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EFEITO DA ADMINISTRAÇÃO INTRAESTRIATAL AGUDA DE ÁCIDO QUINOLÍNICO SOBRE METABOLISMO ENERGÉTICO EM RATOS JOVENS

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“Ser imperador sobre si mesmo é a primeira condição para imperar sobre os demais.”

José Ortega y Gasset

Àquela que logo estará conosco

e que me transformará de filho em pai.

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Obrigado!

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PARTE I

RESUMO

O ácido quinolínico (AQ) é um metabólito do metabolismo do triptofano produzido na via das quinureninas. Demonstrou-se ser um fraco agonista de receptores NMDA, porém capaz de produzir morte em neurônios susceptíveis via ativação à sua ação excitatória. O envolvimento do AQ tem sido sugerido como um mecanismo patológico em diversas doenças neurodegenerativas, incluindo-se as doenças de Huntington, Alzheimer e no complexo de demência induzida pela síndrome da imunodeficiência adquirida. As propriedades neurotóxicas do AQ têm sido atribuídas principalmente à excitotoxicidade e ao estresse oxidativo, enquanto muito pouco se sabe sobre seus efeitos sobre o metabolismo energético cerebral. Assim, no presente estudo investigamos o efeito da administração intraestriatal de AQ (150 nmol) em ratos de 30 dias de vida sobre as atividades de enzimas fundamentais do metabolismo energético, tais como os complexos I-IV da cadeia respiratória, creatina quinase e citrato sintase, além da produção de $^{14}CO_2$ a partir de [1-^{14}C]acetato 3, 6 ou 12 horas após a injeção de AQ. Observamos que a injeção de AQ inibiu significativamente a atividade dos complexos II (50%)**,** III (46%) e II-III (35%), bem como da enzima creatina quinase (27%), mas não alterou as atividades dos complexos I e IV em estriados preparados 12 horas após a injeção de AQ. Além disso, não observamos quaisquer alterações nessas atividades enzimáticas 3 ou 6 horas após o tratamento com AQ. A produção de $^{14}CO_2$ a partir de [1-^{14}C]acetato também foi inibida significativamente (27 %) pelo AQ somente em estriados preparados 12 horas após a injeção. As atividades dos complexos da cadeia respiratória e da creatina quinase foram também medidas em homogeneizados de estriado expostos a 100 μM de AQ. Não foram observadas alterações nessas atividades na presença de AQ. Essas observações *in vitro* juntamente com os achados *ex vivo* sugerem que a ação do AQ comprometendo o metabolismo energético em estriado de ratos é provavelmente indireta. Novos experimentos foram realizados para elucidar os mecanismos envolvidos nas inibições das atividades das enzimas do metabolismo energético induzidas pelo AQ. O pré-tratamento dos ratos com MK-801, um antagonista de receptores NMDA, ou com creatina preveniu completamente os efeitos inibitórios induzidos pelo AQ. Além disso, os seqüestradores de radicais livres α-tocoferol mais ascorbato e o inibidor da óxido nítrico sintase L-NAME preveniram completamente as inibições provocadas pelo AQ nas atividades do complexo III e da creatina quinase, indicando que esses efeitos inibitórios são provavelmente devido à geração de espécies reativas. Por outro lado, o pré-tratamento com piruvato bloqueou completamente os efeitos inibitórios causados pela injeção de AQ na atividade do complexo II e parcialmente a inibição da atividade da creatina quinase indicando que esses efeitos foram provavelmente devido à formação de espécies reativas Tomadas em conjunto, esses resultados indicam fortemente que a fosforilação oxidativa e a transferência energética celular estão comprometidas por altas concentrações de AQ no estriado de ratos jovens e que os efeitos inibitórios em enzimas chave do metabolismo energético causados pela injeção de AQ são provavelmente devido à ativação de receptores NMDA.

ABSTRACT

Quinolinic acid (QA), an endogenous metabolite produced in kynurenine pathway of tryptophan metabolism, is a weak agonist at the NMDA receptor, and selectively kills susceptible neurons via activation of the NMDA receptors The neurotoxic capacity of QA *in vivo* has implicated it in a variety of neurodegenerative disorders, including Huntington's disease, Alzheimer's disease and AIDS dementia complex. Most of QA toxic properties have been attributed to excitotoxicity and oxidative stress, whereas very little is known about its effects on brain energy metabolism. Therefore, in the present study we investigated the *ex vivo* effect of intrastriatal administration of 150 nmol QA to 30-day-old rats on critical enzyme activities of energy metabolism, including the respiratory chain complexes I-IV, creatine kinase and citrate synthase, as well as on $^{14}CO_2$ production from *[1-^{14}C]acetate* at distinct periods after QA injection. We observed that QA injection significantly inhibited complexes II (50%)**,** III (46%) and II-III (35%), as well as creatine kinase (27 %), but not complexes I and IV activities in striatum prepared 12 hours after QA treatment. In contrast, no alterations of these enzyme activities were observed 3 or 6 hours after QA treatment. $^{14}CO_2$ production from [1-^{14}C]acetate was also significantly inhibited (27 %) by QA only in rat striatum prepared 12 hours after injection. The respiratory chain complexes and creatine kinase activities were also measured in striatum homogenates exposed to 100 μM QA. No alterations of these activities were observed in the presence of QA. These *in vitro* observations allied to the *ex vivo* findings suggest that QA compromises energy metabolism in the rat striatum indirectly rather than due to a direct action of QA on the enzymes. New experiments were therefore designed to elucidate the involved mechanisms of QA-induced inhibition on the enzymatic activities of energy metabolism. Pretreatment with the NMDA receptor antagonist MK-801 and with creatine totally prevented the inhibitory effects elicited by QA. In addition, the free-radical scavengers α-tocopherol plus ascorbate and the nitric oxide synthase inhibitor L-NAME completely abolished the inhibitions provoked by QA on CK and complex III, indicating that these effects were probably due to generation of reactive species. On the other hand, pyruvate pretreatment totally blocked the inhibitory effects of QA injection on complex II activity and partially prevented QA-induced CK inhibition. Taken together, these observations strongly indicate that oxidative phosphorylation and cellular energy transfer are compromised by high concentrations of QA in the striatum of young rats and that the inhibitory effects caused by QA injection on critical steps of energy metabolism were probably mediated by NMDA stimulation.

LISTA DE ABREVIATURAS

- AQ, ácido quinolínico
- ADP, difosfato de adenosina
- ATP, trifosfato de adenosina
- CK, creatina quinase
- CoQ, coenzima Q
- CTE, cadeia de transporte de elétrons
- DH, doença de Huntington
- DNA, ácido desoxirribonucléico
- $FADH_2$, flavina adenina dinucleotídeo, forma reduzida
- GABA, ácido γ-aminobutírico
- GSH, glutationa reduzida
- L-NAME, éster metílico da N^{ω}-nitro-L-arginina
- MK-801, maleato de dizocilpina
- NAD^+, nicotinamida adenina dinucleotídeo, forma oxidada
- NADP, nicotinamida adenina dinucleotídeo fosfato
- NMDA, N-metil-D-aspartato
- NO, óxido nítrico
- PCr, fosfocreatina
- SIDA, síndrome da imunodeficiência adquirida
- SNC, sistema nervoso central

1- INTRODUÇÃO

1.1- Metabolismo do Triptofano – Via das Quinureninas

O triptofano é um aminoácido essencial, sendo usado por todas as formas de vida. Em seres humanos, aproximadamente 30% do triptofano da dieta é incorporado em proteínas. Além disso, é substrato para a produção de diversas moléculas com atividade biológica (ALLEGRI *et al*. 2003), conforme esquematizado na Figura 1. No sistema nervoso central e no intestino, o triptofano é substrato para síntese de serotonina, enquanto na glândula pineal é utilizado para síntese de melatonina. Em situações em que o conteúdo de niacina na dieta é insuficiente para as necessidades metabólicas, o triptofano é utilizado na síntese do cofator essencial nicotinamida adenina dinucleotídeo (NAD^+) (MOFFETT *et al*. 2003). O triptofano também é intermediário na via das quinureninas. Nesse particular, a via das quinureninas é a principal rota de catabolismo do triptofano, resultando na produção de NAD^+ e outros intermediários neuroativos. Entre elas, destacam-se a quinurenina, o ácido quinurênico, a 3-hidroxiquinurenina e os ácidos picolínico e quinolínico (Figura 2).

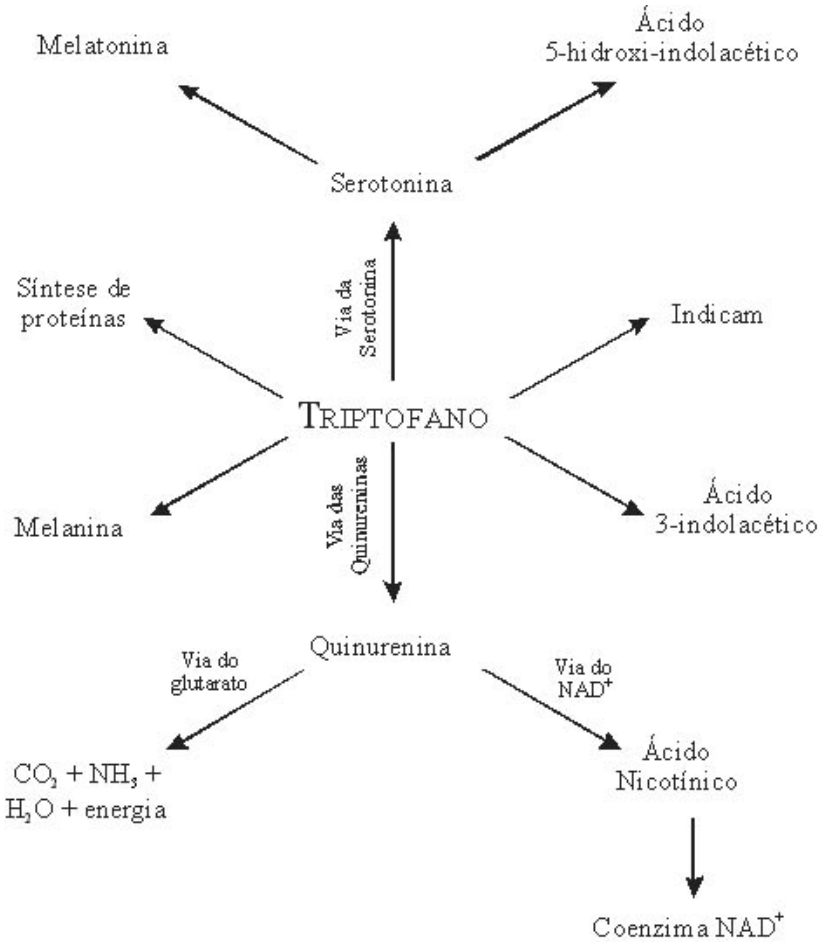

Figura 1. Principais vias metabólicas do triptofano.

No cérebro, a rota metabólica das quinureninas leva à formação de ácido quinolínico e ocorre principalmente nas células da microglia (células semelhantes a macrófagos residentes no cérebro) (HEYES *et al*. 1996). Em astrócitos, não há a expressão da enzima quinurenina 3-hidroxilase, o que direciona a L-quinurenina formada a partir de triptofano para a formação de ácido quinurênico. O ácido quinurênico produzido pelos astrócitos pode ser captado pelas células da microglia para seguir a via das quinureninas e produzir ácido quinolínico (GUILLEMIN *et al*. 2001a).

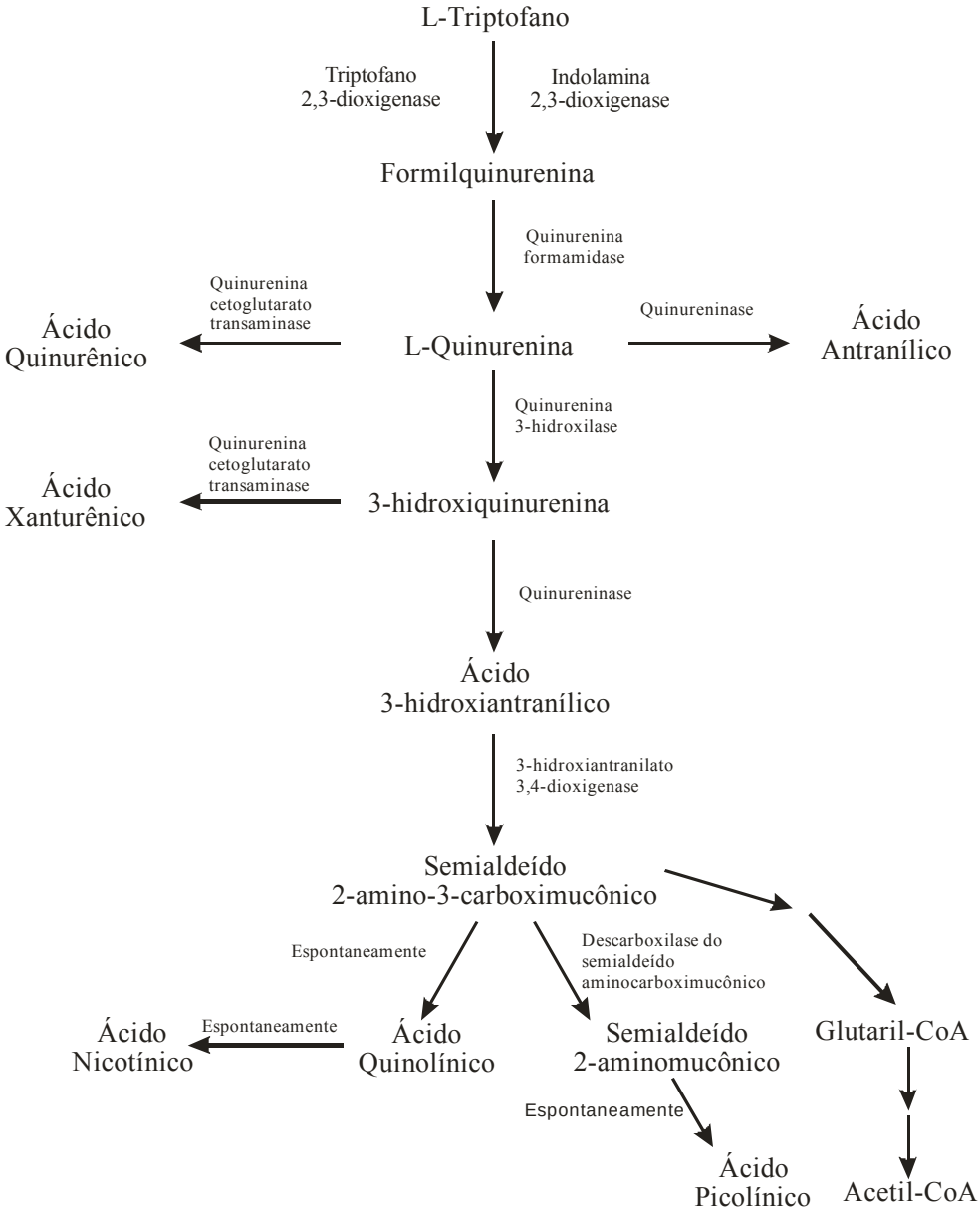

Figura 2. Metabolismo do triptofano: via das quinureninas. A conversão de triptofano em quinurenina se dá no fígado por ação da enzima triptofano 2,3-dioxigenase e nos outros tecidos por ação da enzima indolamina 2,3-dioxigenase.

Nos últimos anos a via das quinureninas tem despertado considerável interesse, visto que diversos dos seus metabólitos apresentam atividades biológicas (ALLEGRI *et al*. 2003), interferindo no comportamento, no sono, na termo-regulação e na gestação (STONE, 1993; CURZON, 1996). Essa rota está bastante relacionada à resposta inflamatória, sendo que seus intermediários são capazes de induzir a expressão de várias

citocinas, quimiocinas e seus receptores em astrócitos (GUILLEMIN *et al*. 2000, 2001a), promovendo a inflamação. Além disso, encontram-se aumentados em diversas condições neuropatológicas (STONE, 2001a).

Dentre os intermediários da via das quinureninas, de fundamental importância é o ácido quinolínico (AQ). Seu potencial fisiológico e significância farmacológica foram reconhecidos com a descoberta da sua capacidade de ativação seletiva de receptores glutamatérgicos excitatórios sensíveis ao N-metil-D-aspartato (NMDA) (STONE, 2001a).

Também foi sugerido recentemente que o acúmulo de intermediários da rota das quinureninas estaria envolvido no processo patogênico presente na acidemia glutárica tipo I (HEYES, 1987; VARADKAR E SURTEES, 2004).

1.2- Ácido Quinolínico e Doenças Neurodegenerativas

O envolvimento do AQ em diversas desordens neurodegenerativas, tais como doenças de Alzheimer (WIDNER *et al*. 2000), Huntington (SCHWARCZ *et al*. 1984; WHETSELL & SCHWARCZ, 1989; STONE, 2001b) e demência associada à síndrome da imunodeficiência adquirida (SIDA) tem sido sugerido (HEYES *et al*. 1989, 1991)

A concentração de AQ no cérebro de pacientes afetados por essas doenças está elevado. Embora essas concentrações raramente excedam 1 μM, esses níveis podem ser suficientes para causar dano neuronal significante por ativação de receptores NMDA ou através da liberação de glutamato endógeno (CONNICK & STONE, 1986, 1988). Nesse particular, a excitotoxicidade produzida pela constante ativação de receptores NMDA causada pelo AQ está relacionada com o aumento das concentrações citosólicas de cálcio, depleção de ATP e GABA e morte específica de neurônios colinérgicos e GABAérgicos (FOSTER *et al*. 1983, SCHWARCZ *et al*. 1984, STONE, 1993).

Fortes evidências indicam que a ativação de receptores NMDA é crítica para a produção do dano cerebral na demência associada à SIDA (LIPTON, 1998). Nesses pacientes os níveis de AQ no líquido cefalorraquidiano estão aumentadas em até 20 vezes, sendo correlacionados com a severidade das disfunções motora e cognitiva apresentadas pelos mesmos (HEYES *et al*. 1991, MARTIN *et al*. 1992, STONE, 2001a). Os níveis de AQ no cérebro encontram-se aumentados em até 60 vezes em pacientes com várias formas de disfunção neurológica associada com inflamação quando comparados com outros pacientes em que o dano cerebral não envolve inflamação (HEYES *et al*. 1992).

Recentemente, foi demonstrado que o peptídeo β-amilóide, cujo acúmulo na forma de placas (conhecidas como placas senis) está associado à doença de Alzheimer, induz a produção de ácido quinolínico por macrófagos e, principalmente, pela microglia (GUILLEMIN *et al*. 2001b). Diminuição nos níveis de ácido quinurênico e elevações nas concentrações de triptofano e seu metabólito L-quinurenina foram encontradas nessa doença, sendo o aumento desses níveis proporcional ao grau do déficit cognitivo (BARAN *et al*. 1999; WIDNER *et al*. 1999, 2000, HEYES *et al*. 1992).

Além disso, diversos estudos demonstraram recentemente que a formação de espécies reativas e a redução das defesas antioxidantes cerebrais também estão envolvidas nas ações neurotóxicas do AQ (RIOS & SANTAMARIA, 1991; BEHAN *et al*. 1999; SANTAMARIA *et al*. 2001a,b, LEIPNITZ *et al*. 2005).

Por outro lado, o AQ tem sido usado como modelo animal de indução química da doença de Huntington (DH), visto que as alterações neuroquímicas e degeneração neuronal induzidas pela infusão de AQ são similares àquelas observadas no cérebro de pacientes com essa doença (SCHWARCZ *et al*. 1983, 1984; BEAL *et al*. 1986; FERRANTE *et al*. 1993).

A DH é uma desordem neurodegenerativa autossômica dominante, caracterizada por depleção neuronal progressiva, particularmente dos gânglios da base, cujos

mecanismos envolvidos ainda não estão completamente elucidados. Entretanto, evidências recentes têm relacionado excitotoxicidade, estresse oxidativo e alterações no metabolismo energético em cérebro de pacientes com DH (BRENNAN *et al*. 1985; MANN *et al*. 1990; GU *et al*. 1996, BEAL, 2000). Outras evidências mostram que a anormalidade mitocondrial no cérebro de pacientes com DH é confinada ao estriado (BROWNE *et al*. 1997) e similar àquela induzida por malonato ou ácido 3-nitropropiônico (BEAL *et al*. 1993a; LUDOLPH *et al*. 1992).

1.3- Metabolismo Energético Cerebral

O cérebro possui uma intensa atividade metabólica, porém suas reservas energéticas são extremamente pequenas em relação à sua demanda. Assim, há necessidade contínua de substratos energéticos para o cérebro de mamíferos, sendo a glicose o principal deles, onde, em contraste com outros tecidos, não necessita de insulina para ser captada e oxidada (DICKINSON, 1996). No entanto, o padrão de utilização deste nutriente varia conforme a etapa de desenvolvimento do sistema nervoso central (SNC), o estado nutricional do indivíduo e o destino de sua cadeia de átomos de carbono (MARKS *et al*. 1996). Situações de jejum prolongado fazem com que o SNC passe a utilizar corpos cetônicos para a obtenção de energia, a fim de poupar o organismo de um catabolismo protéico exacerbado resultante da necessidade da manutenção da glicemia via gliconeogênese (MARKS *et al*. 1996). A glicose captada pelo cérebro é, também, fonte de carbono para a síntese de diversas outras biomoléculas essenciais (por exemplo, neurotransmissores), o que reforça a idéia de que a utilização de glicose não está atrelada somente à produção de energia.

Mitocôndrias de mamíferos são organelas intracelulares ubíquas, responsáveis pela produção de ATP pelo metabolismo aeróbico, mas também desempenham outras funções

intracelulares além da produção de ATP, tendo um papel crítico no processo de apoptose e servindo como um tampão de cálcio. Tecidos com alta atividade aeróbica, tais como cérebro, músculos esquelético e cardíaco, apresentam altas concentrações de mitocôndrias (ORTH & SCHAPIRA, 2001).

O ciclo do ácido cítrico é a via comum de oxidação dos glicídios, aminoácidos e ácidos graxos. O metabolismo energético cerebral se mostra essencialmente aeróbico, sendo a glicose o principal substrato utilizado (CLARK *et al*. 1993), entrando no ciclo sob a forma de acetil-CoA, que é então oxidada completamente a CO_2. Quando não há hipóxia, a fosforilação oxidativa é dependente da concentração de ATP, ADP e fosfato inorgânico (Pi) e da razão mitocondrial de $NADH/NAD^+$, que é determinada pela atividade da cadeia transportadora de elétrons e pela transferência de elétrons provenientes de reações catalisadas por enzimas mitocondriais. A cadeia transportadora de elétrons oxida o NADH e o $FADH_2$ e bombeia prótons para o espaço intermembrana da mitocôndria, formando assim um gradiente de prótons que através da passagem pela ATP-sintase, produz ATP na fosforilação oxidativa (ERECINSKA & SILVER, 1994).

1.3.1- Fosforilação Oxidativa

A fosforilação oxidativa é o processo principal da produção de energia celular. Todos os passos oxidativos na degradação de carboidratos, gorduras e aminoácidos convergem a esse estágio final da respiração celular, em que a energia da oxidação, provida pelo fluxo de elétrons através das enzimas da cadeia respiratória mitocondrial, promove a síntese de ATP (NELSON & COX, 2004). A cadeia de transporte de elétrons (CTE) mitocondrial é composta por quatro complexos enzimáticos (complexos I-IV), recebe elétrons das coenzimas NADH e $FADH_2$, e os transfere através de uma série de reações de oxidação-redução até o oxigênio molecular e simultaneamente acopla essa

reação exoergônica à translocação de prótons através da membrana mitocondrial interna (WALLACE, 1999). O fluxo de prótons (gradiente eletroquímico de prótons) gerado durante o transporte de elétrons na CTE leva à formação de ATP a partir de ADP e Pi pelo complexo V (ATP sintase) (BABCOCK & WIKSTROM, 1992; WALLACE, 1999).

Os complexos da cadeia de transporte de elétrons são complexos protéicos: NADH desidrogenase (complexo I), sucinato: ubiquininona oxirredutase (complexo II), complexo citocromo b-c_1 (complexo III) e citocromo c oxidase (complexo IV), além de elementos móveis que se localizam entre os complexos. São eles a coenzima Q (CoQ), um componente não protéico lipossolúvel que carreia elétrons entre os complexos I e III, e o citocromo *c*, uma proteína localizada na face externa da membrana que transfere os elétrons do complexo III para o complexo IV (MARKS *et al*. 1996).

1.3.2- Creatina Quinase

A creatina quinase (CK) consiste de um grupo de isoenzimas com um papel central no metabolismo energético, principalmente para tecidos com alta demanda energética, como cérebro, músculo cardíaco e esquelético, onde funciona como um efetivo sistema de tampão para os níveis celulares de ATP. A CK catalisa a transfosforilação reversível entre ATP e creatina a ADP e fosfocreatina [$MgATP^-$ + creatina $\leftrightarrow$ $(fosfocreatina)^-$ + $MgADP^-$ + H^+], ajudando a manter os níveis dos substratos fosforilados. Sabe-se que durante a excitação nervosa e neuromuscular ocorre um aumento de dez vezes no *turnover* celular de ATP, e que durante essas mudanças rápidas, o sistema creatina/fosfocreatina é necessário tanto como um tampão energético quanto como um sistema de transporte entre os locais de produção e consumo de ATP pelas ATPases para evitar grandes flutuações nos níveis de ATP/ADP celulares nesses tecidos excitáveis (BESSMAN & CARPENTER, 1985; SCHNYDER *et al*. 1991; WALLIMANN *et al*. 1992).

As isoformas da CQ estão localizadas em sítios de demanda e produção energética. A isoforma citosólica (Ci-CQ) consiste de dímeros e é expressa de uma maneira tecido-específica, isto é, cérebro-específica (BB-CQ), músculo esquelético-específica (MM-CQ) e um heterodímero músculo cardíaco-específico (MB-CQ) (SCHNYDER *et al*. 1991; WALLIMANN *et al*. 1992; O'GORMAN *et al*. 1996; HAMMAN *et al*. 1995). As formas mitocondriais da CQ (Mi-CQ) são dispostas em octâmeros e são compostas da isoforma sarcomérica músculo-específica Mib-CQ e da forma ubíqua Mia-CQ, que é encontrada nas mitocôndrias do tecido cerebral (SAKS *et al*. 1985; SCHELEGEL *et al*. 1988; WALLIMANN *et al*. 1992; GROSS *et al*. 1996).

Devido à sua localização próxima a sítios onde se dá a geração de energia e transporte de íons através de membranas, o sistema CQ/fosfocreatina desempenha um papel fundamental na homeostase energética neuromuscular. Assim, é presumível que alterações na função da CQ levem ao desenvolvimento de vários estados patológicos envolvendo o cérebro, músculo esquelético e músculo cardíaco (HAMMAN *et al*. 1995; DAVID *et al*. 1998, AKSENOV *et al*. 1999; AKSENOV *et al*. 2000).

1.4- Metabolismo Energético nas Doenças Neurodegenerativas

Numerosas hipóteses têm sido propostas para explicar a fisiopatologia das doenças de Alzheimer, Huntington e Parkinson, sem, no entanto, se obter até o momento uma explicação satisfatória para o dano cerebral causado por essas doenças. No entanto, acredita-se que possíveis mecanismos envolvam deficiência no metabolismo energético, estresse oxidativo e neurotoxicidade mediada por receptores glutamatérgicos do tipo NMDA, ou, possivelmente, um somatório desses fatores (ROSE & HENNEBERRY, 1994). Uma das hipóteses é de que alterações na cadeia transportadora de elétrons seria o

evento etiológico primário na maioria dessas doenças (PARKER, 1989; PARKER *et al*. 1989, 1990, 1994; SWERDLOW *et al*. 1996, 1997).

O cérebro é altamente dependente de energia para seu funcionamento normal (ROSSEN *et al*. 1943) e a mitocôndria é a estrutura intracelular que mantém os suprimentos de energia para o cérebro. Uma alteração funcional nessa estrutura poderá levar, portanto, a alterações patológicas nos neurônios e astrócitos (BEAL *et al*. 1993b; BEAL, 1995; BOWLING & BEAL, 1995; DAVIS *et al*. 1995). Mutações no DNA mitocondrial e reações envolvendo geração de espécies reativas podem danificar a mitocôndria e diminuir a atividade dos complexos da cadeia respiratória. Um prejuízo no transporte de elétrons, além de causar um prejuízo na produção de ATP, leva a uma dispersão dos elétrons na forma de radicais livres potencialmente danosos à célula. Visto que os sistemas de defesa antioxidantes, como as enzimas superóxido dismutase, glutationa peroxidase, glutationa redutase e catalase, são relativamente deficientes no cérebro (MARKLUND *et al*. 1982; MARTILLA *et al*. 1988), um ciclo vicioso pode ocorrer, aumentando o dano oxidativo a neurônios e levando à morte neuronal característica de doenças neurodegenerativas. Um prejuízo na fosforilação oxidativa também reduz o bombeamento de prótons através da membrana mitocondrial, diminuindo o potencial da membrana e, conseqüentemente, levando à abertura do poro de transição da permeabilidade mitocondrial. Evidências sugerem que uma diminuição no potencial de membrana mitocondrial seja um evento primário na iniciação do processo apoptótico (WADIA *et al*. 1998).

Por outro lado, dano neuronal e estresse oxidativo podem levar a mudanças reativas em astrócitos situados nas proximidades, levando a um aumento na produção de citocinas pró-apoptóticas e pró-inflamatórias, bem como a uma alteração na homeostase de íons extracelulares e de aminoácidos neurotransmissores (MACCIONI *et al*. 2001). Além disso, mecanismos patogênicos das doenças neurodegenerativas podem estimular células da

microglia, levando à produção de substâncias potencialmente neurotóxicas (MACCIONI *et al*. 2001).

Numerosas evidências associam doenças neurodegenerativas a uma diminuição no metabolismo energético. Estudos demonstraram uma diminuição na atividade do complexo I da cadeia respiratória *postmortem* em cérebros de pacientes portadores de doença de Parkinson (GU *et al*. 1998; JANETZKY *et al*. 1994; SCHAPIRA *et al*. 1989, 1990a,b). Também há relatos de defeitos nos complexos II e III da cadeia respiratória e na enzima α-cetoglutarato desidrogenase, importante enzima do ciclo do ácido cítrico, nessa doença (MIZUNO *et al*. 1990, 1994).

Na doença de Alzheimer, a mais comum dentre as doenças neurodegenerativas, é encontrada principalmente uma redução na atividade do complexo IV da cadeia respiratória (MAURER *et al*. 2000). Estudos de imagem *in vivo* demonstraram uma redução no metabolismo da glicose em pacientes portadores da doença de Alzheimer, e essa diminuição aparece precocemente, precedendo os sintomas clínicos, e se acentua com a severidade da doença (JAGUST *et al*. 1988; SMITH *et al*. 1992; KENNEDY *et al*. 1995; SMALL *et al*. 1995). Estudos *postmortem* em cérebros também demonstraram uma diminuição na atividade do complexo enzimático da piruvato desidrogenase e na atividade da enzima α-cetoglutarato desidrogenase na doença de Alzheimer (PERRY *et al*. 1980; GIBSON *et al*. 1988; MASTROGIACOMO *et al*. 1993).

Na esclerose lateral amiotrófica, observou-se uma diminuição na atividade dos complexos I, III e IV da cadeia respiratória em mitocôndrias de células híbridas (SWERDLOW *et al*. 1998).

Por outro lado, vários estudos têm demonstrado uma diminuição na utilização de glicose em estriado e córtex cerebral de pacientes portadores da doença de Huntington (KUHL *et al*. 1982; HAYDEN *et al*. 1986; YOUNG *et al*. 1986; GRAFTON *et al*. 1992; MARTIN *et al*. 1992; KUWERT *et al*. 1993), além de um aumento nos níveis cerebrais de

lactato (JENKINS *et al*. 1993). Também se observou uma redução na utilização de oxigênio, bem como na atividade dos complexos II, III e IV da cadeia respiratória no núcleo caudato de pacientes portadores dessa doença (BRENNAN *et al*. 1985; GU *et al*. 1996).

2- OBJETIVOS

2.1- Objetivo Geral

Este trabalho tem por objetivo avaliar o efeito da administração aguda intraestriatal de ácido quinolínico (AQ) sobre o metabolismo energético em estriado de ratos jovens bem como os mecanismos envolvidos na disfunção energética estriatal induzida pela administração intraestriatal de AQ.

2.2- Objetivos Específicos

* Avaliar o efeito da administração intraestriatal de AQ sobre a produção de $^{14}CO_2$ a partir de [1-^{14}C]acetato em homogeneizado de estriado de ratos 3, 6 ou 12 horas após a injeção.

* Avaliar o efeito da administração intraestriatal de AQ sobre a atividade dos complexos I, II, III e IV da cadeia respiratória em homogeneizado de estriado de ratos 3, 6 ou 12 horas após a injeção.

* Avaliar o efeito da administração intraestriatal de AQ sobre a atividade da enzima creatina quinase em homogeneizado de estriado de ratos 3, 6 ou 12 horas após a injeção.

* Estudar os mecanismos envolvidos nas possíveis alterações do metabolismo energético estriatal causada pela administração intraestriatal de AQ através da:

- pré-administração intraperitoneal de antioxidantes, tais α-tocoferol (100 mg/kg) mais ácido ascórbico (40 mg/kg) ou da pré-administração intraperitoneal de L-NAME (2 mg/kg);

- avaliação do efeito da pré-administração intraperitoneal de substratos energéticos, tais como ácido pirúvico (500 mg/kg) ou creatina (40 mg/kg).

- pré-administração intraperitoneal do antagonista de receptores NMDA, MK-801 (0,25 mg/kg).

PARTE II

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EVIDENCE THAT QUINOLINIC ACID SEVERELY IMPAIRS ENERGY METABOLISM THROUGH ACTIVATION OF THE NMDA RECEPTOR IN RAT STRIATUM

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Running title: Quinolinic acid and brain energy metabolism

Abbreviations used:

CK, creatine kinase; HD, Huntington's disease; L-NAME, N^{ω}-nitro-L-arginine methyl esther; MK-801, (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate; NO, nitric oxide; NOS, nitric oxide synthase; PCr, phosphocreatine; QA, quinolinic acid

Abstract

Most of quinolinic acid (QA) toxic properties have been attributed to excitotoxicity and oxidative stress, whereas very little is known about its effects on brain energy metabolism. Therefore, in the present study we investigated the *in vivo* effect of intrastriatal administration of 150 nmol QA to 30-day-old rats on critical enzyme activities of energy metabolism, including the respiratory chain complexes I-IV, creatine kinase and citrate synthase, as well as on $^{14}CO_2$ production from [1-^{14}C]acetate at distinct periods after QA injection. We observed that QA injection significantly inhibited complexes II (50%)**,** III (46%) and II-III (35%), as well as creatine kinase (27 %), but not complexes I and IV activities in striatum prepared 12 hours after QA treatment. In contrast, no alterations of these enzyme activities were observed 3 or 6 hours after QA treatment. $^{14}CO_2$ production from [1-^{14}C]acetate was also significantly inhibited (27 %) by QA only in rat striatum prepared 12 hours after injection. The respiratory chain complexes and creatine kinase activities were also measured in striatum homogenates exposed to 100 μmol QA. No alterations of these activities were observed in the presence of QA. These *in vitro* observations allied to the ex vivo findings suggest that QA compromises energy metabolism in the rat striatum indirectly rather than due to a direct action of QA on the enzymes. New experiments were therefore designed to elucidate the involved mechanisms of QA-induced inhibition on the enzymatical activities of energy metabolism. Pretreatment with the NMDA receptor antagonist MK-801 and with creatine totally prevented the inhibitory effects elicited by QA. In addition, the free-radical scavengers α-tocopherol plus ascorbate and the nitric oxide synthase inhibitor L-NAME completely abolished the inhibitions provoked by QA on CK and complex III, indicating that these effects were probably due to generation of reactive species. On the other hand, pyruvate pretreatment totally blocked the inhibitory effects of QA injection on complex II activity and partially prevented QA-induced CK inhibition. Taken together, these observations strongly indicate

that oxidative phosphorylation and cellular energy transfer are compromised by high concentrations of QA in the striatum of young rats and that the inhibitory effects caused by QA injection on critical steps of energy metabolism were probably mediated by NMDA stimulation.

Key words: quinolinic acid, respiratory chain, creatine kinase, energy metabolism

Introduction

Quinolinic acid (QA; 2,3-pryridine dicarboxylic acid), an endogenous tryptophan metabolite produced at the kynurenine pathway, is an excitotoxin acting as an agonist at the N–methyl-D-aspartate (NMDA) receptor (Stone, 1993). Experiments in rodents have demonstrated that intrastriatal injection of QA mimics many of the neurochemical and histological features of Huntington´s disease (HD) (Beal *et al*. 1986), and therefore this neurotoxin has been used as a chemically-induced animal model of this disease (Schwarz *et al*. 1983, 1984; Beal *et al*. 1986; Ferrante *et al*. 1993).

HD is a degenerative neurological disorder in which there is progressive neuronal depletion, particularly in the basal ganglia, whose underlying mechanisms are still poorly established. However, recent evidence has implicated excitotoxicity, oxidative stress and metabolic impairment as important factors of the neuronal degeneration in HD and in other neurodegenerative diseases (Beal *et al*. 2000). In this context, various studies have demonstrated abnormal energy metabolism in postmortem HD brain, most notably reduced activity of succinate-linked oxidation in the caudate nucleus and severe deficiency in activities of the respiratory chain complexes (Brennan *et al*. 1985; Mann *et al*. 1990; Gu *et al*. 1996). Further evidence has shown that the mitochondrial abnormality in HD brain is confined to the striatum (Browne *et al*. 1997), being similar to that induced by the complex II inhibitors of the respiratory chain malonate and 3-nitropropionic acid (Beal *et al*. 1993; Ludolph *et al*. 1992).

Most studies performed to examine the *in vitro* and *in vivo* effect of QA on brain tissue demonstrated excitotoxicity and oxidative damage caused by this neurotoxin (Stone, 1993, Tavares *et al*. 2000, Santamaria *et al*. 2001; Leipnitz *et al*. 2005). It has been demonstrated that excitotoxicity produced by sustained activation of NMDA receptors by QA is related to increased cytosolic Ca^{2+} concentrations, ATP and GABA depletion, and specific GABAergic and cholinergic neuronal death (Foster *et al*. 1983, Schwartz *et al*.

1984, Stone, 1993). In addition, oxidative stress produced by free radical formation has been also documented as an important mechanism of QA toxicity in the brain (Rios and Santamaria, 1991; Santamaria and Rios, 1993; Perez-Severiano *et al*. 1998, 2004; Leipnitz *et al*. 2005). In this context, not only oxygen-derived radical species, but also nitrogen-derived species have been shown to be induced by QA in the rodent brain (Noack *et al*. 1998, Ryu *et al*. 2004). Surprisingly, very few studies were designed to examine the role of quinolinic acid on brain energy metabolism, despite the fact that QA has been found at increased concentrations in brain of HD patients and energy dysfunction and more specifically deficiency of the activities of the respiratory chain complexes were also observed in these patients. To our knowledge, only two works investigated the influence of QA on striatum energetics in adult rats. It was demonstrated that intrastriatal injection of QA provokes a progressive time-dependent mitochondrial dysfunction reflected by a low respiratory chain ratio (decreased respiration) and a reduction of ATP, NAD^+, aspartate and glutamate concentrations (Bordelon *et al*. 1997). Disturbances in neuronal activity and ion gradients secondarily to metabolic impairment have been also attributed to QA (Bordelon *et al*. 1998). Although these studies revealed that electrical activity and cellular respiration are reduced by QA in the striatum, they did not evaluate the underlying mechanisms.

Therefore, the present study was undertaken to investigate the influence of QA intrastriatal injection to young rats on important parameters of energy metabolism, including the activities of the various respiratory chain complexes, as well as on creatine kinase and citrate synthase activities and $^{14}CO_2$ production from [1-^{14}C]acetate at distinct periods after QA injection. We also examined the ability of a variety of administered compounds (energetic substrates, antioxidants and an NMDA-receptor antagonist) to modify the energetic deficit induced by intrastriatal QA injection in order to clarify the mechanisms involved.

Material and Methods

Animals and Reagents

A total of 140 30-day-old Wistar rats obtained from the Central Animal House of the Department of Biochemistry, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, were used in the *in vitro* and *ex vivo* studies. The animals were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature ($22^{\circ}C \pm 1^{\circ}C$) colony room, with food and water *ad libitum*. All reagents used were of analytical grade and purchased from Sigma Co. (St Louis, MO, USA), except for dizocilpine maleate (MK-801), which was purchased from Tocris (Ballwin, MO, USA).

Quinolinic Acid Administration

The rats were deeply anesthetized with sodium thiopental (30 mg/kg i.p.) and placed in a stereotaxic apparatus. A small hole was drilled in the skull for microinjection, and 0.5 μL of 300 mM quinolinic acid (150 nmol, pH 7.4 adjusted with NaOH) or NaCl (controls), at the same concentration, was slowly injected over 4 min into the left striatum via a needle connected by a polythene tube to a 10 μL Hamilton syringe. The needle was left in place for another 1 min before been softly removed, so that the total procedure lasted 5 min. The coordinates for injection were as follows: 0.6 mm posterior to bregma, 2.6 mm lateral to midline and 4.5 mm ventral from dura (Paxinos and Watson, 1986). The correct position of the needle was tested by injecting 0.5 μL of methylene blue injection (4% in saline solution) and carrying out histological analysis. The dose and method of QA administration were based in previous works (Qin *et al*. 1992). The experimental protocol was approved by the Ethics Committee for animal research of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil and followed the NIH *Guide for the Care and Use*

of Laboratory Animals (NIH publication 85-23, revised 1985). All efforts were made to minimize the number of animals used and their suffering.

In some experiments the animals were pretreated during 7 days with daily i.p. administration of α-tocopherol (40 mg/kg) plus ascorbic acid (100 mg/kg) or with NaCl (0.9% 10 mL/kg, i.p.), as described previously (Franzon *et al*. 2003). Some animals were also pretreated with N^{ω}-nitro-L-arginine methyl esther (L-NAME, 2 mg/kg) or NaCl (0.9% 10 mL/kg, i.p.) 30 minutes before QA intrastriatal injection. The L-NAME dose used was shown to inhibit cerebral nitric oxide synthase (NOS) by more than 70% (Nishikawa *et al*. 1993), without significantly altering blood flow (Buisson *et al*. 1992). In another experiment, rats were pretreated with sodium pyruvate i.p. at a dose of 500 mg/kg or NaCl (0.9% 10 mL/kg, i.p.)1 hour before QA injection (Ryu *et al*. 2003). The effect of creatine administration on QA-induced energetic dysfunction was also evaluated by preinjecting the animals for 7 days, two injections per day, with creatine (50 mg/kg, i.p.) or NaCl (0.9% 10 mL/kg, i.p.), after which the animals received one intrastriatal QA injection (Vasques *et al*. 2005). Finally, in the experiments designed to evaluate the participation of glutamatergic mechanisms mediated by NMDA-glutamate receptors, the animals received MK-801 (0.25 mg/kg, i.p.) or NaCl (0.9% 10 mL/kg, i.p.) 30 min before they were injected with QA.

Tissue Preparation

Animals were sacrificed by decapitation 3, 6 or 12 hours after intrastriatal injection of either quinolinic acid or NaCl, the brain was rapidly excised on a Petri dish placed on ice. The olfactory bulb, pons, medulla, cerebral cortex and cerebellum were discarded, and the ipsilateral and contralateral striata were dissected, weighed and kept chilled until homogenization with a ground glass type Potter-Elvehjem homogenizer in the specific buffer used for each technique. For the determination of the electron transfer chain complexes and creatine kinase activities, contralateral and ipsilateral striata were

homogenized in 20 volumes of SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, and 50 IU/mL heparin). The homogenates were centrifuged at 800 X g for 10 min at 4ºC, the pellet was discarded and the supernatants kept at -70°C until enzyme activity determination (Fischer *et al*. 1985). The maximal period between homogenate preparation and enzyme analysis was always less than 7 days.

Mitochondria from striatum were also purified for measurement of complex I activity. Briefly, the cerebral cortex was homogenized with a ground glass type Potter-Elvehjem homogenizer in 10 volumes of phosphate buffer pH 7.4 containing 0.3M sucrose, 5mM MOPS, 1mM EGTA and 0.1% bovine serum albumin. The homogenates were centrifuged at 1500×*g* for 10 min at 4°C and the pellet was discarded. The supernatant was centrifuged at 15,000×*g* in order to isolate mitochondria present in the pellet, which was finally dissolved in the same buffer (Latini *et al*. 2005)

For the experiments carried out to measure $^{14}CO_2$ production, striatum was homogenized (1:10, w/v) in Krebs-Ringer bicarbonate buffer pH 7.4 using an ice-chilled glass homogenizing vessel at 900 rpm and total homogenates were used in these experiments.

For the *in vitro* studies, striatum from non-treated rats was dissected and homogenized in a similar manner as that for the *ex vivo* experiments to obtain supernatants and purified mitochondrial fractions. The supernatants or mitochondrial preparations were them incubated in the presence of 100 µM QA.

Respiratory Chain Complexes Activities

Mitochondrial respiratory chain enzyme activities were measured in striatum supernatants 3, 6 or 12 hours after QA or NaCl intrastriatal injection. The activity of NADH dehydrogenase (Complex I) was assessed as described by Cassina and Radi (1996) in mitochondrial preparations. The activities of succinate: DCIP-oxidoreductase (complex

II) and succinate:cytochrome c oxidoreductase (complex II-III) were determined according to the method of Fischer *et al* (1985). The activity of ubiquinol:cytochrome *c* oxireductase (complex III) was assayed according to the method described by Birch-Machin *et al*. (1995) and that of cytochrome c oxidase (complex IV) according to Rustin *et al*. (1994). The methods described to measure these activities were slightly modified, as described in details in previous reports (Brusque *et al*. 2002, da Silva *et al*. 2002). The activities of the respiratory chain complexes were expressed as nmol/(min mg protein). For the *in vitro* studies, 100 μM QA was supplemented to the incubation medium, whereas the control group did not contain the metabolite.

Creatine Kinase activity (CK) determination

CK activity was measured in striatum homogenates 3, 6 or 12 hours after QA or NaCl intrastriatal injection in a reaction mixture consisting of 60 mM Tris-HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM $MgSO_4$, 0.625 mM lauryl maltoside and approximately 0.4-1.2 μg protein in a final volume of 100 μL. For the *in vitro* studies, 100 μM QA was supplemented to the incubation medium, whereas the control group did not contain the metabolite. After 10 min of pre-incubation at 37ºC, the reaction was started by the addition of 0.3 μmol ADP. The reaction was stopped after 10 min by the addition of 1 μmol of *p*-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962) with slight modifications as described previously (da Silva *et al*. 2004). The color was developed by the addition of 100 μL 2% α-naphtol and 100 μL *0.05*% diacetyl in a final volume of 1 mL and read spectrophotometrically at 540 nm after 20 min. Results were expressed as percentage of controls as μmol creatine formed/(min mg protein).

Citrate Synthase activity

The activity of citrate synthase, a marker enzyme of mitochondrial viability, was assayed according to Shepherd and Garland (1973) in a medium containing 75 mM Tris-HCl pH 8.0, *0.01*% Triton X-100, 0.1 mM DTNB, 0.5 mM oxaloacetic acid and 20 μg protein. The reaction was started with the addition of 0.42 mM acetyl-CoA and monitored spectrophotometrically at 412 nm for 3 min. Results were expressed as nmol TNB formed/(min mg protein).

$^{14}CO_2$ production from [1-^{14}C]acetate

For the experiments designed to evaluate $^{14}CO_2$ production, the animals were sacrificed 3 or 12 hours after intrastriatal QA or NaCl (0.9 %) injection, the contralateral and ipsilateral striata were dissected and homogenized (1:10, w/v) in Krebs-Ringer bicarbonate buffer pH 7.4 using an ice-chilled glass homogenizing vessel at 900 rpm. Total striatal homogenates were added to small flasks (11 cm^3). Flasks were pre-incubated in a metabolic shaker at 37°C for 15 min. After pre-incubation, 0.1 μCi [1-^{14}C]-acetate and 0.5 mM of unlabeled acetate were added to the incubation medium. The flasks were gassed with a O_2:CO_2 (95:5) mixture and sealed with rubber stoppers and Parafilm M. Glass center wells containing a folded 65 mm/5 mm piece of Whatman 3 filter paper were hung from the stoppers. After 60 min of incubation at 37°C, 0.1 mL of 50% trichloroacetic acid was added to the medium and 0.1 mL of benzethonium hydroxide was added to the center wells with needles introduced through the rubber stopper. The flasks were left to stand for 30 min to complete $^{14}CO_2$ trapping and then opened. The filter papers were removed and added to vials containing scintillation fluid, and radioactivity was measured (Dutra-Filho *et al*. 1995).

Protein determination

Protein was measured by the method of Lowry *et al* (1951), using bovine serum albumin as standard. The protein concentration in the supernatants varied from 1.5-4.0 mg protein/mL in all experiments.

Statistical analysis

Data were expressed as means ± SEM for absolute values or percentage of control. Assays were performed in duplicate or triplicate and the mean was used for statistical analysis. Results were assessed with a computerized statistical program (Statistical Package for the Social Sciences, SPSS) on an IBM-compatible PC. Comparisons between values were calculated using the unpaired and paired Student's *t*-tests. Differences between the groups were considered statistical significant when $P < 0.05$.

Results

The activities of the respiratory chain complexes were determined in homogenates from the left striatum of rats sacrificed 3, 6 or 12 hours after QA or NaCl administration. It can be seen in Table 1 that QA did not affect these enzymatic activities when rats were killed 3 and 6 hours after intrastriatal injection, as compared to NaCl-injected animals. In contrast, significant inhibitions on complex II (50%, $P < 0.05$), complex III (46%, $P < 0.05$) and complex II-III (35%, $P < 0.01$) activities, but not of complex I ($P = 0.273$), and complex IV ($P = 0.945$) activities, were observed in striatum prepared 12 hours after QA treatment (Figure 1).

The activity of citrate synthase was also determined in striatum homogenates prepared 12 hours after QA or NaCl injection. No significant difference was found between groups, indicating that the number of mitochondria and/or the mitochondrial integrity was not affected by intrastriatal injection of QA ($P = 0.456$). These data

corroborate our findings of no alteration of complexes I and IV activities of the respiratory chain at 12 hours after QA intrastriatal administration.

The activities of creatine kinase (CK) in striatum homogenates taken 3, 6 or 12 hours after QA or NaCl administration are shown in Figure 2. No significant differences between QA and NaCl-injected striata were found in CK activity at 3 or 6 hours after QA injection. However, CK activity was significantly reduced by about 27% after 12 hours of QA intrastriatal administration ($P < 0.05$).

We then examined the influence of QA treatment on CO_2 production from [1-^{14}C]acetate in total homogenates prepared from striatum of rats that received QA or NaCl 3, 6 or 12 hours after injection. No differences in ^{14}CO2 synthesis were found 3 or 6 hours after QA-injection. However, CO_2 production from acetate was found significantly reduced by about 30% in striatum of QA-injected animals sacrificed 12 hours after QA administration ($P < 0.05$) (Figure 3).

We then examined the *in vitro* effect of QA (100 μM) on the respiratory chain complexes and creatine kinase activities in striatal homogenates of young rats. Table 2 shows that no significant changes were found when QA was added to the incubation medium, as compared to controls. Taken together the *in vitro* and the ex vivo observations, it may be presumed that QA-induced impairment of energy production was mediated by indirect mechanisms, rather due to a direct action of this organic acid on the enzymes.

Therefore, the next set of experiments was designed to elucidate the mechanisms involved in impairment of striatum bioenergetics caused by QA administration. The animals were pretreated with antioxidants, energetic substrates or an NMDA receptor antagonist and sacrificed 12 hours after QA injection. In these experiments, the activities of the respiratory chain complexes and of creatine kinase were evaluated in striatum homogenates prepared from the ipsilateral and the contralateral striatum (controls) in order to minimize the number of animals used. Preliminary experiments revealed no significant

changes in these activities between the NaCl-injected ipsilateral and the noninjected contralateral striatum prepared 12 hours after injection (data not shown), indicating that NaCl injection *per se* did not alter the energy metabolism parameters evaluated. Furthermore, we also observed that QA injection in the ipsilateral striatum did not change the energy metabolism parameters in the contralateral striatum (data not shown).

We initially pretreated the animals with daily i.p. injections of the free-radical scavengers α-tocopherol (40 mg/kg) plus ascorbate (100 mg/kg) for 7 days, while controls received saline solution. As depicted in Figure 4, pretreatment with these drugs totally prevented the inhibitory effect of QA on CK (*P < 0.01 vs saline*) and complex III (*P < 0.05 vs saline*) activities, but did not alter the inhibition provoked by this neurotoxin on complexes II and II-III.

We then pretreated the animals with one i.p. injection of the NOS inhibitor L-NAME (2 mg/kg) 30 min prior to QA intrastriatal administration (Figure 5). This drug was administered in a dose that inhibits cerebral nitric oxide synthase up to 70% (Nishikawa *et al*. Stroke 1993) without altering blood flow (Buisson *et al*. Br. J. Pharmacol, 1992). L-NAME pretreatment only abolished the inhibitory effect of QA administration on CK activity (*P < 0.01 vs saline*) (Figure 5), indicating that NO or/and peroxynitrite generation is probably involved in such inhibitory effect. Taken together, these results indicate that CK and complex III inhibition induced by QA injection is probably mediated by the generation of reactive species.

Animals were also pretreated with the energetic substrates pyruvate and creatine, Pretreatment of rats 1 hour prior to intrastriatal injection of QA with 500 mg/kg pyruvate, a dose previously reported to be efficient to reduce QA-induced striatum toxicity (Ryu *et al*. 2004), did not alter the inhibitory pattern of complex II-III and III, but partially reduced (*P < 0.05 vs contralateral; P < 0.05 vs saline*) the inhibitory effect of QA on CK activity and completely prevented the inhibitory effect of this neurotoxin on complex II activity (*P <*

0.05 vs saline) (Figure 6).

The effect of i.p. creatine (50 mg/kg) or saline (0.9 % NaCl) pretreatment for 7 days, twice a day, on the inhibitory properties of QA was examined. As shown in figure 7, creatine pretreatment completely prevented all inhibitory effects of QA. Moreover, the activity of CK of animals receiving creatine was even higher (25%, $P < 0.01$) in the ipsilateral, as compared with the contralateral striatum.

Finally, we observed that intraperitoneal preadministration of the NMDA antagonist MK-801 (0.25 mg/kg) fully prevented the inhibitory effects elicited by QA intrastriatal injection on all parameters evaluated (Figure 8). These results indicate that the inhibitions of enzymatic activities caused by QA administration were probably mediated by NMDA overstimulation.

Discussion

Quinolinic acid is presumably involved in the pathogenesis of neurodegenerative disorders (Schwarcz *et al*. 1984, Whetsell and Schwarcz, 1989; Widner *et al*. 2000a,b; Obrenovitch, 2001; Stone, 2001), infectious, inflammatory and non-inflammatory diseases (Heyes *et al*. 1991, 1995, Halperin and Heyes, 1992; Brouwers *et al*. 1993) It has been generally considered that the toxic actions of QA are predominantly related with NMDA receptor overactivation (Stone *et al*. 1993; Susel *et al*. 1989), although other investigators do not support the presumption that this mechanism is responsible for most of the brain damage exerted by QA (Obrenovitch *et al*. 2001). In this context, QA is capable to induce oxidative stress through reactive oxygen and nitrogen species formation by NMDA-dependent and NMDA-independent manners, therefore involving a pattern of toxicity distinct from excitotoxicity (Behan *et al*. 1999, Santamaria *et al*. 2001; Leipnitz *et al*. 2005). Furthermore, quinolinic acid (QA) and the inhibitors of complex II of the respiratory chain malonate and 3-nitropropionic acid reproduce the neurochemical and

histopathological features of Huntington´s disease characterized by basal ganglia lesions and QA has been used as an animal model of because it this disorder (Schwarcz *et al*. 1983; Beal *et al*. 1986; Beal *et al*. 1993; Greene *et al*. 1993; Brouillet *et al*. 1995).

On the other hand, intracerebral administration of QA has been shown to cause energetic dysfunction in striatum of adult rats but the involved mechanisms were not unraveled (Bordelon *et al*. 1997, 1998).The present investigation demonstrates that *in vivo* intrastriatal administration of QA to young rats decreases aerobic respiration, by significantly blocking the respiratory chain and the citric acid cycle, and also compromises energy transfer inside the cell, by inhibiting creatine kinase (CK) activity, in a time-dependent manner. The alterations of energy metabolism were observed 12 hours, but not 3 or 6 hours after QA injection, suggesting that these effects could be mediated by indirect mechanisms such as NMDA receptor activation and/or oxidative stress. This assumption is reinforced by the fact that doses of 100 μmol QA were unable to inhibit *in vitro* the activities of the respiratory chain complexes and of creatine kinase, indicating that no direct effect of QA on these enzymes occurred.

Significant reductions of the activities of complexes II (50 %), II-III (35 %), III (46 %) of the respiratory chain and of CK (27 %) were detected in the striatum 12 hours after QA injection. Labeled CO_2 production from [1-^{14}C]acetate, which reflects the activity of the Krebs cycle, was also found reduced (27 %) only at 12 hours after QA infusion. Taken together, these results strongly indicate that the striatum aerobic capacity was compromised by QA.

Young animals (30 day-old rats) were used in our experiments because very little is known on the effects of QA on intermediary metabolism at an early animal age. In this context, changes in temporal and spatial expression of NMDA receptor subtypes (QA is an agonist of these subtype of glutamate receptors) occur during brain development (Monyer *et al*. 1994, Portera-Cailliau *et al* 1996, Wenzel *et al* 1997). Thus, it could be presumed

that differential age-specific effects may be achieved in case QA acts on a specific subtype of NMDA receptor.

We could hypothetically attribute our results of reduced activities of crucial enzymatic steps of energy production as due to tissue damage and cell death caused by QA infusion since reduced ATP production or utilization can result in loss of cell viability by disrupting energy-dependent vital cellular processes, and it has been shown that QA provokes lower ATP synthesis in the striatum (Bordelon *et al*. 1997). In this regard, ATP is essential to fuel ionic pumps that generate and maintain ionic and voltage gradients across neuronal membranes, including Na^+,K^+,ATPase pump that controls the resting membrane potential and other ATPases that regulate intracellular Ca^{2+} levels (Novelli *et al*. 1988, Haneberry *et al*. 1989, Zeevalk *et al*. 1991). Increased intracellular Ca^{2+} may lead to reactive species formation and in turn attack important enzyme activities of the respiratory chain and of the Krebs cycle (Dykens, 1994,). Therefore, impaired energy metabolism and oxidative stress may form a self-amplifying cycle of cell toxicity. However, this was probably not the case i.e. the changes in energy metabolism parameters as reported here are not likely to be due to cell loss, since the activities of NADH dehydrogenase (complex I), complex IV and citrate synthase, other enzyme activities that reflect the mitochondrial content in the samples, were not altered by QA injection. This is in agreement with previous studies showing that there is no significant decrease in cell number after similar injections of this toxin (Bordelon *et al*. 1994).

The animal data obtained in the current study agree to a certain extend to other reports performed in humans and showing that various activities of the respiratory chain complexes, particularly complex II, are markedly and selectively reduced in *postmortem* striatum from HD patients (Tabrizi *et al*. 1999, Gu *et al*. 1996). Thus, our results may provide further support for the usefulness of intrastriatal QA injection as an animal model of HD.

Considering that oxidative stress can be induced by QA *in vitro* and *in vivo* (Leipnitz *et al*. 2005; Rios and Santamaria, 1991, Perez-Severiano *et al*. 2004) and that the respiratory chain complexes II and III and CK can be attacked by free radicals (Cardoso *et al*. 1999; Wendt *et al*. JBC 2003, Stachowiack JBC 1998), we evaluated whether free radical generation could be involved in the energetic impairment provoked by QA intrastriatal injection by pretreating animals with the free radical scavengers α-tocopherol plus ascorbate or the NOS inhibitor L-NAME and then assaying the energetic parameters. We observed that pretreatment with α-tocopherol plus ascorbate was able to fully prevent CK and complex III inhibitions induced by QA infusion, indicating that these inhibitory effects were mediated by reactive species. In this particular, it is well established that α-tocopherol is a lipophilic antioxidant that acts as a free radical scavenger, donating hydrogen to radicals usually peroxyl and alkoxyl radicals, thus preventing the propagation of lipid peroxidation. Because it is localized into the hydrocarbon core of the phospholipids bilayer, it interacts with cell membrane, protecting polyunsaturated fatty acids and thiol groups of membrane proteins, keeping the integrity and function of the biomembranes (Trappel, 1973; Ames *et al*. 1993; Burton *et al*. 1990). During this process, α-tocopherol is converted to the tocopheryl radical, requiring ascorbate for its regeneration back to reduced tocopherol (Frei *et al*. 1990; McCay, 1985; Carr and Frei, 1999). Regarding complex III, it has been recently reported that this complex contains tightly bound cardiolipin molecules that appear to be essential for its catalytic function (Gomez Jr and Robinson, 1999; Lange *et al*. 2001) and that reactive oxygen species generated by the mitochondrial respiratory chain affect complex III activity via cardiolipin peroxidation (Paradies *et al*. 2001). Our results also demonstrated lack of preventive effect of α-tocopherol on complex II activity, and this is in accordance with other study reporting an inability of α-tocopherol to protect complex II in an oxidative stress situation (Cardoso *et al*. 1999). Therefore, it is likely that a direct damage of free radicals towards the complex proteins of this complex occurs,

rather than an indirect action due to lipid peroxidation of membrane proteins in which this complex is embbebed. Further experiments using other antioxidants, such as glutathione esters should be carried out to unravel the involved mechanisms.

Nitric oxide (NO) has been implicated in the neurotoxicity associated with glutamate receptor stimulation (Dawson *et al*. 1991) and more recently with QA-induced neurotoxicity in the striatum (Peres-Severiano *et al*. 1998). NMDA receptor activation induces NOS (Ayata *et al*. 1997) by calcium influx, leading to increase of NO and its highly toxic reactive nitrogen species derivative peroxynitrite. In this context, increase in microglia-derived iNOS and neuronal NOS have been reported in QA-injected striatum (Ryu, 2004, Schmidt *et al*. 1995). Our results revealed that pretreatment with the NOS inhibitor L-NAME was able to totally prevent the inhibitory effect of QA infusion on CK activity, without altering the other parameters of energy metabolism, indicating therefore that CK activity is especially vulnerable to NO and/or peroxynitrite attack . Our findings are in agreement with previous reports showing that CK activity is inhibited by peroxynitrite (Wendt *et al*. 2003, Stachowiack JBC 1998). The absence of a preventive effect of L-NAME on QA-induced inhibition of the respiratory chain complexes II, III and II-III indicates that inhibition of these complexes were probably not mediated by nitrogen reactive species. Taken together, these results indicate that the impairment of striatum bioenergetics induced by QA injection is partially mediated by generation of reactive species.

Since pyruvate serves as a cellular energy substrate (Tsacopoulos and Magistretti, 1996), exhibits antioxidant activity (Giandomenico *et al*. 1997) and protects against NMDA-mediated cell death by enhancing energy metabolism (Maus *et al*. 1999), in this work we also tested the effect of pyruvate pretreatment on the energetic dysfunction caused by QA intrastriatal infusion. Pyruvate completely prevented the inhibitory effect on complex II activity and attenuated the inhibition of CK activity provoked by QA. These

results agree with previous reports showing that this compound is neuroprotective against QA-induced damage in different types of striatal neurons (Ryu *et al*. 2003, 2004). Ryu and coworkers (2004) also reported that pyruvate treatment decreased by about 50% iNOS expression in astrocytes, a fact that may explain the partial prevention of CK activity inhibition and reinforces our present data indicating that this inhibition is at least in part mediated by formation of NO or its metabolites. In addition, since pyruvate can directly neutralize H_2O_2 and lipid peroxides in a non-enzymatic reaction (Constantopoulos and Barranger, 1984; DeBoer *et al*. 1993; Crestanello *et al*. 1995), it may be presumed that these properties may also be related to the inhibition of complex II and CK elicited by QA. Regarding complex II, it has been shown that pyruvate induces *in vitro* synthesis of citrate, NADPH and GSH (Tejero-Taldo *et al*. 1999) and that GSH protects complex II against inhibition induced by oxidative stress (Cardoso *et al*. 1999). Although we did not measure GSH levels after pyruvate pretreatment, it may be presumed that pyruvate may have prevented the inhibitory QA-induced inhibition of complex II of the respiratory chain by increasing GSH levels. This is in agreement with the observations that inhibitions of mitochondrial activities are related to the deficient regeneration of the endogenous naturally-occurring antioxidant GSH that depend on the supply of hydrogen from respiratory substrates (Glinka and Youdim, 1994).

Moreover, considering that various studies have shown neuroprotective effects of creatine supplementation against energy deficit caused by malonate and 3-nitropropionic acid toxicity *in vivo* (Matthews *et al*. 1998; Malcon *et al*. 2000) and in cultivated striatal and hippocampal neurons (Brustovetsky *et al*. 2001), we examined whether creatine administration could change the pattern of energy dysfunction provoked by intrastriatal injection of QA. We found that pretreatment with creatine completely abolished all inhibitory effects induced by QA injection on striatum bioenergetics.

Despite its broad neuroprotective properties *in vivo* and *in vitro*, the mechanisms of action of creatine-mediated neuroprotection are not well understood yet. Current hypotheses include enhanced energy storage, as well as stabilization of the mitochondrial permeability transition pore by the octameric conformation of CK (O'Gorman *et al*. 1997; Brdiczka *et al*. 1998; Wyss and Kaddurah-Daouk, 2000). Thus, it is well established that creatine is an excellent stimulant of mitochondrial respiration, resulting in the generation of phosphocreatine (PCr) and secondarily of ATP (Kernec *et al*. 1996; O'Gorman *et al*. 1996). Furthermore, rise in PCr/ATP ratio may serve to power ion pumps, especially the energetically demanding sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase pump (Wallimann and Hemmer, 1994), whose activities are required to maintain ion homeostasis, excitability, and Ca^{2+} signaling. These ion pumps are coupled to CK and require a high local ATP/ADP ratio for efficient function. In addition, it should be noted that creatine exposure may upregulate transcription of the brain cytosolic CK (Kuzhikandathil and Molloy, 1994), as has been observed by protein kinase A upregulation of the brain isozyme of CK (Kuzhikandahtil and Molloy, 1994). Taken together, these effects may act synergistically in order to improve the overall bioenergetic status of the cell, making it more resistant to injury (Zhu *et al*. 2004).

Creatine also protects against NMDA overstimulation, which may be attributed to its capacity to rise PCr levels and the ability of PCr to stimulate synaptic glutamate uptake and thereby to reduce extracellular glutamate (Xu *et al*. 1996). This hypothesis is reinforced by the fact that QA was previously shown to stimulate synaptosomal glutamate release and to inhibit astrocyte glutamate uptake (Tavares, *et al*. 2002).

Previous reports have demonstrated that QA administration and NMDA excitotoxic lesions are associated with reduction of energy reserves (Mitani, 1994; Tsujii *et al*. 1994; Bordelon *et al*. 1997). Excitotoxicity is accompanied by Ca^{2+} surge (Khodorov *et al*. 1996, Stout *et al*. 1998), which leads to a deterioration of the cellular energy status, if energy

reserves are insufficient for Ca^{2+} pumping (Brewer and Wallimann, 2000). Bioenergetics failure could be the result of reactive species-induced oxidation and inactivation of several of the mitochondrial transport chain complexes (Zhang *et al*. 1990), mitochondrial aconitase (Li *et al*. 2001) and/or ATP synthase (Beal *et al*. 1986). Therefore, we tested whether the noncompetitive NMDA antagonist MK-801 could block QA effect on striatum bioenergetics. We observed that this glutamate antagonist was able to fully prevent all QA-induced inhibitory effects on energy metabolism. These findings are in agreement with a report describing that MK-801 blocks QA-induced degeneration in rat striatum (Woodruff *et al*. 1987) and strongly support the hypothesis that the blockage of the respiratory chain and CK caused by QA intrastriatal injection was secondary to NMDA receptor stimulation.

As regards to the pathophysiological importance of our findings, it should be emphasized that QA concentrations that provoked the effects here observed were similar to those found during neuroinflammatory processes (Beagles *et al*. 1998, Obrenovitch, 2001; Obrenovitch and Urenjak, 2003). Furthermore, although we did not measure ATP levels, a previous experiment using a similar approach demonstrated that ATP synthesis was markedly blocked by QA intrastriatal administration (Bordelon *et al*. 1997). In this scenario, our present investigation extended this study and unraveled some of the involved mechanisms of energy depletion, showing also that the Krebs cycle (CO_2 production) was also blocked by QA, as well as CK activity, indicating that energy transfer is also compromised by intrastriatal high levels of QA.

In conclusion, our present results indicate that energy dysfunction must be also considered as a QA-inducing mechanism of cell damage. Since creatine is well tolerated in man, the present findings are of particular interest because use of oral treatment with creatine may represent a potential novel adjuvant therapeutic strategy against QA toxicity, particularly in neurodegenerative diseases in which brain QA concentrations are increased and/or defects in energy metabolism are implicated (Beal, 1992).

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References

Ames B.N., Shigenaga M.K. and Hagen T.M. (1993) Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl Acad. Sci. U.S.A.* **90**, 7915–7922.

Ayata C., Ayata G., Hara H., *et al*. (1997) Mechanisms of reduced striatal NMDA excitotoxicity in type I nitric oxide synthase knock-out mice. *J. Neurosci.* **17**, 6908-6917.

Beagles K.E., Morrison P.F., and Heyes M.P. (1998) Quinolinic acid *in vivo* synthesis rates, extracellular concentrations, and intercompartmental distributions in normal and immune-activated brain as determined by multiple-isotope microdialysis. *J. Neurochem.* **70**, 281-291.

Beal M.F., Kowall N.W., Ellison D.W., Mazurek M.F., Swartz K.J. and Martin J.B. (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* **321**, 168-171.

Beal M.F. (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses?. *Ann. Neurol.* **31**, 119-130.

Beal M.F., Brouillet E., Jenkins G.G., Henshaw R., Rosen B. and Hyman B.T. (1993) Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. *J. Neurochem.* **61**, 1147-1150.

Beal M.F. (2000) Energetics in the pathogenesis of neurodegenerative diseases. *Trends Neurosci*. **23**, 298-304.

Behan W.M., McDonald M., Darlington L.G. and Stone T.W. (1999) Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl. *Br. J. Pharmacol,* **128**, 1754-1760.

Birch-Machin M.A., Briggs H.L., Saborido A.A., Bindoff L.A. and Turnbull D.M. (1994) An evaluation of the measurement of the activities of complexes I-IV in the respiratory chain of human skeletal muscle mitochondria. *Biochem. Med. Metab. Biol.* **51**, 35-42.

Bordelon Y., MacKenzie L., McLaughlin B.-A. and Chesselet M.-F. (1994) Excitotoxic lesions of rat striatum: time course of DNA damage assessed by in situ nick translation. *Soc. Neurosci*., Abstr., **20**, 988.

Bordelon Y.M., Chesselet M.F., Erecinska M. and Silver I.A. (1998) Effects of intrastriatal injection of quinolinic acid on electrical activity and extracellular ion concentrations in rat striatum *in vivo*. *Neuroscience* **83**, 459-469.

Bordelon Y.M., Chesselet M.F., Nelson D., Welsh F. and Erecinska M. (1997) Energetic dysfunction in quinolinic acid-lesioned rat striatum. *J. Neurochem.* **69**, 1629-1639.

Brdiczka D., Beutner G., Ruck A., Dolder M. and Wallimann T. (1998) The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. *Biofactors* **8**, 235-242.

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

Brewer G.J. and Wallimann T.W. (2000) Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. *J. Neurochem.* **74**,:1968-1978.

Brouillet E., Hantraye P., Ferrante R.J., Dolan R., Leroy-Willig A., Kowall N.W. and Beal M.F. (1995) Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc. Natl Acad. Sci. USA* **92**, 7105-7109.

Brouwers P., Heyes M.P., Moss H.A., Wolters P.L., Poplack D.G., Markey S.P. and Pizzo P.A. (1993) Quinolinic acid in the cerebrospinal fluid of children with symptomatic human immunodeficiency virus type 1 disease: relationships to clinical status and therapeutic response. *J. Infect. Dis.* **168**, 1380-1386.

Browne S.E., Bowling A.C., MacGravey 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.

Brusque A.M., Borba Rosa R., Schuck P.F. *et al*. (2002). Inhibition of the mitochondrial respiratory chain complex activities in rat cerebral cortex by methylmalonic acid. *Neurochem. Int.* **40**, 593-601.

Brustovetsky N., Brustovetsky T.and Dubinsky J.M. (2001) On the mechanisms of neuroprotection by creatine and phosphocreatine. *J. Neurochem.* **76,** 425-434.

Buisson A., Plotkine M. and Boulu R.G. (1992) The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia, *Br. J. Pharmacol.* **106**, 766-767.

Burton G.W., Wronska U., Stone L., Foster D.O. and Ingold, K.U. (1990) Biokinetics of dietary *RRR*-α-tocopherol in the male guinea pig at three dietary levels of Vitamin C and two levels of Vitamin E. *Lipids* **25**, 199–210.

Cardoso S.M., Pereira C. and Oliveira R. (1999) Mitochondrial function is differentially affected upon oxidative stress. *Free Radic. Biol. Med*. **26**, 3-13.

Carr A. and Frei B., (1999) Does Vitamin C act as a pro-oxidant under physiological conditions?. *FASEB J.* **13**, 1007–1024.

Cassina A. and Radi R. (1996) Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch. Biochem. Biophys.* **328,** 309-316

Constantopoulos G. and Barranger J.A. (1984) Non enzymatic decarboxylation of pyruvate. *Anal. Biochem.* **139**, 353–358.

Crestanello J.A., Kamelgard J. and Whitman G.J.R. (1995) The cumulative nature of pyruvate's dual mechanism for myocardial protection. *J. Surg. Res.* **59,** 198–204.

da Silva C.G., Ribeiro C.A., Leipnitz G., Dutra-Filho C.S., Wyse A.T., Wannmacher C.M., Sarkis J.J., Jakobs C. and Wajner M. (2002) Inhibition of cytochrome c oxidase activity in rat cerebral cortex and human skeletal muscle by D-2-hydroxyglutaric acid *in vitro*. *Biochim. Biophys. Acta* **1586**, 81-91.

da Silva C.G., Bueno A.R., Schuck P.F. *et al*. (2004) Inhibition of creatine kinase activity from rat cerebral cortex by D-2-hydroxyglutaric acid *in vitro*. *Neurochem. Int*. **44**, 45-52.

Dawson V.L., Dawson T.M., London E.D., Bredt D.S. and Snyder S.H. (1991) Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl Acad. Sci. USA* **88**, 6368-6371.

DeBoer L.W.V., Bekx P.A., Han L. and Steinke L. (1993) Pyruvate enhances recovery of rat hearts after ischemia and reperfusion by preventing free radical generation. *Am. J. Physiol.* **265**,H1571–H1576

Dutra-Filho C.S., Wajner M., Wannmacher C.M., Gassen E., Candiago R.H., Wilhelms A.M. and de Malfussi H.F. (1995) 2-hydroxybutyrate and 4-hyddroxybutyrate inhibit CO_2 formation from labeled substrates by rat cerebral cortex. *Biochem. Soc. Trans.* **23**, 228S.

Dykens J.A. (1994) Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na: implications for neurodegeneration. *J. Neurochem*. **63**, 584-591.

Ferrante R.J., Kowall N.W., Cipolloni P.B., Storey E. and Beal M.F. (1993) Excitotoxin lesions in primates as a model for Huntington's disease: histophatologic and neurochemical characterization. *Exp. Neurol.* **119**, 46-71.

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**, 23-26.

Foster A.C., Collins J.F. and Schwarcz R. (1983) On the excitotoxic properties of quinolinic acid, 2,3-piridine dicarboxylic acid and structurally related compounds. *Neuropharmacology* **22**, 1331-1342.

Franzon R., Lamers M.L., Stefanello F.M., Wannmacher C.M., Wajner M. and Wyse A.T. (2003) Evidence that oxidative stress is involved in the inhibitory effect of praline on Na^+,K^+-ATPase activity in synaptic plasma membrane of rat hippocampus. *Int. J. Dev. Neurosci.* **21**, 303-307.

Frei B., Stocker R., England L. and Ames, B.N. (1990) Ascorbate: the most effective antioxidant in the blood. *Adv. Exp. Med. Biol.* **264**, 155–163.

Giandomenico A.R., Cerniglia G.E., Biaglow J.E., Stevens C.W. and Koch C.J. (1997) The importance of sodium pyruvate in assessing damage produced by hydrogen peroxide. *Free Radic. Biol. Med.* **23**, 426–434.

Glinka Y.Y. and Youdim M.B. (1995) Inhibition of mitochondrial complexes I and IV by 6-hydroxydopamine. *Eur. J. Pharmacol.* **292,** 329-332.

Gomez Jr. B. and Robinson N.C. (1999) Phospholipase digestion of bound cardiolipin reversibly inactivates bovine cytochrome bc1. *Biochemistry* **38**, 9031-9038.

Greene J.G., Porter R.H., Eller R.V. and Greenamyre J.T. (1993) Inhibition of succinate dehydrogenase by malonic acid produces an "excitotoxic" lesion in rat striatum. *J. Neurochem.* **61**, 1151-1154.

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

Halperin J.J. and Heyes M.P. (1992) Neuroactive kynurenines in Lyme borreliosis. *Neurology* **42**, 43-50.

Henneberry R.L., Novelli A., Cox J.A. and Lysko P.G. (1989) Neurotoxicity at the N-methyl-D-aspartate recetor in energy-compromised neurons: An hypothesis for cell death in aging and disease. *Ann. NY Acad. Sci*. **568**, 225-233.

Heyes M.P., Brew B.J., Martin A. *et al*. (1991) Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. *Ann. Neurol*. **29**, 202-209.

Heyes M.P., Saito K., Milstien S. and Schiff S.J. (1995) Quinolinic acid in tumors, hemorrhage and bacterial infections of the central nervous system in children. *J. Neurol. Sci.* **133**, 112-118.

Hughes B.P. (1962) A method for estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathological sera. *Clin. Chim. Acta*. **7**, 597-603.

Kernec F., Le Tallec N., Nadal L., Begue J.M. and Le Rumeur E. (1996) Phosphocreatine synthesis by isolated rat skeletal muscle mitochondria is not dependent upon external ADP: a 31P NMR study. *Biochem. Biophys. Res. Commun*. **225**, 819-825

Khodorov B., Pinelis V., Vergun O., Storozhevykh T. and Vinskaya, N. (1996) Mitochondrial deenergization underlies neuronal calcium overload following a prolonged glutamate challenge. *FEBS Lett*. **397**, 230-234.

Kuzhikandathil E.V. and Molloy G.R. (1994) Transcription of the brain creatine kinase gene in glial cells is modulated by cyclic AMP-dependent protein kinase. *J. Neurosci. Res.* **39**, 70-82.

Lange C., Nett J.H., Trumpower B.L. and Hunte C. (2001) Specific roles of protein-phospholipid interactions in the yeast cytochrome bc1 complex structure. *EMBO J*. **20**, 6591-6600.

Latini A, Rodriguez M, Borba Rosa R, *et al*. (2005) 3-Hydroxyglutaric acid moderately impairs energy metabolism in brain of young rats. *Neuroscience* **135**, 111-120.

Leipnitz G., Schumacher C., Scussiato K., Dalcin K.B., Wannmacher C.M., Wyse A.T., Dutra-Filho C.S., Wajner M. and Latini A. (2005) Quinolinic acid reduces the antioxidant defenses in cerebral cortex of young rats. *Int. J. Dev. Neurosci.* **23**, 695-701.

Li Q.Y., Pedersen C., Day B.J and Patel M. (2001) Dependence of excitotoxic neurodegeneration on mitochondrial aconitase inactivation. *J. Neurochem*. **78,** 746-755.

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.

Ludolph A.C., Seelig M., Ludolph A., Novitt P., Allen C.N., Spencer P. and Sabri, M.I. (1992) 3-Nitropropionic acid decreases cellular energy levels and causes neuronal degeneration in cortical explants. *Neurodegeneration* **1**, 155-161.

Malcon C., Kaddurah-Daouk R. and Beal M.F. (2000) Neuroprotective effects of creatine administration against NMDA and malonate toxicity. *Brain Res*. **860**, 195-198.

Mann V.M., Cooper J.M., Javoy-Agid F., Agid Y., Jenner P. and Schapira A.H. (1990) Mitochondrial function and parental sex effect in Huntington's disease. *Lancet* **336**, 749.

Matthews R.T., Yang L., Jenkins B.G. *et al*. (1999) Pyruvate and lactate protect striatal neurons against *N*-methyl-D-aspartate-induced neurotoxicity. *Eur. J. Neurosci.* **11**, 3215–3224.

McCay P.B. (1985) Vitamin E: interactions with free radical and ascorbate. *Annu. Rev. Nutr.* **5**, 323–340.

Mitani A., Takeyasu S., Yanase H., Nakamura Y. and Kataoka K. (1994) Changes in intracellular Ca^{2+} and energy levels during *in vitro* ischemia in the gerbil hippocampal slice. *J. Neurochem*. **62,** 626-634.

Monyer H., Burnashev N., Laurie D.J., Sakmann B. and Seeburg P.H. (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**, 529-540.

Nishikawa T., Kirsch J.R., Koehler R.C., Bredt S.D., Snyder S.H. and Traystman R.J. (1993) Effect of nitric oxide synthase inhibition on cerebral blood flow and injury volume during focal ischemia in cats. *Stroke* **24**, 1718-1724.

Noack H., Lindenau J., Rothe F., Asayama K. and G. Wolf G. (1998) 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**, 285–297.

Novelli A., Reilly J.A., Lysko P.G. and Henneberry R.C. (1998) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular levels are reduced. *Brain Res.* **452**, 205-212.

Obrenovitch T.P. (2001) Quinolinic acid accumulation during neuroinflammation. Does it imply excitotoxicity? *Ann. NY Acad. Sci*. **939**, 1-10.

Obrenovitch T.P. and Urenjak J. (2003) Accumulation of quinolinic acid with neuroinflammation: does it mean excitotoxicity? *Adv. Exp. Med. Biol*. **527**, 147-154.

O'Gorman E., Beutner G., Wallimann T. and Brdiczka D. (1996) Differential effects of creatine depletion on the regulation of enzyme activities and on creatine-stimulated mitochondrial respiration in skeletal muscle, heart, and brain. *Biochim. Biophys. Acta* **1276**, 161-170.

O'Gorman E., Beutner G., Dolder M., Koretsky A.P., Brdiczka D. and Wallimann T. (1997) The role of creatine kinase in inhibition of mitochondrial permeability transition. *FEBS Lett.* **414,** 253–257.

Paradies G., Petrosillo G., Pistolese M. and Ruggiero F.M. (2001) Reactive oxygen species generated by the mitochondrial respiratory chain affect the complex III activity via cardiolipin peroxidation in beef-heart submitochondrial particles. *Mitochondrion* **1**, 151-159.

Paxinos G. and Watson C. (1986) *The rat brain in stereotaxic coordinates* (2nd edition), Academic Press, San Diego.

Perez-Severiano F., Escalante B. and Rios C. (1998) Nitric oxide synthase inhibition prevents acute quinolinate-induced striatal neurotoxicity, *Neurochem. Res.* **23**, 1297–1302.

Perez-Severiano F., Rodriguez-Perez M., Pedraza-Chaverri J. *et al*. (2004) *S*-allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats, *Neurochem. Int.* **45**, 1175–1183.

Portera-Cailliau C., Price D.L. and Martin L.J. (1996) N-methyl-D-aspartate receptor proteins NR2A and NR2B are differentially distributed in the developing rat central nervous system as revealed by subunit-specific antibodies. *J. Neurochem.* **66**, 692-700.

Qin Y, Soghomonian J.-J. and Chesselet M.-F. (1992) Effects of quinolinic acid on messenger RNAs encoding somatostatin and glutamic acid decarboxylases in the striatum of adult rats. *Exp. Neurol.* **115**, 200-211.

Rios C. and Santamaria A. (1991) Quinolinic acid is a potent lipid peroxidant in rat brain homogenates, *Neurochem. Res.* **16**, 1139–1143.

Rustin P., Chretien D., Bourgeron T., Gerard B., Rotig A., Saudubray J.M. and Munnich A. (1994) Biochemical and molecular investigations in respiratory chain deficiencies. *Clin. Chim. Acta* **228**, 35-51.

Ryu J.K., Kim S.U. and McLarnon J.G. (2003) Neuroprotective effects of pyruvate in the quinolinic acid rat model of Huntington's disease. *Exp. Neurol.* **183**, 700-704.

Ryu J.K., Kim S.U. and McLarnon J.G. (2004) 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**, 150-159.

Santamaria A. and Rios C. (1993) MK-801, an N-methyl-D-aspartate receptor antagonist, blocks dquinolinic acid-induced lipid peroxidation in rat corpus striatum. *Neurosci. Lett.* **159,** 51-54.

Santamaria A., Jiménez-Capdeville M.E., Camacho A., Rodríguez-Martínez E., Flores A. and Galván-Arzate, S. (2001) *In vivo* hydroxyl radical formation after quinolinic acid infusion into rat corups striatum. *Neuroreport* **12**, 2693-2696.

Schmidt W., Wolf G., Calka J. and Schmidt H.H. (1995) Evidence for bidirectional changes in nitric oxide synthase activity in rat striatum after excitotoxically (quinolinic acid) induced degeneration. *Neuroscience* **67**, 345-356.

Schwarcz R., Whetsell Jr. W.O. and Mangano R.M. (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* **219**, 316-318.

Schwarcz R., Foster A.C., French E.D., Whetsell Jr. W.O. and Kohler, C. (1984) Excitotoxic models for neurodegenerative disorders. *Life Sci*. **35**, 19-32.

Shepherd D. and Garland P.B., (1973) Citrate synthase from rat liver. *Methods Enzymol.* **13**, 11-16.

Stachowiak O., Dolder M., Wallimann T. and Richter C. (1998) Mitochondrial creatine kinase is a prime target of peroxynitrite-induced modification and inactivation. *J. Biol. Chem*. **273**, 16694-16699.

Stone T.W., (1993) Neuropharmacology of quinolinic and kynurenic acids. *Pharmacol. Rev.,* 45, 309-379, 1993

Stone T.W. (2001) Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. *Prog Neurobiol*, **64** 185-218.

Stout A.K., Raphael H.M., Kanterewicz B.I., Klann E. and Reynolds I.J. (1998) Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nat. Neurosci.* **1**, 366–373.

Susel Z., Engber T.M and Chase T.N. (1989) Behavioral evaluation of the anti-excitotoxic properties of MK-801: comparison with neurochemical measurements. *Neurosci. Lett*. **104**, 125-129.

Tabrizi S.J., Cleeter M.W., Xuereb J., Taanman J.W., Cooper J.M. and Schapira A.H. (1999) Biochemical abnormalities and excitotoxicity in Huntington's disease brain. *Ann. Neurol*. **45**, 25-32.

Tappel A.L. (1973) Lipid peroxidation damage to cell components. *Fed. Proc.* **32**, 1870-1874.

Tavares R.G., Tasca C.I., Santos C.E., Wajner M., Souza D.O. and Dutra-Filho C.S. (2000) Quinolinic acid inhibits glutamate uptake into synaptic vesicles from rat brain. *Neuroreport* **11**, 249-253.

Tavares R.G., Tasca C.I., Santos C.E., Alves L.B., Porciuncula L.O., Emanuelli T. and Souza D.O. (2002) Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochem. Int*. **40,** 621-627.

Tejero-Taldo M.I., Caffrey J.L., Sun J. and Mallet R.T. (1999) Antioxidant properties of pyruvate mediate its potentiation of beta- adrenergic inotropism in stunned myocardium. *J. Mol. Cell. Cardiol*. **31**, 1863-1872.

Tsacopoulos M. and Magistretti P.J. (1996) Metabolic coupling between glia and neurons. *J. Neurosci.* **16**, 877–885.

Tsuji K., Nakamura Y., Ogata T., Shibata T. and Kataoka K. (1994) Rapid decrease in ATP content without recovery phase during glutamate-induced cell death in cultured spinal neurons. *Brain Res.* **662**, 289–292.

Vasques V. de C., Brinco F. and Wajner M. (2005) Intrahippocampal administration of the branched-chain alpha-hydroxy acids accumulating in maple syrup urine disease compromises rat performance in aversive and non-aversive behavioral tasks. *J. Neurol. Sci*. **232**, 11-21.

Wallimann T. and Hemmer W. (1994) Creatine kinase in non-muscle tissues and cells. *Mol. Cell. Biochem*. **133-134**, 193-220.

Wendt S., Schlattner U. and Wallimann T. (2003) Differential effects of peroxynitrite on human mitochondrial creatine kinase isoenzymes. Inactivation, octamer destabilization, and identification of involved residues. *J. Biol. Chem.* **278**, 1125-1130.

Wenzel A., Fritschy J.M., Mohler H. and Benke D. (1997) NMDA receptor heterogeneity during postnatal development of the rat brain: differential expression of the NR2A, NR2B, and NR2C subunit proteins, *J. Neurochem.* **68**, 469-478.

Whetsell Jr. W.O. and Schwarcz R. (1989) Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. *Neurosci. Lett*. **97**, 271-275.

Widner B., Leblhuber F., Walli J., Tilz G.P., Demel U. and Fuchs D. (1999) Degradation of tryptophan in neurodegenerative disorders. *Adv. Exp. Med. Biol*. **467**, 133-138.

Widner B., Leblhuber F., Walli J., Tilz G.P., Demel U. and Fuchs D. (2000) Tryptophan degradation and immune activation in Alzheimer's disease. *J. Neural Transm.* **107**, 343-353.

Woodruff G.N., Foster A.C., Gill R., Kemp J.A., Wong E.H. and Iversen L.L. (1987) The interaction between MK-801 and receptors for N-methyl-D-aspartate: functional consequences. *Neuropharmacology* **26,** 903-909.

Wyss M. and Kaddurah-Daouk R. (2000) Creatine and creatinine metabolism. *Physiol. Rev.* 80, 1107-1213.

Xu C.J, Klunk W.E., Kanfer J.N., Xiong Q., Miller G. and Pettegrew J.W. (1996) Phosphocreatine-dependent glutamate uptake by synaptic vesicles. *J. Biol. Chem*. **271**, 13435-13440.

Zeevalk G.D. and Nicklas W.J. (1989) Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition, *J. Pharm. Exp. Ther*. **257**, 870-878.

Zhang Y., Marcillat O., Giulivi C., Ernster L. and Davies K.J. (1990) The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J. Biol. Chem*. **265**, 16330-16336.

Zhu S., Li M., Figueroa B.E. *et al*. (2004) Prophylactic creatine administration mediates neuroprotection in cerebral ischemia in mice. *J. Neurosci*. **24**, 5909-5912.

Table 1: Respiratory chain complex activities in striatum homogenates (complexes II, II-III, III and IV) or in striatum mitochondrial preparations (complex I) 3 or 6 hours after intrastriatal administration of 150 nmol QA.

	Time after intrastriatal injection			
	3 h		6h	
	NaCl	QA	NaCl	QA
Complex I	257.4 ± 24.3 (n = 3)	305.8 ± 49.1 (n = 3)	261.2 ± 13.9 (n = 3)	242.1 ± 14.7 (n = 3)
Complex II	4.59 ± 0.58 (n = 4)	4.35 ± 0.29 (n = 4)	5.30 ± 0.40 (n = 5)	5.64 ± 0.68 (n = 4)
Complex II- III	19.76 ± 1.89 (n = 4)	25.16 ± 1.86 (n = 4)	23.55 ± 0.80 (n = 5)	25.43 ± 3.04 (n = 4)
Complex IV	148.1 ± 36.1 (n = 5)	174.5 ± 13.8 (n = 3)	114.5 ± 15.6 (n = 5)	121.3 ± 11.7 (n = 4)

Data represent mean ± SEM for 4-6 experiments performed in duplicate and are expressed as nmol/min mg protein. Activities are expressed as $nmol.min^{-1}.mg\ protein^{-1}$ No significant differences were found between the various groups (Student´s *t* test for unpaired samples).

Table 2: *In vitro* effect of QA (100 μM) on the respiratory chain complexes and creatine kinase activities in striatum homogenates of 30-day-old Wistar rats.

	Control	100 μM QA
Complex II	4.31 ± 0.49 (n = 4)	4.56 ± 0.30 (n = 4)
Complex II- III	24.24 ± 1.08 (n = 6)	22.53 ± 0.56 (n = 6)
Complex IV	125.5 ± 11.6 (n = 4)	120.9 ± 8.75 (n = 4)
Creatine Kinase	6.02 ± 0.67 (n = 5)	5.99 ± 0.42 (n = 5)

Data represent mean ± SEM for 4-6 experiments performed in duplicate and are expressed as nmol.min^{-1}.mg protein^{-1} for complexes II, II-III and IV and as μmol creatine.min^{-1}.mg protein^{-1} for creatine kinase. No significant differences were found between the various groups (Student´s *t* test for paired samples)

Legend to Figures

Figure 1: Respiratory chain complex activities in striatum 12 hours after intrastriatal administration of 150 nmol QA. Animals received NaCl or QA in the left striatum as described in Material and Methods. Data are represented as mean ± SEM for 3-6 independent experiments performed in duplicate and are expressed as nmol.min^{-1}.mg protein^{-1} * P < *0.05*; ** P < *0.01* compared to NaCl (Student's t test for unpaired samples).

Figure 2: Creatine kinase activity in striatum obtained at different periods (3-12 hours) after intrastriatal administration of 150 nmol QA or NaCl. Animals received NaCl or QA in the left striatum as described in Material and Methods. Data are represented as mean ± SEM for 4-8 independent experiments performed in triplicate and are expressed as μmol creatine.min^{-1}.mg protein^{-1}. ** P < *0.01* compared to NaCl (Student's t test for unpaired samples).

Figure 3: $^{14}CO_2$ production from [1-^{14}C]acetate in striatum obtained at different periods (3-12 hours) after intrastriatal administration of 150 nmol QA or NaCl. Animals received NaCl or QA in the left striatum as described in Material and Methods. Data are represented as mean ± SEM for 4-5 independent experiments performed in triplicate and are expressed as percentage of controls. * P < *0.05* compared to NaCl (Student's t test for unpaired samples).

Figure 4: Effects of α-tocopherol (40 mg/kg) plus ascorbic acid (100 mg/kg) on QA striatal energy metabolism inhibition. Animals were treated with saline or α-tocopherol plus ascorbic acid i.p. for 7 days prior to QA injection. Data are represented as mean ± SEM for

5 independent experiments (animals) performed in duplicate and are expressed as percentage of controls. * Significantly different from contralateral striatum (control) (* P < *0.05*; ** P < *0.001*). ❖❖ Significantly different from ipsilateral striatum of rats that received saline (❖ P < *0.05*, ❖❖ P < *0.01*). (Student's t test for unpaired samples).

Figure 5: Effects of nitric oxide synthase inhibitor (L-NAME, 2 mg/kg) on QA striatal energy metabolism inhibition. Animals were treated with saline or L-NAME i.p. 30 min prior to QA injection. Data are represented as mean ± SEM for 6 independent experiments (animals) performed in duplicate and are expressed as percentage of controls. * Significantly different from contralateral striatum (control) (* P < *0.05*; ** P < *0.01*). ❖ Significantly different from ipsilateral striatum of rats that received saline (❖❖ P < *0.01*). (Student's t test for unpaired samples).

Figure 6: Effects of pyruvate (500 mg/kg) on QA striatal energy metabolism inhibition. Animals were treated with saline or pyruvate i.p. 1 hour prior to QA injection. Data are represented as mean ± SEM for 4-7 independent experiments (animals) performed in duplicate and are expressed as percentage of controls. * Significantly different from contralateral striatum (control) (* P < *0.05*; ** *P < 0.01*). ❖ Significantly different from ipsilateral striatum of rats that received saline (❖ *P < 0.05*). (Student's t test for unpaired samples).

Figure 7: Effects of creatine (50 mg/kg) on QA striatal energy metabolism inhibition. Animals were treated with saline or creatine i.p. for 7 days, twice a day, prior to QA injection. Data are represented as mean ± SEM for 4-6 independent experiments (animals) performed in duplicate and are expressed as percentage of controls. * Significantly different from contralateral striatum (control) (* P < *0.05*; ** P < *0.01*). ❖ Significantly

different from ipsilateral striatum of rats that received saline (❖ P < *0.05*, ❖❖ P < *0.01*). (Student's t test for unpaired samples).

Figure 8: Effects of pretreatment with MK-801 (0.25 mg/kg) prior to QA striatal energy metabolism inhibition. Animals were treated with saline or MK-801 i.p. 30 min before QA injection. Data are represented as mean ± SEM for 5 independent experiments (animals) performed in duplicate and are expressed as percentage of controls. * Significantly different from contralateral striatum (control) (* P < *0.05*; ** P < *0.01*). ❖ Significantly different from ipsilateral striatum of rats that received saline (❖ P < *0.05*). (Student's t test for unpaired samples).

Figure 1

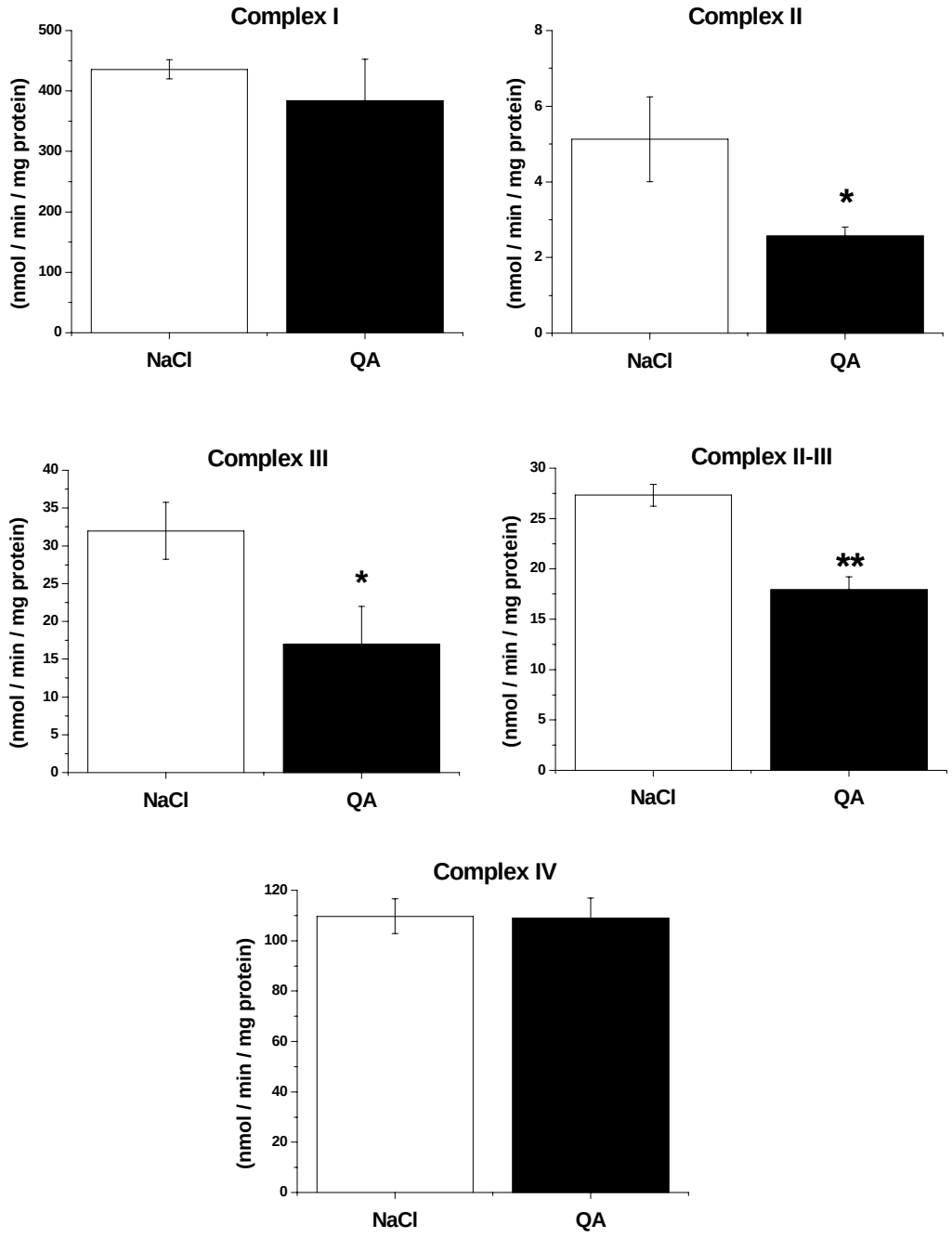

Complex I
(nmol / min / mg protein)
500
400
300
200
100
0
NaCl
QA
Complex II
(nmol / min / mg protein)
8
6
4
2
0
*
NaCl
QA
Complex III
(nmol / min / mg protein)
40
35
30
25
20
15
10
5
0
*
NaCl
QA
Complex II-III
(nmol / min / mg protein)
30
25
20
15
10
5
0
**
NaCl
QA
Complex IV
(nmol / min / mg protein)
120
100
80
60
40
20
0
NaCl
QA

Figure 2

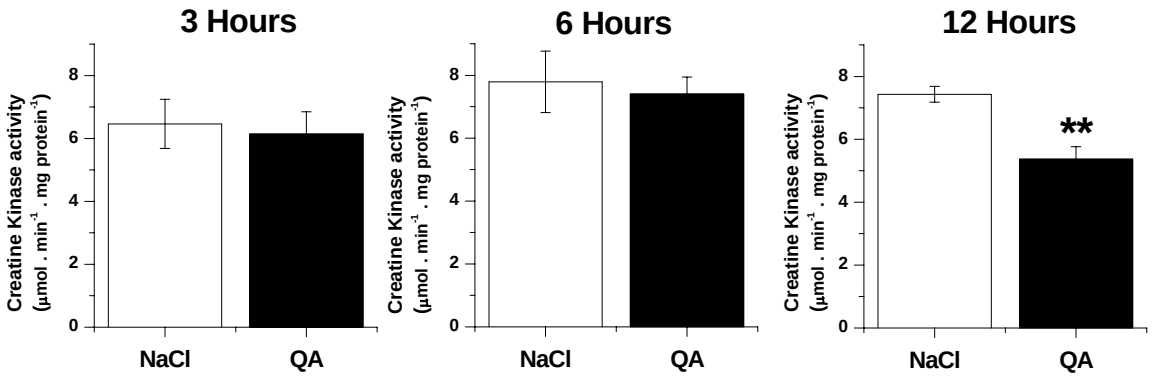

3 Hours
6 Hours
12 Hours
Creatine Kinase activity
(µmol . min-1 . mg protein-1)
0
2
4
6
8
NaCl
QA
**

Figure 3

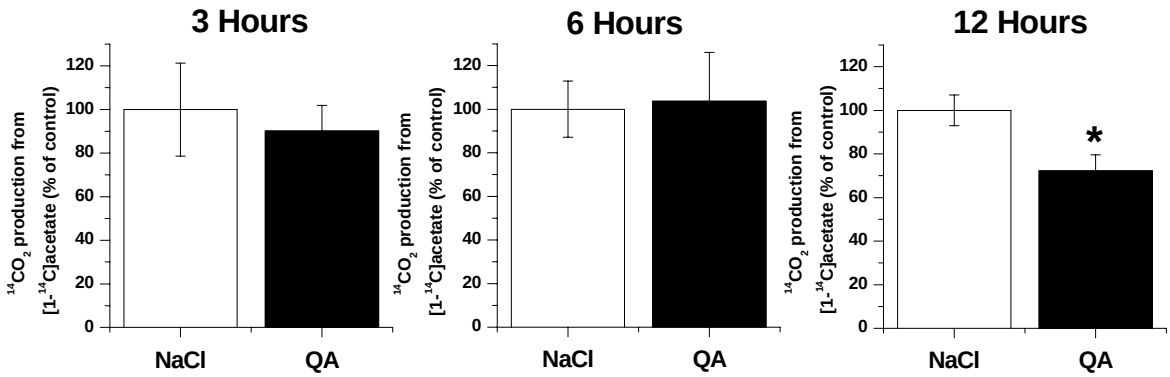

3 Hours
6 Hours
12 Hours
$^{14}CO_2$ production from [1-^{14}C]acetate (% of control)
0
20
40
60
80
100
120
NaCl
QA
*

Figure 4

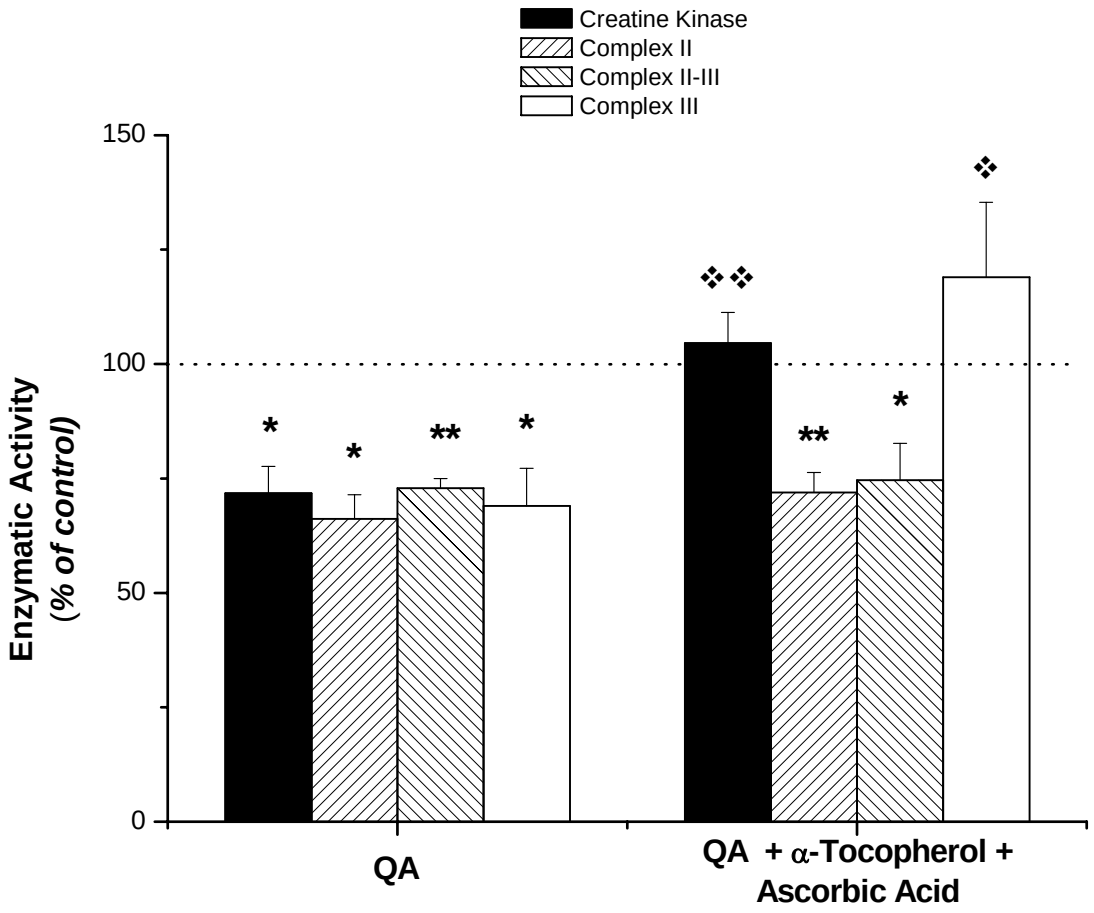

Creatine Kinase
Complex II
Complex II-III
Complex III
150
100
50
0
Enzymatic Activity
(% of control)
*
*
**
*
❖❖
**
*
❖
QA
QA + α-Tocopherol +
Ascorbic Acid

Figure 5

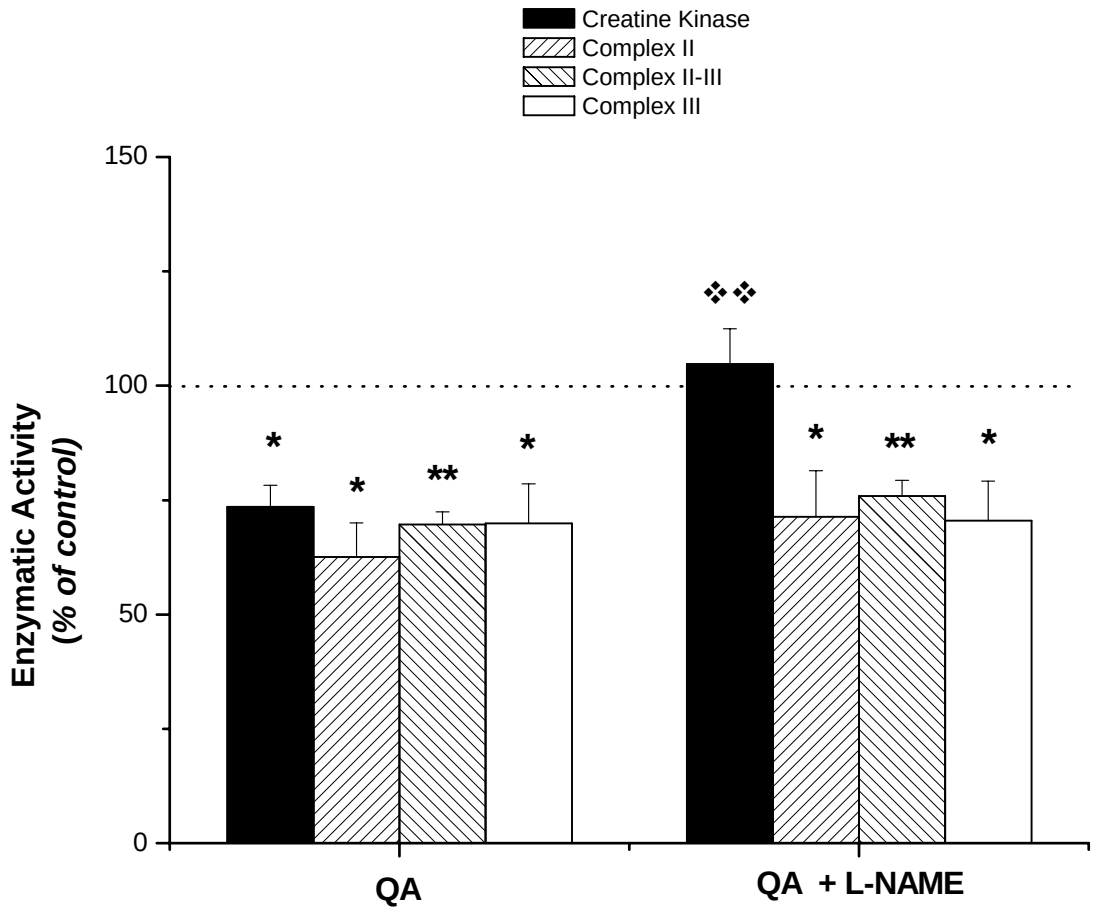

Creatine Kinase
Complex II
Complex II-III
Complex III
150
100
50
0
Enzymatic Activity
(% of control)
*
*
**
*
❖❖
*
**
*
QA
QA + L-NAME

Figure 6

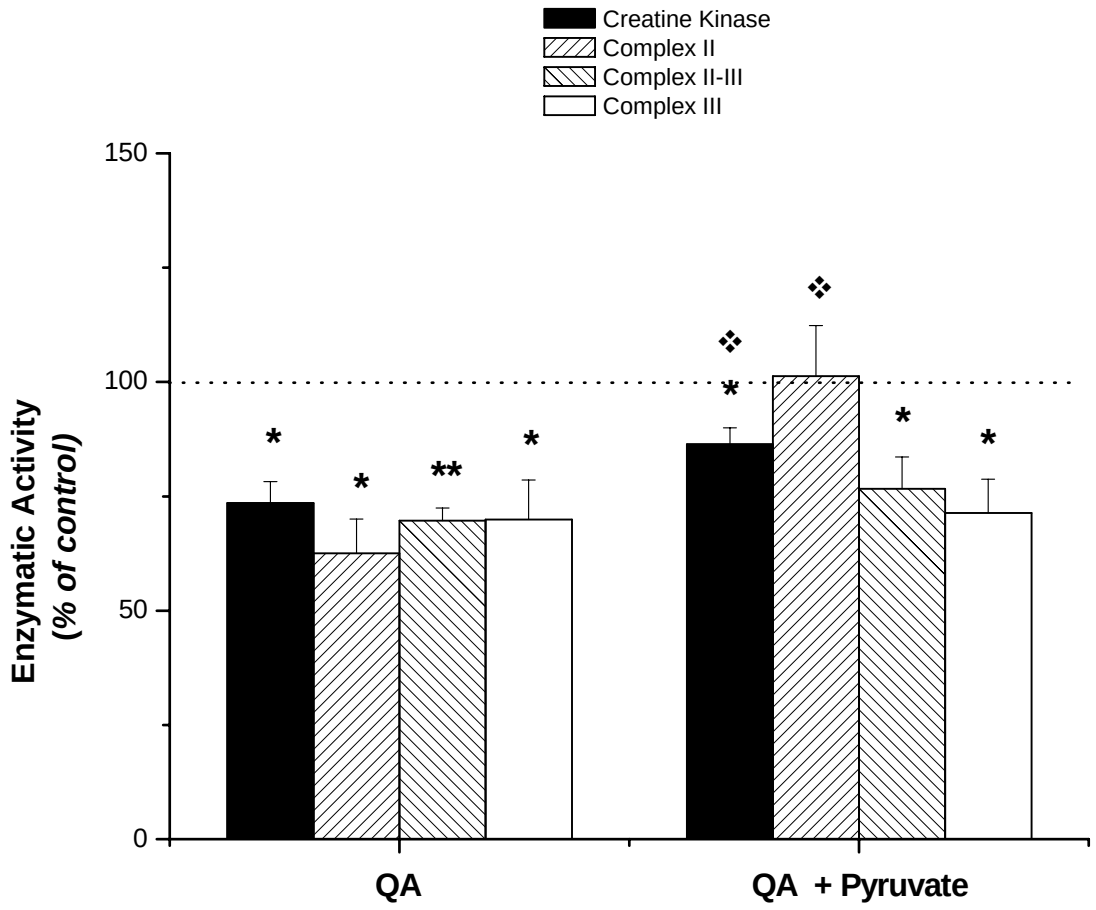

Creatine Kinase
Complex II
Complex II-III
Complex III
150
100
50
0
Enzymatic Activity
(% of control)
*
*
**
*
❖
*
❖
*
*
QA
QA + Pyruvate

Figure 7

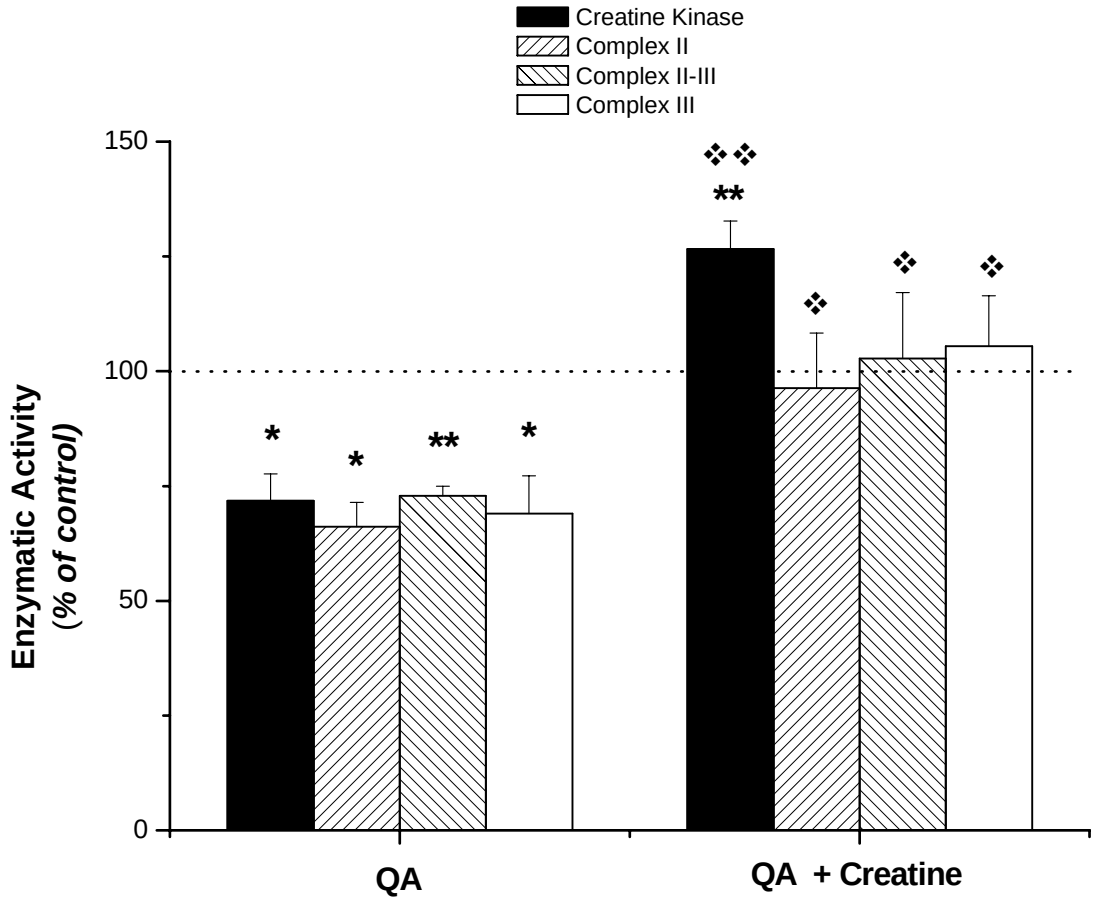
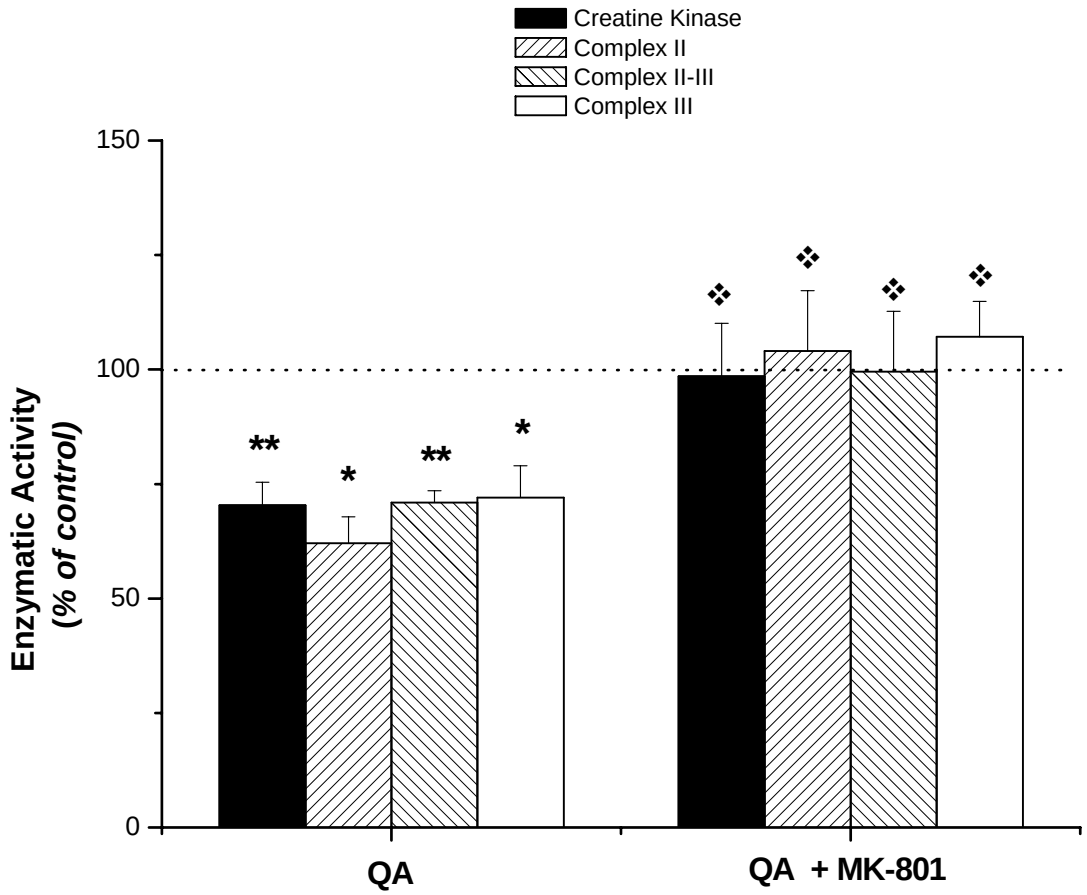

Creatine Kinase
Complex II
Complex II-III
Complex III
150
100
50
0
Enzymatic Activity
(% of control)
*
*
**
*
❖❖
**
❖
❖
❖
QA
QA + Creatine

Figure 8


Creatine Kinase
Complex II
Complex II-III
Complex III
150
100
50
0
Enzymatic Activity
(% of control)
**
*
**
*
❖
❖
❖
❖
QA
QA + MK-801

PARTE III

1- DISCUSSÃO

O ácido quinolínico (AQ) é um agonista NMDA que exerce ações excitotóxicas no sistema nervoso central (SNC) (STONE, 1993). No entanto, uma vez que o AQ é um agonista relativamente fraco desses receptores (SCHWARCZ *et al*. 1983), os mecanismos de toxicidade do AQ sobre o SNC continuam parcialmente esclarecidos. Neste particular foi recentemente relatado que, apesar das concentrações de AQ aumentarem em até 10-100 vezes em células ativadas da microglia e em macrófagos invasores do SNC durante processos neuroinflamatórios, essas quantidades de AQ ainda são insuficientes para resultar em atividade excessiva dos receptores NMDA e morte neuronal (OBRENOVICH, 2001). Assim novos experimentos são necessários para verificar porque o AQ é tão neurotóxico se todos os seus efeitos não podem ser atribuídos à superestimulação do SNC.

Por outro lado, tem sido demonstrado que o AQ induz lesões estriatais semelhantes às encontradas na doença de Huntington, uma vez que o dano produzido por esse ácido poupa os neurônios somatostatina-neuropeptídeo Y (BEAL *et al*. 1986). Daí o fato do AQ estar sendo usado como modelo animal químico da DH para que se possa estudar a patogênese dessa doença com a finalidade de se descobrir agentes terapêuticos para a mesma. Interessante também é a constatação de que as concentrações do AQ estão aumentadas postmortem no cérebro de pacientes afetados pela doença de Huntington e também pela doença de Alzheimer (STONE, 2001). Recentemente a patogênese do dano neurológico dessas patologias tem sido associada à excitotoxicidade, estresse oxidativo e distúrbio metabólico (SALINZKA *et al*. 2005, ZARKOVIC, 2003, BEAL *et al*. 2000).

A presente investigação teve por objetivo estudar o efeito da administração intraestriatal do AQ sobre vários parâmetros do metabolismo energético, com ênfase particular sobre a produção e a transferência celular de energia. Utilizamos em nossos experimentos animais jovens (30 dias de vida) visto que pouco se sabe sobre os efeitos do

AQ em animais dessa idade, sendo a maior parte dos trabalhos com o AQ realizada em ratos de 3 ou mais meses de idade.

Verificamos inicialmente que o AQ não alterou a atividade dos complexos da cadeia respiratória (fosforilação oxidativa) ou da enzima creatina quinase (CK) (transferência de energia) em estriado obtido 3 ou 6 horas após a injeção intraestriatal de AQ. No entanto, 12 horas após a injeção de AQ as atividades dos complexos II, II-III, III e da CK estavam significativamente reduzidos em 50, 35, 46 e 27%, respectivamente. Tais resultados indicam que a cadeia respiratória estava marcadamente bloqueada com reflexos negativos sobre a produção de ATP. Tendo em vista que a CK é fundamental para a transferência do ATP mitocondrial para o citosol, podemos concluir que a produção e a transferência de energia intracelular foram comprometidas pela injeção intraestriatal do AQ. Testamos também a atividade do ciclo do ácido cítrico pela medida da produção de $^{14}CO_2$ a partir de [1-^{14}C]acetato. Observamos que essa medida foi significativamente reduzida 12 horas após a infusão de AQ. Esses resultados, tomados em seu conjunto, indicam que o metabolismo energético no estriado dos animais foi marcadamente afetado pelas concentrações elevadas de AQ após a sua administração intracerebral, mas não informa se esse efeito ocorreu diretamente, ou secundariamente à estimulação de receptores NMDA e/ou à geração aumentada de espécies ativas (RIOS & SANTAMARIA, 1991; BEHAN *et al*. 1999; SANTAMARIA *et al*. 2001a,b, LEIPNITZ *et al*. 2005). O fato de que as alterações no metabolismo energético somente foram observadas após 12 horas da infusão do AQ, podem levantar a suspeita de que esse efeito foi secundário a outras alterações. Então resolvemos testar o efeito *in vitro* do AQ, na concentração de 100 μM, sobre as atividades dos complexos da cadeia respiratória e da CK. Verificamos que o AQ, na concentração de 100 μM, não foi capaz de alterar quaisquer das atividades determinadas, o que sugere que o efeito do mesmo comprometendo o metabolismo energético celular após a administração intraestriatal tenha sido secundário a outros efeitos.

Estudos anteriores demonstraram que lesões excitotóxicas via receptores NMDA estão associadas com redução dos níveis de ATP e fosfocreatina (BORDELON, 1997, MITANI, 1994). A redução da produção ou utilização de ATP pode levar à morte celular via processos dependentes de energia. Assim, o ATP é essencial para várias bombas iônicas que geram e mantêm os gradientes iônicos e elétricos nas membranas neuronais e que são essenciais para o funcionamento das células neurais. Dentre essas bombas, salientamos a bomba Na^+,K^+-ATPase que controla o potencial de membrana e também outras ATPases que regulam os níveis intracelulares de Ca^{2+}. (NOVELLI *et al*. 1988; HENEBERRY *et al*. 1989, ZEEVALK *et al*. 1991).

A redução do metabolismo energético associada com estresse oxidativo podem formar um ciclo de auto-amplificação de toxicidade celular, o que pode explicar o fato de que somente encontramos efeitos 12 horas após a injeção de AQ.

É importante enfatizar que, apesar de não termos analisado histopatologicamente as prováveis lesões provocadas pela administração intraestriatal do AQ, é provável que as alterações no metabolismo energético aqui reportadas não tenham sido devidas à diminuição do número de células neurais, visto que as atividades dos complexos I e IV da cadeia respiratória e da enzima citrato sintase não foram alteradas pela injeção de AQ. Nesse particular, enfatize-se os resultados de BORDELON e colaboradores (1994), demonstrando que não houve diminuição significativa do número de células após horas de injeções similares de AQ.

Assim, nossos dados são consistentes com achados de que as atividades dos complexos da cadeia respiratória são marcada e seletivamente reduzidos no estriado de pacientes com DH (TABRIZI *et al*. 1999, GU *et al*. 1996). Portanto, nossos resultados dão suporte para a utilização de injeção intraestriatal de AQ como modelo químico animal para indução de doenças neurodegenerativas.

Por outro lado, espécies reativas estão envolvidas em vários processos patológicos,

tais como no envelhecimento (BOKOV *et al*., 2004), na hipóxia (CHEN *et al*. 2003) e em várias doenças neurodegenerativas, incluindo a doença de Huntington (STOY *et al*. 2005). Além disso, diversos estudos demonstraram que o AQ pode induzir estresse oxidativo *in vitro* e *in vivo* (LEIPNITZ *et al*. 2005, RIOS e SANTAMARIA, 1991; SANTAMARÍA e RIOS, 1993). Assim, para avaliar o papel da geração de espécies reativas na disfunção energética estriatal encontrada 12 horas após a injeção de AQ, os animais foram pré-tratados com seqüestradores de radicais livres (α-tocoferol mais ascorbato) ou com um inibidor da enzima óxido nítrico sintase (NOS, éster metílico de N^{ω}-nitro-L-arginina, L-NAME) e os parâmetros energéticos foram avaliados.

Observamos que o pré-tratamento com α-tocoferol e ascorbato administrados simultaneamente foi capaz de prevenir a inibição da CK e do complexo III induzida pela injeção de AQ, indicando que esses efeitos inibitórios foram mediados pela geração de espécies reativas. O α-tocoferol interage com as membranas celulares, captura radicais livres e interrompe a cadeia de reações oxidativas que causam dano às células. (AMES *et al*. 1993; BURTON *et al*. 1990) Durante esse processo, α-tocoferol é convertido a radical tocoferil, necessitando de ascorbato para sua regeneração a tocoferol reduzido (FREI *et al*. 1990; McCAY, 1985; CARR & FREI, 1999). Neste contexto, enfatize-se resultados anteriores demonstrando que o complexo III da cadeia respiratória é inibido por espécies ativas de oxigênio (CARDOSO *et al*. 1999), sendo que a CK mitocondrial pode ser atacada por espécies ativas de nitrogênio e de oxigênio (GROSS *et al*. 1996; WENDT, *et al*. 2003; STACHOWIACK *et al*. 1998), sendo que essas atividades foram completamente prevenidas pelo pré-tratamento com α-tocoferol mais ascorbato.

Já o óxido nítrico (NO) está envolvido com a neurotoxicidade causada pela estimulação de receptores glutamatérgicos (DAWSON *et al*. 1991), sendo verificado que a estimulação de receptores glutamatérgicos e a conseqüente geração de NO são fatores

importantes na patogênese de várias doenças neurodegenerativas (MELDRUM & GARTHWAITE, 1990, DAWSON *et al*. 1992). Neste contexto, está bem estabelecido que a ativação de receptores NMDA resulta na ativação da oxido nítrico sintase (iNOS) pela entrada intracelular de cálcio (AYATA *et al*. 1997), o que estimula a síntese de espécies reativas de nitrogênio, como o NO e o seu derivado tóxico peroxinitrito.

Além disso, aumento na iNOS derivada de microglia e NOS neuronal também foram demonstrados em estriado de ratos após a injeção de AQ (RYU *et al*. 2004, SCHMIDT *et al*. 1995).

Nossos resultados mostraram que o pré-tratamento com L-NAME, um inibidor da NOS, foi capaz de prevenir o efeito inibitório da atividade da CK causado pela injeção de AQ, o que está de acordo com resultados prévios de que a atividade da CK é inibida por peroxinitrito (WENDT *et al*. 2003; STACHOWIACK *et al*. 1998). Por outro lado, o pré-tratamento com L-NAME não inibiu a diminuição de atividade dos vários complexos da cadeia respiratória, indicando que as espécies ativas de nitrogênio não estão envolvidas com a inibição da cadeia respiratória. Tomados os resultados em seu conjunto, é possível que a entrada de cálcio na célula secundária à superestimulação dos receptores NMDA pelo AQ possa ter induzido a produção de espécies ativas de oxigênio por parte da mitocôndria que poderiam ser responsáveis pela inibição da cadeia respiratória.

Por outro lado, se o início da cascata patológica é a depleção das reservas energéticas celulares, então agentes que mantêm os estoques energéticos celulares podem atuar como agentes neuroprotetores.

Testamos também o efeito do pré-tratamento com substratos energéticos após a injeção intraestriatal de AQ com o intuito de verificar se esses agentes, que podem ser administrados de forma oral, poderiam prevenir ou atenuar o déficit energético verificado no estriado através das medidas das atividades da cadeia respiratória e da creatina quinase. O ácido pirúvico, metabólito final da glicólise, que é um substrato energético celular

(TSACOPOULOS & MAGISTRETTI, 1996) e também apresenta atividade antioxidante (GIANDOMENICO *et al*. 1997), foi o primeiro substrato a ser testado. Estudos *in vitro* mostraram capacidade neuroprotetora do piruvato sobre morte celular mediada por ativação de receptores NMDA por estimular o metabolismo energético (MAUS *et al*. 1999). Em nosso estudo, demonstramos que o pré-tratamento com 500 mg/kg de piruvato foi capaz de prevenir completamente os efeito inibitório causado pela injeção de AQ sobre a atividade do complexo II e parcialmente sobre a atividade da CK. Nossos resultados concordam com aqueles de RYU e colaboradores (2003, 2004), que observaram efeitos neuroprotetores *in vivo* do piruvato sobre o dano estriatal induzido por AQ. É possível que esses efeitos sejam devidos à capacidade do ácido pirúvico de neutralizar os efeitos do H_2O_2 e de peróxidos lipídicos através de uma reação não-enzimática (CONSTANTOPOULOS & BARRANGER, 1984; DEBOER *et al*. 1993; CRESTANELLO *et al*. 1995), bem como de aumentar as concentrações dos intermediários do ciclo do ácido cítrico (SUNDQVIST *et al*. 1989; RUSSELL & TAEGTMEYER, 1991). Uma vez elevado, o citrato inibe a enzima fosfofrutoquinase (GARLAND *et al*. 1963) e direciona o fluxo glicolítico para a via da hexose monofosfato, a principal fonte de NADPH necessária para regenerar glutationa reduzida (HILTUNEN & DAVIS, 1981). O aumento do fluxo nessa via aumenta a razão NADPH/$NADP^+$ servindo para direcionar a reação da glutationa redutase para a formação de glutationa reduzida, o principal agente antioxidante do SNC. TEJERO-TALDO e colaboradores (1999) confirmaram essa hipótese em um modelo de isquemia/reperfusão cardíaca seguida de tratamento com piruvato, onde encontraram aumento dos níveis de citrato, NADPH e GSH. Embora não tenhamos medido as concentrações de GSH após a administração de piruvato em nosso modelo, e se o piruvato de fato aumenta os níveis de GSH em nosso modelo experimental, isso concordará com os resultados de CARDOSO e colaboradores (1999), que demonstraram que GSH é capaz de proteger a inibição induzida por estresse oxidativo na atividade do

complexo II.

Tendo em vista que estudos prévios demonstraram que GSH é capaz de proteger a inibição da atividade do complexo II induzida por estresse oxidativo (CARDOSO *et al*. 1999), é possível que em nosso estudo o aumento de GSH provocado por piruvato tenha prevenido o efeito inibitório do AQ sobre o complexo II da cadeia respiratória. Além disso, considerando outros resultados, demonstrando que o tratamento com piruvato diminui em aproximadamente 50% a expressão da iNOS em astrócitos, podemos presumir que essa ação possa estar envolvida na prevenção parcial da inibição da atividade da CK provocada pelo piruvato, que demonstramos ser provavelmente devida à formação de NO ou seus metabólitos.

Por outro lado, considerando a gama crescente de evidências indicando efeitos neuroprotetores da creatina sobre a toxicidade induzida por NMDA e malonato *in vivo* (MALCON *et al*. 2000), por glutamato e peptídeo β-amilóide em neurônios hipocampais cultivados (BREWER & WALLIMANN, 2000), e por 3-nitropropionato tanto *in vivo* (MATTHEWS *et al*. 1998) quanto em culturas de neurônios estriatais e hipocampais (BRUSTOVETSKY *et al*. 2001), testamos também o efeito do pré-tratamento com creatina sobre o déficit energético provocado pela administração intraestriatal de AQ. Verificamos que o pré-tratamento com creatina completamente preveniu os efeitos inibitórios sobre o metabolismo energético induzidos pela injeção de AQ. Convém enfatizar que a creatina é um excelente estimulante da respiração mitocondrial, resultando na formação de fosfocreatina (PCr, KERNEC *et al*. 1996; O'GORMAN *et al*. 1996). Além disso, o aumento na relação PCr/ATP estimula as bombas iônicas, especialmente as Ca^{2+}-ATPases do retículo sarcoplasmático-endoplasmático que têm alta demanda energética (WALLIMANN & HEMMER, 1994), necessárias para manter a homeostase iônica, excitabilidade e sinalização de Ca^{2+}. Essas bombas estão acopladas à CK e necessitam uma alta relação local de ATP/ADP para função eficiente. É sabido também que a creatina

aumenta o estado bioenergético celular de uma maneira geral, tornando a célula mais resistente à injúria (ZHU *et al*. 2004).

A capacidade da PCr de aumentar o estado energético celular e, portanto, de estimular a captação sináptica de glutamato, reduzindo a concentração extracelular de glutamato, também pode ser considerado como um fator importante nos efeitos neuroprotetores da creatina (XU *et al*. 1996). Neste particular, foi verificado em nosso laboratório que o AQ estimula a liberação sinaptossomal de glutamato e inibe a captação astrocitária deste neurotransmissor *in vitro* potencialmente levando à excitotoxicidade (TAVARES *et al*. 2002). A excitotoxicidade, por sua vez, é acompanhada por um aumento do influxo intracelular de Ca^{2+} (KHODOROV *et al*. 1996; STOUT *et al*. 1998), levando a um ciclo vicioso por aumento crônico de Ca^{2+} e posterior deterioração do estado energético celular, no caso das reservas energéticas serem insuficientes para o bombeamento de Ca^{2+} (BREWER & WALLIMANN, 2000).

Foi também demonstrado que a exposição por longo tempo com Cr induz a transcrição da CK citosólica (KUZHIKANDATHIL & MOLLOY, 1994). Esse fato, associado com o aumento da demanda energética induzida pela injeção de AQ, pode explicar o aumento das atividades do complexo IV e da CK observados no estriado dos ratos pré-tratados com creatina e que receberam injeção intraestriatal de AQ. Esse resultado não é devido ao aumento da quantidade de mitocôndrias visto que a atividade da citrato sintase permaneceu inalterada.

Além das observações acima referidas quanto às propriedades neuroprotetoras *in vivo* e *in vitro* da creatina, também se tem constatado a estabilização do poro de transição de permeabilidade mitocondrial pela conformação octamérica da CK (O'GORMAN *et al*. 1997, WYSS & KADDURAH-DAOUK, 2000). Assim, alguns dos efeitos da creatina podem ser devidos à estabilização e proteção do poro de transição de permeabilidade e, por conseguinte, da integridade mitocondrial (BRDICZKA *et al*. 1998).

Como o AQ é um agonista de receptores NMDA, resolvemos finalmente testar o efeito do bloqueio desses receptores sobre o déficit energético causado pelo AQ. Neste particular, o MK-801 é um antagonista de receptores NMDA não-competitivo que foi descoberto na triagem de compostos com atividade anticonvulsivante (WONG *et al*. 1986), bloqueando o influxo de cálcio mediado por NMDA (MURPHY *et al*. 1987). No presente estudo, verificamos que o pré-tratamento sistêmico com MK-801 foi capaz de bloquear completamente a toxicidade e todas as alterações do metabolismo energético induzidas pela injeção de AQ. Esses achados estão de acordo com os achados de FOSTER e colaboradores (1987 a,b), em que o MK-801 foi capaz de bloquear a degeneração do estriado e do hipocampo induzida por NMDA, bem como a neurotoxicidade induzida por AQ em estriado de ratos (WOODRUFF *et al*. 1987).

Tomadas essas observações em seu conjunto e aliadas aos resultados do presente trabalho, podemos concluir que a inibição do ciclo do ácido cítrico, da cadeia respiratória e da creatina quinase observadas no presente trabalho foram provavelmente secundárias à superestimulação dos receptores NMDA por AQ. A prevenção da inibição de alguns parâmetros por seqüestradores de radicais livres ou por L-NAME deveu-se possivelmente ao impedimento da formação secundária de espécies reativas devidas à entrada de cálcio conseqüente à superestimulação de receptores NMDA pelo AQ. Enfatize-se que, quando o AQ foi adicionado (experimentos *in vitro*) ao meio de incubação, não houve nenhum efeito do mesmo sobre os parâmetros de metabolismo energético analisados, o que está de acordo com as conclusões de que o déficit energético provocado pela injeção intraestriatal *in vivo* do AQ sobre o estriado é indireto e mediado por estímulo de receptores NMDA.

Apesar das bases moleculares da toxicidade glutamatérgica ainda não estarem completamente elucidadas, há um consenso geral de que é dependente da entrada de cálcio intracelular (CHOI, 1985, 1987, 1995). Além disso, sabe-se que os receptores NMDA são importantes na mediação da neurotoxicidade glutamatérgica, possivelmente devido à sua

alta permeabilidade a cálcio (CHOI, 1988; TYMIANSKI, 1996). Nesse particular, há várias evidências demonstrando a participação de íons cálcio no processo citotóxico. Assim, o aumento do cálcio intracelular leva à ativação de várias enzimas, incluindo-se as calpainas e outras proteases, proteínas quinases, oxido nítrico sintase, calcineurinas e endonucleases. Há também aumento na produção de radicais livres, alterações na organização do citoesqueleto, e ativação de sinais genéticos levando à morte celular (SATTLER & TYMIANSKI, 2000).

2- CONCLUSÕES

Em conclusão, o presente estudo demonstra que a injeção intraestriatal de AQ resulta em uma redução marcada das atividades dos complexos da cadeia respiratória e da enzima CK no SNC de ratos jovens de maneira tempo-dependente. Além disso, nossos achados mostram que os efeitos inibitórios causados pela administração intraestriatal de AQ são totalmente dependentes da ativação de receptores NMDA. Nossos resultados também demonstram que os efeitos inibitórios nas atividades da CK e do complexo III podem ser devidos à geração de espécies reativas, já que tais efeitos inibitórios foram prevenidos quando da pré-administração de seqüestradores de espécies reativas e/ou de um inibidor da síntese de óxido nítrico. O aumento do estado energético pela pré-administração de piruvato parcialmente preveniu os efeitos inibitórios causados pela injeção de AQ, enquanto a pré-administração de creatina foi capaz de prevenir totalmente os efeitos causados pelo AQ. Visto que ações neuroprotetoras da creatina têm sido relatadas, tanto por aumentar o estado energético celular, como por sua capacidade antioxidante, e também por ser bem tolerada em seres humanos, sendo rapidamente absorvida quando administrada por via oral (BALSON *et al*. 1994, DAWSON *et al*. 1995, HAGENFELDT *et al*. 1994), nossos achados sugerem que a suplementação com creatina pode ser uma nova estratégia terapêutica em doenças neurodegenerativas em que ocorre aumento das concentrações intracerebrais do AQ ou durante processos neuroinflamatórios caracterizados quimicamente pelo aumento nas concentrações intracelulares dessa neurotoxina (BEAL, 1992).

REFERÊNCIAS BIBLIOGRÁFICAS

- AKSENOV, M., AKSENOVA, M. V, PAYNE, R. M., TROJANOVSKI, J. Q., SCHMIDT, K. L., CARNEY, J. M., BUTTERFIELD, D. A., MARKESBERY, W. R. Oxidation of cytosolic proteins and expression of creatine kinase BB in frontal lobes of Neurodegenerative disorders. *Dement. Geriatr. Cogn. Disord.* 10, 158-165, 1999.

- AKSENOV, M., AKSENOVA, M. V., BUTTERFIELD, A. D., MARKESBERY, W. R. Oxidative modification of creatine kinase BB in Alzheimer's disease brain. *J. Neurochem.* 74, 2520-2527, 2000.

- ALLEGRI, G.; COSTA, C. V. L.; BERTAZZO, A., BISAIOLO, M., RAGAZZI, E. Enzyme activities of tryptophan metabolism along the kynurenine pathway in various species of animals. *Il Farmaco* **58**, 829-836, 2003

- AMES, B.N., SHIGENAGA, M.K., HAGEN, T.M., Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7915–7922, 1993.

- AYATA C., AYATA G., HARA H., MATTHEWS, R.T., BEAL, M.F., FERRANTE, R.J., ENDRES, M., KIM, A., CHRISTIE, R.H., WAEBER, C., HUANG, P.L., HYMAN, B.T., MOSKOWITZ, M.A. Mechanisms of reduced striatal NMDA excitotoxicity in type I nitric oxide synthase knock-out mice. *J. Neurosci.* **17**, 6908-6917, 1997.

- BABCOCK, G.T., WIKSTROM, M. Oxygen activation and the conservation of energy in cell respiration. *Nature* **356**, 301-309 1992.

- BALSOM, P.D., SODERLUND, K., EKBLOM, B. Creatine in humans with special reference to creatine supplementation. *Sports Med.* **18**, 268-280, 1994.

- BARAN, H.; JELLIGER, K.; DEECKE, L. Kynurenine metabolism in Alzheimer's disease. *J. Neural. Trasm.* **106**, 165-181, 1999.

- BEAL, M. F. Aging, energy, and oxidative stress in neurodegenerative disease. *Ann. Neurol.* **38**, 357-366, 1995.

- BEAL, M. F.; HYMAN, B. T.; KOROSHETZ, W. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? *Trends Neurosci.* **16**, 125-131, 1993b.

- BEAL, M. F.; KOWALL, N. W.; ELLISON, D. W.; MAZUREK, M.F.; SWARTZ, K.J.; MARTIN, J.B. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* **321**, 168-171, 1986.

- BEAL, M.F. Energetics in the pathogenesis of neurodegenerative diseases. *Trends Neurosci*. **23**, 298-304, 2000.

- BEAL, M.F.; BROUILLET, E.; JENKINS. B.; HENSHAW, R.; ROSEN, B.; HYMAN, B.T. Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. *J. Neurochem.* **61**, 1147-1150, 1993a.

- BEHAN W.M., MCDONALD M., DARLINGTON L.G., STONE T.W. Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl. *Br. J. Pharmacol.* **128**, 1754-1760, 1999.

- BESSMAN, S. P., CARPENTER, C. L. The creatine–creatine phosphate energy shuttle. *Ann. Rev. Biochem.* 54: 831-862, 1985.

- BOKOV, A., CHAUDHURI, A., RICHARDSON, A. The role of oxidative damage and stress in aging. *Mech. Ageing Dev.* **125**, 811-826, 2004.

- BORDELON Y., MACKENZIE L., MCLAUGHLIN B.-A. CHESSELET M.-F. Excitotoxic lesions of rat striatum: time course of DNA damage assessed by in situ nick translation. *Soc. Neurosci*., Abstr., **20**, 988, 1994.

- BORDELON, Y.M., CHESSELET, M.F., NELSON, D., WELSH, F., ERECINSKA, M. Energetic dysfunction in quinolinic acid-lesioned rat striatum. *J. Neurochem*. **69**, 1629-1639, 1997.

- BOWLING, A.C.; BEAL, M.F. Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sci.* **56**, 1151-1171, 1995.

- BRDICZKA, D., BEUTNER, G., RUCK, A., DOLDER, M., WALLIMANN, T. The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. *Biofactors* **8**, 235-242, 1998.

- BRENNAN, W. A.; BIRD, E. D.; APRILLE, J. R. Regional mitochondrial respiratory activity in Huntington's disease brain. *J. Neurochem.* **44**,. 1948-1950, 1985.
- BREWER, G.J., WALLIMANN, T.W. Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. *J. Neurochem*. **74**, 1968-1978, 2000.

- BROWNE, S.E., BEAL, M.F. The energetics of Huntington's disease. *Neurochem. Res.* **29**, 531-546, 2004.

- BROWNE, S.E., BOWLING, A.C., MacGARVEY, U., BAIK, M.J., BERGER, S.C., MUQIT, M.M., BIRD, E.D., BEAL, M.F. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann. Neurol.* **41**, 646-653, 1997.

- BRUSTOVETSKY, N., BRUSTOVETSKY, T., DUBINSKY, J.M. On the mechanisms of neuroprotection by creatine and phosphocreatine. *J. Neurochem.* **76**, 425-434, 2001.

- BURTON, G.W., WRONSKA, U., STONE, L., FOSTER, D.O., INGOLD, K.U. Biokinetics of dietary *RRR*-α-tocopherol in the male guinea pig at three dietary levels of Vitamin C and two levels of Vitamin E. *Lipids* **25**, 199–210, 1990.

- CARDOSO, S.M., PEREIRA, C., OLIVEIRA, R. Mitochondrial function is differentially affected upon oxidative stress. *Free Radic. Biol. Med.* **26**, 3-13, 1999.

- CARR, A., FREI, B. Does Vitamin C act as a pro-oxidant under physiological conditions?. *FASEB J.* **13**, 1007–1024, 1999.

- CHEN, Q., VAZQUEZ, E.J., MOGHADDAS, S., HOPPEL, C.L., LESNEFSKY, E.J. Production of reactive oxygen species by mitochondria: central role of complex III. *J. Biol. Chem.* **278**, 36027-36031, 2003.

- CHOI, D.W. Calcium: still center-stage in hypoxic-ischemic neuronal death. *Trends Neurosci.* **18**, 58-60, 1995.

- CHOI, D.W. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci.* **11**, 465-469, 1988.

- CHOI, D.W. Glutamate neurotoxicity in cortical cell cultures is calcium dependent. *Neurosci. Lett.* **58**, 293-297, 1985.

- CHOI, D.W. Ionic dependence of glutamate neurotoxicity. *J. Neurosci.* **7**, 369-379, 1987.

- CLARK, J.B., BATES, T.E., CULLINGFORD, T, Land, J.M. Development of enzymes of energy metabolism in the neonatal mammalian brain. *Dev. Neurosci-Basel.* **17**, 174-180, 1993.

- CONNICK, J.H., STONE, T.W. Quinolinic acid effects on amino acid release from the rat cerebral cortex in vitro and in vivo. *Br. J. Pharmacol.* **93**, 868-876, 1988.

- CONNICK, J.H., STONE, T.W. The effect of kainic, quinolinic and beta-kainic acids on the release of endogenous amino acids from rat brain slices. *Biochem. Pharmacol.* **35**, 3631-3635, 1986.

- CONSTANTOPOULOS, G., BARRANGER, J.A. Non enzymatic decarboxylation of pyruvate. *Anal. Biochem.* **139**, 353–358, 1984.

- CRESTANELLO, J.A., KAMELGARD, J., WHITMAN, G.J.R. The cumulative nature of pyruvate's dual mechanism for myocardial protection. *J. Surg. Res.* **59,** 198–204, 1995.

- CURZON, G. Brain tryptophan. Normal and disturbed control. *Adv. Exp. Med. Biol.* **398**, p. 27-34, 1996.

- DAVID, S.S., SHOEMAKER, M., HALEY, B.E. Abnormal properties of creatine kinase in Alzheimer's disease brain: correlation of reduced enzyme activity and active site photolabelling with aberrant cytosol-membrane partitioning. *Mol. Brain Res.* **54**, 276-287, 1998.

- DAVIS, J.N.; HUNNICUTT, J.C. Jr.; CHISHLOM, A. A mitochondrial bottleneck hypothesis of Alzheimer's disease. *Mol. Med. Today* **1**, 240-247, 1995.

- DAWSON, B., CUTLER, M., MOODY, A., LAWRENCE, S., GOODMAN, C., RANDALL, N. Effects of oral creatine loading on single and repeated maximal short sprints. *Aust. J. Sci. Med. Sport*. **27**, 56-61, 1995.

- DAWSON, T.M., DAWSON, V.L., SNYDER, S.H. A novel neuronal messenger molecule in brain: the free radical, nitric oxide. *Ann. Neurol*. **32**, 297-311, 1992.

- DAWSON, V.L., DAWSON, T.M., LONDON, E.D., BREDT, D.S., SNYDER S.H. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl Acad. Sci. USA* **88**, 6368-6371, 1991.

- DeBOER, L.W.V., BEKX, P.A., HAN, L., STEINKE, L. Pyruvate enhances recovery of rat hearts after ischemia and reperfusion by preventing free radical generation. *Am. J. Physiol.* **265**, H1571–H1576, 1993.

- DICKINSON, C.J. Cerebral oxidative metabolism in hypertension. *Clin. Sci.* **91**, 539-550, 1996.

- ERECINSKA, M.; SILVER, I. A. Íons and energy in mammalian brain. *Prog. Neurobiol.* **43**, 37-71, 1994

- FERRANTE, R.J., KOWALL, N.W., CIPOLLONI, P.B., STOREY, E., BEAL, M.F., Excitotoxin lesions in primates as a model for Huntington's disease: histophatologic and neurochemical characterization. *Exp. Neurol.* **119**, 46-71, 1993.

- FOSTER, A.C., COLLINS, J.F., SCHWARCZ, R., On the excitotoxic properties of quinolinic acid, 2,3-piridine dicarboxylic acid and structurally related compounds. *Neuropharmacology* **22**, 1331-1342, 1983.

- FOSTER, A.C., GILL, R., KEMP, J.A., WOODRUFF, G.N. Systemic administration of MK-801 prevents N-methyl-D-aspartate-induced neuronal degeneration in rat brain. *Neurosci. Lett.* **76**, 307-311, 1987a.

- FOSTER, A.C., WONG, E.H. The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br. J. Pharmacol.* **91**, 403-409, 1987b.

- FREI, B., STOCKER, R., ENGLAND, L. AMES, B.N. Ascorbate: the most effective antioxidant in the blood. *Adv. Exp. Med. Biol.* **264**, 155–163, 1990.

- GARLAND, P.B., RANDLE, P.J., NEWSHOLME, E.A. Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes and starvation. *Nature* **200**, 169–170, 1963.

- GIBSON, G. F.; SHEU, K. F. R.; BLASS, J.P., BAKER, A., CARLSON, K.C., HARDING, B., PERRINO, P. Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease. *Arch. Neurol.* **45**, 836-840, 1988.

- GRAFTON, S.T., MAZZIOTTA, J.C., PAHL, J.J., ST GEORGE-HYSLOP, P., HAINES, J.L., GUSELLA, J., HOFFMAN, J.M., BAXTER, L.R., PHELPS, M.E. Serial changes of cerebral glucose metabolism and caudate size in persons at risk for Huntington's disease. *Arch. Neurol.* **49**, 1161-1167, 1992.

- GROSS, W. L., BAK, M. I., INGWALL, J. S., ARSTALL, M. A., SMITH, T. W., BALLIGAND, J. L., KELLY, R. Nitric oxide inhibits creatine kinase and regulates rat heart contractile reserve. *Proc. Natl Acad. Sci. USA* 93: 5604-5609, 1996.

- GU, M., GASH, M.T., MANN, V.M. JAVOY-AGID F., COOPER J.M., SCHAPIRA A.H.V., Mitochondrial defect in Huntington's disease caudate nucleus. *Ann. Neurol.* **39**, 385-389, 1996.

- GU, M.; OWEN, A. D.; TOFFA, S. E. K. COOPER, J.M., DEXTER, D.T., JENNER, P., MARSDEN, C.D., SCHAPIRA, A.H. Mitochondrial function, GSH and iron in neurodegenerative and Lewy body diseases. *J. Neurol. Sci.* **158**, 24-29, 1998.

- GUILLEMIN, G., CROITORU, J., DORMONT, D. et al. Involvement of quinolinic acid in chemokine production and chemokine receptor expression in astrocytes. *J. Neurovirol.* **6**, 242, 2000.

- GUILLEMIN, G., CROITORU, J., SMITH, D. G. et al. Quinolinic acid up-regulates chemokine production and chemokine recptor expression in astrocytes: implications for brain inflammation and AIDS. *J. Neuroimmunol.* **118**, 119, 2001a.

- GUILLEMIN, G. J., SMITH, D.G., WILLIAMS, K. et al. β-Amyloid peptide 1-42 induces human macrophages to produce the neurotoxin quinolinic acid. *J. Neuroimmunol.* **118**, 336, 2001b.

- HAGENFELDT, L., von DOBELN, U., SOLDERS, G., KAIJSER, L. Creatine treatment in MELAS. *Muscle Nerve.* **17**, 1236-1237, 1994.

- HAGLUND, M.M., STAHL, W.L., KUNKEL, D.D., SCHWARTZKROIN, P.A. Developmental and regional differences in the localization of Na^+,K^+-ATPase activity in the rabbit hippocampus. *Brain Res*. **343**, 198-203, 1985.

- HAMMAN, B.L., BITTL, J.A., JACOBUS, W.E., ALLEN, P.D., SPENCER, R.S., TIAN, R., INGWALL, J.S. Inhibition of the creatine kinase reaction decreases the contractile reserve of isolated rat hearts. *Am. J. Physiol.* **269**, H1030–H1036, 1995.

- HAYAISHI, O. My life with tryptophan – never a dull moment. In: Ishiguro, I. Et al. (Eds.) *Advances in tryptophan research.* Toyoake: Fujita Health University Press, p. 3-16, 1992.

- HAYDEN, M. R.; MARTIN, W. R. W.; STOESSL, A. J. et al. Positron emission tomography in the early diagnosis of Huntington's disease. *Neurology* **36**, 888-894, 1986.

- HENNEBERRY, R.L., NOVELLI, A., COX, J.A., LYSKO, P.G. Neurotoxicity at the N-methyl-D-aspartate recetor in energy-compromised neurons: An hypothesis for cell death in aging and disease. *Ann. NY Acad. Sci.* **568**, 225-233, 1989.

- HEYES, M.P. Hypothesis: a role for quinolinic acid in the neuropathology of glutaric aciduria type I. *Can. J. Neurol. Sci.* **14**, 441–443, 1987.

- HEYES, M.R., BREW, B.J., MARTIN, A., PRICE, R.W., SALAZAR, A.M., SIDTIS, J.J., YERGEY, J.A., MOURDIAN, M.M., SADLER, A.E., KEILP, J., RUBINOW, D., MARKEY, S.P. Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurologic status. *Ann. Neurol.* **29**, 202–209, 1991.

- HEYES, M.P., SAITO, K., CROWLEY, J.S., DAVIS, L.E., DEMITRACK, M.A., DER, M., DILLING, L.A., ELIA, J., KRUESI, M.J.P., LACKNER, A., LARSEN, S.A., LEE, K., LEONARD, H.L., MARKEY, S.P., MARTIN, A., MILSTEIN, S., MOURADIAN, M.M., PRANZATELLI, M.R., QUEARRY, B.J., SALAZAR, A., SMITH, M., STRAUS, S.E., SUNDERLAND, T., SWEDO, S.E., TOURTELLOTTE, W.W. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurologic disease. *Brain* **115**, 1249–1273, 1992.

- HEYES, M.P.; ACHIM, C. L.; WILEY, C. A., MAJOR, E.O., SAITO, K., MARKEY, S.P. Human microglia convert L-tryptophan into the neurotoxin quinolinic acid. *Biochem. J.* **320**, 595-597, 1996.

- HILTUNEN, J.K., DAVIS, E.J. The disposition of citric acid cycle intermediates by isolated rat mitochondria. *Biochim. Biophys. Acta* **678**, 115–121, 1981.

- JAGUST, W. J.; FRIEDLAND, R. P.; BUDINGER, T. F., KOSS, E., OBER, B. Longitudinal studies of regional cerebral metabolism in Alzheimer's disease. *Neurology* **38**, 909-912, 1988.

- JANETZKY, B., HAUCK, S., YOUDIM, M.B.H., RIEDERER, P., JELLINGER, K., PANTUCEK, F., ZOCHLING, R., BOISSL, K.W., REICHMANN, H. Unaltered aconitase activity but decreased complex I activity in substantia nigra pars compacta of patients with Parkinson's disease. *Neurosci. Lett.* **169**, 126-128, 1994.

- JENKINS, B.G., KOROSHETZ, W.J., BEAL, M.F., RODEN, B.R. Evidence for impairment of energy metabolism *in vivo* in Huntington's disease using localized ^{1}H NMR spectroscopy. *Neurology.* **43**, 2689-2695, 1993.

- KENNEDY, A.M., FRACKOWIAK, R.S., NEWMAN, S.K., BLOOMFIELD, P.M., SEAWARD, J., ROQUES, P., LEWINGTON, G., CUNNINGHAM, V.J., ROSSOR, M.N. Deficits in cerebral glucose metabolism demonstrated by position emission tomography in individuals at risk of familial Alzheimer's disease. *Neurosci. Lett.* **186**, 17-20, 1995.

- KHODOROV B., PINELIS V., VERGUN O., STOROZHEVYKH T., VINSKAYA N. Mitochondrial deenergization underlies neuronal calcium overload following a prolonged glutamate challenge. *FEBS Lett.* **397** 230–234, 1996.

- KUHL, D. E.; PHELPS, M. E.; MARKHAM, C. H. METTER, E.J., RIEGE, W.H., WINTER, J. Cerebral metabolism and atrophy in Huntington's disease determined by ^{18}FDG and computed tomographic scan. *Ann. Neurol.* **12**, 425-434, 1982.

- KUWERT, T., LANGE, H.W., BOECKER, H., TITZ, H., HERZOG, H., AULICH, A., WANG, B.C., NAYAK, U., FEINENDEGEN, L.E. Striatal glucose consumption in chorea-free subjects at risk of Huntington's disease. *J. Neurol.* **241**, 31-36, 1993.

- KUZHIKANDATHIL, E.V., MOLLOY, G.R. Transcription of the brain creatine kinase gene in glial cells is modulated by cyclic AMP-dependent protein kinase. *J. Neurosci. Res.* **39** 70–82, 1994.

- LEIPNITZ, G., SCHUMACHER, C., SCUSSIATO, K., DALCIN, K.B., WANNMACHER, C.M., WYSE, A.T., DUTRA-FILHO, C.S., WAJNER, M., LATINI, A. Quinolinic acid reduces the antioxidant defenses in cerebral cortex of young rats. *Int. J. Dev. Neurosci.* **23**, 695-701. 2005.

- LIAO, R., NASCIMBEN, L., FRIEDRICH, J., GAWTHMEY, J.K., INGWALL, J.S. Decrease energy reserve in an animal model of dilated cardiomyopathy. Relationship to contractile performance. *Cir. Res.* **78**, 893-902, 1996.

- LIPTON, S.A. Neuronal injury associated with HIV-1: approaches to treatment. *Ann. Rev. Pharmacol.* **38**, pp. 159–177, 1998.

- LUDOLPH, A.C., SEELIG, M., LUDOLPH, A., NOVITT, P., ALLEN, C.N., SPENCER, P., SABRI, M.I. 3-Nitropropionic acid decreases cellular energy levels and causes neuronal degeneration in cortical explants. *Neurodegeneration* **1**, 155-161, 1992.

- MACCIONI, R.B.; MUNÕZ, P.J.; BARBEITO, L. The molecular bases of Alzheimer's disease and other neurodegenerative disorders. *Arch. Med. Res.* **32**, 367-381, 2001.

- MALCON, C., KADDURAH-DAOUK, R., BEAL, M.F. Neuroprotective effects of creatine administration against NMDA and malonate toxicity. *Brain Res.* **860**, 195-198, 2000.

- MANN, V.M., COOPER, J.M., JAVOY-AGID, F., AGID, Y., JENNER, P., SCHAPIRA, A.H. Mitochondrial function and parental sex effect in Huntington's disease. *Lancet* **336**, 749, 1990.

- MARKLUND, S. L., WESTMAN, N.G., LUNDGREN, E., ROOS, G. Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Res.* **42**, 1955-1961, 1982.

- MARKS, D. B.; MARKS, A. D.; SMITH, C. M. *Basic Medical Biochemisty, A Clinical Approach.* Lippincott Willians & Wilkins, 1996.

- MARTILLA, R. J.; ROYTTA, M.; LORENTZ, U. K. Rinne, U.K. Oxygen toxicity protecting enzymes in the human brain. *J. Neural. Tras.* **74**, 87-95, 1988.

- MARTIN, A., HEYES, M.P., SALAZAR, A.M., KAMPEN, D.L., WILLIAMS, J., LAW, W.A., COATS, M.E., MARKEY, S.P. Progressive slowing of reaction time and increasing cerebrospinal fluid concentrations of quinolinic acid in HIV-infected individuals. *J. Neuropsychiat. Clin. Neurosci.* **4**, 270–279, 1992.

- MASTROGIACOMO, F.; BERGERON, C.; KISH, S. J. Brain alpha ketoglutarate dehydrogenase complex activity in Alzheimer's disease. *J. Neurochem.* **61**, 2007-2014, 1993.

- MATTHEWS, R.T., YANG, L., JENKINS, B.G., FERRANTE, R.J., ROSEN, B.R., KADDURAH-DAOUK, R., BEAL, M.F. Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci.* **18**, 156-163, 1998.

- MAURER, I.; ZIERZ, S.; MÖLLER, H. J. A selective defect of cytochrome *c* oxidase is present in brain of Alzheimer disease patients. *Neurobiol. Aging* **21**, 455-462, 2000.

- McCAY, P.B. Vitamin E: interactions with free radical and ascorbate. *Annu. Rev. Nutr.* **5**, 323–340, 1985.

- MELDRUM, B. GARTHWAITE, J. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.* **11**, 379-387, 1990.

- MITANI, A., TAKEYASU, S., YANASE, H., NAKAMURA, Y., KATAOKA, K. Changes in intracellular Ca^{2+} and energy levels during in vitro ischemia in the gerbil hippocampal slice. *J. Neurochem*. **62**, 626-634, 1994.

- MIZUNO, Y.; MATUDA, S.; YOSHIRO, H., MORI, H., HATTORI, H., IKEGE, S. An immunohistochemical study on α-ketoglutarate dehydrogenase complex in Parkinson's disease. *Ann. Neurol.* **35**, 201-210, 1994.

- MIZUNO, Y.; SUZUKI, K.; OHTA, S. *Postmortem* changes in mitochondrial respiratory enzymes in brain and a preliminary observation in Parkinson's disease. *J. Neurol. Sci.* **96,** 49-57, 1990.

- MOFFETT, J.R., NAMBOODIRI, M.A., Tryptophan and the immune response. *Imunology and Cell Biology*, **81**, 247-265, 2003.

- NELSON, D. L.; COX, M. M. *Lehninger, Principles of Biochemistry.* 4ª ed. New York: Worth Publishers, 2004.

- NOVELLI, A., REILLY, J.A., LYSKO, P.G., HENNEBERRY, R.C. Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular levels are reduced. *Brain Res.* **452**, 205-212, 1988.

- O'GORMAN, E., BEUTNER, G., WALLIMANN, T., BRDICZKA, D. Differential effects of creatine depletion on the regulation of enzyme activities and on creatine-stimulated mitochondrial respiration in skeletal muscle. *Biochim. Biophys. Acta* **1276**, 161-170, 1996.

- OBRENOVITCH, T.P. Quinolinic acid accumulation during neuroinflammation. Does it imply excitotoxicity? *Ann. NY Acad. Sci.* **939**, 1-10, 2001.

- O'GORMAN, E., BEUTNER, G., WALLIMANN, T., BRDICZKA, D. Differential effects of creatine depletion on the regulation of enzyme activities and on creatine-stimulated mitochondrial respiration in skeletal muscle, heart, and brain. *Biochim. Biophys. Acta* **1276**, 161-170, 1996.

- O'GORMAN, E., BEUTNER, G., DOLDER, M., KORETSKY, A.P., BRDICZKA, D., WALLIMANN, T. The role of creatine kinase in inhibition of mitochondrial permeability transition. *FEBS Lett.* **414,** 253–257, 1997.

- ORTH, M., SCHAPIRA, A.H.V. Mitochondria and degenerative disorders. *Am. J. Med. Gen.* **106**, 27-36, 2001.

- PARKER, W.D. Jr. Sporadic neurologic disease and the electron transport chain: a hypothesis. In: Pascuzzi (Ed.) *Proceedings of the 1989 scientific meeting of the American Society for Neurological Investigation: new developments in neuromuscular disease***.** Bloomington: Indiana University Printing Services. **1990**, 59-64, 1989.

- PARKER, W. D.; BOYSON, S. J.; LUDER, A. S. PARKS, J.K. Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology* **40**, 1231-1234, 1990.

- PARKER, W.D. Jr.; PARKS J.K..; FILLEY, C.M., KLEINSCHMIDT-DEMASTERS, B.K. Electron transport defects in Alzheimer's disease brain. *Neurology* **44**, 1090-1096, 1994.

- PERRY, E. K.; PERRY, R. I. I.; TOMLINSON, B. E., BLESSED, G., GIBSON, P.H. Coenzyme A-acetylating enzymes in Alzheimer's disease: possible cholinergic "compartment" of pyuvate dehydrogenase**.** *Neurosci. Lett.* **18**, 105-110, 1980.

- REYNOLDS, I.J., MURPHY, S.N., MILLER, R.J. ^{3}H-labeled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. *Proc. Natl Acad. Sci. USA* **84**, 7744-7748, 1987.

- RIOS, C.; SANTAMARIA, A. Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. *Neurochem. Res.* **16**, 1139-1143, 1991.

- ROSE, C.D.; HENNEBERRY, R.C. Etiology of the neurodegenerative disorders: a critical analysis. *Neurobiol. Aging.* **15**, 233-234, 1994.

- ROSSEN, R.; KABAT, H.; ANDERSON, J. P. Acute arrest of cerebral circulation in man**.** *Arch. Neurol. Psychiatr.* **50**, 510-528, 1943.

- RUSSELL, R.R. III, TAEGTMEYER, H. Pyruvate carboxylation prevents the decline in contractile function of rat hearts oxidizing acetoacetate. *Am. J. Physiol* **261**, H1756–H1762, 1991.

- RYU, J.K., KIM, S.U., MCLARNON, J.G. 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**, 150-159, 2004.

- SAKS, V.A, KUZNETSOV, A.V., KUPRIANOV, V.V., MICELI, M.V., JACOBUS, W.E. Creatine kinase of rat heart mitochondria. The demonstration of functional coupling to oxidative phosphorylation in an inner membrane-matrix preparation. *J. Biol. Chem*. **260**, 7757-7764, 1985.

- SALINSKA, E., DANYSZ, W., LAZAREWICZ, J.W. The role of excitotoxicity in neurodegeneration. *Folia Neuropathol*, **43**, 322-339, 2005.

- SANTAMARÍA A., RIOS C. MK-801, an N-methyl-D-aspartate receptor antagonist, blocks dquinolinic acid-induced lipid peroxidation in rat corpus striatum. *Neurosci. Lett.* **159,** 51-54, 1993.

- SANTAMARIA, A., GALVAN-ARZATE, S., LISY, V., ALI, S.F., DUHART, H.M., OSORIO-RICO, L., RIOS, C., ST'ASTNY, F. Quinolinic acid induces oxidative stress in rat brain synaptosomes. *Neuroreport* **12**, 871-874, 2001a.

- SANTAMARÍA, A., JIMENEZ-CAPDEVILLE, M.E., CAMACHO, A., RODRIGUEZ-MARTINEZ, E., FLORES, A., GALVAN-ARZATE, S. *In vivo* hydroxyl formation after quinolinic acid infusion into rat corpus striatum. *Neuroreport* **12**, 2693-2696, 2001b.

- SATTLER, R., TYMIANSKI, M. Molecular mechanisms of calcium-dependent excitotoxicity. *J. Mol. Med*. **78**, 3-13, 2000.

- SCHAPIRA, A.H., COOPER, J.M., DEXTER, D., JENNER, P., CLARK, J.B., MARSDEN, C.D. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* **1**, 1269, 1989.

- SCHAPIRA, A.H., COOPER, J.M., DEXTER, D., CLARK, J.B., JENNER, P., MARSDEN, C.D. Mitochondrial complex I deficiency in Parkinson's disease. *J. Neurochem.* **54**, 823-827, 1990a.

- SCHAPIRA, A.H., MANN, V.M., COOPER, J.M., DEXTER, D., DANIEL, S.E., JENNER, P., CLARK, J.B., MARSDEN, C.D. Anatomic and disease specificity of NADH CoQ1reductase (complex I) deficiency in Parkinson's disease. *J. Neurochem.* **55**, 2142-2145, 1990b.

- SCHLEGEL, J., ZURBRIGGEN, B. , WEGMANN, G., WYSS, M., EPPENBERGER, H., WALLIMANN, T. Native mitochondrial creatine kinase forms octameric structures. I. Isolation of two interconvertible mitochondrial creatine kinase forms, dimeric and octameric mitochondrial creatine kinase: characterization, localization, and structure-function relationships. *J. Biol. Chem*. **263**, 16942-53, 1988.

- SCHNYDER, T., GROSS, H., WINKLERM H., EPPENBERGER, H. M., WALLIMANN, T. Structure of the mitochondrial creatine kinase octamer: high-resolution shadowing and image averaging of single molecules and formation of linear filaments under specific staining conditions. *J. Cell Biol.* **112**, 95-101, 1991.

- SCHWARCZ, R., FOSTER, A.C., FRENCH, E.D., WHETSELL Jr., W.O., KOHLER, C., Excitotoxic models for neurodegenerative disorders. *Life Sci.* **35**, 19-32, 1984.

- SCHWARCZ. R,, WHETSELL, W.O. Jr., MANGANO, R.M. Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* **219**, 316-318, 1983.

- SMALL, G. W.; MAZZIOTTA, J. C.; COLLINS, M. T. **et al.** Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer's disease. *JAMA* **273**, 942-947, 1995.

- SMITH, G. S.; DE LEON, M.J.; KLUGER, A. Topography of cross sectional and longitudinal glucose metabolic deficits in Alzheimer's disease: Pathophysiologic implications. *Arch. Neurol.* **49**, 1142-1150, 1992.

- STACHOWIAK, O., DOLDER, M., WALLIMANN, T., RICHTER, C.. Mitochondrial creatine kinase is a prime target of peroxynitrite-induced modification and inactivation. *J. Biol. Chem.* **273**, 16694-16699, 1998.

- STONE, T. W. Neuropharmacology of quinolinic and kynurenic acids. *Pharmacol. Rev.* **45**, 309-379, 1993.

- STONE, T.W. Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. *Prog. Neurobiol.* **64**, 185-218, 2001a.

- STONE, T.W. Endogenous neurotoxins from tryptophan. *Toxicon*, **39**, 61-73, 2001b.

- STOUT, A.K., RAPHAEL, H.M., KANTEREWICZ, B.I., KLANN, E., REYNOLDS, I.J. Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nat. Neurosci.* **1** 366–373, 1998.

- STOY, N., MACKAY, G.M., FORREST, C.M., CHRISTOFIDES, J., EGERTON, M., STONE, T.W. Darlington LG. Tryptophan metabolism and oxidative stress in patients with Huntington's disease. *J. Neurochem*. **93**, 611-623, 2005.

- SUNDQVIST, K.E., HILTUNEN, J.K., HASSINEN, I.E. Pyruvate carboxylation in the rat heart. Role of biotin-dependent enzymes. *Biochem. J.* **257**, 913-916, 1989.

- SWERDLOW, R.H., PARKS, J.K., CASSARINO, D.S., MAGUIRE, D.J., MAGUIRE, R.S., BENNETT, J.P. JR, DAVIS, R.E., PARKER, W.D. Jr. Cybrids in Alzheimer's disease: a celular model of the disease? *Neurobiology* **49**, 918-925, 1997.

- SWERDLOW, R.H., PARKS, J.K., MILLER, S.W., TUTTLE, J.B., TRIMMER, P.A., SHEEHAN, J.P., BENNETT, J.P. Jr, DAVIS, R.E., PARKER, W.D. Jr. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann. Neurol.* **40**, 663-671, 1996.

- SWERDLOW, R.H., PARKS, J.K., CASSARINO, D.S., TRIMMER, P.A., MILLER, S.W., MAGUIRE, D.J., SHEEHAN, J.P., MAGUIRE, R.S., PATTEE, G., JUEL, V.C., PHILLIPS, L.H., TUTTLE, J.B., BENNETT, J.P. Jr, DAVIS, R.E., PARKER, W.D. Jr. Mitochondria in sporadic amyotrophic lateral sclerosis. *Exper. Neurol.* **153**, 135-142, 1998.

- TABRIZI, S.J., CLEETER, M.W., XUEREB, J., TAANMAN, J.W., COOPER, J.M., SCHAPIRA, A.H. Biochemical abnormalities and excitotoxicity in Huntington's disease brain. *Ann. Neurol.*, **45**, 25-32, 1999.

- TAVARES, R.G., TASCA, C.I., SANTOS, C.E., ALVES, L.B., PORCIÚNCULA, L.O., EMANUELLI, T., SOUZA, D.O. Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochem. Int.* **40**, 621-627, 2002.

- TEJERO-TALDO, M.I., CAFFREY, J.L., SUN, J., MALLET, R.T. Antioxidant properties of pyruvate mediate its potentiation of beta-adrenergic inotropism in stunned myocardium. *J. Mol. Cell. Cardiol.* **31**, 1863-1872, 1999.

- TYMIANSKI, M. Cytosolic calcium concentrations and cell death in vitro. In: Stiesjo, B.K., Wieloch, T. (eds). *Advances in neurology*. Lippincott-Raven, Philadelphia, 85-105, 1996.

- VARADKAR, S., SURTEES, R. Glutaric aciduria type I and kynurenine pathway metabolites: a modified hypothesis. *J. Inherit. Metab. Dis.* **27**, 835-842, 2004.

- WADIA, J.S., CHALMERS-REDMAN, R.M., JU, W.J., CARLILE, G.W., PHILLIPS, J.L., FRASER, A.D, TATTON, W.G. Mitochondrial membrane potential and nuclear changes in apoptosis caused by trophic withdrawal: time course and modification by (-) deprenyl. *J. Neurosci.* **18**, 932-947, 1998.

- WALLACE, D. C. Mitochondrial diseases in man and mouse. *Science* **283,** 1482-1487, 1999.

- WALLIMANN, T., HEMMER, W. Creatine kinase in non-muscle tissues and cells. *Mol. Cell. Biochem.* **133-134**, 193-220, 1994.

- WALLIMANN, T., WYSS, M., BRDICZKA, D., NICOLAY, K, EPPENBERGER, H.M.. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem. J*. **281,** 21-40, 1992.

- WENDT, S., SCHLATTNER, U., WALLIMANN, T. Differential effects of peroxynitrite on human mitochondrial creatine kinase isoenzymes. Inactivation, octamer destabilization, and identification of involved residues. *J. Biol. Chem.* **278**, 1125-30, 2003.

- WHETSELL, W.O. Jr., SCHWARCZ, R. Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostratal system. *Neurosci. Lett.* **97**, 271-275, 1989.

- WIDNER, B.; LEBLHUBER, F.; WALLI, J. TILZ, G.P., DEMEL, U., FUCHS, D. Degradation of tryptophan in neurodegenerative disorders. *Adv. Exp. Med. Biol.* **467**, 133-138, 1999.

- WIDNER, B., LEBLHUBER, F., WALLI, J., TILZ, G.P., DEMEL, U., FUCHS, D. Tryptophan degradation and immune activation in Alzheimer's disease. *J. Neural Transm.* **107**, 343-353, 2000.

- WONG, E.H., KEMP, J.A., PRIESTLEY, T., KNIGHT, A.R., WOODRUFF, G.N., IVERSEN, L.L. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl Acad. Sci. USA*. **83**, 7104-7108, 1986.

- WOODRUFF, G.N., FOSTER, A.C., GILL, R., KEMP, J.A., WONG, E.H., IVERSEN, L.L. The interaction between MK-801 and receptors for N-methyl-D-aspartate: functional consequences. *Neuropharmacology* **26**, 903-909, 1987.

- WYSS, M., Kaddurah-Daouk, R. Creatine and creatinine metabolism. *Physiol. Rev.* **80,** 1107-213, 2000.

- XU, C.J., KLUNK, W.E., KANFER, J.N., XIONG, Q., MILLER, G., PETTEGREW, J.W. Phosphocreatine-dependent glutamate uptake by synaptic vesicles. A comparison with atp-dependent glutamate uptake. *J. Biol. Chem.* **271**, 13435-13440, 1996.

- YOUNG, A.B., PENNEY, J.B., STAROSTA-RUBINSTEIN, S., MARKEL, D.S., BERENT, S., GIORDANI, B., EHRENKAUFER, R., JEWETT, D., HICHWA, R. PET scan investigations of Huntington's disease: cerebral metabolic correlates of neurological features and functional decline. *Ann. Neurol.* **20**, 296-303, 1986.

- ZARKOVIC, K. 4-hydroxynonenal and neurodegenerative diseases. *Mol. Aspects Med*. **24**, 293-303, 2003

- ZEEVALK, G.D., NICKLAS, W.J. Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition, *J. Pharm. Exp. Ther.*, **257**, 870-878, 1989.