostrado efetivo contra a neurotoxicidade deste composto (Perez-De La Cruz et al., 2005; Perez-Severiano et al., 2004; Santamaria et al., 2003; Tasset et al., 2010).

1.4.2 Ácido 3-nitropropiônico e o modelo do prejuízo no metabolismo energético

O ácido 3-nitropropiônico (3-NP) é uma toxina natural sintetizada por algumas espécies de fungos (*Aspergillus flavus, Astragalus arthrinium)* e plantas (*Indigofera endecapylla)* (Tunez et al., 2010). Entre os anos de 1950 a 1960, o 3-NP foi relacionado a episódios de envenenamento em mamíferos no oeste dos Estados Unidos. Posteriormente, aproximadamente 100 casos de envenenamento com 3-NP foram reportados na China, associados ao consumo de cana-de-açúcar contaminada com o fungo *Arthrinium* (Ludolph et al., 1991). Tais intoxicações foram responsáveis por causar encefalopatia aguda em

adultos e crianças, seguida por casos de distonia e discinesia associados à degeneração do putamen (He et al., 1995).

Estudos em animais de laboratório levaram a caracterização anatomopatológica da toxicidade do 3-NP. No início dos anos 80, Gould e colaboradores demonstraram que o tratamento com 3-NP produzia preferencial degeneração do estriado de ratos e camundongos (Gould e Gustine, 1982; Gould et al., 1985). Posteriormente, foi sugerido que danos neuronais também podiam ocorrer em outras áreas do cérebro, como cerebelo, hipocampo, tálamo e córtex cerebral (Beal et al., 1993; Borlongan et al., 1997; Brouillet et al., 1999).

O 3-NP é capaz de atravessar a barreira hematoencefálica e, portanto, pode causar dano no SNC mesmo após ser administrado sistemicamente por via subcutânea (sc) ou intraperitoneal (ip). Inicialmente, os animais desenvolvem hiperatividade, nas duas primeiras administrações, seguida por hipoatividade a partir da quarta injeção (Borlongan et al., 1997). Tanto a administração sistêmica como intraestriatal é responsável por desenvolver sintomas característicos da DH (Borlongan et al., 1995; Borlongan et al., 1997; Brouillet et al., 1999) como ataxia, movimentos coreiformes, demência, bradicinesia, fraqueza muscular, rigidez, entre outros (Al Mutairy et al., 2010; Silva-Adaya et al., 2008; Tariq et al., 2005).

Diversos estudos vêm caracterizando os efeitos neurotóxicos do 3-NP em modelos animais. Esses trabalhos revelam que a toxicidade do 3-NP *in vivo* pode variar dependendo de múltiplos fatores incluindo espécie animal, sexo, idade ou protocolo de administração (Binienda, 2003; Bizat et al., 2003; Fernagut et al., 2002; Gabrielson et al., 2001; Gopinath et al., 2011; Gould e Gustine, 1982; Ouary et al., 2000; Tasset et al., 2011; Yang et al., 2004). Quando administrado pela via i.p. ou s.c., o 3-NP é mais tóxico para ratos do que para camundongos, bem como a sensibilidade é maior para fêmeas e, de uma maneira geral, é aumenta com a idade (Brouillet et al., 2005).

Administrado sistemicamente, sob condições crônicas, o 3-NP causa lesão bilateral, simétrica e seletiva degeneração do estriado lateral, sendo restrita a área dorso-lateral do caudado-putamen, semelhante ao que ocorre na DH (Blum et al., 2001). Por outro lado, administrações agudas de 3-NP produzem lesões com perda neuronal mais difusa (Tunez et al., 2010), com diminuição da atividade motora, que pode ser seguida por episódios de hiperatividade e movimentos anormais (tremores, movimentos de cabeça, rigidez e elevação de cauda, movimentos em círculo) (Ludolph et al., 1991). Por outro lado, administrações repetidas da toxina (4 semanas) induzem padrão

hipercinético durante as primeiras duas semanas, seguido de pronunciado padrão hipocinético nas últimas semanas de tratamento (Borlongan et al., 1995; Borlongan et al., 1997). Considerando esses achados, o 3-NP pode mimetizar tanto alterações comportamentais vistas no início da doença, como as características das fases mais avançadas (Borlongan et al., 1995), dependendo do tempo de administração.

As projeções neuronais principalmente afetadas pelo 3-NP são neurônios espinhais médios GABAérgicos no estriado (Hassel e Sonnewald, 1995). Dados da literatura demonstraram que a administração de 3-NP em animais de laboratório é capaz de diminuir os níveis de dopamina e a marcação para tirosina hidroxilase (TH) no estriado, sugerindo que as vias dopaminérgicas nigroestriatais são significativamente comprometidas neste modelo (Pei e Ebendal, 1995), (Al Mutairy et al., 2010; Beal et al., 1993; Tariq et al., 2005).

O mecanismo primário de neurotoxicidade do 3-NP está envolvido com a inibição irreversível da enzima mitocondrial SDH, responsável pela oxidação do succinato a fumarato no Ciclo de Krebs e principal constituinte do complexo II da cadeia transportadora de elétrons (Ackrell, 2000; Coles et al., 1979), conforme mencionado previamente (item 1.3.2). O 3-NP possui estrutura química similar ao succinato (substrato da SDH) sendo capaz de bloquear o Ciclo do Krebs, diminuindo a capacidade deste em produzir NADH disponível para o complexo I.. Em 1979, Coles e colaboradores propuseram que o 3-NP é oxidado a nitroacrilato, uma molécula instável que então reage com alguns resíduos no sítio ativo da enzima. Recentemente, Huang e colaboradores (2006) mostraram que o 3-NP, após sofrer oxidação pelo complexo II, forma um aducto covalente com uma arginina no sítio ativo da enzima. o 3-NP bloqueia também o Ciclo do Krebs, diminuindo a capacidade deste em produzir NADH disponível para o complexo I.

Diversos estudos sugerem diferentes mecanismos de toxicidade e morte neuronal induzidos pelo 3-NP, como conseqüência do déficit energético e disfunção mitocondrial devido à inibição do complexo II. Dentre estes estão, prejuízo na fosforilação oxidativa, depleção nos níveis de ATP, diminuição do potencial de membrana mitocondrial, alteração na homeostase do cálcio, geração de ERO, excitotoxicidade e ativação de vias de morte celular (Bizat et al., 2003; Mirandola et al., 2010; Montilla et al., 2004; Rosenstock et al., 2004).

O efeito inibitório do 3-NP sobre a SDH e sua relação com a degeneração estriatal foi bem caracterizado *ex vivo* e indica que a inibição da enzima no estriado é similar a outras regiões do cérebro,

apesar dessa região ser a principal área afetada pela toxina (Alexi et al., 1998; Brouillet et al., 1998). A degeneração estriatal parece ocorrer com uma inibição parcial de 50-60% na atividade da enzima (Alexi et al., 1998; Brouillet et al., 1998). Sob essas condições, estudos *in vitro* utilizando cultura de neurônios demonstraram que a disfunção mitocondrial induzida pelo 3-NP está relacionada com uma rápida diminuição nos níveis de ATP (Liot et al., 2009), perda do potencial de membrana (Lee et al., 2002; Maciel et al., 2004) e aumento na produção de ERO (Liot et al., 2009).

Em 1987, Hamilton e Gould levantaram a hipótese de que a toxicidade mediada pelo glutamato poderia desempenhar um papel importante na degeneração estriatal induzida pelo 3-NP (Hamilton e Gould, 1987). Posteriormente,Novelli e colaboradores (1988) estabeleceram que a disfunção no metabolismo energético pudesse desencadear mecanismos de excitotoxicidade. Esses autores mostraram que a disfunção no metabolismo oxidativo e na enzima Na^+/K^+-ATPase eram capazes de aumentar a neurotoxicidade do glutamato (Novelli et al., 1988).

A hipótese de uma excitotoxicidade indireta ou secundária sugere que a degeneração estriatal induzida pelo 3-NP é, primeiramente, devido a uma depleção nos níveis de ATP produzido pelo déficit no metabolismo energético que diminui a atividade da enzima Na^+/K^+-ATPase e causa despolarização da membrana plasmática, liberando o bloqueio pelos íons Mg^{2+} nos receptores NMDA com conseqüente influxo de Ca^{2+} e Na^{2+} (Beal et al., 1993; Novelli et al., 1988; Perez-De La Cruz e Santamaria, 2007). Sob essas condições, o 3-NP causa excitotoxicidade por tornar os neurônios mais sensíveis a níveis basais de glutamato (Perez-De La Cruz e Santamaria, 2007), produzindo morte neuronal (Pang e Geddes, 1997). Esses achados foram confirmados por Liot e colaboradores (2009), que a excitotoxicidade induzida pelo 3-NP em células neuronais, é capaz de causar uma maior formação de ERO, bem como, significativa fragmentação mitocondrial e morte neuronal, e que o tratamento com um antagonista de receptores NMDA foi capaz de bloquear esses efeitos. Juntos, estes estudos nos fornecem evidências de que a "excitotoxicidade secundária" pode desempenhar importante papel no mecanismo de morte celular induzido pelo 3-NP.

O estresse oxidativo ocasionado pelo aumento na produção de espécies reativas está bem definido no mecanismo de toxicidade induzido pelo 3-NP. De particular importância, um recente estudo mostrou que o tratamento com 3-NP foi capaz de induzir um aumento bifásico na produção de ERO (Liot et al., 2009). Esses autores

demonstraram que o 3-NP induz um rápido aumento na produção de ERO em células neuronais trinta minutos após a exposição, seguido por um segundo pico de ERO algumas horas após (Liot et al., 2009), devido a ativação de receptores NMDA.

Aumentos na geração de ERO, como o $O_2^{\bullet-}$, o H_2O_2 e o $^{\bullet}OH$ foram reportados em estudos *in vitro* e *in vivo* (Bacsi et al., 2006; Liot et al., 2009; Mandavilli et al., 2005; Sandhir et al., 2010). Além disso, marcadores de estresse oxidativo como produtos de peroxidação lipídica (malondialdeído e 4-hidroxinonenal), dano oxidativo ao DNA (8-hidroxi-2-deoxiguanosina) e proteínas carboniladas, foram encontrados em níveis elevados em regiões do cérebro de ratos tratados com 3-NP (Halliwell, 2006; Moncada e Bolanos, 2006; Santamaria et al., 2001), indicando a relação do estresse oxidativo com as manifestações de neurotoxicidade induzidas pela toxina. A administração sistêmica de 3-NP em roedores também pode causar diminuição nos níveis de glutationa (Al Mutairy et al., 2010; Sandhir et al., 2010), depleção das enzimas antioxidantes superóxido dismutase (SOD) (Bhateja et al., 2012; Sandhir et al., 2010) e catalase (Bhateja et al., 2012; Kumar et al., 2012).

Muitos estudos também apontam para o aumento na produção de espécies reativas de nitrogênio (ERN) derivadas do óxido nítrico ($^{\bullet}NO$), através da estimulação da enzima óxido nítrico sintase (NOS) (Deshpande et al., 2006). O tratamento com 3-NP causa aumento do influxo de Ca^{2+} através dos receptores NMDA e conseqüente ativação da NOS. O $^{\bullet}NO$ pode se combinar com ânion superóxido ($O_2^{\bullet-}$) para formar o radical peroxinitrito ($ONOO^-$), uma espécie altamente reativa capaz de induzir citotoxicidade tanto pela nitração de proteínas como pela formação de $^{\bullet}OH$ (Tunez et al., 2010). Tanto o $^{\bullet}NO$ como $ONOO^-$ podem amplificar o inicial bloqueio do complexo II pelo 3-NP, bem como induzir fragmentação mitocondrial (Liot et al., 2009) e consequente morte neuronal. O mecanismo de morte induzido pela toxina também parece estar relacionado com aumentos nas concentrações de Ca^{2+} intracelulares e ativação de caspases e calpaínas (Tunez et al., 2010), resultando em morte celular tanto por necrose como apoptose (Pang e Geddes, 1997; Sandhir et al., 2010; Sato et al., 1997) (Figura 4).

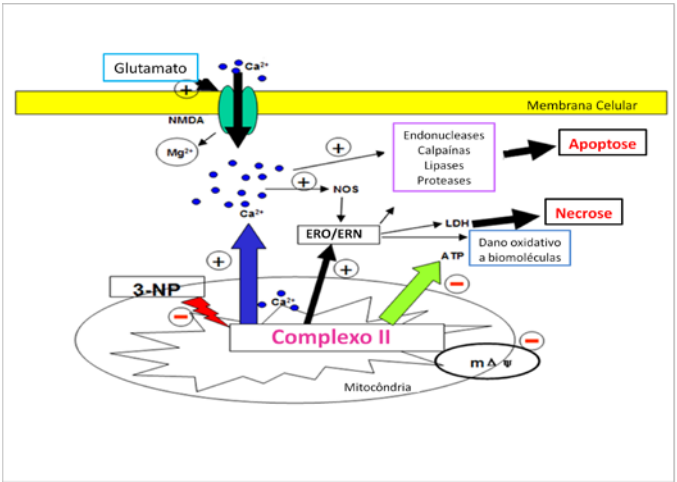

Figura 4: Neurotoxicidade induzida pelo 3-NP**.** O 3-NP inibe o complexo II da cadeia transportadora de elétron causando diminuição na produção de ATP, alterações no potencial de membrana mitocondrial e aumento na produção de espécies reativas. A disfunção no metabolismo energético induzida pelo 3-NP pode desencadear excitotoxicidade secundária por tornar os neurônios mais sensíveis a níveis basais de glutamato. A despolarização da membrana plasmática libera o bloqueio exercido pelos íons Mg^{2+} nos receptores NMDA com conseqüente influxo de Ca^{2+} e ativação de vias morte celular (Adaptado de Tunez et al., 2010).

1.4.3 Ácido quinolínico e ácido 3-nitropropiônico: o modelo combinado

Recentemente foi demonstrado que o déficit energético induzido pelo 3-NP, adicionado a uma moderada toxicidade do AQ, produz uma sinérgica degeneração estriatal, num mecanismo que envolve desregulação no cálcio intracelular (Jacquard et al., 2006; Perez-De La Cruz et al., 2008). Essas evidências estão de acordo com estudos realizados por Pérez-De La Cruz e colaboradores (2010) que demonstraram o importante papel do estresse oxidativo e do déficit energético na morte celular induzida pelo modelo combinado em fatias de estriado de ratos. Além disso, a potenciação dos efeitos tóxicos do AQ pelo tratamento com 3-NP parece ser dependente da porcentagem de inibição do complexo II pelo 3-NP. Jacquard e colaboradores (2006) observaram que a toxicidade do AQ é apenas aumentada quando ocorre uma inibição do complexo II maior do que 35% pelo 3-NP.

Apesar da hipótese da excitotoxicidade secundária ser frequentemente sugerida para explicar como o comprometimento energético potencia ou desencadeia eventos excitotóxicos, achados prévios mostraram que a hipersensibilidade dos receptores NMDA ao AQ parece não aumentar em animais tratados com 3-NP. Neste mesmo trabalho, os autores observaram que o aumento no influxo de Ca^{2+} induzido pelo AQ em cultura de neurônios estriatais não foi aumentado pelo 3-NP, sugerindo a ausência de hipersensibilização de receptores NMDA. Esses achados sugerem que a disfunção mitocondrial que potencia a excitotoxicidade via receptores NMDA, parece não envolver o mecanismo de excitotoxicidade "indireta" ou "secundária". Na verdade, a inibição do complexo II pelo 3-NP pode modificar a capacidade da mitocôndria em captar o Ca^{2+} citosólico. Dessa forma, o tratamento com doses subtóxicas de 3-NP poderia alterar a habilidade da mitocôndria em tamponar o cálcio citosólico que entra através dos receptores NMDA mediado pelo AQ (Jacquard et al., 2006). Isso foi comprovado por um estudo mais recente onde se observou que o Ca^{2+} intracelular parece estar mais envolvido com o dano oxidativo do que o de origem extracelular (Perez-De La Cruz et al., 2008).

Além disso, a ativação de caspases e calpaínas também parece desempenhar um importante papel na toxicidade induzida pela associação de AQ e 3-NP (Perez-De la Cruz et al., 2010). Finalmente, outros mecanismos comuns de dano dessas toxinas como, por exemplo, o potencial inflamatório do AQ aliado a sua ação sobre receptores NMDA, bem como seu efeito pró-oxidante, juntamente com a inibição irreversível do complexo II desempenhada pelo 3-NP, acabam contribuindo para a geração desse modelo peculiar de toxicidade (Tunez et al., 2010). Assim, o mecanismo de toxicidade do modelo combinado parece envolver diferentes eventos e vias de sinalização. Esta questão ainda encontra-se sob investigação e o preciso mecanismo de toxicidade deste modelo ainda não está totalmente elucidado. Porém, vale ressaltar que a disfunção no metabolismo energético e a excitotoxicidade, dois mecanismos comuns na patogênese da DH, quando aliados, torna a associação entre AQ e 3-NP uma ferramenta "mais completa" e precisa como um modelo da patologia de Huntington (Tunez et al., 2010).

1.5 Tratamento da Doença de Huntington e busca de novas terapias

Até o presente momento não existe tratamento efetivo para a DH. Os medicamentos são utilizados, principalmente, com o objetivo de controlar os sintomas motores e melhorar a qualidade de vida dos

pacientes e possuem benefício limitado, não conseguindo conter a progressão da doença.

Sintomas motores como a coréia são tradicionalmente tratados com bloqueadores de receptores de dopamina (neurolépticos típicos e atípicos) ou com seus depletores (tetrabenazina) (Roos, 2010). Neurolépticos típicos, incluindo haloperidol, pimozida, flufenazina, tioridazina, risperidona entre outros são utilizados para o tratamento da coréia e também dos sintomas psicóticos (Bonelli e Hofmann, 2007). Esses medicamentos possuem alta afinidade pelos receptores de dopamina D2, podendo induzir alguns efeitos colaterais como, por exemplo, sintomas parkinsonianos e discinesia tardia, e devem apenas ser utilizados em casos de extrema necessidade. Muitos neurolépticos atípicos, como a clozapina e olanzapina, também são utilizados no tratamento da coréia e dos sintomas psiquiátricos (Adam e Jankovic, 2008).

A tetrabenazina, por sua vez, é atualmente considerada um dos mais efetivos agentes na redução da coréia, sugerindo uma boa tolerabilidade entre os pacientes (Fasano et al., 2008; Frank, 2009; Kenney et al., 2007). É o único medicamento aprovado pela *Food and Drug Administration* nos Estados Unidos para o tratamento da coréia associada à DH. Por outro lado, alguns efeitos adversos foram reportados em tratamentos com a tetrabenazina incluindo insônia, sonolência, depressão, agitação e hipercinesia (Frank, 2010).

Além desses medicamentos, outros tratamentos clínicos podem ser utilizados como antagonistas glutamatérgicos (como amantadina e memantina), inibidores da acetilcolinesterase (rivastigmina e galantamina, para o tratamento da disfunção cognitiva), anticonvulsivantes, entre outros (Adam e Jankovic, 2008; Imarisio et al., 2008; Phillips et al., 2008; Roze et al., 2008). Terapias com antidepressivos são também bastante freqüentes na DH. Os mais prescritos atualmente são amitriptilina, imipramina e antidepressivos tricíclicos (Adam e Jankovic, 2008).

Considerando que os medicamentos utilizados para o tratamento da DH representam uma alternativa meramente paliativa, que proporciona benefícios sintomáticos e discretos sobre os sintomas clínicos da doença, diversas moléculas estão sendo testadas em busca de um tratamento efetivo capaz de retardar a progressão da doença. Neste contexto, a terapia com antioxidantes que é evidentemente efetiva em modelos animais da doença (referncias), é hoje considerada uma estratégia terapêutica para a busca de novos fármacos para o tratamento da patologia de Huntington.

A Coenzima Q10 (CoQ10) é um composto antioxidante que vêm demonstrando eficácia como agente neuroprotetor em modelos animais da DH (Beal et al., 1994; Kasparova et al., 2006; Stack et al., 2006; Yang et al., 2009). Baseado na sua eficácia pré-clínica favorável, a CoQ10 tem também demonstrado resultados positivos em testes em humanos com boa tolerabilidade e segurança (*Huntington's Disease Collaborative Research Group*, 2001; Feigin et al., 1996; Hyson et al., 2010; Koroshetz et al., 1997).

Estudos têm sugerido que a melatonina, um composto *scavenger* de espécies reativas, pode desempenhar um papel terapêutico em doenças neurodegenerativas (Reiter et al., 1999; Stack et al., 2008; Uz et al., 1996). Suas propriedades antioxidantes foram relacionadas ao seu efeito protetor sobre a neurodegeneração induzida em modelos *in vivo* da DH induzidos pelo 3-NP. A melatonina foi capaz de diminuir a peroxidação lipídica e o dano oxidativo ao DNA, bem como aumentou a atividade da enzima antioxidante SOD nesse modelo (Tunez et al., 2004).

Outro composto antioxidante estudado é o ácido lipóico, um co-fator essencial para muitos complexos enzimáticos que tem sido usado no tratamento de doenças associadas com disfunção energética (Henriksen, 2006; Packer et al., 1997). Esse composto se mostrou efetivo em dois modelos genéticos da DH (R6/2 e N171-82Q), aumentando a sobrevivência e prevenindo a perda de peso desses animais (Andreassen et al., 2001). Efeitos neuroprotetores similares também foram demonstrados com o selênio, um elemento essencial para a atividade da enzima GPx. Esse composto foi capaz de melhorar déficits comportamentais, evitar alterações na morfologia neuronal e diminuir a peroxidação lipídica no estriado de animais tratados com AQ (Santamaria et al., 2003).

Apesar de muitos compostos antioxidantes demonstrarem potencial neuroprotetor em modelos animais, até o presente momento, a maioria deles ainda não foram aplicados em testes clínicos em humanos. Dentre estes, o α-tocoferol, ácido ascórbico, creatina já foram testados tanto em animais como em humanos, demonstrando seus benefícios terapêuticos para a DH (Hersch et al., 2006; Peyser et al., 1995; Tabrizi et al., 2003). Um estudo realizado por Peyser e colaboradores, em pacientes com DH com sintomatologia leve à moderada, demonstrou que o tratamento com α-tocoferol, apesar de não ter efeito sobre os sintomas neurológicos e neuropsiquiátricos, diminuiu a taxa de declínio motor no estágio inicial da doença (Peyser et al., 1995).

A creatina é outro candidato com potencial terapêutico para o tratamento de doenças neurodegenerativas como a DH (Beal, 2011). Esse composto além de possuir atividade antioxidante, é um precursor energético que apresentou atividade neuroprotetora em modelos animais da doença (Andreassen et al., 2001; Dedeoglu et al., 2003; Ferrante et al., 2000; Matthews et al., 1998; Shear et al., 2000). A creatina mostrou-se segura, bem tolerável e suas concentrações no cérebro foram aumentadas em pacientes que receberam 10 g/dia durante 12 meses. Além disso, o tratamento com creatina não modificou os escores dos pacientes no *The Unified Huntington Disease Rating Scale (UHRS)*, uma escala desenvolvida para avaliar funções motoras, cognitivas e alterações comportamentais dos pacientes (Siesling et al., 1998), demonstrando que esse composto pode ser efetivo em estabilizar a progressão da doença (Tabrizi et al., 2003). Outro estudo também demonstrou que a creatina (8 g/dia durante 16 semanas) foi bem tolerada, segura e diminuiu os níveis de 8-hidroxi-2-deoxiguonosina, um marcador de dano oxidativo ao DNA que é significativamente elevado em pacientes com a doença (Hersch et al., 2006). Esses achados estão de acordo com estudos realizados em modelos animais transgênicos da doença, onde esse composto mostrou-se efetivo em melhorar o desempenho motor, reduzir a agregação da proteína huntingtina mutante e a atrofia no estriado desses animais (Ferrante et al., 2000).

Considerando o importante papel do estresse oxidativo na patogênese da doença de Huntington, a terapia com moléculas antioxidantes é uma estratégia terapêutica que pode ser efetiva na tentativa de retardar a progressão da doença e melhorar a qualidade de vida dos pacientes.

1.6 Probucol

O probucol é um composto fenólico que foi desenvolvido nos Estados Unidos pela *Consolidation Coal Company* em 1970 (Figura 6). Inicialmente, foi utilizado como antioxidante durante o processo de fabricação da borracha. Posteriormente, as propriedades biológicas do probucol foram descritas por bioquímicos da empresa *Dow Chemical Company*, os quais verificaram os efeitos do mesmo sobre a redução dos níveis plasmáticos de colesterol (Barnhart et al., 1970).

Este composto tem uma longa história de aplicação clínica com eficácia estabelecida e perfis de segurança. Entretanto, em 1995, 18 anos após a introdução do probucol na clínica, vários países interromperam o seu uso em decorrência dos efeitos colaterais desta substância. Neste

período, as agências controladoras retiraram o probucol do mercado devido ao efeito do mesmo sobre a redução dos níveis plasmáticos da lipoproteína de alta densidade (HDL) e também devido à ocorrência de arritmias ventriculares nos usuários deste composto (Tardif et al., 2002). Apesar da demonstração de efeitos colaterais, as propriedades farmacológicas do probucol continuam sendo avaliadas em diversos estudos clínicos (Poirier, 2005; Yamamoto, 2008) e o mesmo é utilizado clinicamente no Japão desde 1985, sendo que atualmente pelo menos 60 mil pacientes japoneses utilizam esse composto como medicamento. Neste contexto, estudos toxicológicos adicionais são necessários para reposicionar o probucol como uma substância terapêutica, visto que os estudos disponíveis acerca dos efeitos colaterais causados pelo mesmo são contraditórios (Tardif et al., 2002; Tardif et al., 2003; Yamashita et al., 2008).

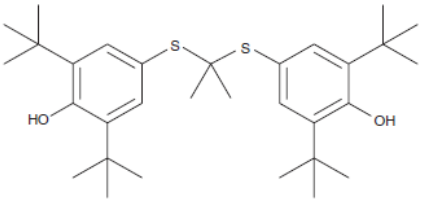

Figura 5. Estrutura química do probucol.

1.6.1 Propriedades farmacológicas

A principal indicação terapêutica do probucol, nas últimas décadas, está associada com o tratamento da aterosclerose e, consequentemente, com a prevenção de doenças cardiovasculares (Yamashita e Matsuzawa, 2009). Estudos experimentais e clínicos têm demonstrado que o probucol diminui a progressão da aterosclerose (Regnstrom et al., 1996), protege a vasculatura (Poirier, 2003), inibe a trombose (Tanous et al., 2006) e atua como uma molécula antioxidante (Liu et al., 2000; Singla et al., 2007). Além disso, um estudo demonstrou que o tratamento por via oral com baixas doses de probucol produz significativos efeitos antioxidantes e reduz os níveis de radicais livres no sangue de pacientes com doença arterial coronariana (Kaminnyi et al., 2007).

Em humanos, o probucol retarda a aterosclerose e diminui a incidência de doenças cardíacas em pacientes hipercolesterolêmicos (Sawayama et al., 2002). Um estudo recente sugeriu que o tratamento com probucol por um longo período previne ataques cardíacos em população japonesa com hipercolesterolemia familiar, sem provocar efeitos adversos significativos (Yamashita et al., 2008).

Além das propriedades sobre o sistema cardiovascular, estudos sugerem que o probucol aumenta a neurogênese (Champagne et al., 2003), modula a atividade de enzimas antioxidantes (Farina et al., 2009) e possui propriedades anti-inflamatórias (Pfuetze e Dujovne, 2000; Uehara et al., 1991). Além disso, um trabalho do nosso grupo de pesquisa (Farina et al., 2009) demonstrou que o probucol é capaz aumentar a atividade da enzima glutationa peroxidase (isoenzima 1) em cultura primária de neurônios cerebelares, sendo que este fenômeno foi responsável pela proteção contra o dano oxidativo induzido por metilmercúrio, um neurotoxicante que induz elevada produção (Franco et al., 2007) e deficiente detoxificação (Farina et al., 2003) de peróxido de hidrogênio. De particular importância, outro recente estudo do nosso grupo demonstrou o efeito protetor do probucol num modelo de doença de Alzheimer induzido por Aβ 1-40 em camundongos. Nesse estudo, o probucol foi efetivo em recuperar a perda sináptica e a disfunção cognitiva induzida pelo βA1 - 40, onde esses efeitos foram atribuídos, ao menos em parte, a sua atividade antioxidante (Santos et al., 2011).

1.6.2 Compostos derivados do Probucol

Alguns derivados do probucol tais como o composto succinobucol (AGI-1067), têm apresentado elevada eficácia terapêutica sem causar os efeitos colaterais decorrentes do uso do probucol (Tanous et al., 2008). O succinobucol (Figura 7) é um éster monosuccínico derivado do probucol que retêm as propriedades antioxidantes deste composto (Meng et al., 2002; Sundell et al., 2003). Estudos demonstraram que o succinobucol previne o desenvolvimento da aterosclerose e possui um efeito benéfico sobre o perfil lipídico plasmático em diferentes modelos experimentais (Sundell et al., 2003). Da mesma forma, um estudo com pacientes cardiopatas demonstrou que o succinobucol é capaz de reduzir significativamente a incidência de infarto do miocárdio e o acidente vascular cerebral (Tardif et al., 2008). Estudos pré-clínicos demonstram que o succinobucol não tem somente efeito antioxidante, mas também possui propriedade anti-inflamatória (Kunsch et al., 2004; Luyendyk et al., 2007) e antidiabética, sendo capaz

de reduzir os níveis de hemoglobina glicada e de glicose de jejum (Tardif et al., 2008). Além disso, esse composto apresentou efeitos benéficos frente à condição de resistência à ação da insulina (Crim et al., 2010). O succinobucol também é capaz de inibir vias de sinalização relacionadas com o processo inflamatório, bem como diminuir a expressão de moléculas de adesão e citocinas inflamatórias em diferentes tipos celulares, incluindo células endoteliais e macrófagos (Kunsch et al., 2004; Luyendyk et al., 2007).

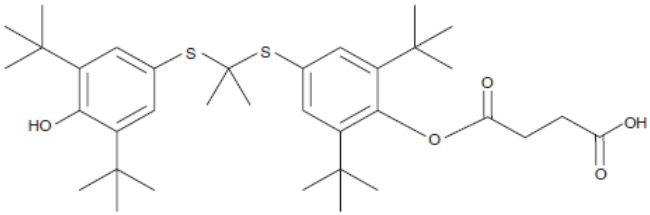

Figura 6: Estrutura química do succinobucol.

2. JUSTIFICATIVA

Apesar de animais transgênicos representarem um modelo mais próximo à condição humana da DH, pela presença da proteína mutante huntingtina, os modelos que empregam o AQ e o 3-NP são de extrema importância para compreender os mecanismos de neurodegeneração da doença, já que esses compostos são capazes de mimetizar alterações comportamentais e bioquímicas observadas em pacientes (Tunez et al., 2010). É evidente que esses modelos possuem limitações, principalmente por não reproduzirem as características genotípicas da doença, embora possam reproduzir o fénotipo da mesma. Vale a pena ressaltar ainda que diversos mecanismos que hoje são compreendidos no desenvolvimento da doença surgiram a partir de estudos em modelos animais *não-genéticos* (Borlongan et al., 1995; Coyle e Schwarcz, 1976; Gould e Gustine, 1982; Gould et al., 1985; Ramaswamy et al., 2007).

A DH é uma patologia neurodegenerativa que está diretamente associada a disfunção no metabolismo energético e ao estresse oxidativo (Zuccato et al., 2010). Considerando o papel importante do estresse oxidativo na patogênese da DH, moléculas antioxidantes têm atraído

considerável atenção da comunidade científica e da indústria farmacêutica como potenciais agentes terapêuticos no tratamento dessa doença (Stack et al., 2008).

Neste sentido, o interesse por estudos químicos e bioquímicos de compostos antioxidantes como o probucol e do seu analógo succinobucol são relevantes, principalmente devido ao fato de que esses compostos apresentam importante atividade antioxidante e anti-inflamatória em outros modelos experimentais. O probucol demonstrou efeito protetor em modelo experimental de doença de Alzhemeir e de isquemia cerebral (Park et al., 2007; Santos et al., 2011). Entretanto, ainda não se sabe seu real efeito neuroprotetor frente a toxicidade e dano oxidativo induzido por mecanismos de excitotoxicidade e disfunção no metabolismo energético. Na verdade, estudos já demonstraram que o probucol protege mitocôndrias isoladas de coração contra o estresse oxidativo (Lemieux et al., 2011), mas seu papel na disfunção mitocondrial no tecido cerebral ainda não foi determinada. Ainda, considerando o importante efeito do probucol em modular a GPx em cultivos de neurônios cerebelares (Farina et al., 2009), torna-se relevante o estudo deste composto em modelos experimentais da DH, uma vez que a GPx e a geração de H_2O_2 parecem estar envolvidos no cenário de estresse oxidativo observado em tal patologia (Hands et al., 2011; Sorolla et al., 2008). Além disso, nenhum estudo investigou, até o presente momento, potenciais efeitos protetores do seu análogo succinobucol no sistema nervoso central. Esse composto desperta atenção porque mantem a atividade antioxidante do probucol sem seus efeitos colaterais, podendo ser uma importante estratégia terapêutica em modelos experimentais de doenças neurodegenerativas.

Tendo em vista que (i) existe um elevado dano oxidativo em determinadas estruturas encefálicas de pacientes com a DH; (ii) ambos os modelos experimentais da DH (3-NP e AQ), assim como o modelo combinado, causam estresse oxidativo; (iii) compostos antioxidantes demonstram papel protetor frente à toxicidade induzida por AQ e 3-NP; (iv) o composto probucol é um potente antioxidante e já demonstrou efeito protetor em modelos de neurotoxicidade/neuropatologia e (v) ainda há uma necessidade de efetivos tratamentos terapêuticos para a DH, acredita-se que a busca de possíveis agentes com atividades neuroprotetoras nestes modelos são amplamente justificáveis. O presente trabalho visa a contribuir com conhecimento básico que pode auxiliar no desenvolvimento de futuros estudos farmacológicos clínicos visando um possível (co) tratamento para retardar a progressão da DH e para melhorar a qualidade de vida dos indivíduos com esta patologia.

3. OBJETIVOS

3.1 Objetivo Geral

O objetivo geral deste trabalho foi investigar o possível efeito protetor do probucol frente à neurotoxicidade e estresse oxidativo em modelos experimentais da Doença de Huntington (*in vitro* e *in vivo*), buscando elucidar possíveis mecanismos de ação.

3.2 Objetivos específicos

(i) Investigar o papel do dano oxidativo nos efeitos neurotóxicos induzidos pelo ácido 3-nitropropiônico, ácido quinolínico e pela associação de ambos em fatia de estriado de ratos;

(ii) Investigar o possível efeito protetor do probucol contra a neurotoxicidade induzida pela administração intraperitoneal do 3-NP em ratos através de parâmetros comportamentais e bioquímicos, bem como o possível mecanismo de neuroproteção;

(iii) Investigar o potencial efeito protetor do probucol e do seu análogo succinobucol sobre a disfunção mitocondrial e estresse oxidativo induzidos pelo 3-NP em preparações mitocondriais de cérebro de ratos, bem como o possível mecanismo de neuroproteção.

4. RESULTADOS

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigo e manuscritos em fase de preparação. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios artigo e manuscritos. O artigo 1 está na forma como foi publicado na revista. O manuscrito 1 está em fase de preparação e não apresenta discussão dos resultados. Uma breve discussão geral de todos os resultados desta dissertação é apresentada no item 5. O manuscrito 2 está na forma como será submetido à revista.

4.1 PROBUCOL MODULA ESTRESSE OXIDATIVO E EXCITOTOXICIDADE EM MODELOS *IN VITRO* DA DOENÇA DE HUNTINGTON

Artigo 1

PROBUCOL MODULATES OXIDATIVE STRESS AND EXCITOTOXICITY IN HUNTINGTON'S DISEASE MODELS *IN VITRO*

Dirleise Colle, Juliana M. Hartwig, Félix A. Antunes Soares, Marcelo Farina

Brain Research Bulletin xxx (2012) xxx-xxx

G Model
BRB-8460; No. of Pages 9

ARTICLE IN PRESS

Brain Research Bulletin xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro

Dirleise Colle[a,*], Juliana M. Hartwig[a], Félix A. Antunes Soares[b], Marcelo Farina[a,*]

[a] Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil
[b] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:
Received 21 October 2011
Received in revised form
27 December 2011
Accepted 1 January 2012
Available online xxx

Keywords:
Huntington's disease
3-Nitropropionic acid
Quinolinic acid
Probucol
Mitochondrial dysfunction
Oxidative stress

ABSTRACT

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease characterized by symptoms attributable to the death of striatal and cortical neurons. The molecular mechanisms mediating neuronal death in HD seem to be related to oxidative stress, excitotoxicity and misbalance in energetic metabolism. In this study we evaluated the potential relationship between energetic impairment, excitotoxicity and oxidative stress in rat striatal slices exposed to quinolinic acid (QA; as an excitotoxic model), 3-nitropropionic acid (3-NP; as an inhibitor of mitochondrial succinate dehydrogenase), as well as a combined model produced by the co-administration of these two toxins at subtoxic concentrations. We took advantage of the direct antioxidant/scavenger properties of Probucol in order to investigate the role of reactive oxygen species (ROS) in mediating the toxicity of both compounds alone or in association. Experiments with MK-801 (a NMDA type glutamate receptor antagonist) and succinate (an energy precursor agent) were also performed in an attempt to better comprehend the mechanisms of damage and neuroprotection. QA (1 mM), 3-NP (1 mM) and QA plus 3-NP (0.1 mM of both) significantly induced mitochondrial dysfunction and produced an increase in ROS generation, as well as a significant increase in lipid peroxidation in striatal slices. Probucol (10 and 30 μM) prevented ROS formation and lipid peroxidation in all used models, but did not protect against the mitochondrial dysfunction induced by 3-NP (only by QA or QA plus 3-NP). Sodium succinate (1 mM) protected the striatal slices only against 3-NP-induced mitochondrial dysfunction. On the other hand, MK-801 protected against mitochondrial dysfunction in all used models. Our data suggest that the two studied toxic models (QA and 3-NP) or the combined model (QA plus 3-NP) can generate complex patterns of damage, which involve metabolic compromise, ROS formation, and oxidative stress. Moreover, a partial inhibition of SDH by subtoxic 3-NP and moderate excitotoxicty by subtoxic QA are potentiated when both agents are associated. The toxic action of QA plus 3-NP seems to be involved with Ca^{2+} metabolism and ROS formation, and can be prevented or attenuated by antioxidant/scavenger compounds and NMDAr antagonists.

1. Introduction

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disorder caused by an abnormal expansion of CAG repeat located in exon 1 of the gene encoding for the Huntingtin protein [8,58]. The CAG repeat expansion leads to an abnormal polyglutamine (polyQ) tract in mutant Htt (mHtt) N-terminal region, which triggers a variety of aberrant interactions leading to pathological gain of toxic functions as well as loss of normal functions [7,59,65]. Moreover, the polyQ expansion can cause conformational changes in the mutant protein leading to intranuclear and intracytoplasmic insoluble aggregates or inclusions, which seem to play important roles in HD pathogenesis [12,37].

HD symptoms consist of motor, cognitive and psychiatric disturbances [58], which are attributable to the death of medium spiny GABAergic striatal neurons and, to a lesser extent, cortical neurons

Abbreviations: DCF, 2′,7′-dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; HD, Huntington's disease; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KRB, Krebs–Ringer bicarbonate buffer; LP, lipid peroxidation; MDA, malonaldehyde-bis-dimethyl acetal; mHtt, mutant huntingtin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMDAr, N-methyl D-aspartate receptors; 3-NP, 3-nitropropionic acid; QA, quinolinic acid; PB, Probucol; RNS, reactive nitrogen species; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TBA, thiobarbituric acid; TBA-RS, thiobarbituric acid-reactive substances.

* Corresponding authors at: Departamento de Bioquímica, Centro de Ciências Biológicas, Campus Universitário, Trindade, Universidade Federal de Santa Catarina, CEP 88040-900, Florianópolis, Santa Catarina, Brazil. Tel.: +55 4837219589; fax: +55 4837219672.
E-mail addresses: dirleise@yahoo.com.br (D. Colle), farina@ccb.ufsc.br (M. Farina).

0361-9230/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2012.01.003

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

[33,55]. Several lines of evidence have proposed that the striatal cell death observed in studies of HD pathogenesis in humans and animal models is mediated by a misbalance in energetic metabolism, as well as oxidative stress and excitotoxicity [8,10,42].

Different genetic and toxin-based protocols have been developed to induce HD-like symptoms in laboratory animals [55]. Of particular importance, the administration of either quinolinic acid (QA) or 3-nitropropionic acid (3-NP) to rodents and non-human primates represents useful experimental models of HD; both biochemical and behavioral characteristics observed in HD patients are reproduced in these models [20,56,60,70].

QA (2,3-pyridinedicarboxylic acid), a tryptophan metabolite at the kynurenine pathway in glial cells, is a well-known agonist of the N-methyl D-aspartate (NMDA) type glutamate receptors that typically produces excitotoxic damage [54,66]. Given its endogenous nature, QA itself has been directly implicated as a potential pathogenic factor in HD [71], since it has been recently demonstrated that neostriatal and cortical levels of this toxicant is significantly enhanced in postmortem brains from HD patients at early stages of the disease [22,77]. QA has been currently shown to exert selective striatal toxicity by means of excitotoxic, pro-inflammatory and oxidative mechanisms [26,29,57,61], and antioxidant compounds have been reported to protect against QA-induced damage [4]. In addition, recent in vivo and in vitro studies showed that QA also causes brain energy impairment, resulting in inhibition of the mitochondrial complexes I, II and IV, as well as oxidative stress [28,62].

3-Nitropropionic acid (3-NP) is a mitochondrial toxin that has been found to effectively produce HD-like symptoms in animals models [35,64,70]. The primary mechanism of 3-NP-induced neurotoxicity involves irreversible inhibition of succinate dehydrogenase (SDH), a key enzyme located at the inner mitochondrial membrane and responsible for succinate oxidation to fumarate [31,70]. SDH inhibition interferes with mitochondrial electron transport cascade and oxidative phosphorylation, which leads to cellular energy deficit [32]. 3-NP treatment causes depletion of ATP levels, alteration in calcium homeostasis, generation of reactive oxygen species (ROS) and neuronal death [34,36,44,50]. Interestingly, some studies have demonstrated that 3-NP-induced neuronal death may also occur as result of excitotoxic events [46,51], which likely represent a secondary response to a primary energetic deficit.

More recently, an emerging line of research has provided interesting models to study integrative toxic events occurring in neurodegenerative disorders, including HD [14,15]. These models comprehend the facilitation of excitotoxic events through the impairment of energy metabolism, and are produced by combination of toxic molecules in different biological systems and under different experimental conditions [70]. Recently, evidence showed that the energy impairment induced by 3-NP, added by a moderate toxic action of QA, produced synergic increase of striatal degeneration, in a mechanism involving intracellular calcium deregulation [24]. These evidences corroborate data from the studies of Pérez-De La Cruz and coworkers, who demonstrated that, in the combined model, both oxidative stress and energy deficit are likely synergically contributing to cell death in slices of striatum [53]. From a molecular point of view, it is noteworthy that impairment in energy metabolism and excitotoxicity, two common elements in HD, seem to affect each other and involve a significant increase in ROS generation and oxidative stress, which modulate pathways mediating neuronal death: the interesting integrative hypothesis for HD is proposed and discussed by Pérez-De La Cruz and Santamaría [51]. Furthermore, some lines of evidence indicate that antioxidants and energy precursor agents may reduce neuronal death in HD models [16,25,68]. Although these different events, namely (i) oxidative stress, (ii) excitotoxicity and/or (iii) energetic deficits affect each other and seem to mediate neuronal death in experimental models of HD [51], the relationship between them in either QA- or 3-NP-based models is not completely understood. In addition, to the best of our knowledge, the understanding about such relationship is significantly scarcer in combined models (e.g., QA plus 3-NP).

Probucol (PB) is a phenolic lipid-lowering agent with antioxidant properties that had been clinically used during the past few decades for the treatment and prevention of cardiovascular diseases [11,74,75]. Of particular importance, previous experimental studies have reported that Probucol plays protective effects in experimental models of neurotoxicity/neuropathology [18,49]. Although the beneficial effects of Probucol under in vivo conditions are mediated by its hypocholesterolemic and anti-inflammatory properties [74], its beneficial roles under short-term incubations in in vitro models are likely related to its direct antioxidant (scavenger) properties [73].

Taking into account that (i) the combined model of HD (QA plus 3-NP) represents an useful tool in studding events mediating HD pathogenesis and that (ii) the potential relationship between energetic impairment, excitotoxicity and oxidative stress in the QA plus 3-NP-based model is not completely understood, we took advantage of the direct antioxidant/scavenger properties of Probucol to comprehend the role of ROS in the neurotoxic effects of QA plus 3-NP, as well as in the synergistic relationship between both challenges. Because of the relevant contribution of astrocytes in the combined model [51], striatal slices were used in this study since neuronal-glial interactions are preserved, thereby resembling the physiological conditions of the brain in a more integrative manner. Markers of energetic metabolism and oxidative stress were evaluated in the slices exposed to 3-NP, QA and/or Probucol in order to investigate the role of ROS in mediating the toxicity of both compounds alone or in association. Additional studies using the NMDA type glutamate receptor antagonist MK-801 and the energy precursor agent succinate were also performed in an attempt to better comprehend the mechanisms of damage and neuroprotection.

2. Methods

2.1. Chemicals

3-Nitropropionic acid, quinolinic acid, Probucol, MK-801, sodium succinate, thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma (St. Louis, MO, USA). All other reagents were obtained from local suppliers.

2.2. Animals

Adult male Wistar rats (200–250 g) ($n = 30$) from our own breeding colony were kept in cages with continuous access to food in a room with controlled temperature (22 ± 3 °C) and a 12 h light/dark cycle, with lights on at 7:00 am. All experiments were conducted in accordance with the Guiding Principles of the Animal Care and Wellness Committee of the Universidade Federal de Santa Catarina (CEUA/UFSC PP00424; 23080.008706/2010-52).

2.3. Preparation and incubation of striatal slices

Rats were killed by decapitation and the striatum was rapidly removed and placed in ice-cold Krebs–Ringer bicarbonate buffer (KRB) (pH 7.4) containing (122 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose). The striatum was removed and slices (0.4 mm) were rapidly prepared using a McIlwain Tissue Chopper, separated in KRB at 4 °C and allowed to recover for 30 min in KRB at 37 °C [47].

2.4. Slice treatment

QA and 3-NP were dissolved in PBS buffer and neutralized to pH 7.4 with NaOH, and they were freshly prepared each time before treatment. Probucol was dissolved

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

G Model
BRB-8460; No. of Pages 9
ARTICLE IN PRESS
D. Colle et al. / Brain Research Bulletin xxx (2012) xxx–xxx 3

in dimethylsulfoxide (DMSO), which was used as control/vehicle and whose concentration did not exceed 0.1%. Sodium succinate and MK-801 were dissolved in PBS buffer.

After the preincubation time striatal slices were incubated with vehicle, QA (0.1, 0.5 and 1 mM), 3-NP (0.1, 0.5 and 1 mM), or with the combination of subtoxic concentrations of both agents (0.1 mM for both) at 37 °C for 2 h in KRB. After this period, the medium was removed, the slices were washed with KRB and the medium was replaced by a nutritive culture medium composed of 50% of KRB, 50% of Dulbecco's modified Eagle's medium (DMEM, Gibco), 20 mM of HEPES and 100 μg/mL of gentamicine in a humidified 5% CO_2/95% air atmosphere at 37 °C [43], and slices were maintained for additional 4 h to evaluate mitochondrial viability, lipid peroxidation and ROS formation.

Some experiments were performed in the presence of Probucol (10 and 30 μM), sodium succinate (1 mM), MK-801 (50 μM) or their respective vehicles. These compounds were co-incubated with the toxins (QA and/or 3-NP) and re-add in the slice medium during the second incubation. The analytical procedures were performed immediately after the last incubation.

2.5. MTT reduction assay

MTT reduction assay was evaluated as an index of mitochondrial function, according to previous reports [16,52]. This method is based in the ability of cells to reduce MTT to a dark violet formazan product by mitochondrial dehydrogenases in viable cells [45].

Striatal mitochondrial viability was evaluated after the second incubation. The slices (one per probe) were added with 15 μL of MTT (5 mg/mL), and re-incubated at 37 °C for 60 min in KRB (750 μL). Then, the medium was removed and the slices were washed for 30 min in 1 mL of dimethylsulfoxide (DMSO) to remove the formazan. Quantification of formazan was estimated by measuring optical density at 540 nm. The slices were solubilized (1% SDS; 0.1 N NaOH) and an aliquot was used for protein determination. Results were expressed as the percentage of MTT reduction with respect to control values. Preliminary experiments showed that 0.1% DMSO (Probucol's vehicle) did not interfere with the analyzed biochemical parameters per se. Data from five experiments per group were collected and analyzed.

2.6. Lipid peroxidation assay

Lipid peroxidation (LP) was assessed in homogenates obtained from the striatal slices (four slices per probe) by the assay of thiobarbituric acid-reactive substances (TBA-RS) formation, according to previous reports [57].

Immediately, after the last incubation, the slices were homogenized in 500 μL of ultra-purified water, and an aliquot of 20 μL of the homogenate was separated for protein determination. The homogenates remaining were mixed with 1 mL of the TBA reagent (containing 15% of trichloroacetic acid, 0.375% of thiobarbituric acid and 2.5%, v/v of HCl) to be re-incubated in a boiling water bath (95 °C) for 30 min. Samples were then centrifuged at 3000 × g, 15 min. The optical density of supernatants was estimated in 540 nm. The concentrations of MDA (expressed as nmol of MDA per mg protein) were calculated by interpolation in a standard curve of MDA (constructed in parallel), corrected by the content of protein per sample and expressed as percent of MDA formed vs. the control values. Data from five experiments per group were collected and analyzed.

2.7. Estimation of reactive oxygen species (ROS) formation

Formation of ROS was estimated with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), as described by [2]. After cellular uptake, DCFH-DA is enzymatically hydrolyzed by intracellular esterase to form non fluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. At the end of incubation, striatal slices were homogenized in order to read the ROS production. An aliquot of 20 μL of the homogenate was separated for protein determination. DCFH-DA (5 μM) was added to supernatants and fluorescence was read after 30 min using excitation and emission wavelengths of 480 and 525 nm, respectively. ROS levels (expressed as nmol of oxidized DCF per mg protein) were calculated by interpolation in a standard curve of oxidized DCF (constructed in parallel), corrected by the content of protein per sample expressed as percent of DCF oxidized formed vs. the control values. Data from five experiments per group were collected and analyzed.

2.8. Protein determination

The protein measurements content of the homogenized slice were assessed according to Lowry method [39].

2.9. Statistical analysis

Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Differences among the groups were analyzed by one-way ANOVA followed by the Tukey's post hoc test. Results are expressed as mean ± SEM. The differences were considered significant when $p < 0.05$.

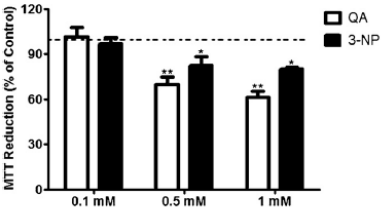

Fig. 1. Mitochondrial dysfunction induced by QA and 3-NP. Striatal slices were incubated with QA (0.1, 0.5 and 1 mM), 3-NP (0.1, 0.5 and 1 mM) or vehicle at 37 °C for 2 h in KRB. After this period, the medium was replaced for fresh culture medium without QA or 3-NP and the slices were maintained for additional 4 h. Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± SEM ($n = 3$). *$p < 0.05$ and **$p < 0.01$ indicate statistical difference from control by one-way ANOVA, following by Tukey's post hoc test.

3. Results

3.1. Probucol protects against mitochondrial dysfunction induced by QA, 3-NP or QA plus 3-NP

In order to investigate the potential deleterious effects of QA and 3-NP on energy metabolism, MTT reduction was assessed as an index of the mitochondrial reductive capacity of striatal slices. Fig. 1 depicts a concentration-response study where slices of striatum were exposed to different concentrations of QA or 3-NP (0–1 mM). QA and 3-NP (at 0.5 and 1 mM, but not 0.1 mM) caused a significant decline in mitochondrial function ($p < 0.05$), as indicated by a decrease in the mitochondrial MTT reductive capacity in striatal slices (Fig. 1). Sub-toxic concentrations of QA (0.1 mM) and 3-NP (0.1 mM), which did not affect mitochondrial function when individually presented in the incubation medium, caused significant

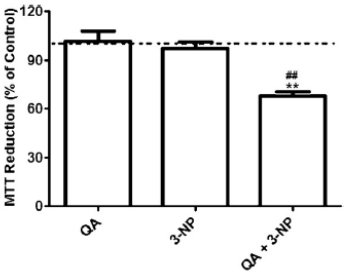

Fig. 2. Mitochondrial dysfunction induced by QA plus 3-NP. Striatal slices were incubated with 0.1 mM QA, 0.1 mM 3-NP, 0.1 mM QA plus 0.1 mM 3-NP or vehicle at 37 °C for 2 h in KRB. After this period, the medium was replaced for fresh culture medium without QA and/or 3-NP and the slices were maintained for additional 4 h. Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± SEM ($n = 3$). **$p < 0.01$ indicates statistical difference from control. ##$p < 0.01$ indicates statistical difference from QA or 3NP by one-way ANOVA, following by Tukey's post hoc test.

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

G Model
BRB-8460; No. of Pages 9
ARTICLE IN PRESS
4 D. Colle et al. / Brain Research Bulletin xxx (2012) xxx–xxx

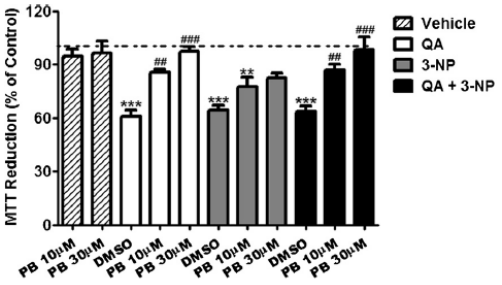

Fig. 3. Protective effect of Probucol against QA, 3-NP or QA plus 3-NP-induced mitochondrial dysfunction. Striatal slices were incubated with 1 mM QA, 1 mM 3-NP, 0.1 mM QA plus 0.1 mM 3-NP or vehicle at 37 °C for 2 h in KRB. After this period, the medium was replaced for fresh culture medium without QA and/or 3-NP and the slices were maintained for additional 4 h. PB (10 and 30 μM) or vehicle were co-incubated with the toxins and re-added in the culture medium during the second incubation. Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± SEM ($n = 5$). $^{**}p < 0.01$ and $^{***}p < 0.001$ indicate statistical difference from control. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ indicate statistical difference from QA, 3NP or QA plus 3NP by one-way ANOVA, following by Tukey's post hoc test.

mitochondrial dysfunction in striatal slices when presented simultaneously (Fig. 2). Because 0.2 mM QA or 3-NP alone also did not affected mitochondrial function (data not shown), it is possible to state that the incubation with QA plus 3-NP induced a synergistic toxicity.

In order to investigate the molecular mechanisms mediating the toxicity induced by QA and 3-NP (alone or in association), the potential protective effect of Probucol (an antioxidant and scavenger compound) was evaluated in the presence of 1 mM of each toxin (alone) or 0.1 mM of both toxins (simultaneously). Fig. 3 show that the three toxic treatments (1 mM QA; 1 mM 3-NP; or 0.1 mM of both) significantly induced mitochondrial dysfunction in striatal slices (Fig. 3). Probucol was effective in protecting the insult elicited by QA in both concentrations ($p < 0.01$ and $p < 0.001$ for 10 and 30 μM, respectively) (Fig. 3). On the other hand, Probucol had no effect in striatal slices against the insult elicited by 3-NP (Fig. 3). However, in QA plus 3-NP model, Probucol (10 and 30 μM) completely preserved the mitochondrial function showed a potent neuroprotective activity, managing to restore the mitochondrial function (Fig. 3).

3.2. ROS production and lipid peroxidation elicited by QA, 3-NP or QA + 3-NP and protective effect of Probucol

Considering that mitochondrial dysfunction and ROS generation are closely related phenomena, which also can contribute to increased lipid peroxidation, ROS levels were investigated in QA- and/or 3-NP-exposed slices. ROS generation was significantly increased in the striatal slices exposed to 1 mM QA, 1 mM 3-NP or 0.1 mM of both compounds (Fig. 4A). As expected, Probucol, which presents scavenger activity, completely prevented the QA, 3-NP and QA plus 3-NP-induced ROS formation in the slices analyzed (Fig. 4A).

Lipid peroxidation, which represents a consequence of increased ROS formation, was assessed as an index of oxidative damage to lipids. The statistical analysis revealed a significant increased of lipid peroxidation by all toxic conditions (1 mM QA, 1 mM 3-NP, or 0.1 mM QA plus 0.1 mM 3-NP) in striatal slices (Fig. 4B). The lipoperoxidative effects induced by either QA, 3-NP or QA plus 3-NP were completely blocked by Probucol 10 and 30 μM (Fig. 4B).

3.3. Protective effect of sodium succinate and MK-801 against QA, 3-NP and QA plus 3-NP-induced mitochondrial dysfunction

Several studies have demonstrated that antioxidant compounds are able to protect against the neurotoxicity elicited by QA, 3-NP and QA plus 3-NP models. However, recently, the particular interest in characterizing the protective properties of energy precursor agents against the toxic insult with 3-NP has been increased [3,30,40,76]. In this study, we used sodium succinate as an energy precursor and its capacity to restore the mitochondrial function was investigate in an attempt to understand the mechanism of damage induced by the toxins and the potential contribution of the energetic metabolism disruption in either QA, 3-NP or QA plus 3-NP-induced toxicity. Sodium succinate (1 mM) was unable to recover the mitochondrial dysfunction induced by QA and QA plus 3-NP treatments (Fig. 5A). On the other hand, sodium succinate effectively protected striatal slices against 3-NP-induced mitochondrial dysfunction ($p < 0.05$, Fig. 5A).

In addition, mitochondrial dysfunction and ROS generation can trigger excitotoxicity and induce massive entry of calcium ions (Ca^{2+}) from the extracellular environment, prompting the activation of cell death pathways [17,72]. Furthermore, QA stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes [69], which could lead to excitotoxic events. MK-801, a well-known NMDA antagonist, has protective action against QA insults [27]. However, studies on the potential protective effect of NMDA receptor antagonists in the combined model (QA plus 3-NP) are lacking in the literature. To further determine the contribution of the excitotoxic events linked with NMDA receptor activation in QA, 3-NP and QA plus 3-NP-induced damage, slices were incubated with the toxins in the absence or presence of MK-801, a non-competitive antagonist of NMDA receptor.

As already demonstrated, MK-801 protected against QA toxicity in striatal slices ($p < 0.001$, Fig. 5B). Interestingly, MK-801 also was effective in protecting the mitochondrial function disrupted by 3-NP and QA plus 3-NP in striatal slices ($p < 0.001$ and $p < 0.01$, Fig. 5B).

4. Discussion

The present study showed that QA (but not 3-NP)-induced mitochondrial dysfunction in striatal slices was prevented by Probucol, an antioxidant compound with scavenger properties in vitro. When

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

G Model
BRB-8460; No. of Pages 9
ARTICLE IN PRESS
D. Colle et al. / Brain Research Bulletin xxx (2012) xxx–xxx

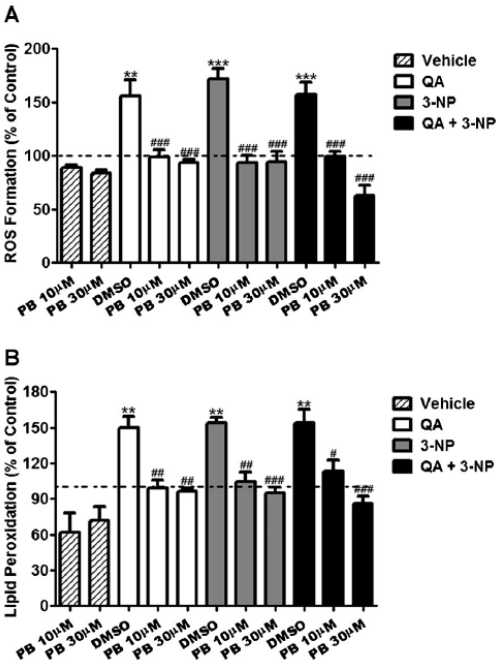

Fig. 4. Protective effect of Probucol against QA, 3-NP or QA plus 3-NP induced ROS formation and lipid peroxidation. Striatal slices were incubated with 1 mM QA, 1 mM 3-NP, 0.1 mM QA plus 0.1 mM 3-NP or vehicle at 37 °C for 2 h in KRB. After this period, the medium was replaced for fresh culture medium without QA and/or 3-NP and the slices were maintained for additional 4 h. PB (10 and 30 μM) or vehicle were co-incubated with the toxins and re-add in the culture medium during the second incubation. Formation of ROS was estimated with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) using excitation and emission wavelengths of 480 and 525 nm, respectively. ROS levels (expressed as nmol of oxidized DCF per mg protein) are expressed as percent of control (dotted line) (A). Lipid peroxidation (LP) was assessed in homogenates obtained from the slices by TBARS formation and expressed as nmol of MDA per mg of protein. Results are expressed as percent of MDA formed vs. the control values (dotted line) (B). Data are represented as mean ± SEM ($n = 5$). $^{**}p < 0.01$ and $^{***}p < 0.001$ indicate statistical difference from control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ indicate statistical difference from 3NP by one-way ANOVA, following by Tukey's post hoc test.

QA and 3-NP were simultaneously present at concentrations that are sub-toxic when incubated individually (0.1 mM), a synergistic mitochondrial toxicity was observed, which was totally protected by Probucol. These results contribute to the understanding on molecular mechanisms mediating QA- and/or 3-NP-induced mitochondrial dysfunction, corroborating previous data pointing to excitotoxicity, oxidative stress and energetic deficit as important events mediating the toxicity [24,53]. In addition, the presented results add new insights on the involvement of ROS in the toxic effects of QA and 3-NP to mitochondria, indicating that the blockade of QA-induced ROS is enough to significantly blunt the decreased mitochondrial dysfunction, although this event was not observed for 3-NP.

Probucol prevented ROS formation and lipid peroxidation in all used models, but did not protect against the mitochondrial dysfunction induced by 3-NP (only by QA or QA plus 3-NP). This data indicates that QA and 3-NP might share similar damage mechanisms (ROS formation), but also may have different mechanisms. Although the predominant hypothesis on QA toxicity is oriented to sustained NMDAr overactivation and excitotoxicity [66], further leading to cell damage produced by enhanced levels of Ca^{2+} [41,63], evidence showed that QA is also able to produce damage via ROS production [33,67] and/or alterations in energy metabolism [28,62]. In this study, we demonstrated that QA was able to induced mitochondrial dysfunction in striatal slices, but this effect may be secondary to excitotoxicity, calcium influx and ROS production. Interestingly, Probucol prevented mitochondrial dysfunction probably by avoiding the secondary effect (energetic deficit) due to the blockade of the primary event (ROS production). Thus, when QA-induced ROS production was prevented by Probucol, there was protection against mitochondrial dysfunction. These results indicate that ROS production and oxidative stress played an important role of QA mediating deleterious effects in striatal slices (mitochondrial dysfunction) and that the antioxidant and

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

G Model
BRB-8460; No. of Pages 9
ARTICLE IN PRESS
6 D. Colle et al. / Brain Research Bulletin xxx (2012) xxx–xxx

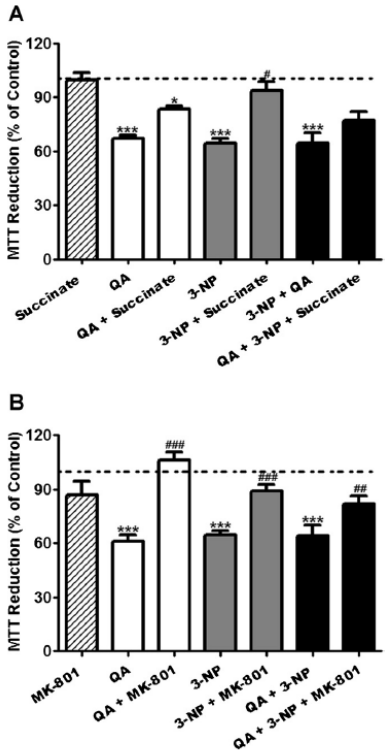

Fig. 5. Protective effect of sodium succinate and MK-801 against QA, 3-NP and QA plus 3-NP-induced mitochondrial dysfunction. Striatal slices were incubated with 1 mM QA, 1 mM 3-NP, 0.1 mM QA plus 0.1 mM 3-NP or vehicle at 37 °C for 2 h in KRB. After this period, the medium was replaced for fresh culture medium without QA and/or 3-NP and the slices were maintained for additional 4 h. Sodium succinate (1 mM) (A) or MK-801 (50 μM) (B) were co-incubated with the toxins and re-add in the slices medium during the second incubation. Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± SEM ($n = 5$). *$p < 0.05$ and ***$p < 0.001$ indicate statistical difference from control. #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$ indicate statistical difference from QA, 3NP or QA plus 3NP by one-way ANOVA, following by Tukey's post hoc test.

scavengers properties of Probucol were important in counteracting these effects. This idea is supported by other studies reporting the involvement of oxidative stress induced by QA [33]. In this regard, the toxicity induced by QA in striatal slices could be linked not just to NMDAr overactivation and excitotoxicity, but also to ROS production-inducing mitochondrial dysfunction.

On the other hand, Probucol, which diminished ROS production and lipid peroxidation induced by 3-NP, did not protect against the mitochondrial dysfunction induced by this toxin. The scavenger property of Probucol protected only against QA probably because it was not able to modulate a direct energetic deficit induced by 3-NP. 3-NP is a suicide inactivator of the mitochondrial Complex II, directly leading to mitochondrial dysfunction [23], decreased ATP levels, membrane depolarization and ROS formation [34,36,44]. Thus, 3-NP-induced ROS formation is a consequence of mitochondrial dysfunction and despite Probucol antioxidant effects, mitochondrial function was not re-established. Thus, this work demonstrated a crucial effect of ROS in mitochondrial dysfunction induced by QA, but also demonstrated that 3-NP-induced ROS production is not the only responsible by mitochondrial dysfunction in this model. Noteworthy, the novelty of this study does not contradict previous data from literature, which show that ROS, excitotoxicity and energetic deficit are mechanisms modulating the toxicity in all the models [24,53].

However, the temporal profile of primary and secondary events seems to be important in these models. This idea was better understood by using MK-801 (an NMDAr antagonist) and succinate (an energy precursor agent). MK-801, which prevents excitotoxicity, also prevented QA, 3-NP and QA plus 3-NP-induced mitochondrial dysfunction. These findings indicate that 3-NP also can induce excitotoxic events. However, the incubation of striatal slices with succinate, an energetic precursor, was able to prevent against 3-NP-induced mitochondrial dysfunction, but did not protect against QA effects. Interesting, succinate also did not protect against QA plus 3-NP. These findings demonstrate the involvement of primary and secondary events in these models. Although oxidative stress, energetic deficit and excitotoxicity represent important events in the models (QA, 3-NP and QA plus 3-NP), their sequences are likely different depending upon the specific model. On the other hand, the prevention of excitotoxicity-induced by 3-NP (by using MK-801) was able to prevent mitochondrial dysfunction. This data prove a critical role of excitotoxicity in mitochondrial dysfunction induced by 3-NP model alone or in association with QA. Studies demonstrated that 3-NP can induce excitotoxicity [46,51]. The hypothesis of an indirect or "secondary" excitotoxicity suggests that 3-NP-induced striatal degeneration is due in first place to depletion in ATP levels produced by a deficit in energy metabolism, further leading to membrane depolarization and sustained voltage-gated NMDAr activation by primary alteration of membrane Na^+, K^+-ATPases [1,5,46,51]. Under these conditions, 3-NP is able to cause excitotoxicity by making neurons vulnerable to endogenous basal levels of glutamate [51], producing neuronal necrotic death [6,48].

Liot et al. [38] showed that 3-NP induced the activation of NMDAr in neuronal cells, leading to ROS formation, as well as a significant mitochondrial fragmentation and cell death [38]. Remarkably, pretreatment with AP5, a glutamate receptor antagonist blocked the 3-NP-induced ROS formation, mitochondrial fragmentation, and neuronal cell death [38]. This study provides evidence that secondary excitotoxicity (caused by primary complex II inhibition) may play an important role in 3-NP-induced cell death. This is in accordance with our study, which indicates that MK-801 was able to protect striatal slices from 3-NP-induced mitochondrial toxicity. The protective effect of MK-801 against 3-NP toxicity may indicate a secondary excitotoxicity with involvement of NMDAr activation. This is in accordance with other studies indicating the involvement of glutamate receptor activation in 3-NP-induced cell death [5,9,13,19]. The results also suggest that 3-NP-induced damage may be partially glutamate receptor-mediated because the energy deficiency induced by this toxin might lead to increases in glutamate release, cellular depolarization, activation of NMDA receptors, and increases in damaging calcium cascades [15,21,38].

Our results also showed that the simultaneous exposure to subtoxic concentrations of QA plus 3-NP (which cannot induce

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

G Model
BRB-8460; No. of Pages 9
ARTICLE IN PRESS
D. Colle et al. / Brain Research Bulletin xxx (2012) xxx–xxx 7

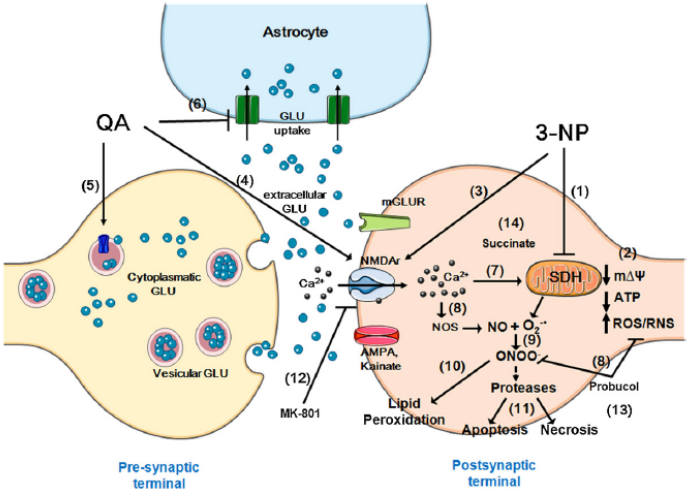

Fig. 6. Schematic representation of the mechanisms of QA plus 3NP-induced toxicity. Low concentrations of 3-NP primarily induce a moderate mitochondrial respiratory complex II inhibition (SDH) (event 1), which in turn triggers ATP drop, decrease in mitochondrial membrane potential ($m\Delta\Psi$) and massive production of ROS/RNS (event 2). The energy deficit makes neurons more vulnerable to endogenous basal levels of glutamate ("secondary" excitotoxicity). This scenario leads to plasma membrane depolarization, which may release the Mg^{2+} blockade of voltage-gated NMDAr (event 3). Opening of NMDAr causes intracellular Ca^{2+} influx. On the other hand, QA induce a moderate activation NMDAr (event 4), thus causing increased intracellular Ca^{2+} concentrations. In addition, QA also stimulates synaptosomal glutamate release (event 5) and affects glutamate re-uptake into astrocytes (event 6), and so increasing extracellular concentrations of glutamate. Increased levels of intracellular calcium, which can directly lead to mitochondrial dysfunction (event 7), might activate nitric oxide synthase (NOS) (event 8) thus increasing nitric oxide (NO) formation. NO can combine with O^{2-} to form $ONOO^-$ (event 9). In addition, ROS/RNS generation might induce lipid peroxidation (event 10). Altogether, these events lead to proteases activation, thus inducing cell death by necrosis and/or apoptosis (event 11). The blockade of NMDAr with MK-801 (event 12) and the scavenging activity of Probucol toward ROS/RNS (event 13) can block mitochondrial dysfunction and neuronal cell death. Succinate, as an energetic precursor, may mitigate mitochondrial dysfunction induced by 3-NP (event 14).

mitochondrial damage alone) induced mitochondrial dysfunction and oxidative stress in a synergistic manner. In fact, when subtoxic concentrations of QA (0.1 mM) and 3-NP (0.1 mM) were combined, there was a significant decrease in the mitochondrial viability in striatal slices, as well as increased in ROS levels and lipid peroxidation, showing the involvement of oxidative stress in the impaired of mitochondrial function induced by this mixed exposure. Interestingly, Probucol (by scavenging ROS) prevented mitochondrial toxicity in the mixed model. Probucol was able to protect striatal slices against QA plus 3-NP-inducing mitochondrial dysfunction, suggesting that oxidative stress played an important role in mediating the deleterious effects of QA plus 3-NP. In addition, MK-801 also provides protection against QA plus 3NP. This is in accordance with Pérez-De La Cruz and coworkers, who reported a reduction of lipid peroxidation by MK-801 in QA plus 3-NP-treated synaptosomal membranes [52]. On the other hand, succinate did not protect striatal slices against mitochondrial dysfunction induced by QA plus 3-NP. Conversely, MK-801 effectively blunted the mitochondrial toxicity induced by the association of both toxins. Altogether, these findings suggest that a cascade of toxic events related with NMDAr overactivation may play a relevant role for cell damage following the toxic insult that involves deficit in energy metabolism and excitotoxicity when QA and 3-NP are associated.

As already mentioned, we found a synergistic toxicity of QA and 3-NP, which produced oxidative damage to striatal slices. Based on literature data [24,51,53,70] and on our current findings, Fig. 6 depicts the main molecular mechanisms of QA plus 3NP-induced toxicity. The primary mechanism mediating such damage may involve a moderate energy metabolism deficit induced by SDH inhibition by 3-NP. The energy metabolism dysfunction makes neuronal cells more vulnerable to be damage by physiologic glutamate levels ("secondary" excitotoxicity) [51]. In addition, QA induces a moderate activation NMDAr, thus likely causing increased intracellular Ca^{2+} concentrations, which in turn can lead to major alterations in synaptic and mitochondrial functions, generation of ROS and RNS and activation of cell death pathways [51]. 3-NP also induces ROS and RNS formation, as well as increases in intracellular Ca^{2+} levels and further activation of proteases [70]. Altogether, these events might cause neuronal cell death (either necrotic or apoptotic) (Fig. 6).

In summary, the findings of this study show that the two studied toxic models (QA and 3-NP) or the combined model (QA plus 3-NP) can generate complex patterns of damage, which involve metabolic compromise, ROS formation, and oxidative stress. These neurotoxic models share common mechanisms of cell damage, despite each model recruits these processes in a differential manner: QA by NMDAr activation and 3-NP by SDH inhibition. These events were counteracted by Probucol, an antioxidant compound with scavenger properties under in vitro conditions. Moreover, a partial inhibition of SDH by subtoxic 3-NP and moderate excitotoxicty by subtoxic QA are potentiated when both agents are associated.

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

G Model
BRB-8460; No. of Pages 9
ARTICLE IN PRESS
8 D. Colle et al. / Brain Research Bulletin xxx (2012) xxx–xxx

The toxic action of QA plus 3-NP seems to involve changes in Ca^{2+} metabolism and ROS/RNS formation, and can be prevented or attenuated by antioxidant/scavenger compounds and NMDAr antagonists. Therefore, oxidative stress remains as a major expression in these toxic models, as well as a potential key target to ameliorate neuronal damage in HD patients.

Disclosures

The authors report no declarations of interest.

Acknowledgements

The financial supports by (i) FINEP Research Grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00, (ii) Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), (iii) Fundação de Apoio à Pesquisa do Estado de Santa Catarina (FAPESC - TO 1346/2010-3), (iv) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and (v) INCT-CNPq-Excitotoxicity and Neuroprotection are gratefully acknowledged. M.F. and F.A.A.S. recipient of CNPq fellowship.

References

[1] R.L. Albin, J.T. Greenamyre, Alternative excitotoxic hypotheses, Neurology 42 (4) (1992) 733–738.
[2] S.F. Ali, C.P. LeBel, S.C. Bondy, Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity, Neurotoxicology 13 (1992) 637–648.
[3] O.A. Andreassen, A. Dedeojlu, R.J. Ferrante, B.G. Jenkins, M. Thomas, Creatine increases survival and delays motor symptoms in a transgenic animal model of Huntington's disease, Neurobiol. Dis. 8 (2001) 479–491.
[4] D.S. Avila, P. Gubert, A. Palma, D. Colle, D. Alves, C.W. Nogueira, J.B. Rocha, F.A. Soares, An organotellurium compound with antioxidant activity against excitotoxic agents without neurotoxic effects in brain of rats, Brain Res. Bull. 76 (2008) 114–123.
[5] M.F. Beal, B.T. Hyman, W. Koroshetz, Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases, Trends Neurosci. 16 (1993) 125–131.
[6] M.I. Behrens, J. Koh, L.M. Canzoniero, S.L. Sensi, C.A. Csernansky, D.W. Choi, 3-Nitropropionic acid induces apoptosis in cultured striatal and cortical neurons, Neuroreport 6 (1995) 545–548.
[7] P.F. Behrens, P. Franz, B. Woodman, K.S. Lindenberg, G.B. Landwehrmeyer, Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation, Brain 125 (2002) 1908–1922.
[8] A. Benchoua, Y.J. Trioulier, D. Zala, M.C. Gaillard, N. Lefort, N. Dufour, F. Saudou, J.M. Elalouf, E. Hirsch, P. Hantraye, N. Déglon, E. Brouillet, Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin, Mol. Biol. Cell 17 (2006) 1652–1663.
[9] E. Brouillet, F. Conde, M.F. Beal, P. Hantraye, Replicating Huntington's disease phenotype in experimental animals, Prog. Neurobiol. 59 (1999) 427–468.
[10] S.E. Browne, M.F. Beal, Oxidative damage in Huntington's disease pathogenesis, Antioxid. Redox Signal. 8 (2006) 2061–2073.
[11] M.M. Buckley, K.L. Goa, A.H. Price, R.N. Brogden, Probucol. A reappraisal of its pharmacological properties and therapeutic use in hypercholesterolaemia, Drugs 37 (1989) 761–800.
[12] C. Chen, Mitochondrial dysfunction, metabolic deficits, and increased oxidative stress in Huntington's disease, Chang Gung Med. J. 34 (2011) 135–152.
[13] D.W. Choi, Cerebral hypoxia: some new approaches and unanswered questions, J. Neurosci. 10 (1990) 2493–2501.
[14] P. Del Río, L. Massieu, Mild mitochondrial inhibition in vivo enhances glutamate-induced neuronal damage through calpain but not caspase activation: role of ionotropic glutamate receptors, Exp. Neurol. 212 (2008) 179–188.
[15] P. Del Río, T. Montinel, V. Chagoya, L. Massieu, Exacebation of excitotoxic neuronal death induced during mitochondrial inhibition in vivo: relation to energy imbalance or ATP depletion? Neuroscience 146 (2007) 1561–1570.
[16] D. Elinos-Calderón, Y. Robledo-Arratia, V. Pérez-De La Cruz, P.D. Maldonado, S. Galván-Arzate, J. Pedraza-Chaverrí, A. Santamaría, Antioxidant strategy to rescue synaptosomes from oxidative damage and energy failure in neurotoxic models in rats: protective role of S-allylcysteine, J. Neural Transm. 117 (1) (2010) 35–44.
[17] G. Ermak, K.J.A. Davies, Calcium and oxidative stress: from cell signaling to cell death, Mol. Immunol. 38 (2001) 713–721.
[18] M. Farina, F. Campos, I. Vendrell, J. Berenguer, M. Barzi, S. Pons, C. Suñol, Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells, Toxicol. Sci. 112 (2) (2009) 416–426.
[19] S.L. Fink, D.Y. HO, R.M. Sapolsky, Energy and glutamate dependency of 3-nitropropionic acid neurotoxicity in culture, Exp. Neurol. 138 (1996) 298–304.
[20] C. Giampà, S. Patassini, A. Borreca, D. Laurenti, F. Marullo, G. Bernardi, F.S. Menniti, F.R. Fusco, Phosphodiesterase 10 inhibition reduces striatal excitotoxicity in the quinolinic acid model of Huntington's disease, Neurobiol. Dis. 34 (2009) 450–456.
[21] J.G. Greene, S.S. Sheu, R.A. Gross, J.T. Greenamyre, 3-Nitropropionic acid exacerbates N-methyl-D-aspartate toxicity in striatal culture by multiple mechanisms, Neuroscience 84 (1998) 503–510.
[22] P. Guidetti, R.E. Luthi-Carter, S.J. Augood, R. Schwarzca, Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease, Neurobiol. Dis. 17 (2004) 455–461.
[23] L. Huang, G. Sun, D. Cobessi, A. Wang, J.T. Shen, E.Y. Tung, V.E. Anderson, E.A. Berry, 3-Nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme, J. Biol. Chem. 281 (9) (2006) 5965–5972.
[24] C. Jacquard, Y. Trioulier, F. Cosker, C. Escartin, N. Bizat, P. Hantraye, J.M. Cancela, G. Bonvento, E. Brouillet, Brain mitochondrial defects amplify intracellular [Ca^{2+}] rise and neurodegeneration but not Ca^{2+} entry during NMDA receptor activation, FASEB J. 20 (2006) 1021–1023.
[25] S. Jang, H.S. Jeong, J.S. Parka, Y.S. Kim, C.Y. Jin, M.B. Seol, B.C. Kim, M.C. Lee, Neuroprotective effects of (−)-epigallocatechin-3-gallate against quinolinic acid-induced excitotoxicity via PI3K pathway and NO inhibition, Brain Res. 1313 (2010) 25–33.
[26] H. Kalonia, A. Kumar, Suppressing inflammatory cascade by cyclo-oxygenase inhibitors attenuates quinolinic acid induced Huntington's disease-like alterations in rats, Life Sci. 88 (2011) 784–791.
[27] H. Kalonia, P. Kumar, B. Nehru, A. Kumar, Neuroprotective effect of MK-801 against intra-striatal quinolinic acid induced behavioral, oxidative stress and cellular alterations in rats, Indian J. Exp. Biol. 47 (11) (2009) 880–892.
[28] H. Kalonia, P. Kumar, A. Kumar, B. Nehru, Protective effect of rofecoxib and nimesulide against intra-striatal quinolinic acid-induced behavioral, oxidative stress and mitochondrial dysfunctions in rats, Neurotoxicology 31 (2010) 195–203.
[29] H. Kalonia, P. Kumar, A. Kumar, Attenuation of proinflammatory cytokines and apoptotic process by verapamil and diltiazem against quinolinic acid induced Huntington like alterations in rats, Brain Res. 1372 (2011) 115–126.
[30] S. Kasparová, Z. Sumbalová, P. Bystrický, J. Kucharská, T. Liptaj, V. Mlynárik, A. Gvozdjáková, Effect of coenzyme Q10 and vitamin E on brain energy metabolism in the animal model of Huntington's disease, Neurochem. Int. 48 (2) (2005) 93–99.
[31] P. Kumar, A. Kumar, Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunction in rat brain, Prog. Neuropsycopharmacol. Prog. Neuropsychopharmacol. Biol. Psych. 33 (1) (2009) 100–108.
[32] P. Kumar, S.S.V. Padis, P.S. Naidu, A. Kumar, Cyclooxygenase inhibition attenuates 3-nitropropionic acid-induced neurotoxicity in rats: possible antioxidant mechanisms, Fundam. Clin. Pharmacol. 21 (2007) 297–306.
[33] P. Kumar, H. Kalonia, A. Kumar, Huntington's disease: pathogenesis to animal models, Pharmacol. Rep. 62 (2010) 1–14.
[34] P. Kumar, H. Kalonia, A. Kumar, Role of LOX/COX pathways in 3-nitropropionic acid-induced Huntington's disease-like symptoms in rats: protective effect of licofenole, Br. J. Pharmacol. 162 (2b) (2011) 644–654.
[35] W.T. Lee, C. Chang, Magnetic resonance imaging and spectroscopy in assessing 3-nitropropionic acid-induced brain lesions: an animal model of Huntington's disease, Prog. Neurobiol. 72 (2004) 87–110.
[36] W.T. Lee, H.S. Yin, Y.Z. Shen, The mechanisms of neuronal death produced by mitochondrial toxin 3-nitropropionic acid: the roles of N-methyl-D-aspartate glutamate receptors and mitochondrial calcium overload, Neuroscience 112 (2002) 707–716.
[37] X.J. Li, S. Li, Proteasomal dysfunction in aging and Huntington disease, Neurobiol. Dis. 43 (2011) 4–8.
[38] G. Liot, B. Bossy, S. Lubitz, Y. Kushnareva, N. Sejbuk, E. Bossy-Wetzel, Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway, Cell Death Differ. 16 (2009) 899–909.
[39] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[40] L. Massieu, P. Del Río, T. Montiel, Neurotoxicity of glutamate uptake inhibition *in vivo*: correlation with succinate dehydrogenase activity and prevention by energy substrates, Neuroscience 106 (4) (2001) 669–677.
[41] E. Matyja, Intracellular calcium overload in a model of quinolinic acid neurotoxicity in organotypic culture of rat hippocampus; inhibited by nimodipine, Folia Neuropathol. 35 (1997) 8–17.
[42] F. Mochel, R.G. Haller, Energy deficit in Huntington disease: why it matters, J. Clin. Invest. 121 (2) (2011) 493–499.
[43] S. Molz, H. Decker, T. Dal-Cim, C. Cremonez, F.M. Cordova, R.B. Leal, C.I. Tasca, Glutamate-induced toxicity in hippocampal slices involves apoptotic features and $p38^{MAPK}$ signaling, Neurochem. Res. 33 (2008) 27–36.
[44] P. Montilla, I. Túnez, M.C. Muñoz, M. Salcedo, M. Feijóo, J.R. Muñoz-Castañeda, I. Bujalance, Effect of glucocorticoids on 3-nitropropionic acid-induced oxidative stressing synaptosomes, Eur. J. Pharmacol. 488 (2004) 19–25.
[45] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1-2) (1983) 55–63.

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

[46] A. Novelli, J.A. Reilly, P.G. Lysko, R.C. Henneberry, Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced, Brain Res. 451 (1988) 205–212.
[47] I.J.L. Oliveira, S. Molz, D.O. Souza, C.I. Tasca, Neuroprotective effect of GMP in hippocampal slices submitted to an *in vitro* model of ischemia, Cell. Mol. Neurobiol. 22 (3) (2002) 335–344.
[48] Z. Pang, J.W. Geddes, Mechanisms of cell death induced by the mitochondrial toxin 3-nitropropionic acid: acute excitotoxic necrosis and delayed apoptosis, J. Neurosci. 7 (1997) 3064–3073.
[49] S.Y. Park, J.H. Lee, C.D. Kim, B.Y. Rhim, K.W. Hong, W.S. Lee, Beneficial synergistic effects of concurrent treatment with cilostazol and probucol against focal cerebral ischemic injury in rats, Brain Res. 1157 (2007) 112–120.
[50] R.P. Pereira, R. Fachinetto, A. de Souza Prestes, R.L. Puntel, G.N. Santos da Silva, B.M. Heinzmann, T.K. Boschetti, M.L. Athayde, M.E. Bürger, A.F. Morel, V.M. Morsch, J.B. Rocha, Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citratus*, Neurochem. Res. 34 (5) (2009) 973–983.
[51] V. Pérez-De La Cruz, A. Santamaría, Integrative hypothesis for Huntington's Disease: a brief review of experimental evidence, Physiol. Res. 56 (2007) 513–526.
[52] V. Pérez-De La Cruz, M. Konigsberg, J. Pedraza-Chaverri, N. Herrera-Mundo, M. Díaz-Muñoz, J. Morán, T. Fortoul-van der Goes, A. Rondán-Zárate, P.D. Maldonado, S.F. Ali, A. Santamaría, Cytoplasmic calcium mediates oxidative damage in an excitotoxic/energetic deficit synergic model in rats, Eur. J. Neurosci. 27 (2008) 1075–1085.
[53] V. Pérez-De La Cruz, D. Elinos-Calderón, P. Carrillo-Mora, D. Silva-Adaya, M. Konigsberg, J. Morán, S.F. Ali, M.E. Chánez-Cárdenas, G. Pérez-De La Cruz, A. Santamaría, Time-course correlation of early toxic events in three models of striatal damage: modulation by proteases inhibition, Neurochem. Int. 56 (2010) 834–842.
[54] R.L. Puntel, C.W. Nogueira, J.B. Rocha, N-methyl-D-aspartate receptors are involved in the quinolinic acid, but not in the malonate pro-oxidative activity in vitro, Neurochem. Res. 30 (3) (2005) 417–424.
[55] S. Ramaswamy, J.L. McBride, J.H. Kordower, Animal models of Huntington's disease, ILAR J. 48 (4) (2007) 356–373.
[56] L. Rekik, V. Daguin-Nerrière, J. Petit, P. Brachet, 3'γ-Aminobutyric acid type B receptor changes in the rat striatum and substantia nigra following intrastriatal quinolinic acid lesions, J. Neurosci. Res. 89 (2011) 524–535.
[57] C. Rios, A. Santamaría, Quinolinic acid is a potent lipid peroxidant in rat brain homogenates, Neurochem. Res. 16 (1991) 1139–1143.
[58] R.A.C. Roos, Huntington's disease: a clinical review, Orphanet J. Rare Dis. 5 (40) (2010) 1–8.
[59] C.A. Ross, S.J. Tabrizi, Huntington's disease: from molecular pathogenesis to clinical treatment, Lancet Neurol. 10 (2011) 83–98.
[60] J. Rossignol, C. Boyer, X. Lévèque, K.D. Fink, R. Thinard, F. Blanchard, G.L. Dunbar, L. Lescaudron, Mesenchymal stem cell transplantation and DMEM administration in a 3NP rat model of Huntington's disease: morphological and behavioral outcomes, Behav. Brain Res. 217 (2011) 369–378.
[61] A. Santamaría, S. Galván-Arzate, V. Lisy, S.F. Ali, H.M. Duhart, L. Osorio-Rico, C. Rios, F. St'Astny, Quinolinic acid induces oxidative stress in rat brain synaptosomes, Neuroreport 12 (2001) 871–874.
[62] P.F. Schuck, A. Tonin, G.C. Ferreira, R.B. Rosa, A. Latini, F. Balestro, M.L.S. Perry, C.M.D. Wannmacher, A.T.S. Wyse, M. Wajner, In vitro effect of quinolinic acid on energy metabolism in brain of young rats, Neurosci. Res. 57 (2007) 277–288.
[63] R. Schwarcz, W.O.J.R. Whetsell, R.M. Mangano, Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain, Science 219 (1983) 316–319.
[64] D. Silva-Adaya, V. Pérez-De La Cruz, M.N. Herrera-Mundo, K. Mendoza-Macedo, J. Villeda-Hernández, Z. Binienda, S.F. Ali, A. Santamaría, Excitotoxic damage, disrupted energy metabolism and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine, J. Neurochem. 105 (2008) 677–689.
[65] A.L. Southwell, P.H. Patterson, Gene therapy in mouse models of Huntington disease, Neuroscientist 17 (2) (2011) 153–162.
[66] T.W. Stone, M.N. Perkins, Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS, Eur. J. Pharmacol. 72 (1981) 411–412.
[67] T.W. Stone, W.M. Behan, M. MacDonald, L.G. Darlington, Possible mediation of quinolinic acid-induced hippocampal damage by reactive oxygen species, Amino Acids 19 (1) (2000) 275–281.
[68] I. Tasset, V. Pérez-De La Cruz, D. Elinos-Calderón, P. Carrillo-Mora, I.G. González-Herrera, A. Luna-López, M. Konigsberg, J. Pedraza-Chaverrí, P. Maldonado, S.F. Ali, I. Túnez, A. Santamaría, Protective effect of tert-butylhydroquinone on the quinolinic-acid-induced toxicity in rat striatal slices: role of the Nrf2-antioxidant response element pathway, Neurosignals 18 (1) (2010) 24–31.
[69] R.G. Tavares, C.I. Tasca, C.E.S. Santos, L.B. Alves, L.O. Porciúncula, T. Emanuelli, D.O. Souza, Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes, Neurochem. Int. 40 (2002) 621–627.
[70] I. Túnez, I. Tasset, V. Pérez-De La Cruz, A. Santamaría, 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future, Molecules 15 (2010) 878–916.
[71] E. Vamos, A. Pardutz, P. Klivenyi, J. Toldi, L. Vecsei, The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection, J. Neurol. Sci. 283 (2009) 21–27.
[72] S. Wang, E.K. Michaelis, Selective neuronal vulnerability to oxidative stress in the brain, Front. Aging Neurosci. 2 (2010) 1–13.
[73] P.K. Witting, B.J. Wu, M. Raftery, P. Southwell-Keely, R. Stocker, Probucol protects against hypochlorite-induced endothelial dysfunction: indentification of a novel pathway of probucol oxidation to a biologically active intermediate, J. Biol. Chem. 280 (16) (2005) 15612–15618.
[74] S. Yamashita, Y. Matsuzawa, Where are we with probucol: a new life for an old drug? Atherosclerosis 207 (2009) 16–23.
[75] S. Yamashita, H. Hbujo, H. Arai, M. Harada-Shiba, S. Matsui, M. Fukushima, Y. Saito, T. Kita, Y. Matsuzawa, Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan, J. Atheroscler. Thromb. 15 (6) (2008) 292–303.
[76] L. Yang, N.Y. Calingasan, E.J. Wille, K. Cormier, K. Smith, R.J. Ferrante, M.F. Beal, Combination therapy with coenzyme Q10 and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's diseases, J. Neurochem. 109 (5) (2009) 1427–1439.
[77] D. Zadóri, P. Klivényi, E. Vámos, F. Fulo, J. Toldi, L. Vécsei, Kynurenines in chronic neurodegenerative disorders: future therapeutic strategies, J. Neural. Transm. 116 (2009) 1403–1409.

Please cite this article in press as: D. Colle, et al., Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro, Brain Res. Bull. (2012), doi:10.1016/j.brainresbull.2012.01.003

4.2 PROBUCOL AUMENTA A ATIVIDADE DA GLUTATIONA PEROXIDASE ESTRIATAL E CORTICAL E PROTEGE CONTRA O DANO OXIDATIVO E O FENÓTIPO DE DOENÇA DE HUNTINGTON INDUZIDO PELO ÁCIDO 3-NITROPROPIÔNICO EM RATOS

Manuscrito 1

PROBUCOL INCREASES STRIATAL AND CEREBRO-CORTICAL GLUTATHIONE PEROXIDASE ACTIVITY AND PROTECTS AGAINST 3-NITROPROPIONIC ACID-INDUCED PRO-OXIDATIVE DAMAGE AND HUNTINTGON´S DISEASE-LIKE PHENOTYPE IN RATS

Dirleise Colle, Danúbia B. dos Santos, Eduardo L.G. Moreira, Alessandra A. dos Santos, Luciana T. Zimmermann, Marcelo Farina

Probucol increases striatal and cerebro-cortical glutathione peroxidase activity and protects against 3-nitropropionic acid-induced pro-oxidative damage and Huntintgon´s disease-like phenotype in rats

Dirleise Colle[a,b,*], Danúbia B. dos Santos[a,b], Eduardo L.G. Moreira[a,c], Alessandra A. dos Santos[a,b], Luciana T. Zimmermann[a,b], Marcelo Farina[a,b,c,*]

[a]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil
[b]Programa de Pós-Graduação em Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil
[c]Programa de Pós-Graduação em Neurociências, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

*__Corresponding author:__ Marcelo Farina (farina@ccb.ufsc.br) or Dirleise Colle (dirleise@yahoo.com.br). Departamento de Bioquímica, Centro de Ciências Biológias, Campus Universitário, Trindade, Bloc C, Universidade Federal de Santa Catarina, CEP 88040-900, Florianópolis, Santa Catarina, Brazil. Fax: +554837219672; Phone: +55 4837212480.

Introduction

Huntington's disease (HD) is a neurodegenerative disorder primarily caused by a mutation in the gene encoding Huntingtin, which leads to the production of a mutated protein (mHtt) (Li e Li, 2011; Roos, 2010; Southwell e Patterson, 2011). mHtt modulates molecular events responsible for a progressive neurodegeneration of the caudate nucleus and putamen in the basal ganglia (Reiner et al., 1988; Vonsattel e DiFiglia, 1998) and in cortical regions (Heinsen et al., 1994; Mann et al., 1993), which manifests with cognitive disturbance, behavioral disorder, and movement incoordination (Roos, 2010).

A large body of evidence from both experimental and clinical studies supports a pivotal role for oxidative stress and attendant mitochondrial dysfunction in mediating the neuronal degeneration observed in HD (Stack et al., 2008). Increased levels of oxidative damage products, including protein nitration, lipid peroxidation, DNA oxidation, and exacerbated lipofuscin accumulation, occur in HD (Browne e Beal, 2006; Browne et al., 1997; Browne et al., 1999; Chen et al., 2007; Hersch et al., 2006). Strong evidence exists for early oxidative stress in HD, coupled with mitochondrial dysfunction, each exacerbating the other and leading to energy deficits (Stack et al., 2008).

The administration of 3-NP to rodents and non-human primates has been proposed as a useful experimental model of HD; both biochemical and behavioral characteristics observed in HD patients are reproduced in this model (Rossignol et al., 2011; Tunez et al., 2010). It is noteworthy that, likewise in HD, striatal GABAergic neurons are particularly affected by 3-NP exposure (Hassel e Sonnewald, 1995). Additionaly, similarly to that observed in HD patients, the dopaminergic nigrostriatal pathways are also significantly impaired following 3-NPA administration (Pei e Ebendal, 1995).

The primary mechanism of 3-NP-induced neurotoxicity involves irreversible inhibition of succinate dehydrogenase (SDH), a key enzyme located at the inner mitochondrial membrane and responsible for succinate oxidation to fumarate (Kumar e Kumar, 2009; Tunez et al., 2010). SDH inhibition interferes with mitochondrial electron transport cascade and oxidative phosphorylation, which leads to cellular energy deficit (Kumar et al., 2007). On the other hand, recent studies clearly demonstrate that impaired energy metabolism can produce oxidative stress as well as formation of reactive oxygen (ROS) and nitrogen species (Liot et al., 2009; Sandhir et al., 2010) which are critically involved in neuronal death induced by 3-NP.

Of particular importance, some lines of evidence have showed that glutathione peroxidase (GPx), an important enzyme mediating the detoxification of peroxides in several tissues (Lubos et al., 2011), including the central nervous system (CNS) (Dringen e Hirrlinger, 2003), should display an important role in HD models and in the pathogenesis of HD. In fact, GPx and peroxide metabolism seem to be important in HD because GPx was induced in the striatum and cortex of HD patients (Sorolla et al., 2008). On the other hand, Chen and co-workers (2007) demonstrated a decreased in GPx activity in erythrocyte of HD patients and no change on GPx activity was observed in R6/1 mice, a transgenic model of HD (Perez-Severiano et al., 2004). However, this topic remains elusive and additional researches in this theme is well warranted.

Probucol, a phenolic lipid-lowering agent with antioxidant and anti-inflammatory properties (Yamashita e Matsuzawa, 2009), had been clinically used during the past few decades for the treatment and prevention of cardiovascular diseases (Buckley et al., 1989; Yamashita et al., 2008; Yamashita e Matsuzawa, 2009). Interestingly, previous experimental studies have reported that probucol plays protective effects in experimental models of neurotoxicity/neuropathology (Farina et al., 2009; Park et al., 2007; Santos et al., 2011). With a particular emphasis on neurodegenerative disorders' models, Santos and co-workers demonstrated beneficial effects of probucol against $A\beta_{1-40}$-induced synaptic loss and cognitive impairment in an Alzheimer's disease *in vivo* study with mice (Santos et al., 2011). In addition, this compound was able to modulate oxidative stress and excitotoxicity in an *in vitro* HD model by decreasing 3-NP-induced ROS production and lipid peroxidation in striatal slices (Colle et al., 2012). Although the molecular mechanisms mediating the beneficial effects of probucol in the aforementioned studies are not well understood, its antioxidant, anti-inflammatory and hypocholesterolemic properties likely account for the observed protection. Of particular importance, an *in vitro* study showed that probucol increased GPx activity in primary cultures of cerebellar neurons (Farina et al., 2009), which was responsible for a protective effect against the toxicity elicited by methylmercury, an environmental pollutant whose mechanisms of toxicity are related, at least partially, to the increased production (Franco et al., 2007) and decreased detoxification (Franco et al., 2009) of peroxides. Although glutathione peroxidase has been reported as an enzyme that seems to be important in the pathogenesis of HD (Chen et al., 2007; Sorolla et al., 2008) and hydrogen peroxide has been proposed as a critical ROS mediating the

deleterious effects observed in HD models (Hands et al., 2011; Tunez et al., 2006), there are no studies in the literature on the potential protective effects of probucol in *in vivo* experimental models of HD.

As already mentioned, oxidative stress represents a crucial event in HD experimental models (Perez-Severiano et al., 2004; Santamaria et al., 2003; Tunez et al., 2006), as well as in the pathogenesis of HD (Chen et al., 2007; Hersch et al., 2006; Sorolla et al., 2008), and glutathione peroxidase has been pointed as an important enzyme in this scenario (Santamaria et al., 2003; Sorolla et al., 2008; Tunez et al., 2006). In addition, a previous *in vitro* study from our group showed a stimulatory effect of probucol toward GPx activity in primary cultured neurons (Farina et al., 2009). Based on these evidences, we hypothesized that probucol could present beneficial effects in an *in vivo* HD's model based on 3-NP intoxication. Our research was motivated by the fact that previous data support the rationale for therapeutic strategies that either potentiate antioxidant defenses or avoid oxidative stress generation to delay HD progression (Sorolla et al., 2008). Behavioral analyses (mainly based on the motor performance) and biochemical alterations (oxidative stress-related parameters, particularly related to glutathione peroxidase activity and hydrogen peroxide metabolism) were evaluated in the striatum and prefrontal cortices of rats exposed to 3-NP in an attempt to elucidate mechanisms of neurotoxicity and neuroprotection.

Materials and Methods

Chemicals

Probucol, 3-nitropropionic acid, β-Nicotinamide adenine dinucleotide phosphate sodium salt reduced from, glutathione reductase from baker's yeast, reduced glutathione and dimethyl sulfoxide were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of the highest grade available commercially.

Animals

Adult Wistar male rats (5 months old), from our own breeding colony, were maintained at 22°C, on a 12 h light: 12 h dark cycle, with free access to food and water. All experiments were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology (1989) and were

approved by our ethics committee for animal use at the Universidade Federal de Santa Catarina (CEUA/UFSC PP00424; 23080.008706/2010-52).

Drug treatment protocol

Forty animals were randomly divided into four groups containing 10 animals each. To investigate the effect of long-term administration of probucol on the behavioral and biochemical impairment, animals received approximately 3.5 mg/Kg/day of probucol or vehicle (10% of DMSO) in drinking water daily during 2 months. After this period, animals received 25 mg/Kg of 3-NP intraperitoneally (i.p.) or vehicle (NaCl 0.9%), once a day during six consecutive days (Al Mutairy et al., 2010; Tariq et al., 2005) with continuous treatment in the drinking water (probucol or vehicle), with some modifications. Water consumption was monitored every 2 days in order to correct probucol dosage if necessary and gain weight was monitored every 2 weeks.

Behavioral analysis

Twenty four hours after the last 3-NP administration, open field and rota-rod tasks were conducted for evaluation of locomotors and motor abilities.

Spontaneous locomotor activity

The animals were evaluated for 5 min in the open field arena (Archer, 1973).The apparatus, made of wood and covered with impermeable Formica, had a 100 cm x 100 cm white floor (divided by black lines into 25 20 cm x 20cm squares) and 40-cm high white walls. Each rat was placed in the center of the open field, and the number of squares crossed and rearings were registered (Moreira et al., 2010).

Rota rod task

The integrity of motor system was evaluated using the rota rod test. Briefly, the rota rod apparatus consists of a rod 30 cm long and 3 cm in diameter that is subdivided into four compartments by discs 24 cm in diameter. The rod rotates at a constant speed of 14 rpm. The animals were given a prior training session before the initialization of 3-NP

administration to acclimate them to the apparatus. The latency for first fall of from the rod and number of falls were noted. The cut-off time was 240 s (Avila et al., 2010).

Biochemical analyses

Tissue preparation

Twenty four hours after of the behavioral analyses, animals (6-5 per group) were anesthetized with isoflurane (1 mL/mL; Abbot Laboratórios do Brasil Ltda., RJ, Brazil) using a vaporizer system (SurgiVet Inc., WI, USA) and the blood was collected by cardiac puncture in heparinized tubes. Then, the animals were killed by decapitation, the brain was removed and the prefrontal cortices and striatum were dissected. The cerebral cortex and striatum (from half hemisphere) were randomly homogenized (1:10w/v) in HEPES buffer (20 mM, pH 7.0). The tissue homogenates were centrifuged 16,000 x *g*, at 4°C for 20 min and the supernatants obtained were used for the determination of enzymatic activities and for the quantification of the levels of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS). Whole blood was centrifuged at 3,000 x *g*, at room temperature for 10 min and the obtained plasma was used to measure total cholesterol levels.

Cholesterol levels

Total cholesterol levels were determined in plasma by an enzymatic method based on the oxidase/peroxidase system using commercial kit reagents (Labtest Diagnostica®, Lagoa Santa-MG, Brazil).

Antioxidant enzymes

Cortical and striatal Glutathione reductase (GR) activity was determined based on the protocol developed by Carlberg e Mannervik, 1985). Briefly, GR reduces GSSG to GSH at the expense of NADPH, the disappearance of which can be followed at 340 nm. Cortical and striatal Glutathione peroxidase (GPx) activity was determined based on the protocol developed by Wendel, 1981) by indirectly measuring the consumption of NADPH at 340 nm. The GPx uses GSH to reduce the

tert-butyl hydroperoxide, producing GSSG, which is readily reduced to GSH by GR using NADPH as a reducing equivalent donor.

Superoxide dismutase (SOD) activity was determined in cortical and striatal homogenates according to the methodology of Misra and Fridowich in 480 nm (Misra e Fridovich, 1972). The addition of samples of tissue containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored during 180 seconds. The amount of enzyme required to produce 50% inhibition was defined as one unit of enzyme activity. Catalase activity was measured by the method of Aebi et al., 1974). The reaction was started by the addition of freshly prepared 30 mM H_2O_2. The rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm.

Reduced Glutathione levels

Reduced glutathione (GSH) levels were determined by a fluorimetric assay as previously described (Hissin e Hilf, 1976). GSH was measured in cerebral cortex and striatum homogenates after precipitation with 1 volume of 0.6 M perchloric acid and centrifuged at 14,000 rpm at 4 ºC for 10 min. A volume of 50 µl of supernatant was incubated with 100 µl of ortho-phthaldehyde (0.1% w/v in methanol) and 1.85 ml of 100 mM Na_2HPO_4 for 15 min at room temperature. Fluorescence intensity was read in a microplate reader at an emission wavelength of 420 nm with an excitation wavelength of 350 nm. GSH content was calculated by using concurrently run standard curves and expressed as nmol GSH.mg protein $^{-1}$.

Determination of thiobarbituric acid reactive substances levels

Thiobarbituric acid reactive substances (TBARS) were determined in the striatal and cortical homogenates using the method described by Ohkawa et al., 1979, in which malondialdehyde (MDA), an end-product of lipid peroxidation, reacts with thiobarbituric acid to form a colored complex. The samples were incubated at 100ºC for 60 minutes in acid medium containing 0.45% sodium dodecyl sulphate and 0.67% thiobarbituric acid. After centrifugation, the reaction product was determined at 532 nm using MDA as standard.

Protein determination

The protein measurements content of the homogenates were assessed according to Lowry method (Lowry et al., 1951).

Respiratory chain complex II activity

Tissue preparation

The cerebral cortex and striatum (from half hemisphere) were homogenized (1:10w/v) in 4.4 mM potassium phosphate buffer pH 7.4, containing 0.3 M sucrose, 5 mM MOPS, 1 mM EGTA and 0.1% bovine serum albumin. The homogenates were centrifuged at 3000×g for 10 min at 4 °C. The pellet was discarded and the supernatants were centrifuged at 17,000×g for 10 min at 4 °C. The obtained pellet was dissolved in the same buffer and kept at -70 °C until enzyme activity determination (Latini et al., 2005).

Measurement of the respiratory chain Complex II activity

The activity of succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) was determined according to the method of Fischer et al., 1985. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm and calculated as nmol .min^{-1} .mg $protein^{-1}$.

Western Blot analyses

Tissue preparation

Cortical and striatal tissues were homogenized (1:10 w/v) in ice-cold lysis buffer (50 mM Tris-HCl pH 7.5, 1% Triton X-100, 100 mM NaCl, 5 mM EDTA pH 8.0, 40 mM β-glycerolphosphate, 50 mM NaF, 200 µM orthovanadate, 5% glycerol and protease inhibitors). The homogenates were centrifuged at 13,000 x *g*, at 4°C for 45 min. Prior to western blot analysis, equivalent amounts of proteins were mixed in buffer (Tris 200 mM, glycerol 10%, SDS 2%, β- mercaptoethanol 2.75 mM and bromophenol blue 0.04%), boiled for 5 minutes and kept at −20 °C until western blot analyses.

Western Blot

Samples (50 µg of protein) were subjected to SDS polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels for inducible nitric oxide synthase (iNOS). Gels were run at 30 mA for about 120 min, with the electrophoresis tank placed in an iced water bath. Separated proteins were electroblotted onto nitrocellulose membranes at a constant current intensity of 400 mA for 90 min. The membranes were blocked for 60 min at room temperature in blocking buffer containing 5% nonfat dry milk. Blots were incubated overnight at 4°C with either the primary polyclonal antibody against iNOS (1:1000, Santa Cruz) or against β-actin (1:2000) in TBS-Tween-BSA buffer (20 mM Tris base, 140 mM NaCl, 0.05% Tween-20). After washing, the blots were incubated for 60 min at room temperature with protein A/G-horseradish peroxidase conjugate in TBS-Tween buffer. Then, membranes were washed and developed with Immun-Star HRP Chemiluminescent reagents (Bio-Rad, Hercules, CA), and chemiluminescence was viewed with the Versadoc Imaging system (Bio-Rad). Band intensity was quantified by using the Scion Image software. Densitometric values from iNOS bands were normalized with respect to β-actin bands.

Statistical analysis

Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Differences among the groups were analyzed by one-way ANOVA followed by the Tukey *post hoc* test. Alternatively, 3-NP vs. probucol interactions was analyzed by two-way ANOVA followed by Bonferroni *post hoc* test. Results are expressed as mean ± SEM. The differences were considered significant when $p < 0.05$.

Results

Probucol prevents 3-NP-induced decrease in body weight, but not modify complex II inhibition

Probucol treatment did not affect physiological parameters such as body weight and food or liquid consumption when compared to non-treated animals before the 3-NP administration (data not shown). Total cholesterol levels decreased in the plasma of animals treated with probucol when compared with the control group, confirming its hipocholesterolemic effects independently from 3-NP administration

[$F_{3,15}$ = 4.7, p<0.05] (data not shown). 3-NP administration did not change total cholesterol levels in plasma.

The 3-NP administration induced a significant decrease in body weight ($F_{3, 30}$ = 7.582, p<0.001), while this decrease was not observed in the animals pretreated with probucol (Figure 1). Two-way ANOVA showed a significant interaction between 3-NP and Probucol [$F_{1,30}$ =6.01, p= 0.02] concerning body weight.

The mitochondrial complex II activity was inhibited by 3-NP treatment in cortex [$F_{3, 17}$ = 4.94, p< 0.01] (Figure 2A) and striatum [$F_{1, 21}$ = 11.88, p< 0.001] (Figure 2B). However, probucol was not able to revert this inhibition.

Probucol pretreatment prevents the motor impairment induced by 3-NP

In order to evaluate the effects of 3-NP administration on motor performance, open field and rota rod tasks were performed. 3-NP treatment was associated with significant alterations in the behavioral tests, characterized by a decrease in the number of crossings and rearing in the open field [$F_{3,32}$ = 4.578; p <0.01 and $F_{3,30}$ = 6.945; p <0.01, respectively] (Figure 3A and 3B), decrease in the latency to the first fall, and increase in the number of falls in the rota rod task [$F_{3,22}$ = 9.543; p <0.05 and $F_{3,24}$ = 10.84 ; p <0.001, respectively] (Figure 3C and 3D). Probucol pretreatment for 60 days improved the performance of the 3-NP-exposed rats in the open-field and in rota rod tasks. Probucol pretreatment totally protected against 3-NP-induced decrease in the number of rearing in the open field and decrease in the latency to the first fall in the rota rod, but the decrease in the number of crossings and the increased in the number of falls were only partially protected by probucol. This data indicate that probucol pretreatment was able to prevent the effect of 3-NP administration.

Probucol pretreatment prevents cortical and striatal oxidative stress induced by 3-NP

As shown in Figure 4, 3-NP administration caused a significant increase in TBARS production in the cortex ($F_{3,19}$ =7.085, p<0.01) and striatum [$F_{3,15}$ =6.271, p<0.01] (Figure 4A and 4B). Pretreatment with probucol led to a decrease in striatal and cortical lipid peroxidation to levels indistinguishable (similar) from controls (Figure 4A and 4B). In agreement, two-way ANOVA showed significant interactions between

3-NP and Probucol in cortex [$F_{1,19}$ =10.21, p= 0.0048] and striatum [$F_{1,15}$ =13.14, p= 0.0025] concerning TBARS levels.

Additionally, the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase were significantly increased by 3-NP in cortex and striatum when compared with control group [$F_{3,15}$ =4.00, p<0.05 in cortex and $F_{3,18}$ =4.972, p<0.05 in striatum] (Figure 5A and 5B), and [$F_{3,16}$ =6.873, p<0.01 in cortex and $F_{3,18}$ =6.046, p<0.05 in striatum] (Figure 5C and 5D), respectively. Probucol treatment significantly attenuated the 3-NP-induced increase in SOD and catalase activities. Moreover, interactions were observed between 3-NP and probucol by two-away ANOVA regarding SOD [$F_{1,16}$ =4.54, p=0.0489 in cortex and $F_{1,18}$ =4.92, p=0.0397 in striatum] and catalase [$F_{1,16}$ =11.44, p=0.0038 in cortex and $F_{1,18}$ =10.66, p=0.0043 in striatum] activities, respectively.

Effects of 3-NP and/or probucol on cortical and striatal glutathione peroxidase activity and other GSH-related parameters

Probucol treatment caused a significant increase in cortical and striatal glutathione peroxidase (GPx) activity independently from 3-NP administration as indicated by a significant one-way ANOVA [$F_{3,13}$ =4.486, p<0.05] (Figure 6A) and [$F_{3,17}$ =7.246, p<0.05] (Figure 6B). 3NP-treatment significantly increased glutathione reductase (GR) activity when compared with control group in cortical tissue [$F_{3,15}$ =4.068, p<0.05] (Figure 7A). In addition, two-way ANOVA showed significant interactions between 3-NP and Probucol [$F_{1,15}$=9.1, p= 0.0087] concerning GR activity in cortex. Striatal GR activity was not significantly different among groups (Figures 7B).

3-NP-treatment significantly decreased GSH levels in cortex when compared with control group [$F_{3,15}$ =5.159, p<0.05], and probucol pretreatment significantly attenuated the 3-NP-induced decrease in GSH levels (Figure 7C). Two-way ANOVA showed significant interaction between 3-NP and Probucol [$F_{1,13}$ =13.3, p= 0.003] concerning GSH levels in cortex. Striatal GSH content was not significantly different among groups by one-away ANOVA, but a significant effect of the pretreatment was observed in two-way ANOVA [$F_{1,14}$ =5.84, p= 0.03] (Figure 7D).

Probucol pretreatment prevents cortical and striatal increase of iNOS expression induced by 3-NP

3-NP treated rats showed higher levels of iNOS expression in cortex [$F_{3,8}$ = 11.35; P<0.05] (Figure 8A) and striatum [$F_{3,8}$ = 7.786; P<0.05] (Figure 8B) compared to the vehicle-treated rats, confirming iNOS increments in the 3-NP model. However, the 3-NP plus probucol groups showed iNOS levels statistically similar to controls, suggesting that probucol blunted the iNOS up-regulation induced by 3-NP administration.

Financial support: The financial supports by (i) FINEP Research Grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00, (ii) Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), (iii) Fundação de Apoio à Pesquisa do Estado de Santa Catarina (FAPESC - TO 1346/2010-3), (iv) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and (v) INCT-CNPq-Excitotoxicity and Neuroprotection are gratefully acknowledged. M.F. recipient of CNPq fellowship.

References

Aebi, H., Wyss, S. R., Scherz, B., and Skvaril, F., 1974. Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. Eur J Biochem 48, 137-145.

Al Mutairy, A., Al Kadasah, S., Elfaki, I., Arshaduddin, M., Malik, D., Al Moutaery, K., and Tariq, M., 2010. Trolox ameliorates 3-nitropropionic acid-induced neurotoxicity in rats. Neurotoxicol Teratol 32, 226-233.

Archer, J., 1973. Tests for emotionality in rats and mice: a review. Anim Behav 21, 205-235.

Avila, D. S., Colle, D., Gubert, P., Palma, A. S., Puntel, G., Manarin, F., Noremberg, S., Nascimento, P. C., Aschner, M., Rocha, J. B., and Soares, F. A., 2010. A possible neuroprotective action of a vinylic telluride against Mn-induced neurotoxicity. Toxicol Sci 115, 194-201.

Browne, S. E., and Beal, M. F., 2006. Oxidative damage in Huntington's disease pathogenesis. Antioxid Redox Signal 8, 2061-2073.

Browne, S. E., Bowling, A. C., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D., and Beal, M. F., 1997. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol 41, 646-653.

Browne, S. E., Ferrante, R. J., and Beal, M. F., 1999. Oxidative stress in Huntington's disease. Brain Pathol 9, 147-163.

Buckley, M. M., Goa, K. L., Price, A. H., and Brogden, R. N., 1989. Probucol. A reappraisal of its pharmacological properties and therapeutic use in hypercholesterolaemia. Drugs 37, 761-800.

Carlberg, I., and Mannervik, B., 1985. Glutathione reductase. Methods Enzymol 113, 484-490.

Chen, C. M., Wu, Y. R., Cheng, M. L., Liu, J. L., Lee, Y. M., Lee, P. W., Soong, B. W., and Chiu, D. T., 2007. Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. Biochem Biophys Res Commun 359, 335-340.

Colle, D., Hartwig, J. M., Antunes Soares, F. A., and Farina, M., 2012. Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro. Brain Res Bull.

Dringen, R., and Hirrlinger, J., 2003. Glutathione pathways in the brain. Biol Chem 384, 505-516.

Farina, M., Campos, F., Vendrell, I., Berenguer, J., Barzi, M., Pons, S., and Sunol, C., 2009. Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells. Toxicol Sci 112, 416-426.

Fischer, J. C., Ruitenbeek, W., Berden, J. A., Trijbels, J. M., Veerkamp, J. H., Stadhouders, A. M., Sengers, R. C., and Janssen, A. J., 1985. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 153, 23-36.

Franco, J. L., Braga, H. C., Stringari, J., Missau, F. C., Posser, T., Mendes, B. G., Leal, R. B., Santos, A. R., Dafre, A. L., Pizzolatti, M. G., and Farina, M., 2007. Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. Chem Res Toxicol 20, 1919-1926.

Franco, J. L., Posser, T., Dunkley, P. R., Dickson, P. W., Mattos, J. J., Martins, R., Bainy, A. C., Marques, M. R., Dafre, A. L., and Farina, M., 2009. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. Free Radic Biol Med 47, 449-457.

Hands, S., Sajjad, M. U., Newton, M. J., and Wyttenbach, A., 2011. In vitro and in vivo aggregation of a fragment of huntingtin protein directly causes free radical production. J Biol Chem 286, 44512-44520.

Hassel, B., and Sonnewald, U., 1995. Selective inhibition of the tricarboxylic acid cycle of GABAergic neurons with 3-nitropropionic acid in vivo. J Neurochem 65, 1184-1191.

Heinsen, H., Strik, M., Bauer, M., Luther, K., Ulmar, G., Gangnus, D., Jungkunz, G., Eisenmenger, W., and Gotz, M., 1994. Cortical and striatal neurone number in Huntington's disease. Acta Neuropathol 88, 320-333.

Hersch, S. M., Gevorkian, S., Marder, K., Moskowitz, C., Feigin, A., Cox, M., Como, P., Zimmerman, C., Lin, M., Zhang, L., Ulug, A. M., Beal, M. F., Matson, W., Bogdanov, M., Ebbel, E., Zaleta, A., Kaneko, Y., Jenkins, B., Hevelone, N., Zhang, H., Yu, H., Schoenfeld, D., Ferrante, R., and Rosas, H. D., 2006. Creatine in Huntington disease is safe, tolerable, bioavailable in brain and reduces serum 8OH2'dG. Neurology 66, 250-252.

Hissin, P. J., and Hilf, R., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74, 214-226.

Kumar, P., and Kumar, A., 2009. Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunctions in rat brain. Prog Neuropsychopharmacol Biol Psychiatry 33, 100-108.

Kumar, P., Padi, S. S., Naidu, P. S., and Kumar, A., 2007. Cyclooxygenase inhibition attenuates 3-nitropropionic acid-induced neurotoxicity in rats: possible antioxidant mechanisms. Fundam Clin Pharmacol 21, 297-306.

Latini, A., da Silva, C. G., Ferreira, G. C., Schuck, P. F., Scussiato, K., Sarkis, J. J., Dutra Filho, C. S., Wyse, A. T., Wannmacher, C. M., and Wajner, M., 2005. Mitochondrial energy metabolism is markedly impaired by D-2-hydroxyglutaric acid in rat tissues. Mol Genet Metab 86, 188-199.

Li, X. J., and Li, S., 2011. Proteasomal dysfunction in aging and Huntington disease. Neurobiol Dis 43, 4-8.

Liot, G., Bossy, B., Lubitz, S., Kushnareva, Y., Sejbuk, N., and Bossy-Wetzel, E., 2009. Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway. Cell Death Differ 16, 899-909.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265-275.

Lubos, E., Loscalzo, J., and Handy, D. E., 2011. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 15, 1957-1997.

Mann, D. M., Oliver, R., and Snowden, J. S., 1993. The topographic distribution of brain atrophy in Huntington's disease and progressive supranuclear palsy. Acta Neuropathol 85, 553-559.

Misra, H. P., and Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247, 3170-3175.

Moreira, E. L., Rial, D., Duarte, F. S., de Carvalho, C. R., Horst, H., Pizzolatti, M. G., Prediger, R. D., and Ribeiro-do-Valle, R. M., 2010. Central nervous system activity of the proanthocyanidin-rich fraction obtained from Croton celtidifolius in rats. J Pharm Pharmacol 62, 1061-1068.

Ohkawa, H., Ohishi, N., and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95, 351-358.

Park, S. Y., Lee, J. H., Kim, C. D., Rhim, B. Y., Hong, K. W., and Lee, W. S., 2007. Beneficial synergistic effects of concurrent treatment with cilostazol and probucol against focal cerebral ischemic injury in rats. Brain Res 1157, 112-120.

Pei, G., and Ebendal, T., 1995. Specific lesions in the extrapyramidal system of the rat brain induced by 3-nitropropionic acid (3-NPA). Exp Neurol 132, 105-115.

Perez-Severiano, F., Rodriguez-Perez, M., Pedraza-Chaverri, J., Maldonado, P. D., Medina-Campos, O. N., Ortiz-Plata, A., Sanchez-Garcia, A., Villeda-Hernandez, J., Galvan-Arzate, S., Aguilera, P., and Santamaria, A., 2004. S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem Int 45, 1175-1183.

Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C. J., Penney, J. B., and Young, A. B., 1988. Differential loss of striatal projection neurons in Huntington disease. Proc Natl Acad Sci U S A 85, 5733-5737.

Roos, R. A., 2010. Huntington's disease: a clinical review. Orphanet J Rare Dis 5, 40.

Rossignol, J., Boyer, C., Leveque, X., Fink, K. D., Thinard, R., Blanchard, F., Dunbar, G. L., and Lescaudron, L., 2011. Mesenchymal stem cell transplantation and DMEM administration in a 3NP rat model of Huntington's disease: morphological and behavioral outcomes. Behav Brain Res 217, 369-378.

Sandhir, R., Mehrotra, A., and Kamboj, S. S., 2010. Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system. Neurochem Int 57, 579-587.

Santamaria, A., Salvatierra-Sanchez, R., Vazquez-Roman, B., Santiago-Lopez, D., Villeda-Hernandez, J., Galvan-Arzate, S., Jimenez-Capdeville, M. E., and Ali, S. F., 2003. Protective effects of the antioxidant selenium on quinolinic acid-induced neurotoxicity in rats: in vitro and in vivo studies. J Neurochem 86, 479-488.

Santos, D. B., Peres, K. C., Ribeiro, R. P., Colle, D., Santos, A. A., Moreira, E. L., Souza, D. O., Figueiredo, C. P., and Farina, M., 2011. Probucol, a lipid-lowering drug, prevents cognitive and

hippocampal synaptic impairments induced by amyloid beta peptide in mice. Exp Neurol.

Sorolla, M. A., Reverter-Branchat, G., Tamarit, J., Ferrer, I., Ros, J., and Cabiscol, E., 2008. Proteomic and oxidative stress analysis in human brain samples of Huntington disease. Free Radic Biol Med 45, 667-678.

Southwell, A. L., and Patterson, P. H., 2011. Gene therapy in mouse models of huntington disease. Neuroscientist 17, 153-162.

Stack, E. C., Matson, W. R., and Ferrante, R. J., 2008. Evidence of oxidant damage in Huntington's disease: translational strategies using antioxidants. Ann N Y Acad Sci 1147, 79-92.

Tariq, M., Khan, H. A., Elfaki, I., Al Deeb, S., and Al Moutaery, K., 2005. Neuroprotective effect of nicotine against 3-nitropropionic acid (3-NP)-induced experimental Huntington's disease in rats. Brain Res Bull 67, 161-168.

Tunez, I., Drucker-Colin, R., Jimena, I., Medina, F. J., Munoz Mdel, C., Pena, J., and Montilla, P., 2006. Transcranial magnetic stimulation attenuates cell loss and oxidative damage in the striatum induced in the 3-nitropropionic model of Huntington's disease. J Neurochem 97, 619-630.

Tunez, I., Tasset, I., Perez-De La Cruz, V., and Santamaria, A., 2010. 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. Molecules 15, 878-916.

Vonsattel, J. P., and DiFiglia, M., 1998. Huntington disease. J Neuropathol Exp Neurol 57, 369-384.

Wendel, A., 1981. Glutathione peroxidase. Methods Enzymol 77, 325-333.

Yamashita, S., Hbujo, H., Arai, H., Harada-Shiba, M., Matsui, S., Fukushima, M., Saito, Y., Kita, T., and Matsuzawa, Y., 2008. Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. J Atheroscler Thromb 15, 292-303.

Yamashita, S., and Matsuzawa, Y., 2009. Where are we with probucol: a new life for an old drug? Atherosclerosis 207, 16-23.

FIGURES AND LEGENDS

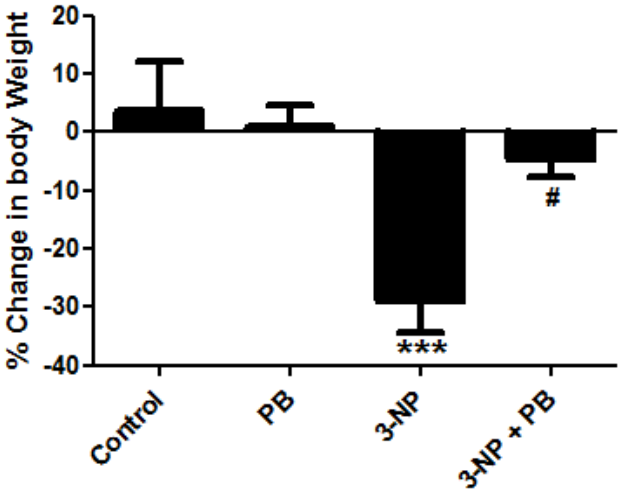

Figure 1: Probucol prevents 3-NP-induced decreased in body weight. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10 % of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. The values are expressed as percentage of change in body weight after 3-NP administrations and expressed as mean ± S.E.M. (n=10-11 rats/group). ***$p < 0.001$ when compared with the control group and # $p< 0.05$ when compared with the 3-NP group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

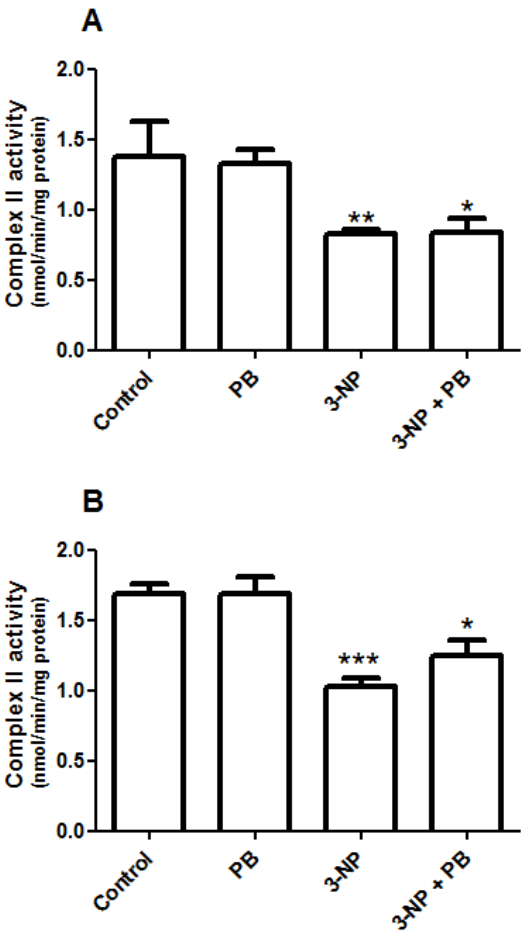

Figure 2: 3-NP treatment reduces complex II activity. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10% of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Complex II activity in cortex (A) and striatum (B) is expressed as nmol.min^{-1}.mg protein^{-1} and presented as mean ± S.E.M. (n=5-6 rats/group). *p < 0.05, ** p<0.01 and *** p<0.001 when compared with the control group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

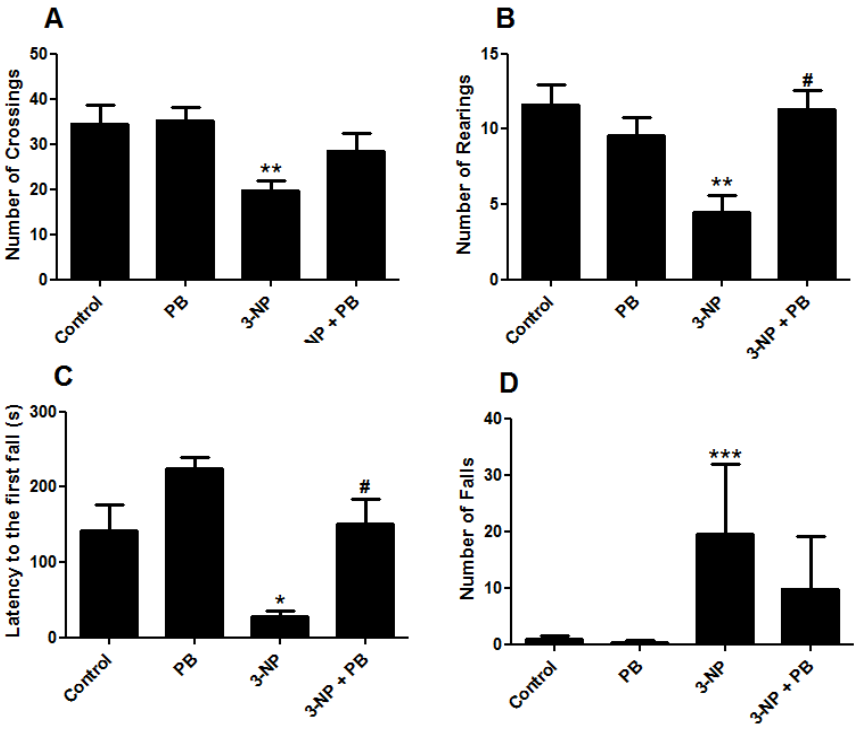

Figure 3: Probucol attenuates motor impairment induced by 3-NP in rats. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10% of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Locomotor (A) and exploratory (B) activities in the open field as well as the latency for the first fall (C) and the number of falls in the rota rod (D) were evaluated 24 h after the last 3-NP administration. The results are expressed as the total number of crossings (A), total number of rearings (B), the latency for the first fall (s) (C) and total number of falls. Data are presented as mean ± S.E.M. (n=9-10 rats/group). *p < 0.05, ** p<0.01 and *** p<0.01 when compared with the control group and # p<0.05 when compared with the 3-NP group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

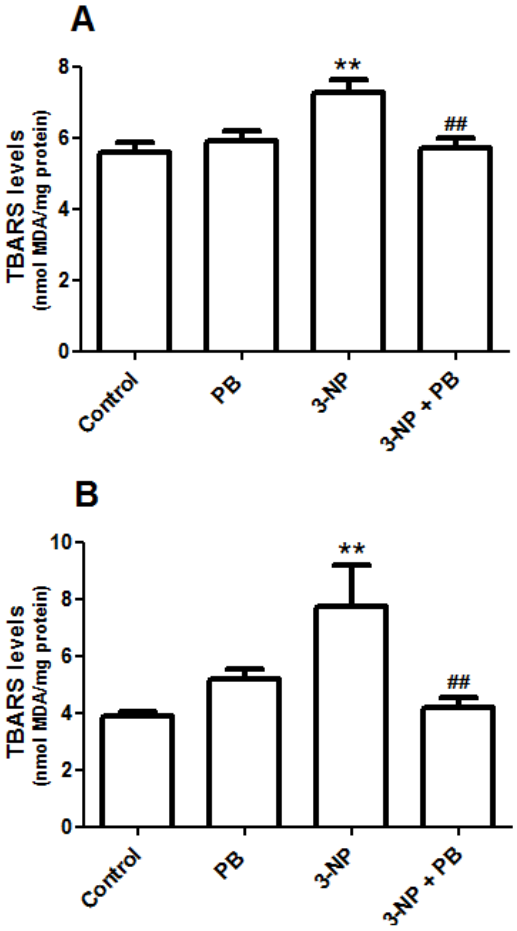

Figure 4: Probucol prevents cortical and striatal lipid peroxidation induced by 3-NP in rats. The animals were pretreated with 3.5 mg/Kg of PB or vehicle (10% of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Cortical (A) and striatal (B) thiobarbituric acid reactive substances (TBARS) levels are expressed as nmol of MDA/mg protein. Data are presented as mean ± S.E.M. (n= 5-6 rats/group). **p< 0.01 when compared with the control group and ## p<0.01 when compared with the 3-NP group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

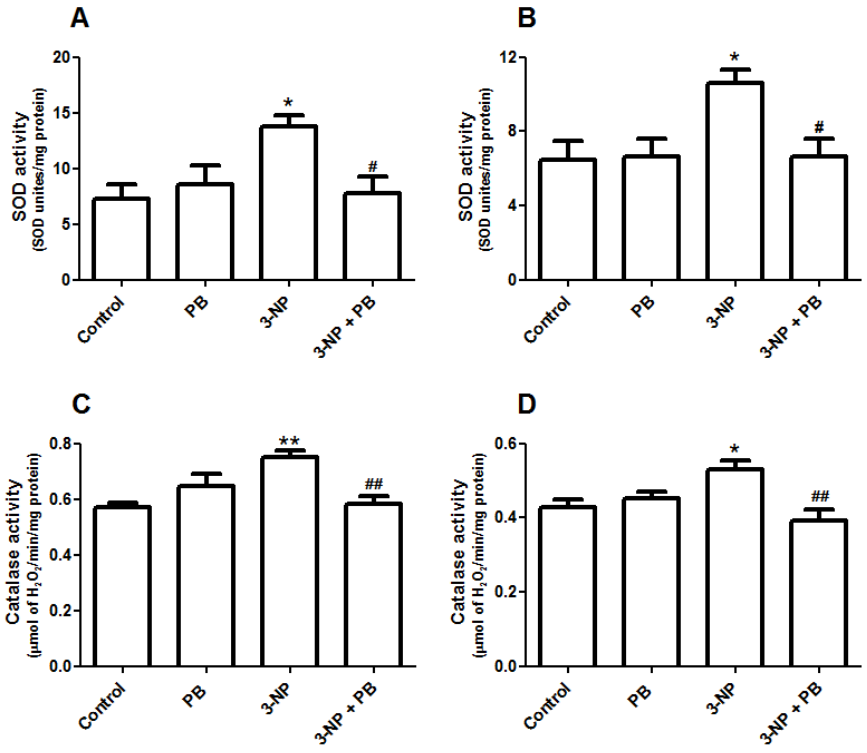

Figure 5: Probucol prevents the increase in superoxide dismutase (SOD) and catalase activities in cortex and striatum of rats. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10% of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Cortical (A) and striatal (B) SOD activity is expressed as SOD unites/mg of protein. Catalase activity in cortex (C) and striatum (D) is expressed as µmol of H_2O_2/min/mg protein. Data are presented as mean ± S.E.M. (n=5-6 rats/group). *p< 0.05 and **p<0.01 when compared with the control group, and #p<0.05 and ##p<0.01 when compared with the 3-NP group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

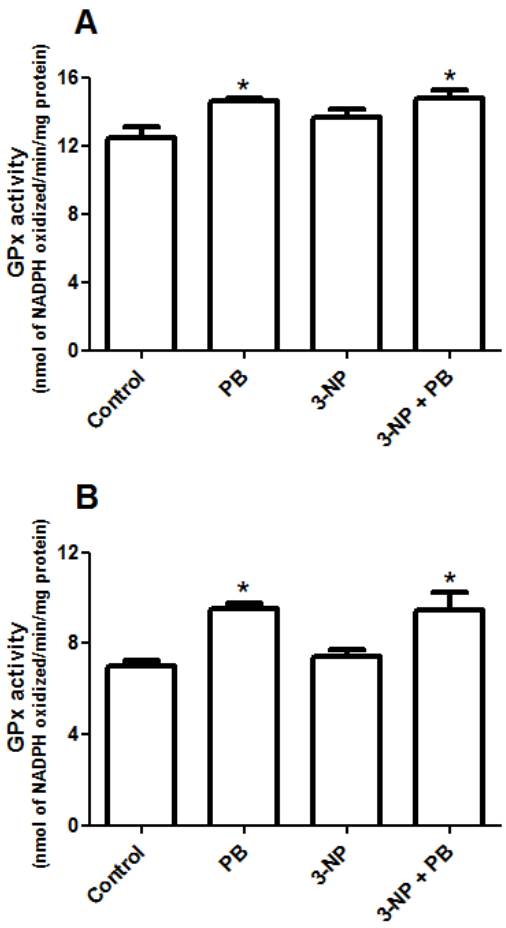

Figure 6: Probucol modulates the cortical and striatal glutathione peroxidase activity. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10 % of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Cortical (A) and striatal (B) GPx activity is expressed as nmol of NADPH oxidized/min/mg protein. Data are presented as mean ± S.E.M. (n=5-6 rats/group). *p< 0.05 when compared with the control group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

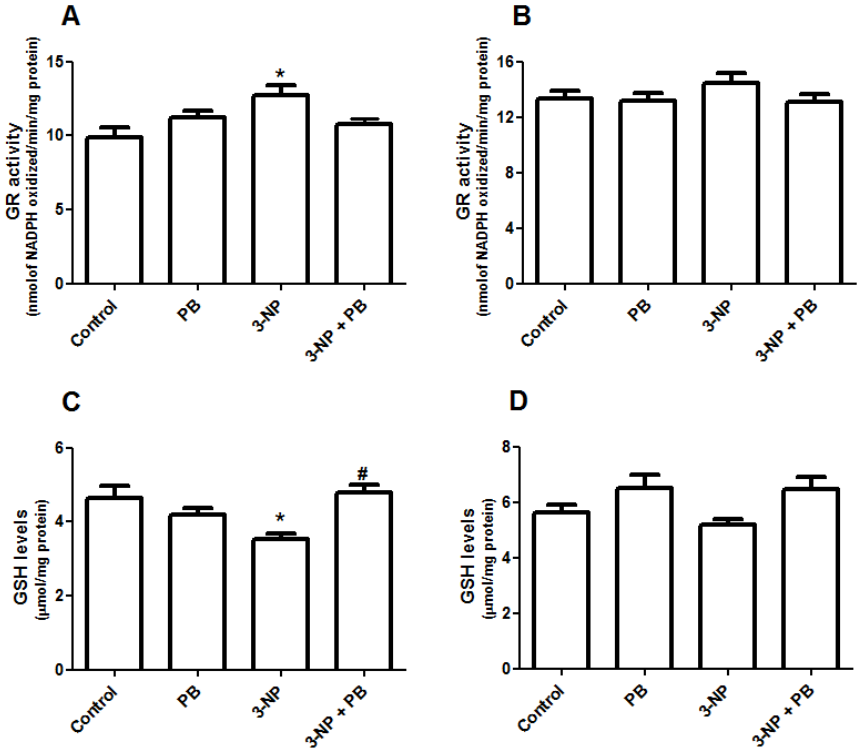

Figure 7: Probucol prevents the increase in glutathione reductase activity and the decreased in reduced glutathione levels induced by 3-NP in the cortex of rats. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10% of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Cortical (A) and striatal (B) GR activity is expressed as nmol of NADPH oxidized/min/mg protein. GSH levels in cortex (C) and striatum (D) are expressed as µmol GSH.mg protein^{-1}. Data are presented as mean ± S.E.M. (n=5-6 rats/group). *$p< 0.05$ when compared with the control group and # $p<0.05$ when compared with the 3-NP group by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

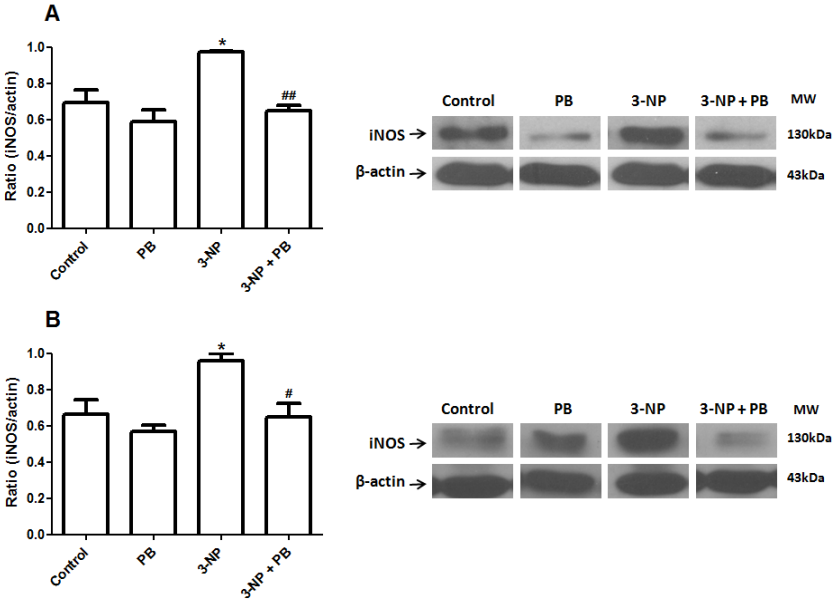

Figure 8: Probucol prevents cortical and striatal increase in iNOS expression induced by 3-NP. The animals were pretreated with 3.5 mg/Kg of probucol or vehicle (10% of DMSO) in drinking water daily for 2 months and administered intraperitoneally with 3-NP (25 mg/Kg) or vehicle, once a day, during 6 consecutive days. Cortical (A) and striatal (B) iNOS expression was determined by *Western blot* and expressed as optical density related to actin. Data are presented as mean ± S.E.M. (n=3 rats/group). *$p < 0.05$ when compared with the control group, and #$p<0.05$ and $p<0.01$ when compared with the 3-NP group by one-way analysis of variance (ANOVA) followed by
Tukey's multiple comparison test.

4.3 PROBUCOL E SUCCINOBUCOL PREVINEM O DANO MITOCONDRIAL E O ESTRESSE OXIDATIVO INDUZIDO PELO 3-NP *IN VITRO*

Manuscrito 2

PROBUCOL AND SUCCINOBUCOL PREVENTS 3-NP-INDUCED MITOCHONDRIAL DAMAGE AND OXIDATIVE STRESS *IN VITRO*

Dirleise Colle, Danúbia B. dos Santos, Juliana M. Hartwig, Marcelo Godoi, Antonio L. Braga, Marcelo Farina

Probucol and succinobucol prevent 3-NP-induced mitochondrial damage and oxidative stress *in vitro*

Dirleise Colle[1], Danúbia B. Santos[1], Juliana M. Hartwig[1], Marcelo Godoi[2], Antonio L. Braga[2], Marcelo Farina[1,*]

[1]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil
[2] Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.

[*]**Corresponding author:** Marcelo Farina (farina@ccb.ufsc.br), Departamento de Bioquímica, Centro de Ciências Biológicas, Campus Universitário, Trindade, Bloco C, Universidade Federal de Santa Catarina, CEP 88040-900, Florianópolis, Santa Catarina, Brazil. Fax: +554837219672; Phone: +55 4837219589.

Abstract

Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by symptoms attributable to the death of striatal and cortical neurons. Mitochondrial dysfunction and oxidative damage have been related with neurodegeneration and cell death in HD. This study evaluated and compared the potential protective effects of probucol and succinobucol, two compounds with anti-inflammatory and antioxidant properties, on oxidative stress and mitochondrial dysfunction induced by 3-nitropropionic acid (3-NP, an inhibitor of succinate dehidrogenase) in rat brain mitochondria-enriched preparation. 3-NP caused significant inhibition of mitochondrial complex II activity, induced mitochondrial dysfunction and increased reactive oxygen species (ROS) generation, as well as significantly increased lipid peroxidation in mitochondria. Probucol and succinobucol pretreatment (1, 3 and 10 μM) did not prevent 3-NP-induced mitochondrial complex II inhibition. On the other hand, both compounds prevented ROS formation and lipid peroxidation, but only succinobucol was able to prevent the mitochondrial dysfunction induced by 3-NP. The present results indicate that probucol and succinobucol are able to counteract the oxidative stress induced by 3-NP. In addition, mitochondrial dysfunction induced by 3-NP was completely protected by succinobucol in a mechanism independent of complex II inhibition. This is the first study reporting the beneficial effects of succinobucol in an *in vitro* experimental model of a neurodegenerative disease. The present findings suggest that succinobucol might be a novel strategy to slow or halt oxidative process in neurodegenerative process, but further studies are needed to fully elucidate its mechanism of action.

Keywords: Huntington's disease, 3-nitropropionic acid, probucol, succinobucol, mitochondrial dysfunction, oxidative stress.

.

1. Introduction

Mitochondria are responsible for major biochemical functions needed for cellular homeostasis, and represent the main source of ATP. Basic research has demonstrated that mitochondria play crucial roles in cell survival by controlling energy metabolism, apoptosis pathways, calcium (Ca^{2+}) homeostasis and free radicals production (Mattson et al., 2008). With a particular emphasis on the central nervous system (CNS), it is well known that an adequate balance in these processes is essential for neuronal signaling, plasticity and neurotransmitter release. On the other hand, mitochondrial dysfunction and oxidative damage have been related with the pathogenesis of numerous neurodegenerative disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD) (de Moura et al., 2010). In Huntington's disease, recent studies have highlighted the particular mechanisms that directly link mitochondrial defects with neurodegeneration and cell death (Damiano et al., 2010; Mochel e Haller, 2011; Oliveira, 2010).

HD is a progressive neurodegenerative disorder caused by mutation in gene encoding Huntingtin protein leading to production of neurotoxic form of mutated Huntingtin (m-Htt) protein (Roos, 2010; Ross e Tabrizi, 2011; Southwell e Patterson, 2011), and is characterized by a progressive neurodegeneration mainly in the basal ganglia and cortical regions (Jackson et al., 1991).

A considerable amount of evidence suggests that mitochondrial dysfunction is directly or indirectly involved in HD (Reddy et al., 2009). For example, biochemical studies demonstrated decreased glucose metabolism (Andrews e Brooks, 1998; Kuwert et al., 1990) and elevated lactate production (Browne, 2008; Reynolds et al., 2005) in the basal ganglia and cerebral cortex in HD patients. In addition, analysis of *postmortem* HD brain showed reduced activity in complex II, III, and IV of the electron transport chain (Benchoua et al., 2006; Brennan et al., 1985; Browne et al., 1997; Gu et al., 1996; Lim et al., 2008). Mitochondrial complex II dysfunction in HD seems to be intimately associated with oxidative stress and excitotoxicity that leads to cell death (Benchoua et al., 2006; Browne, 2008; Mochel e Haller, 2011). Reactive oxygen species (ROS) generated by mitochondria have shown to target different molecules, including diverse mitochondrial components (lipids, proteins, DNA, etc.) (Perez-De la Cruz et al., 2010).

The administration of the mitochondrial toxin 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehidrogenase (SDH, complex II), was shown to elicit cortical and striatal neuronal

degeneration and behavioral abnormalities in animals similarly to those observed in HD patients (Beal et al., 1993; Brouillet et al., 1998; Rossignol et al., 2011; Tunez et al., 2010). The mechanism of 3-NP-induced neurotoxicity involves depletion of ATP levels, mitochondrial membrane despolarization, dysregulation of intracellular calcium homeostasis, generation of ROS and activation of death pathways (Bizat et al., 2003; Kumar et al., 2010; Lee et al., 2002; Lee et al., 2002; Montilla et al., 2004).

Probucol (PB) is a phenolic lipid-lowering agent with powerful antioxidant properties, clinically used during the past few decades for the treatment and prevention of cardiovascular diseases (Buckley et al., 1989; Yamashita et al., 2008; Yamashita e Matsuzawa, 2009); due to its hypocholesterolemic and anti-inflammatory properties (Yamashita e Matsuzawa, 2009). Probucol is an important agent in promotion endogenous antioxidant reserve and protecting against increased in oxidative stress (Singla et al., 2007). Furthermore, a recent study has shown that this compound is able to prevent the oxidative stress in heart mitochondria (Lemieux et al., 2011). Of particular importance, previous experimental studies have also reported that probucol plays protective effects in experimental models of neurotoxicity/neuropathology (Colle et al., 2012; Farina et al., 2009; Park et al., 2007; Santos et al., 2011). Despite the benefits of the probucol, this compound was associated to adverse and undesirable effects including lowering of HDL cholesterol (Tardif et al., 2002) and prolongation of cardiac repolarization (McDowell et al., 1994; Tardif et al., 2003). Alternatively, succinobucol (AGI-1067), the monosuccinic acid ester of probucol ("*probucol-derivative*"), is a metabolically stable modification that retains antioxidant and anti-inflammatory properties equipotent to those of probucol but did not cause its collateral effects (Kunsch et al., 2004). Although some studies have evaluated the potential beneficial effects of probucol and its derivative (succinobucol) in different pathological conditions (Al-Majed, 2011; Sia et al., 2002; Tardif et al., 2008; Tardif et al., 2008), no studies have evaluated the potential protective effects of succinobucol in models of neurophatology.

As mentioned previously, mitochondrial damage and oxidative stress seem to be involved in the neurodegeneration observed in HD (Damiano et al., 2010; Mochel e Haller, 2011; Oliveira, 2010). On the other hand, probucol and succinobucol have notably shown anti-inflammatory and antioxidant properties (Kunsch et al., 2004; Pallebage-Gamarallage et al., 2010) and the pattern compound has been reported to present neuroprotective effects in the experimental models

(Colle et al., 2012; Farina et al., 2009; Park et al., 2007; Santos et al., 2011). However, there is a lack of evidence concerning the potential protective effect of succinobucol in experimental models of neurotoxicity/neuropathology in the literature. This kind of evidence seems to be well-warranted because of the absence of adverse effects of succinobucol in humans, in sharp opposition to its pattern compound (probucol). Thus, we used an *in vitro* approach with mitochondria-enriched preparations derived from rat brain in order to tested the hypothesis whether probucol and its analogue succinobucol could prevent the potential deleterious effects of 3-NP, comparing their effects. Biochemical parameters related to energetic metabolism and oxidative stress in mitochondria-enriched preparations exposed to 3-NP, as well as the potential protective effects of probucol and succinobucol against 3-NP-induced mitochondrial dysfunction were investigated in an attempt to elucidate mechanisms of protection.

2. Material and Methods

2.1. Compounds

The chemical structures of the compounds tested in the present study are shown in the Figure 1: Probucol was purchased from Sigma Aldrich and Succinobucol was synthesized according to previous literature (Haibin et al., 2006).

2.2. Chemicals

3-Nitropropionic acid, sodium succinate, thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2',7'-dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma (St. Louis, MO, USA). All other reagents were obtained from local suppliers.

2.3. Animals

Adult male Wistar rats (200-250 g) from our own breeding colony were kept in cages with continuous access to food in a room with controlled temperature (22 ± 3°C) and a 12 h light/dark cycle, with lights on at 7:00 am. All experiments were conducted in accordance with the Guiding Principles of the Animal Care and Wellness

Committee of the Universidade Federal de Santa Catarina (CEUA/UFSC PP00424; 23080.008706/2010-52).

2.4. Preparation of brain mitochondrial-enriched fractions

Rat brain mitochondria were isolated as previously described Brustovetsky e Dubinsky, 2000) with some modifications. Rats were killed by decapitation and the brain was rapidly removed and homogenized (1:5 w/v) in ice-cold ‘‘isolation buffer I’’ (225 mM mannitol, 75mM sucrose, 1mM EGTA, 0.1% bovine serum albumin (BSA), and 10mM HEPES, pH 7.2). The resulting suspension was centrifuged for 7 min at 2000 X g at 2°C. After centrifugation, the supernatant was recentrifuged for 10 min at 12,000 X g. The pellet was resuspended in 5 mL of ‘‘isolation buffer II’’ containing 225 mM mannitol, 75 mM sucrose, 1 mM EGTA, and 10 mM HEPES, pH 7.2, and recentrifuged at 12,000 X g for 10 min. The supernatant was decanted, and the final pellet was gently washed and resuspended in “incubation buffer” (65 mM KCl, 100 mM sucrose, 50 µM EGTA, and 10 mM HEPES, pH 7.2), to a protein concentration of approximately 10 mg/mL. These enriched mitochondrial fractions were used for measurement of MTT reduction assay, lipid peroxidation and ROS production.

For measuring the respiratory chain complex II activity, tissue mitochondrial suspensions were prepared as previously described (Latini et al., 2005). Briefly, tissues were homogenized in 10 volumes of 4.4 mM potassium phosphate buffer pH 7.4, containing 0.3 M sucrose, 5 mM MOPS, 1 mM EGTA and 0.1% bovine serum albumin. The homogenates were centrifuged at 3000×g for 10 min at 4 °C. The pellet was discarded and the supernatants were centrifuged at 17,000×g for 10 min at 4 °C. The obtained pellet was dissolved in the same buffer.

2.5. Standard incubation procedure

3-NP was dissolved in PBS buffer and neutralized to pH 7.4 with NaOH, and they were freshly prepared each time before treatment. Probucol and succinobucol were dissolved in dimethylsulfoxide (DMSO), which was used as control/vehicle and whose concentration did not exceed 0.1%.

Mitochondria-enriched fractions (0.5 mg protein) were added to 200 µl standard incubation buffer. Sodium succinate (2 mM) was added in the incubation medium, and mitochondria-enriched fractions were

incubated with vehicle or 3-NP (1, 2 or 3 mM) at 37°C for 30, 60 or 120 min. Some experiments were performed in the presence of probucol or succinobucol (1, 3 and 10 µM), or vehicles. These compounds were pre-incubated for 2.5 h and maintained during 3-NP incubation.

Some experiments were performed in the presence of by *tert*-butyl hydroperoxide (*t*BuOOH) in which mitochondria were incubated with this organic peroxide (300 µM) at 37°C for 30 min after probucol or succinobucol pre-incubations. The analytical procedures were performed immediately after the last incubation. The results shown are representative of a series of four to five independent experiments, using independently isolated mitochondrial-enriched fractions.

2.6. MTT reduction assay

MTT reduction assay was evaluated as an index of mitochondrial function, according to previous reports (Elinos-Calderon et al., 2010; Perez-De La Cruz et al., 2009). This method is based in the ability of cells to reduce MTT to a dark violet formazan product by mitochondrial dehydrogenases in viable cells (Mosmann, 1983).

Mitochondrial-enriched fractions were incubated with 200 µL of MTT (0.5 mg/mL), and re-incubated at 37°C for 20 min. Samples were then centrifuged at 16,000 X g, 10 min and the pellet were washed for 60 minutes in 300 µL of dimethylsulfoxide (DMSO) to remove the formazan. Quantification of formazan was estimated by measuring optical density at 540 nm. Results were expressed as the percentage of MTT reduction with respect to control values.

2.7. Estimation of reactive oxygen species (ROS) production

Formation of ROS was estimated with the fluorescent probe, 2',7'-dichlorofluorescein diacetate (DCFH-DA), as described by Ali et al., 1992. DCFH-DA is enzymatically hydrolyzed by intracellular esterase to form non fluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2', 7'-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. At the end of incubation, DCFH-DA (5 µM) was added to the mitochondrial-enriched fractions and re-incubated for 20 min. Mitochondria-enriched fractions were centrifuged at 1,000 X g, 5 min and the pellet were resuspended in PBS for determination of intra-mitochondrial ROS levels. Fluorescence was read using excitation and emission wavelengths of 480 and 525 nm, respectively, using

oxidized DCF as standard. The values are expressed as percent of control in the absence of treatment.

2.8. Lipid peroxidation assay

Lipid peroxidation (LP) was assessed in the mitochondrial-enriched fractions by the assay of thiobarbituric acid-reactive substances (TBARS) formation, according to previous reports (Ohkawa et al., 1979). Mitochondria-enriched fractions were centrifuged at 3,000 X g, 10 min. The pellet was washed with PBS, re-centrifuged and then, resuspended in 75 µL of PBS. The samples were incubated at 100°C for 60 minutes in acid medium containing 0.45% sodium dodecyl sulphate and 0.67% thiobarbituric acid. After centrifugation, the reaction product was determined at 532 nm using MDA as standard. Data were expressed as percent of MDA formed vs. the control values.

2.9. Measurement of the respiratory chain complex II activity

The activity of succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) was determined according to the method of Fischer et al., 1985. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm and calculated as nmol .min^{-1} .mg $protein^{-1}$.

2.10. Protein determination

The protein measurements content of the mitochondrial-enriched fractions were assessed according to Lowry method (Lowry et al., 1951).

2.11. Statistical analysis

Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Differences among the groups were analyzed by one-way ANOVA followed by the Tukey *post hoc* test. Results are expressed as mean ± SEM. The differences were considered significant when $p < 0.05$.

3. Results

3.1. 3-NP-induced mitochondrial dysfunction and oxidative stress

In order to investigate the potential deleterious effects of 3-NP on energy metabolism, MTT reduction was assessed as an index of the mitochondrial reductive capacity. Figure 2 depicts concentration and time-response studies. 3-NP (1, 2 and 3 mM) caused a significant decline in mitochondrial function (approximately 25, 30 and 45%, respectively) ($p<0.05$, $p<0.01$ and $p<0.001$, respectively) (Fig. 2A). In figure 2B, 2 mM 3-NP (60 and 120 min of incubation) caused a significant decline in mitochondrial function (approximately 30% for both times) ($p<0.05$), as indicated by a decrease in the mitochondrial MTT reductive capacity in mitochondrial-enriched fractions.

3-NP (2 mM), which did not affect mitochondrial function in 30 min of incubation, caused significant inhibition of mitochondrial complex II activity (up to 80%) in brain preparation ($p<0.001$, Fig. 3). Mitochondrial-enriched fractions treated with 3-NP at 120 min, showed 95% of complex II inhibition ($p<0.001$, Fig. 3). Moreover, 3-NP-exposed mitochondrial-enriched fractions exhibited a fast increase in the ROS production in the first 30 min ($p<0.05$, Fig. 4A), which was accompanied by a significant increase in lipid peroxidation in 120 min of incubation ($p<0.001$, Fig. 4B). Respiratory complex II inhibition by 3-NP likely caused this increase in ROS.

3.2. Probucol and succinobucol prevent 3-NP-induced oxidative stress

Several studies have demonstrated that antioxidant compounds are able to protect against the neurotoxicity elicited by 3-NP. However, recently, we demonstrated that probucol, an antioxidant compound with scavenger properties under *in vitro* conditions, prevented ROS formation and lipid peroxidation, but did not protect against the mitochondrial dysfunction induced by 3-NP (Colle et al., 2012). So, we decided to study if succinobucol, an analogue of probucol, could prevent against 3-NP-incuced toxicity, as well as comparing the effects of both compounds.

The pre-treatment with probucol (1 μM) partially prevented the mitochondrial damage induced by 3-NP treatment (Figure 5A). On the other hand, succinobucol (1, 3 and 10 μM) effectively prevented 3-NP-induced mitochondrial damage (Figure 5B).

In addition, probucol and succinobucol, which present scavenger activity, completely prevented 3-NP-induced ROS formation

in mitochondrial-enriched fractions in all used concentrations (Figure 6A and 6B). Lipid peroxidation induced by 3-NP was also completely blocked by both compounds in all concentrations tested (Figure 7A and 7B).

To better understand mechanisms mediating the protective effects of probucol and succinobucol, additional experiments on complex II activity were performed. Figure 8 shows that 2 mM 3-NP induced a significant inhibition of mitochondrial complex II activity. Probucol and succinobucol did not prevent complex II inhibition induced by 3-NP ($p<0.001$, Fig 8A and 8B). However, probucol and succinobucol effectively prevented mitochondrial dysfunction induced by *tert*-butyl hydroperoxide (*t*BuOOH) ($p<0.05$, Fig 9A and 9B). These data confirm that the protective effects of both compounds in mitochondrial function are not involved with the recovery of complex II activity; the antioxidant and scavenger properties of these compounds are likely involved with their protective effects against 3-NP.

4. Discussion

The present results show that probucol and succinobucol, two agents with anti-inflammatory and antioxidant properties, protected against 3-NP-induced oxidative stress in mitochondria-enriched fractions derived from rat brain. In addition, mitochondrial dysfunction induced by 3-NP was totally protected by succinobucol in a mechanism independent of complex II inhibition. This is the first study reporting the beneficial effects of succinobucol in an experimental model of neurotoxicity. On the other hand, probucol, which diminished ROS production and lipid peroxidation induced by 3-NP, did not protect against the mitochondrial dysfunction induced by this toxin. This is in accordance with another study from our group, which demonstrated that probucol was not able to protect striatal slices from 3-NP-induced mitochondrial dysfunction (Colle et al., 2012). Although both compounds (probucol and succinobucol) did not prevent 3-NP-induced inhibition of the activity of the mitochondrial complex II, succinobucol protected against 3-NP-induced mitochondrial dysfunction, when evaluated by the MTT assay.

3-NP is a suicide inactivator of the mitochondrial Complex II, directly leading to mitochondrial dysfunction (Huang et al., 2006) and ROS formation (Kumar et al., 2010; Lee et al., 2002; Montilla et al., 2004). It was proposed that 3-NP is oxidized to 3-nitroacrylate, an unstable molecule which then reacts with some residue in the active site

of SDH (Coles et al., 1979). In fact, Huang and co-workers reported that 3-NP forms a covalent adduct with the side chain of Arg297 (Huang et al., 2006). In the present study, 3-NP induced immediate complex II inhibition and ROS production at 30 min, but mitochondrial dysfunction (evaluated by MTT reduction assay) was observed only at 60 min after 3-NP treatment. This is in accordance with Liot and co-workers (2009), who reported that the inhibition of complex II by 3-NP in cortical neurons was able to evokes an immediate ATP drop and sustained ROS increased at 30 min after 3-NP exposure, but neither mitochondrial damage nor cell death were observed in that time. Other studies also demonstrated that mitochondrial dysfunction induced by 3-NP, measured by MTT assay, occurs after 60 min of exposure in synaptosomal preparations (Elinos-Calderon et al., 2010; Perez-De La Cruz et al., 2006). Based on our data and on the previous mentioned evidences, one could suppose that 3-NP-induced ROS generation is responsible, at least in part, for the delayed decrease observed in MTT reduction.

In this study, we used enriched mitochondrial fractions from brain tissue obtained from a differential centrifugation methodology. However, this approach removes only nuclei, undisrupted cells and the cytosolic fraction from the tissue homogenates (Kristian, 2010). Additionally, the mitochondrial fractions are heavily contaminated with synaptosomes and myelin (Graham, 2001). Removal of these contaminants required gradient centrifugation using a density media (Kristian, 2010). Thus, as previously mentioned, the findings of this study show that SDH might be not necessarily the only enzyme responsible for the MTT reduction, since that other factors present in the enriched mitochondrial fraction might be involved in the formation of formazan when SDH was inhibited by 3-NP. Thus, although there is increasing evidence showing that SDH is an important enzyme mediating the reduction of MTT to its formazan metabolite (Staler et al., 1963; Berridge and Tan, 1992), our finding suggests that SDH contribution in this process (MTT reduction) might have been overestimated in the current literature.

MTT is a water soluble tetrazolium salt, which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria (Slater et al., 1963). In addition, some lines of evidence suggest that MTT is reduced to form a formazan product by accepting electrons from cellular reducing equivalents (i.e. NADH, NADPH, or succinate), indicating that MTT reduction is dependent on cellular redox activity, thus a reflection of

mitochondrial function (Berridge e Tan, 1992; Musser e Oseroff, 1994). Accordingly, MTT reduction could has been used as an indicator for cellular oxidative metabolic activity (Ahmadian et al., 2009; Takahashi et al., 2002).

On the other hand, MTT can be reduced by receiving electrons not only in mitochondria but other cellular organelles, even though the exact sites of reduction or responsible enzymes remain elusive (Takahashi et al., 2002). In addition, evidence shows that the first step of mitochondrial oxidative phosphorylation, which is catalyzed by pyruvate dehydrogenase complex, might play a key role in MTT reduction rather than SDH (Takahashi et al., 2002). Recently, McKenna and co-works (2000) reported which the activity of malic enzyme (malate-$NADP^+$-oxidoreductase decarboxylating) that converts malate to pyruvate, is high in cortical synaptic mitochondria of adult rat brain. Based on this evidence, one could suppose that this enzyme might play a role in MTT reduction in mitochondria. This idea is supported by the findings of Takahashi and co-workers, who demonstrated that malate causes small increases in MTT reduction by cultured neurons, although the activity of malic enzyme is much lower in mitochondria from cultured cortical neurons than those from cortical synaptic mitochondria of adult rat brain (Takahashi et al., 2002). This findings can explain the MTT reduction even with complex II inhibition by 3-NP. Accordingly, succinobucol, which did not recover SDH inhibition, was able to protect against 3-NP-induced decreased of MTT reduction.

The protective effect afforded by succinobucol could be related, at least in part, to its antioxidant properties. From a mechanistic point of view, it is hypothesized that 3-NP-induced SDH inhibition could lead to the generation of ROS (Liot et al., 2009), which might subsequently cause a decrease in mitochondrial function (evaluated by MTT assay) due to their hazardous effects toward different dehydrogenases (located either in mitochondria or in the intra-synaptosomal milieu). Succinobucol, which presents scavenger properties, could prevent the secondary events (decreased MTT reduction) due to its ability to neutralize the ROS generated after SDH inhibition. This idea is reinforced by the fact thar succinobucol preventes *tert*-butyl hydroperoxide-induced mitochondrial dysfunction. However, a potential relationship between the observed protective effects of succinobucol and its capacity in modulating energetic factors could be not ruled out.

An intriguing (and not solved) question from our study is why just succinobucol, but not probucol, protected against 3-NP-induced mitochondrial dysfunction. In fact, both compounds protected against 3-

NP-induced ROS generation and lipid peroxidation. Thus, what makes succinobucol a better protective agent (MTT reduction) in our experimental model? Based on its molecular structure, one could hypothesize that the succinobucol might modulate proteins involved in the mitochondrial homeostasis. These effects could be done by the whole molecule (not by a metabolite) because succinobucol is not metabolized to probucol neither to other Muldrew e Franks, 2009. Taking into account the modulatory (and beneficial) effects of succinate in Huntington experimental model (Colle et al., 2012), the potential advantage of succinobucol when compared to probucol could be due its succinyl moety. However, additional studies are necessary to solve this question.

As already mentioned, mitochondrial dysfunction and oxidative stress represents a crucial event in HD experimental models (Perez-Severiano et al., 2004; Santamaria et al., 2003; Tunez et al., 2006), as well as in the pathogenesis of HD (Chen et al., 2007; Klepac et al., 2007; Sorolla et al., 2008). Thus the search for therapeutic strategies that can either potentiate antioxidant defenses or avoid oxidative stress generation are extremely relevant to retard HD progression. The present findings suggest that the antioxidant succinobucol, might be a novel strategy to slow or halt oxidative process in neurodegenerative process, but further studies are needed to elucidate its potential mechanism of action.

Financial support: The financial supports by (i) FINEP Research Grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00, (ii) Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), (iii) Fundação de Apoio à Pesquisa do Estado de Santa Catarina (FAPESC - TO 1346/2010-3), (iv) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and (v) INCT-CNPq-Excitotoxicity and Neuroprotection are gratefully acknowledged. M.F. recipient of CNPq fellowship.

5. References

Ahmadian, S., Barar, J., Saei, A. A., Fakhree, M. A., and Omidi, Y., 2009. Cellular toxicity of nanogenomedicine in MCF-7 cell line: MTT assay. J Vis Exp.

Al-Majed, A. A., 2011. Probucol attenuates oxidative stress, energy starvation, and nitric acid production following transient forebrain ischemia in the rat hippocampus. Oxid Med Cell Longev 2011**,** 471590.

Ali, S. F., LeBel, C. P., and Bondy, S. C., 1992. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology 13**,** 637-648.

Andrews, T. C., and Brooks, D. J., 1998. Advances in the understanding of early Huntington's disease using the functional imaging techniques of PET and SPET. Mol Med Today 4**,** 532-539.

Beal, M. F., Brouillet, E., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Miller, J. M., Storey, E., Srivastava, R., Rosen, B. R., and Hyman, B. T., 1993. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci 13**,** 4181-4192.

Benchoua, A., Trioulier, Y., Zala, D., Gaillard, M. C., Lefort, N., Dufour, N., Saudou, F., Elalouf, J. M., Hirsch, E., Hantraye, P., Deglon, N., and Brouillet, E., 2006. Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. Mol Biol Cell 17**,** 1652-1663.

Berridge, M. V., and Tan, A. S., 1992. The protein kinase C inhibitor, calphostin C, inhibits succinate-dependent mitochondrial reduction of MTT by a mechanism that does not involve protein kinase C. Biochem Biophys Res Commun 185**,** 806-811.

Bizat, N., Hermel, J. M., Humbert, S., Jacquard, C., Creminon, C., Escartin, C., Saudou, F., Krajewski, S., Hantraye, P., and Brouillet, E., 2003. In vivo calpain/caspase cross-talk during 3-nitropropionic acid-induced striatal degeneration: implication of a calpain-mediated cleavage of active caspase-3. J Biol Chem 278**,** 43245-43253.

Brennan, W. A., Jr., Bird, E. D., and Aprille, J. R., 1985. Regional mitochondrial respiratory activity in Huntington's disease brain. J Neurochem 44**,** 1948-1950.

Brouillet, E., Guyot, M. C., Mittoux, V., Altairac, S., Conde, F., Palfi, S., and Hantraye, P., 1998. Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. J Neurochem 70**,** 794-805.

Browne, S. E., 2008. Mitochondria and Huntington's disease pathogenesis: insight from genetic and chemical models. Ann N Y Acad Sci 1147**,** 358-382.

Browne, S. E., Bowling, A. C., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D., and Beal, M. F., 1997. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol 41**,** 646-653.

Brustovetsky, N., and Dubinsky, J. M., 2000. Dual responses of CNS mitochondria to elevated calcium. J Neurosci 20**,** 103-113.

Buckley, M. M., Goa, K. L., Price, A. H., and Brogden, R. N., 1989. Probucol. A reappraisal of its pharmacological properties and therapeutic use in hypercholesterolaemia. Drugs 37**,** 761-800.

Chen, C. M., Wu, Y. R., Cheng, M. L., Liu, J. L., Lee, Y. M., Lee, P. W., Soong, B. W., and Chiu, D. T., 2007. Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. Biochem Biophys Res Commun 359**,** 335-340.

Coles, C. J., Edmondson, D. E., and Singer, T. P., 1979. Inactivation of succinate dehydrogenase by 3-nitropropionate. J Biol Chem 254**,** 5161-5167.

Colle, D., Hartwig, J. M., Antunes Soares, F. A., and Farina, M., 2012. Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro. Brain Res Bull.

Damiano, M., Galvan, L., Deglon, N., and Brouillet, E., 2010. Mitochondria in Huntington's disease. Biochim Biophys Acta 1802**,** 52-61.

de Moura, M. B., dos Santos, L. S., and Van Houten, B., 2010. Mitochondrial dysfunction in neurodegenerative diseases and cancer. Environ Mol Mutagen 51**,** 391-405.

Elinos-Calderon, D., Robledo-Arratia, Y., Perez-De La Cruz, V., Maldonado, P. D., Galvan-Arzate, S., Pedraza-Chaverri, J., and Santamaria, A., 2010. Antioxidant strategy to rescue synaptosomes from oxidative damage and energy failure in

neurotoxic models in rats: protective role of S-allylcysteine. J Neural Transm 117**,** 35-44.

Farina, M., Campos, F., Vendrell, I., Berenguer, J., Barzi, M., Pons, S., and Sunol, C., 2009. Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells. Toxicol Sci 112**,** 416-426.

Fischer, J. C., Ruitenbeek, W., Berden, J. A., Trijbels, J. M., Veerkamp, J. H., Stadhouders, A. M., Sengers, R. C., and Janssen, A. J., 1985. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 153**,** 23-36.

Graham, J. M., 2001. Isolation of mitochondria from tissues and cells by differential centrifugation. Curr Protoc Cell Biol Chapter 3**,** Unit 3 3.

Gu, M., Gash, M. T., Mann, V. M., Javoy-Agid, F., Cooper, J. M., and Schapira, A. H., 1996. Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 39**,** 385-389.

Haibin, Y., Yan, Y., and Jinjin, D., 2006. Preparation of probucol monosuccinate. Patent No CN 1970536.

Huang, L. S., Sun, G., Cobessi, D., Wang, A. C., Shen, J. T., Tung, E. Y., Anderson, V. E., and Berry, E. A., 2006. 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme. J Biol Chem 281**,** 5965-5972.

Jackson, R. L., Barnhart, R. L., and Mao, S. J., 1991. Probucol and its mechanisms for reducing atherosclerosis. Adv Exp Med Biol 285**,** 367-372.

Klepac, N., Relja, M., Klepac, R., Hecimovic, S., Babic, T., and Trkulja, V., 2007. Oxidative stress parameters in plasma of Huntington's disease patients, asymptomatic Huntington's disease gene carriers and healthy subjects : a cross-sectional study. J Neurol 254**,** 1676-1683.

Kristian, T., 2010. Isolation of mitochondria from the CNS. Curr Protoc Neurosci Chapter 7**,** Unit 7 22.

Kumar, P., Kalonia, H., and Kumar, A., 2010. Huntington's disease: pathogenesis to animal models. Pharmacol Rep 62**,** 1-14.

Kunsch, C., Luchoomun, J., Grey, J. Y., Olliff, L. K., Saint, L. B., Arrendale, R. F., Wasserman, M. A., Saxena, U., and Medford, R. M., 2004. Selective inhibition of endothelial and monocyte

redox-sensitive genes by AGI-1067: a novel antioxidant and anti-inflammatory agent. J Pharmacol Exp Ther 308**,** 820-829.

Kuwert, T., Lange, H. W., Langen, K. J., Herzog, H., Aulich, A., and Feinendegen, L. E., 1990. Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. Brain 113 (Pt 5)**,** 1405-1423.

Latini, A., da Silva, C. G., Ferreira, G. C., Schuck, P. F., Scussiato, K., Sarkis, J. J., Dutra Filho, C. S., Wyse, A. T., Wannmacher, C. M., and Wajner, M., 2005. Mitochondrial energy metabolism is markedly impaired by D-2-hydroxyglutaric acid in rat tissues. Mol Genet Metab 86**,** 188-199.

Lee, W. T., Itoh, T., and Pleasure, D., 2002. Acute and chronic alterations in calcium homeostasis in 3-nitropropionic acid-treated human NT2-N neurons. Neuroscience 113**,** 699-708.

Lee, W. T., Yin, H. S., and Shen, Y. Z., 2002. The mechanisms of neuronal death produced by mitochondrial toxin 3-nitropropionic acid: the roles of N-methyl-D-aspartate glutamate receptors and mitochondrial calcium overload. Neuroscience 112**,** 707-716.

Lemieux, H., Bulteau, A. L., Friguet, B., Tardif, J. C., and Blier, P. U., 2011. Dietary fatty acids and oxidative stress in the heart mitochondria. Mitochondrion 11**,** 97-103.

Lim, D., Fedrizzi, L., Tartari, M., Zuccato, C., Cattaneo, E., Brini, M., and Carafoli, E., 2008. Calcium homeostasis and mitochondrial dysfunction in striatal neurons of Huntington disease. J Biol Chem 283**,** 5780-5789.

Liot, G., Bossy, B., Lubitz, S., Kushnareva, Y., Sejbuk, N., and Bossy-Wetzel, E., 2009. Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway. Cell Death Differ 16**,** 899-909.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193**,** 265-275.

Mattson, M. P., Gleichmann, M., and Cheng, A., 2008. Mitochondria in neuroplasticity and neurological disorders. Neuron 60**,** 748-766.

McDowell, I. F., Brennan, G. M., McEneny, J., Young, I. S., Nicholls, D. P., McVeigh, G. E., Bruce, I., Trimble, E. R., and Johnston, G. D., 1994. The effect of probucol and vitamin E treatment on the oxidation of low-density lipoprotein and forearm vascular responses in humans. Eur J Clin Invest 24**,** 759-765.

McKenna, M. C., Stevenson, J. H., Huang, X., Tildon, J. T., Zielke, C. L., and Hopkins, I. B., 2000. Mitochondrial malic enzyme activity is much higher in mitochondria from cortical synaptic terminals compared with mitochondria from primary cultures of cortical neurons or cerebellar granule cells. Neurochem Int 36**,** 451-459.

Mochel, F., and Haller, R. G., 2011. Energy deficit in Huntington disease: why it matters. J Clin Invest 121**,** 493-499.

Montilla, P., Espejo, I., Munoz, M. C., Bujalance, I., Munoz-Castaneda, J. R., and Tunez, I., 2004. Effect of red wine on oxidative stress and hypercholesterolemia induced by feeding a high-cholesterol diet in rat. J Physiol Biochem 60**,** 259-264.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65**,** 55-63.

Muldrew, K. M., and Franks, A. M., 2009. Succinobucol: review of the metabolic, antiplatelet and cardiovascular effects. Expert Opin Investig Drugs 18**,** 531-539.

Musser, D. A., and Oseroff, A. R., 1994. The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II. Photochem Photobiol 59**,** 621-626.

Ohkawa, H., Ohishi, N., and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95**,** 351-358.

Oliveira, J. M., 2010. Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum. J Neurochem 114**,** 1-12.

Pallebage-Gamarallage, M. M., Takechi, R., Lam, V., Galloway, S., Dhaliwal, S., and Mamo, J. C., 2010. Post-prandial lipid metabolism, lipid-modulating agents and cerebrovascular integrity: implications for dementia risk. Atheroscler Suppl 11**,** 49-54.

Park, S. Y., Lee, J. H., Kim, C. D., Rhim, B. Y., Hong, K. W., and Lee, W. S., 2007. Beneficial synergistic effects of concurrent treatment with cilostazol and probucol against focal cerebral ischemic injury in rats. Brain Res 1157**,** 112-120.

Perez-De la Cruz, V., Carrillo-Mora, P., and Santamaria, A., 2010. Huntington's disease and mitochondrial alterations: emphasis on experimental models. J Bioenerg Biomembr 42**,** 207-215.

Perez-De La Cruz, V., Elinos-Calderon, D., Robledo-Arratia, Y., Medina-Campos, O. N., Pedraza-Chaverri, J., Ali, S. F., and Santamaria, A., 2009. Targeting oxidative/nitrergic stress ameliorates motor impairment, and attenuates synaptic mitochondrial dysfunction and lipid peroxidation in two models of Huntington's disease. Behav Brain Res 199**,** 210-217.

Perez-De La Cruz, V., Gonzalez-Cortes, C., Pedraza-Chaverri, J., Maldonado, P. D., Andres-Martinez, L., and Santamaria, A., 2006. Protective effect of S-allylcysteine on 3-nitropropionic acid-induced lipid peroxidation and mitochondrial dysfunction in rat brain synaptosomes. Brain Res Bull 68**,** 379-383.

Perez-Severiano, F., Santamaria, A., Pedraza-Chaverri, J., Medina-Campos, O. N., Rios, C., and Segovia, J., 2004. Increased formation of reactive oxygen species, but no changes in glutathione peroxidase activity, in striata of mice transgenic for the Huntington's disease mutation. Neurochem Res 29**,** 729-733.

Reddy, P. H., Mao, P., and Manczak, M., 2009. Mitochondrial structural and functional dynamics in Huntington's disease. Brain Res Rev 61**,** 33-48.

Reynolds, N. C., Jr., Prost, R. W., and Mark, L. P., 2005. Heterogeneity in 1H-MRS profiles of presymptomatic and early manifest Huntington's disease. Brain Res 1031**,** 82-89.

Roos, R. A., 2010. Huntington's disease: a clinical review. Orphanet J Rare Dis 5**,** 40.

Ross, C. A., and Tabrizi, S. J., 2011. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 10**,** 83-98.

Rossignol, J., Boyer, C., Leveque, X., Fink, K. D., Thinard, R., Blanchard, F., Dunbar, G. L., and Lescaudron, L., 2011. Mesenchymal stem cell transplantation and DMEM administration in a 3NP rat model of Huntington's disease: morphological and behavioral outcomes. Behav Brain Res 217**,** 369-378.

Santamaria, A., Salvatierra-Sanchez, R., Vazquez-Roman, B., Santiago-Lopez, D., Villeda-Hernandez, J., Galvan-Arzate, S., Jimenez-Capdeville, M. E., and Ali, S. F., 2003. Protective effects of the antioxidant selenium on quinolinic acid-induced neurotoxicity in rats: in vitro and in vivo studies. J Neurochem 86**,** 479-488.

Santos, D. B., Peres, K. C., Ribeiro, R. P., Colle, D., Santos, A. A., Moreira, E. L., Souza, D. O., Figueiredo, C. P., and Farina, M.,

2011. Probucol, a lipid-lowering drug, prevents cognitive and hippocampal synaptic impairments induced by amyloid beta peptide in mice. Exp Neurol.

Sia, Y. T., Parker, T. G., Liu, P., Tsoporis, J. N., Adam, A., and Rouleau, J. L., 2002. Improved post-myocardial infarction survival with probucol in rats: effects on left ventricular function, morphology, cardiac oxidative stress and cytokine expression. J Am Coll Cardiol 39**,** 148-156.

Singla, D. K., Kaur, K., Sharma, A. K., Dhingra, S., and Singal, P. K., 2007. Probucol promotes endogenous antioxidant reserve and confers protection against reperfusion injury. Can J Physiol Pharmacol 85**,** 439-443.

Slater, T. F., Sawyer, B., and Straeuli, U., 1963. Studies on Succinate-Tetrazolium Reductase Systems. Iii. Points of Coupling of Four Different Tetrazolium Salts. Biochim Biophys Acta 77**,** 383-393.

Sorolla, M. A., Reverter-Branchat, G., Tamarit, J., Ferrer, I., Ros, J., and Cabiscol, E., 2008. Proteomic and oxidative stress analysis in human brain samples of Huntington disease. Free Radic Biol Med 45**,** 667-678.

Southwell, A. L., and Patterson, P. H., 2011. Gene therapy in mouse models of huntington disease. Neuroscientist 17**,** 153-162.

Takahashi, S., Abe, T., Gotoh, J., and Fukuuchi, Y., 2002. Substrate-dependence of reduction of MTT: a tetrazolium dye differs in cultured astroglia and neurons. Neurochem Int 40**,** 441-448.

Tardif, J. C., Gregoire, J., and L'Allier, P. L., 2002. Prevention of restenosis with antioxidants: mechanisms and implications. Am J Cardiovasc Drugs 2**,** 323-334.

Tardif, J. C., Gregoire, J., L'Allier, P. L., Ibrahim, R., Anderson, T. J., Reeves, F., Title, L. M., Schampaert, E., LeMay, M., Lesperance, J., Scott, R., Guertin, M. C., Brennan, M. L., Hazen, S. L., and Bertrand, O. F., 2008. Effects of the antioxidant succinobucol (AGI-1067) on human atherosclerosis in a randomized clinical trial. Atherosclerosis 197**,** 480-486.

Tardif, J. C., Gregoire, J., Schwartz, L., Title, L., Laramee, L., Reeves, F., Lesperance, J., Bourassa, M. G., L'Allier, P. L., Glass, M., Lambert, J., and Guertin, M. C., 2003. Effects of AGI-1067 and probucol after percutaneous coronary interventions. Circulation 107**,** 552-558.

Tardif, J. C., McMurray, J. J., Klug, E., Small, R., Schumi, J., Choi, J., Cooper, J., Scott, R., Lewis, E. F., L'Allier, P. L., and Pfeffer,

M. A., 2008. Effects of succinobucol (AGI-1067) after an acute coronary syndrome: a randomised, double-blind, placebo-controlled trial. Lancet 371**,** 1761-1768.

Tunez, I., Drucker-Colin, R., Jimena, I., Medina, F. J., Munoz Mdel, C., Pena, J., and Montilla, P., 2006. Transcranial magnetic stimulation attenuates cell loss and oxidative damage in the striatum induced in the 3-nitropropionic model of Huntington's disease. J Neurochem 97**,** 619-630.

Tunez, I., Tasset, I., Perez-De La Cruz, V., and Santamaria, A., 2010. 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. Molecules 15**,** 878-916.

Yamashita, S., Hbujo, H., Arai, H., Harada-Shiba, M., Matsui, S., Fukushima, M., Saito, Y., Kita, T., and Matsuzawa, Y., 2008. Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. J Atheroscler Thromb 15**,** 292-303.

Yamashita, S., and Matsuzawa, Y., 2009. Where are we with probucol: a new life for an old drug? Atherosclerosis 207**,** 16-23.

FIGURES AND LEGENDS

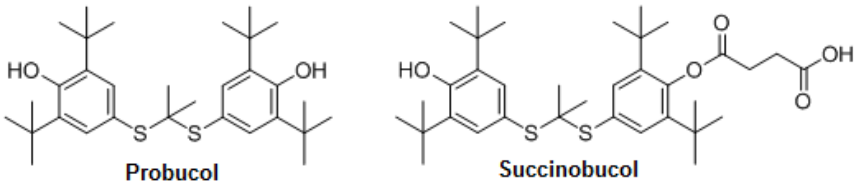

Figure 1. Structures of the compounds.

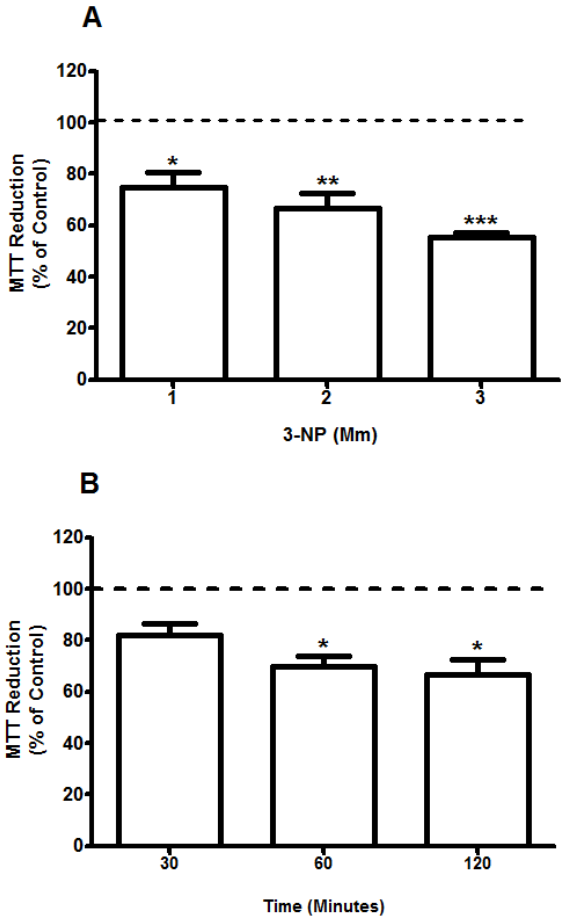

Figure 2: Mitochondrial dysfunction induced by 3-NP. Mitochondria-enriched fractions were exposed to different concentrations of 3-NP (1, 2 and 3 mM) for 2 h (A) or 2 mM 3-NP for 30, 60 and 120 min (B). Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± S.E.M. (n=4). * $p< 0.05$, ** $p<0.01$ and $p<0.001$ indicates statistical difference from control by one-way ANOVA, following by Tukey *post-hoc* test.

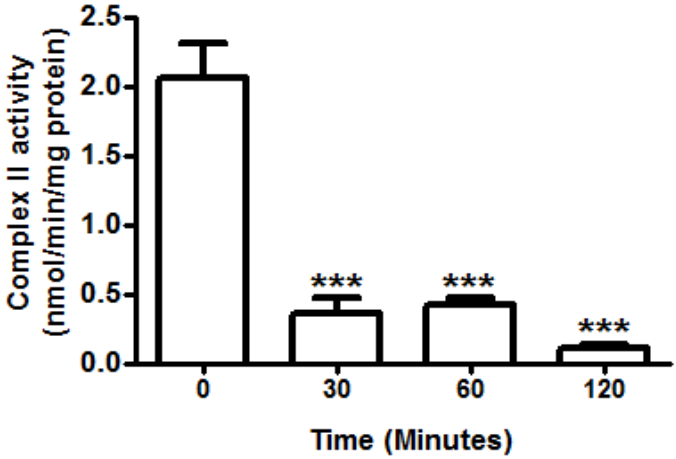

Figure 3: Effects of 3-NP on Complex II activity. Mitochondria-enriched fractions were exposed to 2 mM 3-NP and the activity of complex II was measured after 30, 60 and 120 min of incubation. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm, calculated as nmol/min/mg of protein and presented as mean ± S.E.M. (n= 4-5). *** $p<0.001$ indicates statistical difference from control by one-way ANOVA, following by Tukey *post-hoc* test.

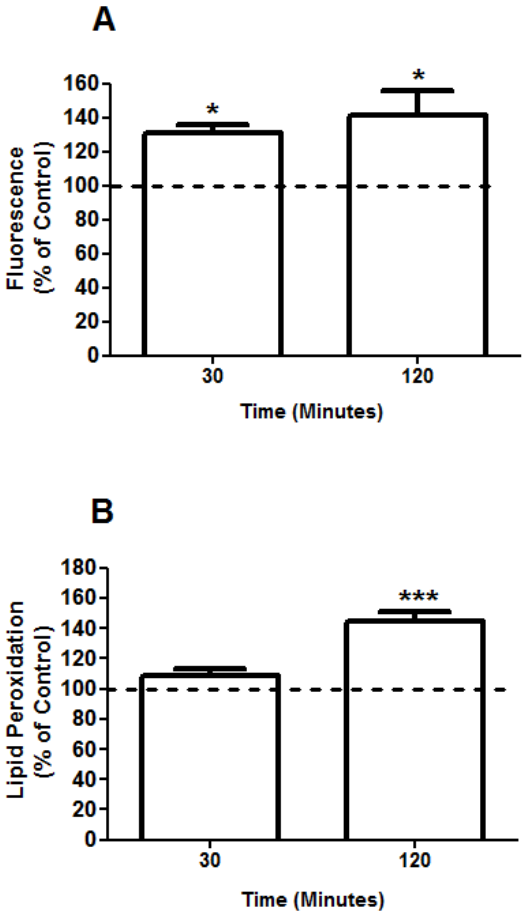

Figure 4: Effects of 3-NP on ROS production and lipid peroxidation in brain mitochondria. Mitochondria-enriched fractions were exposed to 2 mM 3-NP at 37°C for 30 and 120 min. Production of ROS was estimated with the fluorescent probe, 2',7'-dichlorofluorescein diacetate (DCFH-DA) using excitation and emission wavelengths of 480 and 525 nm, respectively. ROS levels (expressed as nmol of oxidized DCF per mg protein) are expressed as percent of control (dotted line) (A). Lipid peroxidation (LP) was assessed by TBARS formation (expressed as nmol of MDA per mg of protein) are expressed as percent of control (dotted line) (B). Data are represented as mean ± S.E.M. (n= 4-5). * $p<0.05$ and *** $p<0.001$ indicates statistical difference from by one-way ANOVA, following by Tukey *post-hoc* test.

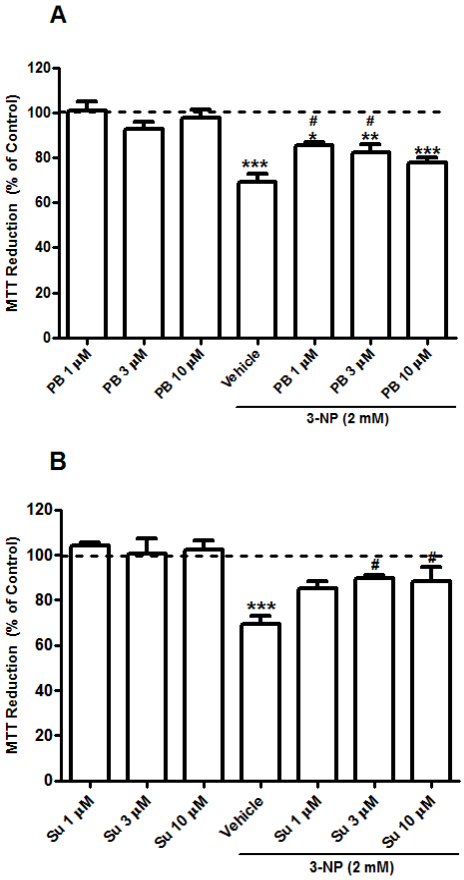

Figure 5: Protective effect of probucol and succinobucol against 3-NP induced mitochondrial dysfunction. Mitochondria-enriched fractions were pre-incubated with probucol (1, 3 and 10 µM) (A) or succinobucol (1, 3 and 10 µM) (B) or vehicle at 37°C for 2.5 h. After this period, mitochondria-enriched fractions were subjected to damage with 2 mM 3-NP for 120 min. Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± S.E.M. (n=5-7). * $p<0.05$, ** $p< 0.01$ and *** $p<0.001$ indicates statistical difference from control. # $p< 0.05$ indicates statistical difference from 3-NP by one-way ANOVA, following by Tukey *post-hoc* test.

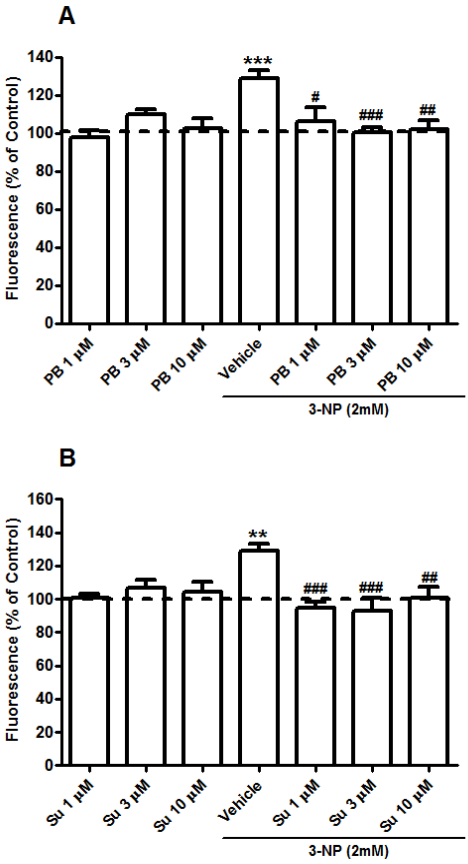

Figure 6: Protective effect of probucol and succinobucol on the production of reactive oxygen species (ROS) induced by 3-NP in brain mitochondria. Mitochondria-enriched fractions were pre-incubated with vehicle and probucol (1, 3 and 10 µM) (A) or succinobucol (1, 3 and 10 µM) (B) at 37°C for 2.5 h. After this period, mitochondria-enriched fractions were subjected to damage with 2 mM 3-NP for 30 min. ROS levels (expressed as nmol of oxidized DCF per mg protein) are expressed as percent of control (dotted line). Data are represented as mean ± S.E.M. (n= 5-6). ** $p< 0.01$ and *** $p<0.001$ indicates statistical difference from control. # $p< 0.05$; ## $p< 0.01$ and ### $p<0.001$ indicates statistical difference from 3-NP by one-way ANOVA, following by Tukey *post-hoc* test.

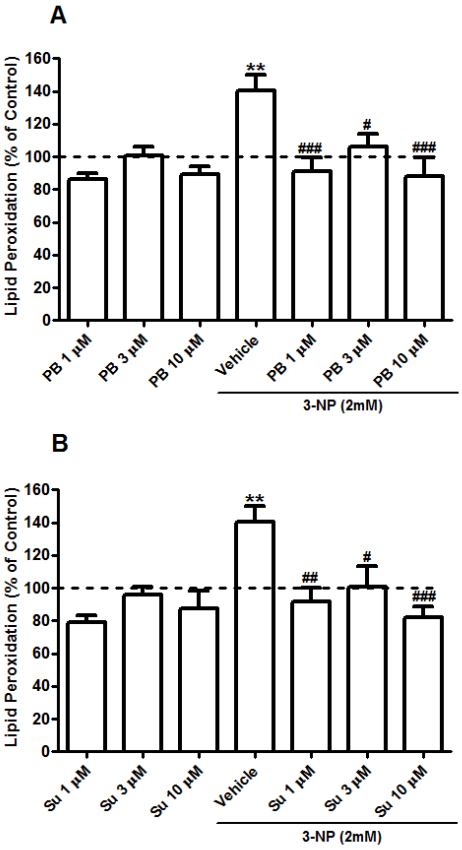

Figure 7: Protective effect of Probucol and succinobucol on lipid peroxidation induced by 3-NP in brain mitochondria. Mitochondria-enriched fractions were pre-incubated with vehicle and probucol (1, 3 and 10 µM) (A) or succinobucol (1, 3 and 10 µM) (B) at 37°C for 2.5 h. After this period, mitochondria-enriched fractions were subjected to damage with 2 mM 3-NP for 120 min. Lipid peroxidation (LP) was assessed by TBARS formation and expressed as nmol of MDA per mg of protein. Results are expressed as percent of MDA formed vs. the control values (dotted line). Basal lipid peroxidation was 1.847 ± 0.0338 nmol MDA/mg protein. Data are represented as mean ± S.E.M. (n= 4-5). ** $p< 0.01$ indicates statistical difference from control. # $p< 0.05$; ## $p< 0.01$ and ### $p<0.001$ indicates statistical difference from 3-NP by one-way ANOVA, following by Tukey *post-hoc* test.

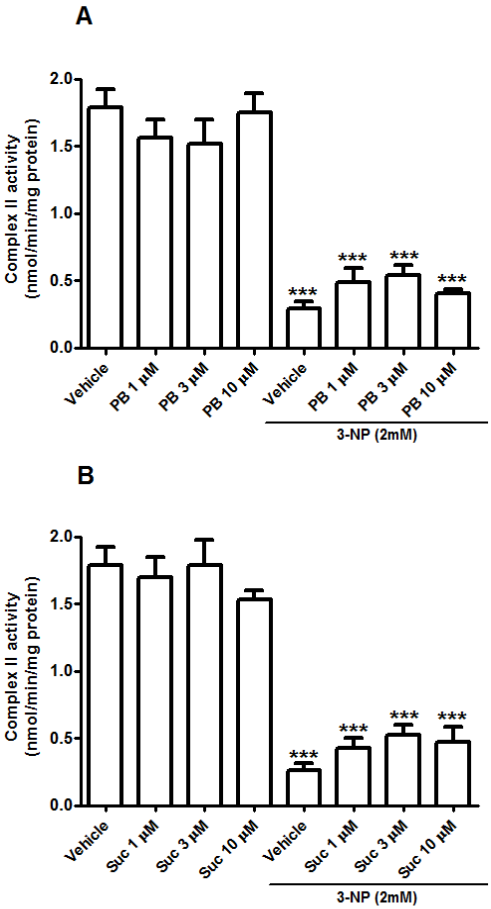

Figure 8: Effects of probucol and succinobucol on 3-NP-inducing inhibition of Complex II activity in mitochondria brain. Mitochondria-enriched fractions were pre-incubated with vehicle and probucol (1, 3 and 10 µM) (A) or succinobucol (1, 3 and 10 µM) (B) at 37°C for 2.5 h. After this period, mitochondria-enriched fractions were subjected to damage with 2 mM 3-NP for 30 min. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm, calculated as nmol/min/mg of protein and presented as mean ± S.E.M. (n= 4-5). *** $p<0.001$ indicates statistical difference from control by one-way ANOVA, following by Tukey *post-hoc* test.

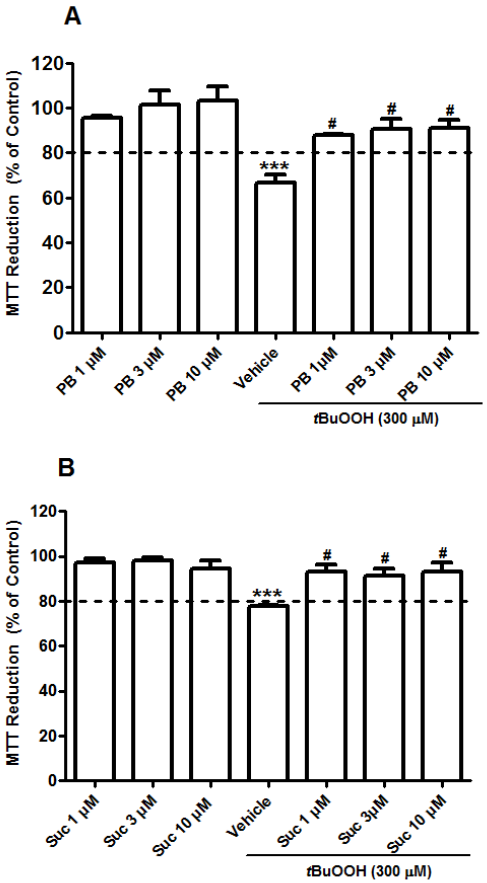

Figure 9: Protective effect of probucol and succinobucol against *t*BuOOH-induced mitochondrial dysfunction. Mitochondria-enriched fractions were pre-incubated with probucol (1, 3 and 10 µM) (A) or succinobucol (1, 3 and 10 µM) (B) or vehicle at 37°C for 2.5 h. After this period, mitochondria-enriched fractions were subjected to damage with 300 µM *t*BuOOH for 30 min. Mitochondrial viability was evaluated by the MTT reduction method. Results are expressed as the percentage of MTT reduction with respect to control values (dotted line). Data are represented as mean ± S.E.M. (n=5-7). *** $p<0.001$ indicates statistical difference from control. # $p< 0.05$ indicates statistical difference from 3-NP by one-way ANOVA, following by Tukey *post-hoc* test.

5. DISCUSSÃO GERAL

Desde a sua descrição inicial, feita 1872, a doença de Huntington (HD) vem sendo caracterizada como uma desordem neurodegenerativa progressiva com sintomas atribuídos à morte de neurônios das projeções estriatais e de neurônios corticais do cérebro (Heinsen et al., 1994; Mann et al., 1993; Vonsattel e DiFiglia, 1998). Diversos fatores parecem estar envolvidos na patogênese da doença como a excitotoxicidade, a disfunção mitocondrial e o estresse oxidativo (Benchoua et al., 2006; Browne e Beal, 2006; Mochel e Haller, 2011).

O entendimento dos mecanismos moleculares relacionados com a patogênese da DH tem crescido bastante nos últimos anos, graças ao desenvolvimento de modelos animais que mimetizam características fenotípicas ou genotípicas da doença. Dentre os modelos utilizados para estudar o comprometimento mitocondrial e a morte celular induzida por mecanismos excitotóxicos, os mais empregados são o modelo do ácido 3-nitropropiônico e o modelo do ácido quinolínico, respectivamente (Perez-De La Cruz e Santamaria, 2007). Além disso, recentemente algumas linhas de pesquisa vêm utilizando o AQ e o 3-NP em associação, ou seja, administrados concomitantemente, no intuito de se obter um modelo mais completo e com elevada relevância para o estudo dos mecanismos envolvidos na patogênese da doença (Tunez et al., 2010), porém os mecanismos envolvidos na toxicidade do modelo combinado ainda não estão totalmente esclarecidos.

Neste contexto, o objetivo do primeiro estudo foi avaliar a potencial relação entre déficit energético, excitotoxicidade e estresse oxidativo no mecanismo de dano induzido pela associação entre AQ e 3-NP em fatias de estriado de ratos *in vitro*.

Neste estudo foi observado que a toxicidade induzida pelo AQ não está apenas relacionada ao seu efeito excitotóxico primário, mas a disfunção mitocondrial e o estresse oxidativo também contribuem para o mecanismo de dano induzido por este composto. O probucol preveniu a disfunção mitocondrial induzida pelo AQ provavelmente por prevenir o efeito deste composto sobre a produção de ERO, mostrando o importante papel das ERO e do estresse oxidativo na toxicidade do AQ.

Por outro lado, o 3-NP induziu disfunção mitocondrial como um mecanismo primário de toxicidade devido à inibição do complexo II da cadeia transportadora de elétrons (Huang et al., 2006), porém as propriedades antioxidantes do probucol não foram capazes de proteger contra esse efeito. Já o tratamento com MK-801, reverteu a disfunção mitocondrial induzida pelo 3-NP, mostrando o envolvimento de eventos

excitotóxicos (secundários à inibição do complexo II) no mecanismo de toxicidade deste composto.

Os resultados também mostraram que a exposição simultânea a concentrações subtóxicas de AQ e 3-NP induzem disfunção mitocondrial e estresse oxidativo de uma maneira sinérgica. O probucol foi capaz de prevenir a disfunção mitocondrial induzida pela associação de ambos os compotos, reforçando a importância das ERO nos danos induzidos por este modelo. Ainda, o efeito protetor do MK-801 no modelo combinado sugere que eventos tóxicos mediados pela super-estimulação de receptores NMDA também possuem um relevante papel no mecanismo de dano celular induzido pela associação do AQ e 3-NP. Assim, a ação tóxica da associação desses compostos parece envolver mecanismos de excitotoxicidade e geração de ERO e pode ser prevenida por antagonistas de receptores NMDA e por compostos antioxidantes/scavengers como o probucol.

Considerando a capacidade do probucol em modular o estresse oxidativo induzido pelo 3-NP em fatias de estriado de ratos *in vitro*, bem como seu efeito benéfico ou protetor em outros modelos de neurotoxicidade/neuropatologia previamente descritos na literatura (Farina et al., 2009; Park et al., 2007; Santos et al., 2011), o objetivo do segundo estudo foi investigar o potencial efeito benéfico do probucol em um modelo *in vivo* de DH induzido pelo 3-NP em ratos.

A administração 3-NP (25 mg/kg, i.p.) durante 6 dias induziu significativa perda de peso, alterações comportamentais e bioquímicos nos animais. A excessiva perda de peso induzida por repetidas administrações de 3-NP é bem evidenciada na literatura (Kumar et al., 2012; Kumar e Kumar, 2009; Ramaswamy et al., 2007) e pode estar diretamente relacionada ao déficit energético induzido pela inibição do complexo II induzido pela toxina (Kumar e Kumar, 2009). De fato, em nosso estudo, houve uma diminuição significativa na atividade do complexo II. O pré-tratamento com probucol (aproximadamente 3,5 mg/kg/dia) durante 2 meses foi capaz de prevenir a perda de peso nos animais, mas não reverteu a inibição do complexo II induzida pela toxina.

Além disso, a administração de 3-NP induziu significativas alterações motoras nos animais. Esse efeito é bem evidenciado na literatura, onde os animais tratados com 3-NP podem desenvolver hipoatividade seguido de administrações repetidas da toxina (Borlongan et al., 1997). Estudos sugerem que o comprometimento do sistema motor está relacionado à inibição da SDH e a conseqüente diminuição nos níveis energéticos nas células (Seaman, 2000). O pré-tratamento

com probucol preveniu o prejuízo no sistema locomotor induzido pela toxina, apesar de não reverter a inibição do complexo II.

O tratamento com 3-NP também induziu estresse oxidativo, o qual foi evidenciado pelo aumento nos níveis de TBARS e na atividade das enzimas antioxidantes superóxido dismutase (SOD) e catalase no córtex e no estriado dos animais tratados. Além disso, foi observado um aumento significativo na atividade da enzima glutationa redutase e diminuição nos níveis de GSH no córtex dos animais tratados com 3-NP. Ainda, o tratamento com 3-NP induziu um aumento na expressão da iNOS, uma enzima responsável pela síntese de $^{\bullet}$ON. Este estresse oxidativo está relacionado à disfunção mitocondrial induzida pelo 3-NP, que é responsável por causar uma diminuição nos níveis de ATP e geração de ERO.

O probucol demonstrou uma potente atividade antioxidante e conseguiu diminuir o estresse oxidativo induzido pelo 3-NP. De maneira interessante, o probucol aumentou a atividade da GPx no estriado e no córtex dos animais, um efeito que foi independente do 3-NP. Essa capacidade do probucol foi evidenciada em prévios estudos *in vitro*, onde esse composto aumentou a atividade da GPx em cultivos de neurônios cerebelares (Farina et al., 2009), o que foi responsável pelo efeito protetor desse composto frente ao metilmercúrio, um composto altamente tóxico no sistema nervoso central (Farina et al., 2011).

Algumas linhas de evidência vêm monstrando que a GPx pode desempenhar um importante papel na DH. Estudos recentes demonstraram um aumento na atividade da GPx no estriado e no córtex (Sorolla et al., 2008), e uma diminuição nos eritrócitos (Chen et al., 2007) de pacientes. Além disso, foi demonstrado que a presença da proteína huntingtina mutante está relacionada com excessiva produção de peróxido de hidrogênio (Hands et al., 2011). Neste contexto, os dados obtidos com o probucol tornam-se relevante sugerindo que o efeito protetor desse composto em retardar o estresse oxidativo induzido pelo 3-NP pode estar relacionado, ao menos em parte, à sua capacidade de modular a GPx. A importância desta enzima frente ao estresse oxidativo induzido pelo 3-NP foi demonstrada em um estudo onde camundongos *knock-out* para a GPx apresentaram degeneração estriatal muito mais pronunciada do que animais selvagens, seguido da administração de 3-NP (Klivenyi et al., 2000). Além disso, Santamaría e colaboradores demonstraram que o tratamento com selenito de sódio, um composto que também aumenta a atividade da GPx, foi capaz proteger contra a toxicidade induzida pelo AQ (Santamaria et al., 2003), mostrando a importância dessa enzima em conter o dano oxidativo.

Por outro lado, outros mecanismos de ação do probucol não podem ser descartados, como o efeito direto desse composto como um antioxidante/*scavenger*, bem como seu efeito anti-inflamatório. A atividade antiinflamatória do probucol tem sido importante na sua proteção principalmente sobre o sistema cardiovascular (Poirier, 2003; Tanous et al., 2006; Yamashita e Matsuzawa, 2009), porém esse efeito pode também ser importante em modelos envolvendo o sistema nervoso central. O aumento da expressão da iNOS está relacionado com processo inflamatório. Essa enzima é comumente regulada por mediadores inflamatórios e sua persistente ativação pode causar o aumento na produção de $^{\bullet}$ON (Napolitano et al., 2008). Um estudo recente mostrou aumentos na expressão de alguns mediadores inflamatórios no estriado e no córtex de pacientes com DH (Silvestroni et al., 2009), demonstrando que o processo inflamatório parece ser importante na patogênese da doença.

Em resumo, o tratamento com 3-NP inibiu o complexo II, o que não foi prevenido pelo probucol. Porém, o estresse oxidativo subseqüente a essa inibição foi atenuado pelo composto, provavelmente devido a sua capacidade de aumentar a atividade da GPx. Por outro lado, as propriedades anti-inflamatórias do probucol não podem ser descartadas sendo que mais estudos são necessários pra elucidar o possível mecanismo de ação deste composto neste modelo de DH induzido pelo 3-NP.

No terceiro trabalho desta dissertação, avaliou-se os efeitos protetores do probucol e do succinobucol sobre o dano oxidativo induzido pelo 3-NP em preparações mitocondriais de cérebro de ratos, objetivando-se comparar ambos os compostos.

Tanto o probucol como o succinobucol foram capazes de prevenir o estresse oxidativo induzido pelo 3-NP, mas apenas o succinobucol foi capaz de proteger contra a disfunção mitocondrial induzida pela toxina. Por outro lado, esses compostos não tiveram nenhum efeito sobre a inibição do complexo II induzida pelo 3-NP. Esses dados estão de acordo com os resultados apresentados anteriormente no artigo 1 desta dissertação, onde foi demonstrado que o probucol é capaz de conter apenas o estresse oxidativo induzido pelo 3-NP, mas não protege a disfunção mitocondrial.

O efeito protetor do succinobucol evidenciado no presente estudo está relacionado, pelo menos em parte, a sua atividade antioxidante. A inibição da SDH pelo 3-NP é responsável pela geração de ERO, podendo causar diminuição da função mitocondrial (avaliada pelo método da redução do MTT). As propriedades scavenger do

succinobucol poderiam proteger esse evento secundário à inibição da SDH devido a sua habilidade de neutralizar espécies reativas geradas após a inibição do complexo II. Essa idéia é reforçada pelos dados que demonstram que o succinobucol previne a disfunção mitocondrial induzida pelo *t*BuOOH.

No entanto, o mecanismo de proteção do succinobucol parece não estar somente envolvido com as propriedades antioxidantes e *scavenger* deste composto, uma vez que o probucol, que apresenta propriedades similares e que também foi capaz de proteger contra a geração de ERO e peroxidação lipídica induzida pelo 3-NP, não protegeu da disfunção mitocondrial induzida pela toxina. Uma hipótese para explicar esse mecanismo pode estar relacionada à estrututra química desses compostos, onde o succinobucol poderia ser responsável por modular proteínas envolvidas na homeostase mitocondrial e, portanto, ser mais efetivo quando comparado ao probucol devido ao grupamento succinato presente na sua estrutura. Entretanto estudos adicionais são necessários para determinar o real mecanismo de proteção do succinobucol.

Dessa maneira, o presente estudo evidencia os possíveis efeitos benéficos que podem ser obtidos pela administração do probucol e do seu análogo succinobucol em modelos experimentais *in vivo* e *in vitro* da DH. Terapias preventivas capazes de aumentar as defesas antioxidantes ou de reduzir o estresse oxidativo podem ser de extrema importância no intuito de retardar a progressão da DH.

6. CONCLUSÕES

1) O 3-NP e o AQ, bem como a associação de ambos os compostos em concentrações sub-tóxicas, são capazes de induzir um complexo mecanismo de dano envolvendo comprometimento metabólico, formação de ERO e estresse oxidativo em fatias teciduais de estriado de ratos;

2) O probucol foi capaz de modular o estresse oxidativo induzido pelas três condições acima-mencionadas, o que parece ocorrer devido à sua capacidade antioxidante;

3) O probucol preveniu a perda de peso, as alterações motoras e o estresse oxidativo induzido pela administração de 3-NP, e este efeito pode ser atribuído, ao menos em parte, à sua capacidade de aumentar a atividade da enzima GPx no estriado e no córtex

dos animais, bem como às suas propriedades antioxidante direta (*scavenger)* e anti-inflamatória;

4) O probucol e o sucinobucol preveniram o estresse oxidativo induzido pelo 3-NP em preparações mitocondriais de cérebro de ratos; além disso, o succinobucol foi capaz de prevenir o dano mitocondrial induzido pelo 3-NP num mecanismo independente da inibição do complexo II.

7. PERSPECTIVAS

Alguns aspectos relacionados a este trabalho ainda precisam ser melhor elucidados. Desta forma, algumas perspectivas são propostas ao final desta dissertação:

1. Avaliar o efeito do tratamento com 3-NP sobre marcadores de sinapses nervosas (níveis da proteína sinaptofisina) em córtex e estriado de ratos, bem como o papel do pré-tratamento com probucol;

2. Investigar a possível contribuição das propriedades antiinflamatórias do probucol no mecanismo de proteção desse composto frente à neurotoxicidade do 3-NP através da determinação de marcadores de inflamação como níveis de TNF-α, interleucina-6 e interleucina-1β, astrócito e microglia ativados;

3) Avaliar melhor o mecanismo de ação do succinobucol na proteção contra o dano mitocondrial induzido pelo 3-NP.

8. REFERÊNCIAS BIBLIOGRÁFICAS

Ackrell BA. Progress in understanding structure-function relationships in respiratory chain complex II. **FEBS Lett**, 466(1): 1-5, 2000.

Adam OR; Jankovic J. Symptomatic treatment of Huntington disease. **Neurotherapeutics**, 5(2): 181-97, 2008.

Aebi H; Wyss SR; Scherz B; Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. **Eur J Biochem**, 48(1): 137-45, 1974.

Ahmadian S; Barar J; Saei AA; Fakhree MA; Omidi Y. Cellular toxicity of nanogenomedicine in MCF-7 cell line: MTT assay. **J Vis Exp**, (26): 2009.

Al-Majed AA. Probucol attenuates oxidative stress, energy starvation, and nitric acid production following transient forebrain ischemia in the rat hippocampus. **Oxid Med Cell Longev**, 2011(471590, 2011.

Al Mutairy A; Al Kadasah S; Elfaki I; Arshaduddin M; Malik D; Al Moutaery K; Tariq M. Trolox ameliorates 3-nitropropionic acid-induced neurotoxicity in rats. **Neurotoxicol Teratol**, 32(2): 226-33, 2010.

Alexi T; Hughes PE; Knusel B; Tobin AJ. Metabolic compromise with systemic 3-nitropropionic acid produces striatal apoptosis in Sprague-Dawley rats but not in BALB/c ByJ mice. **Exp Neurol**, 153(1): 74-93, 1998.

Ali SF; LeBel CP; Bondy SC. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. **Neurotoxicology**, 13(3): 637-48, 1992.

Alston TA; Mela L; Bright HJ. 3-Nitropropionate, the toxic substance of Indigofera, is a suicide inactivator of succinate dehydrogenase. **Proc Natl Acad Sci U S A**, 74(9): 3767-71, 1977.

Andreassen OA; Dedeoglu A; Ferrante RJ; Jenkins BG; Ferrante KL; Thomas M; Friedlich A; Browne SE; Schilling G; Borchelt DR; Hersch SM; Ross CA; Beal MF. Creatine increase survival and delays motor

symptoms in a transgenic animal model of Huntington's disease. **Neurobiol Dis**, 8(3): 479-91, 2001.

Andreassen OA; Ferrante RJ; Dedeoglu A; Beal MF. Lipoic acid improves survival in transgenic mouse models of Huntington's disease. **Neuroreport**, 12(15): 3371-3, 2001.

Andrew S; Theilmann J; Almqvist E; Norremolle A; Lucotte G; Anvret M; Sorensen SA; Turpin JC; Hayden MR. DNA analysis of distinct populations suggests multiple origins for the mutation causing Huntington disease. **Clin Genet**, 43(6): 286-94, 1993.

Andrews TC; Brooks DJ. Advances in the understanding of early Huntington's disease using the functional imaging techniques of PET and SPET. **Mol Med Today**, 4(12): 532-9, 1998.

Archer J. Tests for emotionality in rats and mice: a review. **Anim Behav**, 21(2): 205-35, 1973.

Arzberger T; Krampfl K; Leimgruber S; Weindl A. Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GLT1) mRNA expression in Huntington's disease--an in situ hybridization study. J **Neuropathol Exp Neurol**, 56(4): 440-54, 1997.

Avila DS; Colle D; Gubert P; Palma AS; Puntel G; Manarin F; Noremberg S; Nascimento PC; Aschner M; Rocha JB; Soares FA. A possible neuroprotective action of a vinylic telluride against Mn-induced neurotoxicity. **Toxicol Sci**, 115(1): 194-201, 2010.

Bacsi A; Woodberry M; Widger W; Papaconstantinou J; Mitra S; Peterson JW; Boldogh I. Localization of superoxide anion production to mitochondrial electron transport chain in 3-NPA-treated cells. **Mitochondrion**, 6(5): 235-44, 2006.

Barnhart JW; Sefranka JA; McIntosh DD. Hypocholesterolemic effect of 4,4'-(isopropylidenedithio)-bis(2,6-di-t-butylphenol) (probucol). **Am J Clin Nutr**, 23(9): 1229-33, 1970.

Bates GP. History of genetic disease: the molecular genetics of Huntington disease - a history. **Nat Rev Genet**, 6(10): 766-73, 2005.

Bazzett TJ; Falik RC; Becker JB; Albin RL. Synergistic effects of chronic exposure to subthreshold concentrations of quinolinic acid and malonate in the rat striatum. **Brain Res**, 718(1-2): 228-32, 1996.

Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. **Ann Neurol**, 38(3): 357-66, 1995.

Beal MF. Neuroprotective effects of creatine. **Amino Acids**, 40(5): 1305-13, 2011.

Beal MF; Brouillet E; Jenkins BG; Ferrante RJ; Kowall NW; Miller JM; Storey E; Srivastava R; Rosen BR; Hyman BT. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. **J Neurosci**, 13(10): 4181-92, 1993.

Beal MF; Henshaw DR; Jenkins BG; Rosen BR; Schulz JB. Coenzyme Q10 and nicotinamide block striatal lesions produced by the mitochondrial toxin malonate. **Ann Neurol**, 36(6): 882-8, 1994.

Beal MF; Hyman BT; Koroshetz W. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? **Trends Neurosci**, 16(4): 125-31, 1993.

Beal MF; Kowall NW; Ellison DW; Mazurek MF; Swartz KJ; Martin JB. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. **Nature**, 321(6066): 168-71, 1986.

Behan WM; McDonald M; Darlington LG; Stone TW. Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl. **Br J Pharmacol**, 128(8): 1754-60, 1999.

Behrens PF; Franz P; Woodman B; Lindenberg KS; Landwehrmeyer GB. Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. **Brain**, 125(8): 1908-22, 2002.

Benchoua A; Trioulier Y; Zala D; Gaillard MC; Lefort N; Dufour N; Saudou F; Elalouf JM; Hirsch E; Hantraye P; Deglon N; Brouillet E. Involvement of mitochondrial complex II defects in neuronal death

produced by N-terminus fragment of mutated huntingtin. **Mol Biol Cell**, 17(4): 1652-63, 2006.

Berardelli A; Noth J; Thompson PD; Bollen EL; Curra A; Deuschl G; van Dijk JG; Topper R; Schwarz M; Roos RA. Pathophysiology of chorea and bradykinesia in Huntington's disease. **Mov Disord**, 14(3): 398-403, 1999.

Berridge MV; Tan AS. The protein kinase C inhibitor, calphostin C, inhibits succinate-dependent mitochondrial reduction of MTT by a mechanism that does not involve protein kinase C. **Biochem Biophys Res Commun**, 185(3): 806-11, 1992.

Bhateja DK; Dhull DK; Gill A; Sidhu A; Sharma S; Reddy BV; Padi SS. Peroxisome proliferator-activated receptor-alpha activation attenuates 3-nitropropionic acid induced behavioral and biochemical alterations in rats: possible neuroprotective mechanisms. **Eur J Pharmacol**, 674(1): 33-43, 2012.

Binienda ZK. Neuroprotective effects of L-carnitine in induced mitochondrial dysfunction. **Ann N Y Acad Sci**, 993(289-95), 2003.

Bizat N; Hermel JM; Boyer F; Jacquard C; Creminon C; Ouary S; Escartin C; Hantraye P; Kajewski S; Brouillet E. Calpain is a major cell death effector in selective striatal degeneration induced in vivo by 3-nitropropionate: implications for Huntington's disease. **J Neurosci**, 23(12): 5020-30, 2003.

Bizat N; Hermel JM; Humbert S; Jacquard C; Creminon C; Escartin C; Saudou F; Krajewski S; Hantraye P; Brouillet E. In vivo calpain/caspase cross-talk during 3-nitropropionic acid-induced striatal degeneration: implication of a calpain-mediated cleavage of active caspase-3. **J Biol Chem**, 278(44): 43245-53, 2003.

Blum D; Gall D; Cuvelier L; Schiffmann SN. Topological analysis of striatal lesions induced by 3-nitropropionic acid in the Lewis rat. **Neuroreport**, 12(8): 1769-72, 2001.

Bonelli RM; Hofmann P. A systematic review of the treatment studies in Huntington's disease since 1990. **Expert Opin Pharmacother**, 8(2): 141-53, 2007.

Bordelon YM; Chesselet MF; Nelson D; Welsh F; Erecinska M. Energetic dysfunction in quinolinic acid-lesioned rat striatum. **J Neurochem**, 69(4): 1629-39, 1997.

Borlongan CV; Koutouzis TK; Freeman TB; Cahill DW; Sanberg PR. Behavioral pathology induced by repeated systemic injections of 3-nitropropionic acid mimics the motoric symptoms of Huntington's disease. **Brain Res**, 697(1-2): 254-7, 1995.

Borlongan CV; Koutouzis TK; Freeman TB; Hauser RA; Cahill DW; Sanberg PR. Hyperactivity and hypoactivity in a rat model of Huntington's disease: the systemic 3-nitropropionic acid model. **Brain Res Brain Res Protoc**, 1(3): 253-7, 1997.

Brennan WA, Jr.; Bird ED; Aprille JR. Regional mitochondrial respiratory activity in Huntington's disease brain. **J Neurochem**, 44(6): 1948-50, 1985.

Brouillet E; Conde F; Beal MF; Hantraye P. Replicating Huntington's disease phenotype in experimental animals. **Prog Neurobiol**, 59(5): 427-68, 1999.

Brouillet E; Guyot MC; Mittoux V; Altairac S; Conde F; Palfi S; Hantraye P. Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. **J Neurochem**, 70(2): 794-805, 1998.

Brouillet E; Jacquard C; Bizat N; Blum D. 3-Nitropropionic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease. **J Neurochem**, 95(6): 1521-40, 2005.

Browne SE. Mitochondria and Huntington's disease pathogenesis: insight from genetic and chemical models. **Ann N Y Acad Sci**, 1147(358-82, 2008.

Browne SE; Beal MF. Oxidative damage in Huntington's disease pathogenesis. **Antioxid Redox Signal**, 8(11-12): 2061-73, 2006.

Browne SE; Bowling AC; MacGarvey U; Baik MJ; Berger SC; Muqit MM; Bird ED; Beal MF. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. **Ann Neurol**, 41(5): 646-53, 1997.

Browne SE; Ferrante RJ; Beal MF. Oxidative stress in Huntington's disease. **Brain Pathol**, 9(1): 147-63, 1999.

Brustovetsky N; Dubinsky JM. Dual responses of CNS mitochondria to elevated calcium. **J Neurosci**, 20(1): 103-13, 2000.

Buckley MM; Goa KL; Price AH; Brogden RN. Probucol. A reappraisal of its pharmacological properties and therapeutic use in hypercholesterolaemia. **Drugs**, 37(6): 761-800, 1989.

Butterworth J; Yates CM; Reynolds GP. Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase and gamma-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. **J Neurol Sci**, 67(2): 161-71, 1985.

Calabresi P; Centonze D; Pisani A; Bernardi G. Metabotropic glutamate receptors and cell-type-specific vulnerability in the striatum: implication for ischemia and Huntington's disease. **Exp Neurol**, 158(1): 97-108, 1999.

Carlberg I; Mannervik B. Glutathione reductase. **Methods Enzymol**, 113(484-90, 1985.

Champagne D; Pearson D; Dea D; Rochford J; Poirier J. The cholesterol-lowering drug probucol increases apolipoprotein E production in the hippocampus of aged rats: implications for Alzheimer's disease. **Neuroscience**, 121(1): 99-110, 2003.

Chang DT; Rintoul GL; Pandipati S; Reynolds IJ. Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. **Neurobiol Dis**, 22(2): 388-400, 2006.

Chen CM; Wu YR; Cheng ML; Liu JL; Lee YM; Lee PW; Soong BW; Chiu DT. Increased oxidative damage and mitochondrial abnormalities

in the peripheral blood of Huntington's disease patients. **Biochem Biophys Res Commun**, 359(2): 335-40, 2007.

Choo YS; Johnson GV; MacDonald M; Detloff PJ; Lesort M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. **Hum Mol Genet**, 13(14): 1407-20, 2004.

Coles CJ; Edmondson DE; Singer TP. Inactivation of succinate dehydrogenase by 3-nitropropionate. **J Biol Chem**, 254(12): 5161-7, 1979.

Colle D; Hartwig JM; Antunes Soares FA; Farina M. Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro. **Brain Res Bull**, 2012.

Connick JH; Carla V; Moroni F; Stone TW. Increase in kynurenic acid in Huntington's disease motor cortex. **J Neurochem**, 52(3): 985-7, 1989.

Coyle JT; Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. **Science**, 262(5134): 689-95, 1993.

Coyle JT; Schwarcz R. Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. **Nature**, 263(5574): 244-6, 1976.

Crim WS; Wu R; Carter JD; Cole BK; Trace AP; Mirmira RG; Kunsch C; Nadler JL; Nunemaker CS. AGI-1067, a novel antioxidant and anti-inflammatory agent, enhances insulin release and protects mouse islets. **Mol Cell Endocrinol**, 323(2): 246-55, 2010.

Crossman AR. Primate models of dyskinesia: the experimental approach to the study of basal ganglia-related involuntary movement disorders. **Neuroscience**, 21(1): 1-40, 1987.

Crossman AR; Mitchell IJ; Sambrook MA; Jackson A. Chorea and myoclonus in the monkey induced by gamma-aminobutyric acid antagonism in the lentiform complex. The site of drug action and a hypothesis for the neural mechanisms of chorea. **Brain**, 111 (1211-33) 1988.

Cui L; Jeong H; Borovecki F; Parkhurst CN; Tanese N; Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. **Cell**, 127(1): 59-69, 2006.

Damiano M; Galvan L; Deglon N; Brouillet E. Mitochondria in Huntington's disease. **Biochim Biophys Acta**, 1802(1): 52-61, 2010.

Danbolt NC. Glutamate uptake. **Prog Neurobiol**, 65(1): 1-105, 2001.

de Moura MB; dos Santos LS; Van Houten B. Mitochondrial dysfunction in neurodegenerative diseases and cancer. **Environ Mol Mutagen**, 51(5): 391-405, 2010.

Dedeoglu A; Kubilus JK; Yang L; Ferrante KL; Hersch SM; Beal MF; Ferrante RJ. Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. **J Neurochem**, 85(6): 1359-67, 2003.

Del Rio P; Massieu L. Mild mitochondrial inhibition in vivo enhances glutamate-induced neuronal damage through calpain but not caspase activation: role of ionotropic glutamate receptors. **Exp Neurol**, 212(1): 179-88, 2008.

Del Rio P; Montiel T; Chagoya V; Massieu L. Exacerbation of excitotoxic neuronal death induced during mitochondrial inhibition in vivo: relation to energy imbalance or ATP depletion? **Neuroscience**, 146(4): 1561-70, 2007.

Deshpande SB; Hida H; Takei-Io N; Masuda T; Baba H; Nishino H. Involvement of nitric oxide in 3-nitropropionic acid-induced striatal toxicity in rats. **Brain Res**, 1108(1): 205-15, 2006.

Dewhurst K; Oliver JE; McKnight AL. Socio-psychiatric consequences of Huntington's disease. **Br J Psychiatry**, 116(532): 255-8, 1970.

Dhami GK; Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. **Pharmacol Ther**, 111(1): 260-71, 2006.

DiFiglia M. Excitotoxic injury of the neostriatum: a model for Huntington's disease. **Trends Neurosci**, 13(7): 286-9, 1990.

DiFiglia M; Sapp E; Chase KO; Davies SW; Bates GP; Vonsattel JP; Aronin N. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. **Science**, 277(5334): 1990-3, 1997.

Dingledine R; Borges K; Bowie D; Traynelis SF. The glutamate receptor ion channels. **Pharmacol Rev**, 51(1): 7-61, 1999.

Djousse L; Knowlton B; Cupples LA; Marder K; Shoulson I; Myers RH. Weight loss in early stage of Huntington's disease. **Neurology**, 59(9): 1325-30, 2002.

Dringen R; Hirrlinger J. Glutathione pathways in the brain. **Biol Chem**, 384(4): 505-16, 2003.

During MJ; Heyes MP; Freese A; Markey SP; Martin JB; Roth RH. Quinolinic acid concentrations in striatal extracellular fluid reach potentially neurotoxic levels following systemic L-tryptophan loading. **Brain Res**, 476(2): 384-7, 1989.

Edmonds B; Gibb AJ; Colquhoun D. Mechanisms of activation of glutamate receptors and the time course of excitatory synaptic currents. **Annu Rev Physiol**, 57:95-519, 1995.

Elinos-Calderon D; Robledo-Arratia Y; Perez-De La Cruz V; Maldonado PD; Galvan-Arzate S; Pedraza-Chaverri J; Santamaria A. Antioxidant strategy to rescue synaptosomes from oxidative damage and energy failure in neurotoxic models in rats: protective role of S-allylcysteine. **J Neural Transm**, 117(1): 35-44, 2010.

Emerich DF; Thanos CG; Goddard M; Skinner SJ; Geany MS; Bell WJ; Bintz B; Schneider P; Chu Y; Babu RS; Borlongan CV; Boekelheide K; Hall S; Bryant B; Kordower JH. Extensive neuroprotection by choroid plexus transplants in excitotoxin lesioned monkeys. **Neurobiol Dis**, 23(2): 471-80, 2006.

Estrada Sanchez AM; Mejia-Toiber J; Massieu L. Excitotoxic neuronal death and the pathogenesis of Huntington's disease. **Arch Med Res**, 39(3): 265-76, 2008.

Farina M; Aschner M; Rocha JB. Oxidative stress in MeHg-induced neurotoxicity. **Toxicol Appl Pharmacol**, 256(3): 405-17, 2011.

Farina M; Campos F; Vendrell I; Berenguer J; Barzi M; Pons S; Sunol C. Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells. **Toxicol Sci**, 112(2): 416-26, 2009.

Farina M; Frizzo ME; Soares FA; Schwalm FD; Dietrich MO; Zeni G; Rocha JB; Souza DO. Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. **Toxicol Lett**, 144(3): 351-7, 2003.

Fasano A; Cadeddu F; Guidubaldi A; Piano C; Soleti F; Zinzi P; Bentivoglio AR. The long-term effect of tetrabenazine in the management of Huntington disease. **Clin Neuropharmacol**, 31(6): 313-8, 2008.

Feigin A; Kieburtz K; Como P; Hickey C; Claude K; Abwender D; Zimmerman C; Steinberg K; Shoulson I. Assessment of coenzyme Q10 tolerability in Huntington's disease. **Mov Disord**, 11(3): 321-3, 1996.

Fernagut PO; Diguet E; Jaber M; Bioulac B; Tison F. Dopamine transporter knock-out mice are hypersensitive to 3-nitropropionic acid-induced striatal damage. **Eur J Neurosci**, 15(12): 2053-6, 2002.

Ferraguti F; Crepaldi L; Nicoletti F. Metabotropic glutamate 1 receptor: current concepts and perspectives. **Pharmacol Rev**, 60(4): 536-81, 2008.

Ferrante RJ; Andreassen OA; Jenkins BG; Dedeoglu A; Kuemmerle S; Kubilus JK; Kaddurah-Daouk R; Hersch SM; Beal MF. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. **J Neurosci**, 20(12): 4389-97, 2000.

Ferrante RJ; Beal MF; Kowall NW; Richardson EP, Jr.; Martin JB. Sparing of acetylcholinesterase-containing striatal neurons in Huntington's disease. **Brain Res**, 411(1): 162-6, 1987.

Ferrante RJ; Gutekunst CA; Persichetti F; McNeil SM; Kowall NW; Gusella JF; MacDonald ME; Beal MF; Hersch SM. Heterogeneous topographic and cellular distribution of huntingtin expression in the normal human neostriatum. **J Neurosci**, 17(9): 3052-63, 1997.

Fischer JC; Ruitenbeek W; Berden JA; Trijbels JM; Veerkamp JH; Stadhouders AM; Sengers RC; Janssen AJ. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. **Clin Chim Acta**, 153(1): 23-36, 1985.

Folstein S; Abbott MH; Chase GA; Jensen BA; Folstein MF. The association of affective disorder with Huntington's disease in a case series and in families. **Psychol Med**, 13(3): 537-42, 1983.

Foster AC; Collins JF; Schwarcz R. On the excitotoxic properties of quinolinic acid, 2,3-piperidine dicarboxylic acids and structurally related compounds. **Neuropharmacology**, 22(12): 1331-42, 1983.

Franco JL; Braga HC; Stringari J; Missau FC; Posser T; Mendes BG; Leal RB; Santos AR; Dafre AL; Pizzolatti MG; Farina M. Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. **Chem Res Toxicol**, 20(12): 1919-26, 2007.

Franco JL; Posser T; Dunkley PR; Dickson PW; Mattos JJ; Martins R; Bainy AC; Marques MR; Dafre AL; Farina M. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. **Free Radic Biol Med**, 47(4): 449-57, 2009.

Frank S. Tetrabenazine as anti-chorea therapy in Huntington disease: an open-label continuation study. Huntington Study Group/TETRA-HD Investigators. **BMC Neurol**, 9(62), 2009.

Frank S. Tetrabenazine: the first approved drug for the treatment of chorea in US patients with Huntington disease. **Neuropsychiatr Dis Treat**, 6:57-65, 2010.

Furtado JC; Mazurek MF. Behavioral characterization of quinolinate-induced lesions of the medial striatum: relevance for Huntington's disease. **Exp Neurol**, 138(1): 158-68, 1996.

Fusco FR; Chen Q; Lamoreaux WJ; Figueredo-Cardenas G; Jiao Y; Coffman JA; Surmeier DJ; Honig MG; Carlock LR; Reiner A. Cellular localization of huntingtin in striatal and cortical neurons in rats: lack of correlation with neuronal vulnerability in Huntington's disease. **J Neurosci**, 19(4): 1189-202, 1999.

Gabrielson KL; Hogue BA; Bohr VA; Cardounel AJ; Nakajima W; Kofler J; Zweier JL; Rodriguez ER; Martin LJ; de Souza-Pinto NC; Bressler J. Mitochondrial toxin 3-nitropropionic acid induces cardiac and neurotoxicity differentially in mice. **Am J Pathol**, 159(4): 1507-20, 2001.

Gafni J; Ellerby LM. Calpain activation in Huntington's disease. **J Neurosci**, 22(12): 4842-9, 2002.

Garcia Ruiz PJ; Gomez Tortosa E; Sanchez Bernados V; Rojo A; Fontan A; Garcia de Yebenes J. Bradykinesia in Huntington's disease. **Clin Neuropharmacol**, 23(1): 50-2, 2000.

Gil-Mohapel JM; Rego AC. Huntington’s Disease: A Review on the Physiopathological Aspects. Rev Neurocienc, 1-11, 2011.

Gines S; Seong IS; Fossale E; Ivanova E; Trettel F; Gusella JF; Wheeler VC; Persichetti F; MacDonald ME. Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. **Hum Mol Genet**, 12(5): 497-508, 2003.

Gizatullina ZZ; Lindenberg KS; Harjes P; Chen Y; Kosinski CM; Landwehrmeyer BG; Ludolph AC; Striggow F; Zierz S; Gellerich FN. Low stability of Huntington muscle mitochondria against Ca2+ in R6/2 mice. **Ann Neurol**, 59(2): 407-11, 2006.

Goldberg YP; Nicholson DW; Rasper DM; Kalchman MA; Koide HB; Graham RK; Bromm M; Kazemi-Esfarjani P; Thornberry NA; Vaillancourt JP; Hayden MR. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. **Nat Genet**, 13(4): 442-9, 1996.

Gopinath K; Prakash D; Sudhandiran G. Neuroprotective effect of naringin, a dietary flavonoid against 3-nitropropionic acid-induced neuronal apoptosis. **Neurochem Int**, 59(7): 1066-73, 2011.

Gould DH; Gustine DL. Basal ganglia degeneration, myelin alterations, and enzyme inhibition induced in mice by the plant toxin 3-nitropropanoic acid. Neuropathol **Appl Neurobiol**, 8(5): 377-93, 1982.

Gould DH; Wilson MP; Hamar DW. Brain enzyme and clinical alterations induced in rats and mice by nitroaliphatic toxicants. **Toxicol Lett**, 27(1-3): 83-9, 1985.

Grafton ST; Mazziotta JC; Pahl JJ; St George-Hyslop P; Haines JL; Gusella J; Hoffman JM; Baxter LR; Phelps ME. Serial changes of cerebral glucose metabolism and caudate size in persons at risk for Huntington's disease. **Arch Neurol**, 49(11): 1161-7, 1992.

Graham JM. Isolation of mitochondria from tissues and cells by differential centrifugation. **Curr Protoc Cell Biol**, Chapter 3(Unit 33) 2001.

Graybiel AM. Neurotransmitters and neuromodulators in the basal ganglia. **Trends Neurosci**, 13(7): 244-54, 1990.

Greene JG; Greenamyre JT. Exacerbation of NMDA, AMPA, and L-glutamate excitotoxicity by the succinate dehydrogenase inhibitor malonate. **J Neurochem**, 64(5): 2332-8, 1995.

Greene JG; Sheu SS; Gross RA; Greenamyre JT. 3-Nitropropionic acid exacerbates N-methyl-D-aspartate toxicity in striatal culture by multiple mechanisms. **Neuroscience**, 84(2): 503-10, 1998.

Gu M; Gash MT; Mann VM; Javoy-Agid F; Cooper JM; Schapira AH. Mitochondrial defect in Huntington's disease caudate nucleus. **Ann Neurol**, 39(3): 385-9, 1996.

Guidetti P; Luthi-Carter RE; Augood SJ; Schwarcz R. Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. **Neurobiol Dis**, 17(3): 455-61, 2004.

Gusella JF; Wexler NS; Conneally PM; Naylor SL; Anderson MA; Tanzi RE; Watkins PC; Ottina K; Wallace MR; Sakaguchi AY; et al. A polymorphic DNA marker genetically linked to Huntington's disease. **Nature**, 306(5940): 234-8, 1983.

Haibin Y; Yan Y; Jinjin D. Preparation of probucol monosuccinate. Patent No CN 1970536, 2006.

Halliwell B. Oxidative stress and neurodegeneration: where are we now? **J Neurochem**, 97(6): 1634-58, 2006.

Hamilton BF; Gould DH. Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage. **Acta Neuropathol**, 72(3): 286-97, 1987.

Han I; You Y; Kordower JH; Brady ST; Morfini GA. Differential vulnerability of neurons in Huntington's disease: the role of cell type-specific features. **J Neurochem**, 113(5): 1073-91, 2010.

Hands S; Sajjad MU; Newton MJ; Wyttenbach A. In vitro and in vivo aggregation of a fragment of huntingtin protein directly causes free radical production. **J Biol Chem**, 286(52): 44512-20, 2011.

Harjes P; Wanker EE. The hunt for huntingtin function: interaction partners tell many different stories. **Trends Biochem Sci**, 28(8): 425-33, 2003.

Hassel B; Sonnewald U. Selective inhibition of the tricarboxylic acid cycle of GABAergic neurons with 3-nitropropionic acid in vivo. **J Neurochem**, 65(3): 1184-91, 1995.

Hassel B; Tessler S; Faull RL; Emson PC. Glutamate uptake is reduced in prefrontal cortex in Huntington's disease. **Neurochem Res**, 33(2): 232-7, 2008.

He F; Zhang S; Qian F; Zhang C. Delayed dystonia with striatal CT lucencies induced by a mycotoxin (3-nitropropionic acid). **Neurology**, 45(12): 2178-83, 1995.

Heinsen H; Strik M; Bauer M; Luther K; Ulmar G; Gangnus D; Jungkunz G; Eisenmenger W; Gotz M. Cortical and striatal neurone number in Huntington's disease. **Acta Neuropathol**, 88(4): 320-33, 1994.

Henriksen EJ. Exercise training and the antioxidant alpha-lipoic acid in the treatment of insulin resistance and type 2 diabetes. **Free Radic Biol Med**, 40(1): 3-12, 2006.

Hersch SM; Gevorkian S; Marder K; Moskowitz C; Feigin A; Cox M; Como P; Zimmerman C; Lin M; Zhang L; Ulug AM; Beal MF; Matson W; Bogdanov M; Ebbel E; Zaleta A; Kaneko Y; Jenkins B; Hevelone N; Zhang H; Yu H; Schoenfeld D; Ferrante R; Rosas HD. Creatine in Huntington disease is safe, tolerable, bioavailable in brain and reduces serum 8OH2'dG. **Neurology**, 66(2): 250-2, 2006.

Hissin PJ; Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. **Anal Biochem**, 74(1): 214-26, 1976.

Ho LW; Carmichael J; Swartz J; Wyttenbach A; Rankin J; Rubinsztein DC. The molecular biology of Huntington's disease. **Psychol Med**, 31(1): 3-14, 2001.

Hodgson JG; Agopyan N; Gutekunst CA; Leavitt BR; LePiane F; Singaraja R; Smith DJ; Bissada N; McCutcheon K; Nasir J; Jamot L; Li XJ; Stevens ME; Rosemond E; Roder JC; Phillips AG; Rubin EM; Hersch SM; Hayden MR. A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. **Neuron**, 23(1): 181-92, 1999.

Hoffner G; Kahlem P; Djian P. Perinuclear localization of huntingtin as a consequence of its binding to microtubules through an interaction with beta-tubulin: relevance to Huntington's disease. **J Cell Sci**, 115(Pt 5): 941-8, 2002.

Huang LS; Sun G; Cobessi D; Wang AC; Shen JT; Tung EY; Anderson VE; Berry EA. 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme. **J Biol Chem**, 281(9): 5965-72, 2006.

Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. **Cell**, 72(6): 971-83, 1993.

Huntington's Disease Collaborative Research Group. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. **Neurology**, 57(3): 397-404, 2001.

Hyson HC; Kieburtz K; Shoulson I; McDermott M; Ravina B; de Blieck EA; Cudkowicz ME; Ferrante RJ; Como P; Frank S; Zimmerman C; Ferrante K; Newhall K; Jennings D; Kelsey T; Walker F; Hunt V; Daigneault S; Goldstein M; Weber J; Watts A; Beal MF; Browne SE; Metakis LJ. Safety and tolerability of high-dosage coenzyme Q10 in Huntington's disease and healthy subjects. **Mov Disord**, 25(12): 1924-8, 2010.

Imarisio S; Carmichael J; Korolchuk V; Chen CW; Saiki S; Rose C; Krishna G; Davies JE; Ttofi E; Underwood BR; Rubinsztein DC. Huntington's disease: from pathology and genetics to potential therapies. **Biochem J**, 412(2): 191-209, 2008.

Isacson O; Brundin P; Kelly PA; Gage FH; Bjorklund A. Functional neuronal replacement by grafted striatal neurones in the ibotenic acid-lesioned rat striatum. **Nature**, 311(5985): 458-60, 1984.

Jackson RL; Barnhart RL; Mao SJ. Probucol and its mechanisms for reducing atherosclerosis. **Adv Exp Med Biol**, 285(367-72, 1991.

Jacquard C; Trioulier Y; Cosker F; Escartin C; Bizat N; Hantraye P; Cancela JM; Bonvento G; Brouillet E. Brain mitochondrial defects amplify intracellular [Ca2+] rise and neurodegeneration but not Ca2+ entry during NMDA receptor activation. **FASEB J**, 20(7): 1021-3, 2006.

Jenkins BG; Koroshetz WJ; Beal MF; Rosen BR. Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized 1H NMR spectroscopy. **Neurology**, 43(12): 2689-95, 1993.

Jenkins BG; Rosas HD; Chen YC; Makabe T; Myers R; MacDonald M; Rosen BR; Beal MF; Koroshetz WJ. 1H NMR spectroscopy studies of Huntington's disease: correlations with CAG repeat numbers. **Neurology**, 50(5): 1357-65, 1998.

Kalonia H; Kumar A. Suppressing inflammatory cascade by cyclo-oxygenase inhibitors attenuates quinolinic acid induced Huntington's disease-like alterations in rats. **Life Sci**, 88(17-18): 784-91, 2011.

Kalonia H; Kumar P; Kumar A; Nehru B. Protective effect of rofecoxib and nimesulide against intra-striatal quinolinic acid-induced behavioral, oxidative stress and mitochondrial dysfunctions in rats. **Neurotoxicology**, 31(2): 195-203, 2010.

Kaminnyi AI; Lankin VZ; Perepelitsa EI; Konovalova GG; Samko AN; Tikhaze AK; Kukharchuk VV; Belenkov YN. Relationship between free-radical lipid oxidation and efficiency of coronary angioplasty in coronary patients. **Bull Exp Biol Med**, 144(5): 664-6, 2007.

Kasparova S; Sumbalova Z; Bystricky P; Kucharska J; Liptaj T; Mlynarik V; Gvozdjakova A. Effect of coenzyme Q10 and vitamin E on brain energy metabolism in the animal model of Huntington's disease. **Neurochem Int**, 48(2): 93-9, 2006.

Kassubek J; Gaus W; Landwehrmeyer GB. Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. **Neurology**, 62(3): 523-4; author reply 524, 2004.

Kendall AL; David F; Rayment G; Torres EM; Annett LE; Dunnett SB. The influence of excitotoxic basal ganglia lesions on motor performance in the common marmoset. **Brain**, 123 (7):442-58, 2000.

Kenney C; Hunter C; Jankovic J. Long-term tolerability of tetrabenazine in the treatment of hyperkinetic movement disorders. **Mov Disord**, 22(2): 193-7, 2007.

Kim YJ; Yi Y; Sapp E; Wang Y; Cuiffo B; Kegel KB; Qin ZH; Aronin N; DiFiglia M. Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. **Proc Natl Acad Sci U S A**, 98(22): 12784-9, 2001.

Klepac N; Relja M; Klepac R; Hecimovic S; Babic T; Trkulja V. Oxidative stress parameters in plasma of Huntington's disease patients,

asymptomatic Huntington's disease gene carriers and healthy subjects : a cross-sectional study. **J Neurol**, 254(12): 1676-83, 2007.

Klivenyi P; Andreassen OA; Ferrante RJ; Dedeoglu A; Mueller G; Lancelot E; Bogdanov M; Andersen JK; Jiang D; Beal MF. Mice deficient in cellular glutathione peroxidase show increased vulnerability to malonate, 3-nitropropionic acid, and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. **J Neurosci**, 20(1): 1-7, 2000.

Koroshetz WJ; Jenkins BG; Rosen BR; Beal MF. Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. **Ann Neurol**, 41(2): 160-5, 1997.

Kristian T. Isolation of mitochondria from the CNS. **Curr Protoc Neurosci**, Chapter7(Unit 7)22, 2010.

Krobitsch S; Kazantsev AG. Huntington's disease: From molecular basis to therapeutic advances. **Int J Biochem Cell Biol**, 43(1): 20-4, 2011.

Kuhl DE; Markham CH; Metter EJ; Riege WH; Phelps ME; Mazziotta JC. Local cerebral glucose utilization in symptomatic and presymptomatic Huntington's disease. **Res Publ Assoc Res Nerv Ment Dis**, 63(199-209), 1985.

Kumar P; Kalonia H; Kumar A. Huntington's disease: pathogenesis to animal models. **Pharmacol Rep**, 62(1): 1-14, 2010.

Kumar P; Kalonia H; Kumar A. Possible GABAergic mechanism in the neuroprotective effect of gabapentin and lamotrigine against 3-nitropropionic acid induced neurotoxicity. **Eur J Pharmacol**, 674(2-3): 265-74, 2012.

Kumar P; Kumar A. Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunctions in rat brain. **Prog Neuropsychopharmacol Biol Psychiatry**, 33(1): 100-8, 2009.

Kumar P; Padi SS; Naidu PS; Kumar A. Cyclooxygenase inhibition attenuates 3-nitropropionic acid-induced neurotoxicity in rats: possible antioxidant mechanisms. **Fundam Clin Pharmacol**, 21(3): 297-306, 2007.

Kunsch C; Luchoomun J; Grey JY; Olliff LK; Saint LB; Arrendale RF; Wasserman MA; Saxena U; Medford RM. Selective inhibition of endothelial and monocyte redox-sensitive genes by AGI-1067: a novel antioxidant and anti-inflammatory agent. **J Pharmacol Exp Ther**, 308(3): 820-9, 2004.

Kuwert T; Lange HW; Langen KJ; Herzog H; Aulich A; Feinendegen LE. Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. **Brain**, 113 (Pt 5)(1405-23, 1990.

Kuwert T; Noth J; Scholz D; Schwarz M; Lange HW; Topper R; Herzog H; Aulich A; Feinendegen LE. Comparison of somatosensory evoked potentials with striatal glucose consumption measured by positron emission tomography in the early diagnosis of Huntington's disease. **Mov Disord**, 8(1): 98-106, 1993.

Landles C; Bates GP. Huntingtin and the molecular pathogenesis of Huntington's disease. Fourth in molecular medicine review series. **EMBO Rep**, 5(10): 958-63, 2004.

Latini A; da Silva CG; Ferreira GC; Schuck PF; Scussiato K; Sarkis JJ; Dutra Filho CS; Wyse AT; Wannmacher CM; Wajner M. Mitochondrial energy metabolism is markedly impaired by D-2-hydroxyglutaric acid in rat tissues. **Mol Genet Metab**, 86(1-2): 188-99, 2005.

Lee WT; Itoh T; Pleasure D. Acute and chronic alterations in calcium homeostasis in 3-nitropropionic acid-treated human NT2-N neurons. **Neuroscience**, 113(3): 699-708, 2002.

Lee WT; Yin HS; Shen YZ. The mechanisms of neuronal death produced by mitochondrial toxin 3-nitropropionic acid: the roles of N-methyl-D-aspartate glutamate receptors and mitochondrial calcium overload. **Neuroscience**, 112(3): 707-16, 2002.

Lemieux H; Bulteau AL; Friguet B; Tardif JC; Blier PU. Dietary fatty acids and oxidative stress in the heart mitochondria. **Mitochondrion**, 11(1): 97-103, 2011.

Leung AW; Halestrap AP. Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore. **Biochim Biophys Acta**, 1777(7-8): 946-52, 2008.

Levine MS; Klapstein GJ; Koppel A; Gruen E; Cepeda C; Vargas ME; Jokel ES; Carpenter EM; Zanjani H; Hurst RS; Efstratiadis A; Zeitlin S; Chesselet MF. Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. **J Neurosci Res**, 58(4): 515-32, 1999.

Li SH; Li XJ. Huntingtin-protein interactions and the pathogenesis of Huntington's disease. **Trends Genet**, 20(3): 146-54, 2004.

Li XJ; Li S. Proteasomal dysfunction in aging and Huntington disease. **Neurobiol Dis**, 43(1): 4-8, 2011.

Li XJ; Orr AL; Li S. Impaired mitochondrial trafficking in Huntington's disease. **Biochim Biophys Act**a, 1802(1): 62-5, 2010.

Lievens JC; Woodman B; Mahal A; Spasic-Boscovic O; Samuel D; Kerkerian-Le Goff L; Bates GP. Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. **Neurobiol Dis**, 8(5): 807-21, 2001.

Lim D; Fedrizzi L; Tartari M; Zuccato C; Cattaneo E; Brini M; Carafoli E. Calcium homeostasis and mitochondrial dysfunction in striatal neurons of Huntington disease. **J Biol Chem**, 283(9): 5780-9, 2008.

Liot G; Bossy B; Lubitz S; Kushnareva Y; Sejbuk N; Bossy-Wetzel E. Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway. **Cell Death Differ**, 16(6): 899-909, 2009.

Liu CS; Cheng WL; Kuo SJ; Li JY; Soong BW; Wei YH. Depletion of mitochondrial DNA in leukocytes of patients with poly-Q diseases. **J Neurol Sci**, 264(1-2): 18-21, 2008.

Liu GX; Ou DM; Liu JH; Huang HL; Liao DF. Probucol inhibits lipid peroxidation of macrophage and affects its secretory properties. **Acta Pharmacol Sin**, 21(7): 637-40, 2000.

Lodi R; Schapira AH; Manners D; Styles P; Wood NW; Taylor DJ; Warner TT. Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. **Ann Neurol**, 48(1): 72-6, 2000.

Lowry OH; Rosebrough NJ; Farr AL; Randall RJ. Protein measurement with the Folin phenol reagent. **J Biol Chem**, 193(1): 265-75, 1951.

Lubos E; Loscalzo J; Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. **Antioxid Redox Signal**, 15(7): 1957-97, 2011.

Ludolph AC; He F; Spencer PS; Hammerstad J; Sabri M. 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. **Can J Neurol Sci**, 18(4): 492-8, 1991.

Luyendyk JP; Piper JD; Tencati M; Reddy KV; Holscher T; Zhang R; Luchoomun J; Chen X; Min W; Kunsch C; Mackman N. A novel class of antioxidants inhibit LPS induction of tissue factor by selective inhibition of the activation of ASK1 and MAP kinases. **Arterioscler Thromb Vasc Biol**, 27(8): 1857-63, 2007.

Maciel EN; Kowaltowski AJ; Schwalm FD; Rodrigues JM; Souza DO; Vercesi AE; Wajner M; Castilho RF. Mitochondrial permeability transition in neuronal damage promoted by Ca2+ and respiratory chain complex II inhibition. **J Neurochem**, 90(5): 1025-35, 2004.

Mandavilli BS; Boldogh I; Van Houten B. 3-nitropropionic acid-induced hydrogen peroxide, mitochondrial DNA damage, and cell death are attenuated by Bcl-2 overexpression in PC12 cells. **Brain Res Mol Brain Res**, 133(2): 215-23, 2005.

Mangiarini L; Sathasivam K; Seller M; Cozens B; Harper A; Hetherington C; Lawton M; Trottier Y; Lehrach H; Davies SW; Bates GP. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. **Cell**, 87(3): 493-506, 1996.

Mann DM; Oliver R; Snowden JS. The topographic distribution of brain atrophy in Huntington's disease and progressive supranuclear palsy. **Acta Neuropathol**, 85(5): 553-9, 1993.

Marsh JL; Pallos J; Thompson LM. Fly models of Huntington's disease. **Hum Mol Genet**, 12 (2):187-93, 2003.

Matthews RT; Yang L; Jenkins BG; Ferrante RJ; Rosen BR; Kaddurah-Daouk R; Beal MF. Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. **J Neurosci**, 18(1): 156-63, 1998.

Mattson MP; Gleichmann M; Cheng A. Mitochondria in neuroplasticity and neurological disorders. **Neuron**, 60(5): 748-66, 2008.

McDowell IF; Brennan GM; McEneny J; Young IS; Nicholls DP; McVeigh GE; Bruce I; Trimble ER; Johnston GD. The effect of probucol and vitamin E treatment on the oxidation of low-density lipoprotein and forearm vascular responses in humans. **Eur J Clin Invest**, 24(11): 759-65, 1994.

McKenna MC; Stevenson JH; Huang X; Tildon JT; Zielke CL; Hopkins IB. Mitochondrial malic enzyme activity is much higher in mitochondria from cortical synaptic terminals compared with mitochondria from primary cultures of cortical neurons or cerebellar granule cells. **Neurochem Int**, 36(4-5): 451-9, 2000.

McLin JP; Thompson LM; Steward O. Differential susceptibility to striatal neurodegeneration induced by quinolinic acid and kainate in inbred, outbred and hybrid mouse strains. **Eur J Neurosci**, 24(11): 3134-40, 2006.

Meng CQ; Somers PK; Rachita CL; Holt LA; Hoong LK; Zheng XS; Simpson JE; Hill RR; Olliff LK; Kunsch C; Sundell CL; Parthasarathy S; Saxena U; Sikorski JA; Wasserman MA. Novel phenolic antioxidants as multifunctional inhibitors of inducible VCAM-1 expression for use in atherosclerosis. **Bioorg Med Chem Lett**, 12(18): 2545-8, 2002.

Milakovic T; Johnson GV. Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. **J Biol Chem**, 280(35): 30773-82, 2005.

Mirandola SR; Melo DR; Saito A; Castilho RF. 3-nitropropionic acid-induced mitochondrial permeability transition: comparative study of

mitochondria from different tissues and brain regions. **J Neurosci Res**, 88(3): 630-9, 2010.

Misra HP; Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. **J Biol Chem**, 247(10): 3170-5, 1972.

Mitchell IJ; Cooper AJ; Griffiths MR. The selective vulnerability of striatopallidal neurons. **Prog Neurobiol**, 59(6): 691-719, 1999.

Mochel F; Haller RG. Energy deficit in Huntington disease: why it matters. **J Clin Invest**, 121(2): 493-9, 2011.

Moncada S; Bolanos JP. Nitric oxide, cell bioenergetics and neurodegeneration. **J Neurochem**, 97(6): 1676-89, 2006.

Montilla P; Espejo I; Munoz MC; Bujalance I; Munoz-Castaneda JR; Tunez I. Effect of red wine on oxidative stress and hypercholesterolemia induced by feeding a high-cholesterol diet in rat. **J Physiol Biochem**, 60(4): 259-64, 2004.

Moreira EL; Rial D; Duarte FS; de Carvalho CR; Horst H; Pizzolatti MG; Prediger RD; Ribeiro-do-Valle RM. Central nervous system activity of the proanthocyanidin-rich fraction obtained from Croton celtidifolius in rats. **J Pharm Pharmacol**, 62(8): 1061-8, 2010.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. **J Immunol Methods**, 65(1-2): 55-63, 1983.

Muldrew KM; Franks AM. Succinobucol: review of the metabolic, antiplatelet and cardiovascular effects. **Expert Opin Investig Drugs**, 18(4): 531-9, 2009.

Musser DA; Oseroff AR. The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II. **Photochem Photobiol**, 59(6): 621-6, 1994.

Napolitano M; Zei D; Centonze D; Palermo R; Bernardi G; Vacca A; Calabresi P; Gulino A. NF-kB/NOS cross-talk induced by mitochondrial

complex II inhibition: implications for Huntington's disease. **Neurosci Lett**, 434(3): 241-6, 2008.

Nicholls DG. Mitochondrial calcium function and dysfunction in the central nervous system. **Biochim Biophys Acta**, 1787(11): 1416-24, 2009.

Noack H; Lindenau J; Rothe F; Asayama K; Wolf G. Differential expression of superoxide dismutase isoforms in neuronal and glial compartments in the course of excitotoxically mediated neurodegeneration: relation to oxidative and nitrergic stress. **Glia**, 23(4): 285-97, 1998.

Novelli A; Reilly JA; Lysko PG; Henneberry RC. Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. **Brain Res**, 451(1-2): 205-12, 1988.

Ohkawa H; Ohishi N; Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Anal Biochem**, 95(2): 351-8, 1979.

Oliveira JM. Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum. **J Neurochem**, 114(1): 1-12, 2010.

Oliveira JM; Chen S; Almeida S; Riley R; Goncalves J; Oliveira CR; Hayden MR; Nicholls DG; Ellerby LM; Rego AC. Mitochondrial-dependent Ca2+ handling in Huntington's disease striatal cells: effect of histone deacetylase inhibitors. **J Neurosci**, 26(43): 11174-86, 2006.

Olney JW. New mechanisms of excitatory transmitter neurotoxicity. **J Neural Transm Suppl**, 43(47-51, 1994.

Orr AL; Li S; Wang CE; Li H; Wang J; Rong J; Xu X; Mastroberardino PG; Greenamyre JT; Li XJ. N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. **J Neurosci**, 28(11): 2783-92, 2008.

Ouary S; Bizat N; Altairac S; Menetrat H; Mittoux V; Conde F; Hantraye P; Brouillet E. Major strain differences in response to chronic systemic administration of the mitochondrial toxin 3-nitropropionic acid

in rats: implications for neuroprotection studies. **Neuroscience**, 97(3): 521-30, 2000.

Packer L; Tritschler HJ; Wessel K. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. **Free Radic Biol Med**, 22(1-2): 359-78, 1997.

Pallebage-Gamarallage MM; Takechi R; Lam V; Galloway S; Dhaliwal S; Mamo JC. Post-prandial lipid metabolism, lipid-modulating agents and cerebrovascular integrity: implications for dementia risk. **Atheroscler Suppl**, 11(1): 49-54, 2010.

Pang Z; Geddes JW. Mechanisms of cell death induced by the mitochondrial toxin 3-nitropropionic acid: acute excitotoxic necrosis and delayed apoptosis. **J Neurosci**, 17(9): 3064-73, 1997.

Panov A; Obertone T; Bennett-Desmelik J; Greenamyre JT. Ca(2+)-dependent permeability transition and complex I activity in lymphoblast mitochondria from normal individuals and patients with Huntington's or Alzheimer's disease. **Ann N Y Acad Sci**, 893(365-8), 1999.

Panov AV; Gutekunst CA; Leavitt BR; Hayden MR; Burke JR; Strittmatter WJ; Greenamyre JT. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. **Nat Neurosci**, 5(8): 731-6, 2002.

Park SY; Lee JH; Kim CD; Rhim BY; Hong KW; Lee WS. Beneficial synergistic effects of concurrent treatment with cilostazol and probucol against focal cerebral ischemic injury in rats. **Brain Res**, 1157(112-20), 2007.

Parker JA; Connolly JB; Wellington C; Hayden M; Dausset J; Neri C. Expanded polyglutamines in Caenorhabditis elegans cause axonal abnormalities and severe dysfunction of PLM mechanosensory neurons without cell death. **Proc Natl Acad Sci U S A**, 98(23): 13318-23, 2001.

Pearson SJ; Reynolds GP. Increased brain concentrations of a neurotoxin, 3-hydroxykynurenine, in Huntington's disease. **Neurosci Lett**, 144(1-2): 199-201, 1992.

Pei G; Ebendal T. Specific lesions in the extrapyramidal system of the rat brain induced by 3-nitropropionic acid (3-NPA). **Exp Neurol**, 132(1): 105-15, 1995.

Perez-De la Cruz V; Carrillo-Mora P; Santamaria A. Huntington's disease and mitochondrial alterations: emphasis on experimental models. **J Bioenerg Biomembr**, 42(3): 207-15, 2010.

Perez-De La Cruz V; Elinos-Calderon D; Robledo-Arratia Y; Medina-Campos ON; Pedraza-Chaverri J; Ali SF; Santamaria A. Targeting oxidative/nitrergic stress ameliorates motor impairment, and attenuates synaptic mitochondrial dysfunction and lipid peroxidation in two models of Huntington's disease. **Behav Brain Res**, 199(2): 210-7, 2009.

Perez-De La Cruz V; Gonzalez-Cortes C; Galvan-Arzate S; Medina-Campos ON; Perez-Severiano F; Ali SF; Pedraza-Chaverri J; Santamaria A. Excitotoxic brain damage involves early peroxynitrite formation in a model of Huntington's disease in rats: protective role of iron porphyrinate 5,10,15,20-tetrakis (4-sulfonatophenyl)porphyrinate iron (III). **Neuroscience**, 135(2): 463-74, 2005.

Perez-De La Cruz V; Gonzalez-Cortes C; Pedraza-Chaverri J; Maldonado PD; Andres-Martinez L; Santamaria A. Protective effect of S-allylcysteine on 3-nitropropionic acid-induced lipid peroxidation and mitochondrial dysfunction in rat brain synaptosomes. **Brain Res Bull**, 68(5): 379-83, 2006.

Perez-De La Cruz V; Konigsberg M; Pedraza-Chaverri J; Herrera-Mundo N; Diaz-Munoz M; Moran J; Fortoul-van der Goes T; Rondan-Zarate A; Maldonado PD; Ali SF; Santamaria A. Cytoplasmic calcium mediates oxidative damage in an excitotoxic /energetic deficit synergic model in rats. **Eur J Neurosci**, 27(5): 1075-85, 2008.

Perez-De La Cruz V; Santamaria A. Integrative hypothesis for Huntington's disease: a brief review of experimental evidence. **Physiol Res**, 56(5): 513-26, 2007.

Perez-Severiano F; Rodriguez-Perez M; Pedraza-Chaverri J; Maldonado PD; Medina-Campos ON; Ortiz-Plata A; Sanchez-Garcia A; Villeda-Hernandez J; Galvan-Arzate S; Aguilera P; Santamaria A. S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-

induced neurotoxicity and oxidative damage in rats. **Neurochem Int**, 45(8): 1175-83, 2004.

Perez-Severiano F; Santamaria A; Pedraza-Chaverri J; Medina-Campos ON; Rios C; Segovia J. Increased formation of reactive oxygen species, but no changes in glutathione peroxidase activity, in striata of mice transgenic for the Huntington's disease mutation. **Neurochem Res**, 29(4): 729-33, 2004.

Peyser CE; Folstein M; Chase GA; Starkstein S; Brandt J; Cockrell JR; Bylsma F; Coyle JT; McHugh PR; Folstein SE. Trial of d-alpha-tocopherol in Huntington's disease. **Am J Psychiatry**, 152(12): 1771-5, 1995.

Pfuetze KD; Dujovne CA. Probucol. **Curr Atheroscler Rep**, 2(1): 47-57, 2000.

Phillips W; Shannon KM; Barker RA. The current clinical management of Huntington's disease. **Mov Disord**, 23(11): 1491-504, 2008.

Poirier J. Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. **Trends Mol Med**, 9(3): 94-101, 2003.

Poirier J. Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. **Neurobiol Aging**, 26(3): 355-61, 2005.

Popoli P; Blum D; Martire A; Ledent C; Ceruti S; Abbracchio MP. Functions, dysfunctions and possible therapeutic relevance of adenosine A2A receptors in Huntington's disease. **Prog Neurobiol**, 81(5-6): 331-48, 2007.

Powers WJ; Videen TO; Markham J; McGee-Minnich L; Antenor-Dorsey JV; Hershey T; Perlmutter JS. Selective defect of in vivo glycolysis in early Huntington's disease striatum. **Proc Natl Acad Sci U S A**, 104(8): 2945-9, 2007.

Puigserver P; Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. **Endocr Rev**, 24(1): 78-90, 2003.

Ramaswamy S; McBride JL; Kordower JH. Animal models of Huntington's disease. I**LAR J**, 48(4): 356-73, 2007.

Reddy PH; Mao P; Manczak M. Mitochondrial structural and functional dynamics in Huntington's disease. **Brain Res Rev**, 61(1): 33-48, 2009.

Reiner A; Albin RL; Anderson KD; D'Amato CJ; Penney JB; Young AB. Differential loss of striatal projection neurons in Huntington disease. **Proc Natl Acad Sci U S A**, 85(15): 5733-7, 1988.

Reiter RJ; Cabrera J; Sainz RM; Mayo JC; Manchester LC; Tan DX. Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and parkinsonism. **Ann N Y Acad Sci**, 890(471-85, 1999.

Reynolds NC, Jr.; Prost RW; Mark LP. Heterogeneity in 1H-MRS profiles of presymptomatic and early manifest Huntington's disease. **Brain Res**, 1031(1): 82-9, 2005.

Ribeiro CA; Grando V; Dutra Filho CS; Wannmacher CM; Wajner M. Evidence that quinolinic acid severely impairs energy metabolism through activation of NMDA receptors in striatum from developing rats. **J Neurochem**, 99(6): 1531-42, 2006.

Ribeiro FM; Paquet M; Cregan SP; Ferguson SS. Group I metabotropic glutamate receptor signalling and its implication in neurological disease. **CNS Neurol Disord Drug Targets**, 9(5): 574-95, 2010.

Ribeiro FM; Pires RG; Ferguson SS. Huntington's disease and Group I metabotropic glutamate receptors. **Mol Neurobiol**, 43(1): 1-11, 2011.

Roos RA. Huntington's disease: a clinical review. **Orphanet J Rare Dis**, 5(1): 40, 2010.

Rosenstock TR; Carvalho AC; Jurkiewicz A; Frussa-Filho R; Smaili SS. Mitochondrial calcium, oxidative stress and apoptosis in a neurodegenerative disease model induced by 3-nitropropionic acid. **J Neurochem**, 88(5): 1220-8, 2004.

Ross CA; Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. **Lancet Neurol**, 10(1): 83-98, 2011.

Rossignol J; Boyer C; Leveque X; Fink KD; Thinard R; Blanchard F; Dunbar GL; Lescaudron L. Mesenchymal stem cell transplantation and DMEM administration in a 3NP rat model of Huntington's disease: morphological and behavioral outcomes. **Behav Brain Res**, 217(2): 369-78, 2011.

Roze E; Saudou F; Caboche J. Pathophysiology of Huntington's disease: from huntingtin functions to potential treatments. **Curr Opin Neurol**, 21(4): 497-503, 2008.

Ruddick JP; Evans AK; Nutt DJ; Lightman SL; Rook GA; Lowry CA. Tryptophan metabolism in the central nervous system: medical implications. **Expert Rev Mol Med**, 8(20): 1-27, 2006.

Rustin P; Munnich A; Rotig A. Succinate dehydrogenase and human diseases: new insights into a well-known enzyme. **Eur J Hum Genet**, 10(5): 289-91, 2002.

Ryu JK; Kim SU; McLarnon JG. Blockade of quinolinic acid-induced neurotoxicity by pyruvate is associated with inhibition of glial activation in a model of Huntington's disease. **Exp Neurol**, 187(1): 150-9, 2004.

Saft C; Zange J; Andrich J; Muller K; Lindenberg K; Landwehrmeyer B; Vorgerd M; Kraus PH; Przuntek H; Schols L. Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease. **Mov Disord**, 20(6): 674-9, 2005.

Sanberg PR; Fibiger HC; Mark RF. Body weight and dietary factors in Huntington's disease patients compared with matched controls. **Med J Aust**, 1(8): 407-9, 1981.

Sanchez-Pernaute R; Kunig G; del Barrio Alba A; de Yebenes JG; Vontobel P; Leenders KL. Bradykinesia in early Huntington's disease. **Neurology**, 54(1): 119-25, 2000.

Sandhir R; Mehrotra A; Kamboj SS. Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system. **Neurochem Int**, 57(5): 579-87, 2010.

Santamaria A; Perez-Severiano F; Rodriguez-Martinez E; Maldonado PD; Pedraza-Chaverri J; Rios C; Segovia J. Comparative analysis of superoxide dismutase activity between acute pharmacological models and a transgenic mouse model of Huntington's disease. **Neurochem Res**, 26(4): 419-24, 2001.

Santamaria A; Rios C. MK-801, an N-methyl-D-aspartate receptor antagonist, blocks quinolinic acid-induced lipid peroxidation in rat corpus striatum. **Neurosci Lett**, 159(1-2): 51-4, 1993.

Santamaria A; Salvatierra-Sanchez R; Vazquez-Roman B; Santiago-Lopez D; Villeda-Hernandez J; Galvan-Arzate S; Jimenez-Capdeville ME; Ali SF. Protective effects of the antioxidant selenium on quinolinic acid-induced neurotoxicity in rats: in vitro and in vivo studies. **J Neurochem**, 86(2): 479-88, 2003.

Santos DB; Peres KC; Ribeiro RP; Colle D; Santos AA; Moreira EL; Souza DO; Figueiredo CP; Farina M. Probucol, a lipid-lowering drug, prevents cognitive and hippocampal synaptic impairments induced by amyloid beta peptide in mice. **Exp Neurol**, 233: 765-775, 2011.

Sato S; Gobbel GT; Honkaniemi J; Li Y; Kondo T; Murakami K; Sato M; Copin JC; Chan PH. Apoptosis in the striatum of rats following intraperitoneal injection of 3-nitropropionic acid. **Brain Res**, 745(1-2): 343-7, 1997.

Sawayama Y; Shimizu C; Maeda N; Tatsukawa M; Kinukawa N; Koyanagi S; Kashiwagi S; Hayashi J. Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. Fukuoka Atherosclerosis Trial (FAST). **J Am Coll Cardiol**, 39(4): 610-6, 2002.

Schuck PF; Tonin A; da Costa Ferreira G; Rosa RB; Latini A; Balestro F; Perry ML; Wannmacher CM; de Souza Wyse AT; Wajner M. In vitro effect of quinolinic acid on energy metabolism in brain of young rats. **Neurosci Res**, 57(2): 277-88, 2007.

Schwarcz R; Okuno E; White RJ; Bird ED; Whetsell WO, Jr. 3-Hydroxyanthranilate oxygenase activity is increased in the brains of Huntington disease victims. **Proc Natl Acad Sci U S A**, 85(11): 4079-81, 1988.

Seaman RL. Effects of acute systemic 3-nitropropionic acid administration on rat activity and acoustic startle. **Neurosci Lett**, 280(3): 183-6, 2000.

Seong IS; Ivanova E; Lee JM; Choo YS; Fossale E; Anderson M; Gusella JF; Laramie JM; Myers RH; Lesort M; MacDonald ME. HD CAG repeat implicates a dominant property of huntingtin in mitochondrial energy metabolism. **Hum Mol Genet**, 14(19): 2871-80, 2005.

Shear DA; Dong J; Haik-Creguer KL; Bazzett TJ; Albin RL; Dunbar GL. Chronic administration of quinolinic acid in the rat striatum causes spatial learning deficits in a radial arm water maze task. **Exp Neurol**, 150(2): 305-11, 1998.

Shear DA; Haik KL; Dunbar GL. Creatine reduces 3-nitropropionic-acid-induced cognitive and motor abnormalities in rats. **Neuroreport**, 11(9): 1833-7, 2000.

Shin JY; Fang ZH; Yu ZX; Wang CE; Li SH; Li XJ. Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. **J Cell Biol**, 171(6): 1001-12, 2005.

Shinomol GK; Muralidhara. Effect of Centella asiatica leaf powder on oxidative markers in brain regions of prepubertal mice in vivo and its in vitro efficacy to ameliorate 3-NPA-induced oxidative stress in mitochondria. **Phytomedicine**, 2008.

Sia YT; Parker TG; Liu P; Tsoporis JN; Adam A; Rouleau JL. Improved post-myocardial infarction survival with probucol in rats: effects on left ventricular function, morphology, cardiac oxidative stress and cytokine expression. **J Am Coll Cardiol**, 39(1): 148-56, 2002.

Siesling S; van Vugt JP; Zwinderman KA; Kieburtz K; Roos RA. Unified Huntington's disease rating scale: a follow up. **Mov Disord**, 13(6): 915-9, 1998.

Silva-Adaya D; Perez-De La Cruz V; Herrera-Mundo MN; Mendoza-Macedo K; Villeda-Hernandez J; Binienda Z; Ali SF; Santamaria A. Excitotoxic damage, disrupted energy metabolism, and oxidative stress

in the rat brain: antioxidant and neuroprotective effects of L-carnitine. **J Neurochem**, 105(3): 677-89, 2008.

Silvestroni A; Faull RL; Strand AD; Moller T. Distinct neuroinflammatory profile in post-mortem human Huntington's disease. **Neuroreport**, 20(12): 1098-103, 2009.

Simpson JR; Isacson O. Mitochondrial impairment reduces the threshold for in vivo NMDA-mediated neuronal death in the striatum. **Exp Neurol**, 121(1): 57-64, 1993.

Singla DK; Kaur K; Sharma AK; Dhingra S; Singal PK. Probucol promotes endogenous antioxidant reserve and confers protection against reperfusion injury. **Can J Physiol Pharmacol**, 85(3-4): 439-43, 2007.

Slater TF; Sawyer B; Straeuli U. Studies on Succinate-Tetrazolium Reductase Systems. Iii. Points of Coupling of Four Different Tetrazolium Salts. **Biochim Biophys Acta**, 77(383-93, 1963.

Sorolla MA; Reverter-Branchat G; Tamarit J; Ferrer I; Ros J; Cabiscol E. Proteomic and oxidative stress analysis in human brain samples of Huntington disease. **Free Radic Biol Med**, 45(5): 667-78, 2008.

Southwell AL; Patterson PH. Gene therapy in mouse models of huntington disease. **Neuroscientist**, 17(2): 153-62, 2011.

Squitieri F; Cannella M; Sgarbi G; Maglione V; Falleni A; Lenzi P; Baracca A; Cislaghi G; Saft C; Ragona G; Russo MA; Thompson LM; Solaini G; Fornai F. Severe ultrastructural mitochondrial changes in lymphoblasts homozygous for Huntington disease mutation. **Mech Ageing Dev**, 127(2): 217-20, 2006.

St-Pierre J; Drori S; Uldry M; Silvaggi JM; Rhee J; Jager S; Handschin C; Zheng K; Lin J; Yang W; Simon DK; Bachoo R; Spiegelman BM. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell, 127(2): 397-408, 2006.

Stack EC; Matson WR; Ferrante RJ. Evidence of oxidant damage in Huntington's disease: translational strategies using antioxidants. **Ann N Y Acad Sci**, 1147(79-92, 2008.

Stack EC; Smith KM; Ryu H; Cormier K; Chen M; Hagerty SW; Del Signore SJ; Cudkowicz ME; Friedlander RM; Ferrante RJ. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. **Biochim Biophys Acta**, 1762(3): 373-80, 2006.

Stahl WL; Swanson PD. Biochemical abnormalities in Huntington's chorea brains. **Neurology**, 24(9): 813-9, 1974.

Stone TW; Behan WM; MacDonald M; Darlington LG. Possible mediation of quinolinic acid-induced hippocampal damage by reactive oxygen species. **Amino Acids**, 19(1): 275-81, 2000.

Stone TW; Perkins MN. Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. **Eur J Pharmacol**, 72(4): 411-2, 1981.

Storey E; Beal MF. Neurochemical substrates of rigidity and chorea in Huntington's disease. **Brain**, 116 (Pt 5)(1201-22, 1993.

Strand AD; Aragaki AK; Shaw D; Bird T; Holton J; Turner C; Tapscott SJ; Tabrizi SJ; Schapira AH; Kooperberg C; Olson JM. Gene expression in Huntington's disease skeletal muscle: a potential biomarker. **Hum Mol Genet**, 14(13): 1863-76, 2005.

Sun B; Fan W; Balciunas A; Cooper JK; Bitan G; Steavenson S; Denis PE; Young Y; Adler B; Daugherty L; Manoukian R; Elliott G; Shen W; Talvenheimo J; Teplow DB; Haniu M; Haldankar R; Wypych J; Ross CA; Citron M; Richards WG. Polyglutamine repeat length-dependent proteolysis of huntingtin. **Neurobiol Dis**, 11(1): 111-22, 2002.

Sun Y; Savanenin A; Reddy PH; Liu YF. Polyglutamine-expanded huntingtin promotes sensitization of N-methyl-D-aspartate receptors via post-synaptic density 95. **J Biol Chem**, 276(27): 24713-8, 2001.

Sundell CL; Somers PK; Meng CQ; Hoong LK; Suen KL; Hill RR; Landers LK; Chapman A; Butteiger D; Jones M; Edwards D; Daugherty A; Wasserman MA; Alexander RW; Medford RM; Saxena U. AGI-1067: a multifunctional phenolic antioxidant, lipid modulator, anti-inflammatory and antiatherosclerotic agent. **J Pharmacol Exp Ther**, 305(3): 1116-23, 2003.

Tabrizi SJ; Blamire AM; Manners DN; Rajagopalan B; Styles P; Schapira AH; Warner TT. Creatine therapy for Huntington's disease: clinical and MRS findings in a 1-year pilot study. **Neurology**, 61(1): 141-2, 2003.

Tabrizi SJ; Cleeter MW; Xuereb J; Taanman JW; Cooper JM; Schapira AH. Biochemical abnormalities and excitotoxicity in Huntington's disease brain. **Ann Neurol**, 45(1): 25-32, 1999.

Takahashi S; Abe T; Gotoh J; Fukuuchi Y. Substrate-dependence of reduction of MTT: a tetrazolium dye differs in cultured astroglia and neurons. **Neurochem Int**, 40(5): 441-8, 2002.

Tang TS; Tu H; Chan EY; Maximov A; Wang Z; Wellington CL; Hayden MR; Bezprozvanny I. Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. **Neuron**, 39(2): 227-39, 2003.

Tanous D; Brasen JH; Choy K; Wu BJ; Kathir K; Lau A; Celermajer DS; Stocker R. Probucol inhibits in-stent thrombosis and neointimal hyperplasia by promoting re-endothelialization. **Atherosclerosis**, 189(2): 342-9, 2006.

Tanous D; Hime N; Stocker R. Anti-atherosclerotic and anti-diabetic properties of probucol and related compounds. **Redox Rep**, 13(2): 48-59, 2008.

Tardif JC; Gregoire J; L'Allier PL. Prevention of restenosis with antioxidants: mechanisms and implications. **Am J Cardiovasc Drugs**, 2(5): 323-34, 2002.

Tardif JC; Gregoire J; L'Allier PL; Ibrahim R; Anderson TJ; Reeves F; Title LM; Schampaert E; LeMay M; Lesperance J; Scott R; Guertin MC; Brennan ML; Hazen SL; Bertrand OF. Effects of the antioxidant succinobucol (AGI-1067) on human atherosclerosis in a randomized clinical trial. **Atherosclerosis**, 197(1): 480-6, 2008.

Tardif JC; Gregoire J; Schwartz L; Title L; Laramee L; Reeves F; Lesperance J; Bourassa MG; L'Allier PL; Glass M; Lambert J; Guertin MC. Effects of AGI-1067 and probucol after percutaneous coronary interventions. **Circulation**, 107(4): 552-8, 2003.

Tardif JC; McMurray JJ; Klug E; Small R; Schumi J; Choi J; Cooper J; Scott R; Lewis EF; L'Allier PL; Pfeffer MA. Effects of succinobucol (AGI-1067) after an acute coronary syndrome: a randomised, double-blind, placebo-controlled trial. **Lancet**, 371(9626): 1761-8, 2008.

Tariq M; Khan HA; Elfaki I; Al Deeb S; Al Moutaery K. Neuroprotective effect of nicotine against 3-nitropropionic acid (3-NP)-induced experimental Huntington's disease in rats. **Brain Res Bull**, 67(1-2): 161-8, 2005.

Tasset I; Aguera E; Olmo-Camacho R; Escribano B; Sanchez-Lopez F; Delgado MJ; Cruz AH; Gascon F; Luque E; Pena J; Jimena IM; Tunez I. Melatonin improves 3-nitropropionic acid induced behavioral alterations and neurotrophic factors levels. **Prog Neuropsychopharmacol Biol Psychiatry**, 35(8): 1944-9, 2011.

Tasset I; Perez-De La Cruz V; Elinos-Calderon D; Carrillo-Mora P; Gonzalez-Herrera IG; Luna-Lopez A; Konigsberg M; Pedraza-Chaverri J; Maldonado PD; Ali SF; Tunez I; Santamaria A. Protective effect of tert-butylhydroquinone on the quinolinic-acid-induced toxicity in rat striatal slices: role of the Nrf2-antioxidant response element pathway. **Neurosignals**, 18(1): 24-31, 2010.

Tavares RG; Schmidt AP; Abud J; Tasca CI; Souza DO. In vivo quinolinic acid increases synaptosomal glutamate release in rats: reversal by guanosine. **Neurochem Res**, 30(4): 439-44, 2005.

Tavares RG; Tasca CI; Santos CE; Alves LB; Porciuncula LO; Emanuelli T; Souza DO. Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. **Neurochem Int**, 40(7): 621-7, 2002.

Thompson PD; Berardelli A; Rothwell JC; Day BL; Dick JP; Benecke R; Marsden CD. The coexistence of bradykinesia and chorea in Huntington's disease and its implications for theories of basal ganglia control of movement. **Brain**, 111 (Pt 2)(223-44, 1988.

Trushina E; Dyer RB; Badger JD, 2nd; Ure D; Eide L; Tran DD; Vrieze BT; Legendre-Guillemin V; McPherson PS; Mandavilli BS; Van Houten B; Zeitlin S; McNiven M; Aebersold R; Hayden M; Parisi JE; Seeberg

E; Dragatsis I; Doyle K; Bender A; Chacko C; McMurray CT. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. **Mol Cell Biol**, 24(18): 8195-209, 2004.

Tunez I; Drucker-Colin R; Jimena I; Medina FJ; Munoz Mdel C; Pena J; Montilla P. Transcranial magnetic stimulation attenuates cell loss and oxidative damage in the striatum induced in the 3-nitropropionic model of Huntington's disease. **J Neurochem**, 97(3): 619-30, 2006.

Tunez I; Montilla P; Del Carmen Munoz M; Feijoo M; Salcedo M. Protective effect of melatonin on 3-nitropropionic acid-induced oxidative stress in synaptosomes in an animal model of Huntington's disease. **J Pineal Res**, 37(4): 252-6, 2004.

Tunez I; Tasset I; Perez-De La Cruz V; Santamaria A. 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. **Molecules**, 15(2): 878-916, 2010.

Uehara Y; Shimizu H; Sato N; Shimomura Y; Mori M; Kobayashi I. Probucol partially prevents development of diabetes in NOD mice. **Diabetes Res**, 17(3): 131-4, 1991.

Uz T; Giusti P; Franceschini D; Kharlamov A; Manev H. Protective effect of melatonin against hippocampal DNA damage induced by intraperitoneal administration of kainate to rats. **Neuroscience**, 73(3): 631-6, 1996.

van der Burg JM; Bjorkqvist M; Brundin P. Beyond the brain: widespread pathology in Huntington's disease. **Lancet Neurol**, 8(8): 765-74, 2009.

van Duijn E; Kingma EM; van der Mast RC. Psychopathology in verified Huntington's disease gene carriers. **J Neuropsychiatry Clin Neurosci**, 19(4): 441-8, 2007.

Vazey EM; Chen K; Hughes SM; Connor B. Transplanted adult neural progenitor cells survive, differentiate and reduce motor function impairment in a rodent model of Huntington's disease. **Exp Neurol**, 199(2): 384-96, 2006.

Vezzani A; Sangalli L; Wu HQ; Schwarcz R. ATP as a marker of excitotoxin-induced nerve cell death in vivo. **J Neural Transm**, 70(3-4): 349-56, 1987.

Vonsattel JP. Huntington disease models and human neuropathology: similarities and differences. **Acta Neuropathol**, 115(1): 55-69, 2008.

Vonsattel JP; DiFiglia M. Huntington disease. **J Neuropathol Exp Neurol**, 57(5): 369-84, 1998.

Vonsattel JP; Myers RH; Stevens TJ; Ferrante RJ; Bird ED; Richardson EP, Jr. Neuropathological classification of Huntington's disease. **J Neuropathol Exp Neurol**, 44(6): 559-77, 1985.

Wendel A. Glutathione peroxidase. **Methods Enzymol**, 77(325-33), 1981.

Weydt P; Pineda VV; Torrence AE; Libby RT; Satterfield TF; Lazarowski ER; Gilbert ML; Morton GJ; Bammler TK; Strand AD; Cui L; Beyer RP; Easley CN; Smith AC; Krainc D; Luquet S; Sweet IR; Schwartz MW; La Spada AR. Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. **Cell Metab**, 4(5): 349-62, 2006.

Wheelock VL; Tempkin T; Marder K; Nance M; Myers RH; Zhao H; Kayson E; Orme C; Shoulson I. Predictors of nursing home placement in Huntington disease. **Neurology**, 60(6): 998-1001, 2003.

Yamamoto A. A uniqe antilipidemic drug--probucol. **J Atheroscler Thromb**, 15(6): 304-5, 2008.

Yamashita S; Hbujo H; Arai H; Harada-Shiba M; Matsui S; Fukushima M; Saito Y; Kita T; Matsuzawa Y. Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. **J Atheroscler Thromb**, 15(6): 292-303, 2008.

Yamashita S; Matsuzawa Y. Where are we with probucol: a new life for an old drug? **Atherosclerosis**, 207(1): 16-23, 2009.

Yang L; Calingasan NY; Wille EJ; Cormier K; Smith K; Ferrante RJ; Beal MF. Combination therapy with coenzyme Q10 and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's diseases. **J Neurochem**, 109(5): 1427-39, 2009.

Yang L; Sugama S; Mischak RP; Kiaei M; Bizat N; Brouillet E; Joh TH; Beal MF. A novel systemically active caspase inhibitor attenuates the toxicities of MPTP, malonate, and 3NP in vivo. **Neurobiol Dis**, 17(2): 250-9, 2004.

Young AB; Greenamyre JT; Hollingsworth Z; Albin R; D'Amato C; Shoulson I; Penney JB. NMDA receptor losses in putamen from patients with Huntington's disease. **Science**, 241(4868): 981-3, 1988.

Yu ZX; Li SH; Evans J; Pillarisetti A; Li H; Li XJ. Mutant huntingtin causes context-dependent neurodegeneration in mice with Huntington's disease. **J Neurosci**, 23(6): 2193-202, 2003.

Zuccato C; Valenza M; Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. **Physiol Rev**, 90(3): 905-81, 2010.

Zucker B; Luthi-Carter R; Kama JA; Dunah AW; Stern EA; Fox JH; Standaert DG; Young AB; Augood SJ. Transcriptional dysregulation in striatal projection- and interneurons in a mouse model of Huntington's disease: neuronal selectivity and potential neuroprotective role of HAP1. **Hum Mol Genet**, 14(2): 179-89, 2005.