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GISLAINE TEZZA REZIN

AVALIAÇÃO DO METABOLISMO ENERGÉTICO CEREBRAL EM RATOS SUBMETIDOS À ADMINISTRAÇÃO DE FEMPROPOREX E ESTABILIZADORES DE HUMOR

CRICIÚMA, AGOSTO DE 2011

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde para obtenção do título de Doutora em Ciências da Saúde.

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Os membros da Banca Examinadora designada pelo Colegiado de Coordenação do Programa de Pós-Graduação em Ciências da Saúde (Mestrado e Doutorado) reuniram-se para realizar a argüição da Tese de DOUTORADO apresentada pela candidata Gislaine Tezza Rezin sob o título **"Avaliação do metabolismo energético cerebral em ratos submetidos à administração de femproporex e tratados com estabilizadores de humor"** para obtenção do grau de **DOUTOR EM CIÊNCIAS DA SAÚDE** do Curso de Pós-Graduação em Ciências da Saúde da Universidade do Extremo Sul Catarinense – UNESC.

Após haver analisado o referido trabalho e argüido á candidata, os membros são de parecer pela **"APROVAÇÃO"** da Tese, com conceito A.

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DEDICATÓRIA

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... Um dia você aprende...

...E você aprende que realmente pode suportar...

...Que realmente é forte, e que pode ir muito mais
longe depois de pensar que não se pode mais.
E que realmente a vida tem valor e
que você tem valor diante da vida!

(William Shakespeare)

RESUMO

O femproporex é o segundo anorexígeno derivado de anfetamina mais consumido no mundo. Estudos sugerem que a anfetamina induz neurotoxicidade através da geração de radicais livres e apoptose mitocondrial. Além disso, a anfetamina é utilizada para induzir modelo animal de mania. Neste trabalho nós avaliamos parâmetros bioquímicos em animais jovens submetidos à administração aguda e crônica de femproporex. Nós também avaliamos parâmetros comportamentais e bioquímicos em animais adultos submetidos à administração aguda e crônica de femproporex. Também foram avaliados parâmetros comportamentais e bioquímicos em animais submetidos à administração crônica de femproporex, lítio e valproato. Ratos jovens e adultos receberam uma simples ou repetidas injeções intraperitoneal de veículo ou femproporex. Duas horas após a última administração, os animais jovens foram mortos para avaliar parâmetros bioquímicos. Também após a última administração, os animais adultos foram submetidos ao teste de campo aberto e, em seguida, foram mortos para avaliar parâmetros bioquímicos. Os animais adultos também foram submetidos ao experimento de reversão e prevenção dos efeitos causados pelo femproporex. No experimento de reversão, os animais receberam primeiro femproporex ou veículo, e em seguida, lítio, valproato ou veículo. No experimento de prevenção, os animais receberam primeiro lítio, valproato ou veículo, e em seguida, femproporex ou veículo. A atividade locomotora e o metabolismo energético foram avaliados. Nossos resultados mostraram que a atividade das enzimas do ciclo de Krebs, cadeia respiratória mitocondrial e creatina quinase foram ativadas após a administração aguda e crônica de femproporex nos animais jovens. Nossos resultados também mostraram que a administração aguda e crônica de femproporex em animais adultos aumentou o número de *crossings*, *rearings* e visita ao centro. Nas altas doses, repetidas administrações de femproporex aumentaram o comportamento estereotípico e o conteúdo fecal. Além disso, o peso corporal dos animais adultos foi medido continuamente durante o experimento crônico. Nenhuma diferença do peso corporal foi encontrada entre os grupos no primeiro dia de experimento. Houve um ganho de peso do grupo controle no sétimo e décimo quarto dia. Porém, como esperado, a administração de femproporex diminuiu o peso corporal, de acordo com a dose, no sétimo e décimo quarto dia. O femproporex também diminuiu a atividade das enzimas do ciclo de Krebs, cadeia respiratória mitocondrial e creatina quinase. Nossos resultados também mostraram que no experimento de reversão e prevenção, o femproporex aumentou a atividade locomotora, mas a administração de lítio e valproato preveniu e reverteu esta ativação. No experimento de reversão, o peso corporal não foi alterado no primeiro e sétimo dia. Porém na segunda semana de administração de femproporex houve uma diminuição do peso corporal dos animais, mas foi revertido pelo lítio e valproato. No experimento de prevenção o peso corporal não foi alterado. Além disso, no experimento de reversão e prevenção houve uma inibição das enzimas do metabolismo energético. No entanto, os estabilizadores de humor reverteram e preveniram esta inibição, exceto o lítio e valproato preveniram a inibição da atividade do complexo IV. Nossos resultados estão consistentes com a literatura que demonstra mudanças comportamentais e disfunção mitocondrial causada por psicoestimulantes.

Palavras-Chave: Femproporex; Comportamento; Ciclo de Krebs; Cadeia respiratória mitochondrial; Creatina quinase.

ABSTRACT

Fenproporex is the second most consumed amphetamine-based anorectic worldwide. Studies suggest that amphetamine induces neurotoxicity through generation of free radicals and mitochondrial apoptotic. Besides, amphetamine is used to induced animal model of mania. In this work, we evaluated the activity of energy metabolism enzymes in brain of young animals submitted to acute and chronic administration of fenproporex. Furthermore, we evaluated behavioral and energy metabolism parameters in adult animals submitted to acute and chronic administration of fenproporex. Finally, we also evaluated the behavior activity and energy metabolism in animals submitted to chronic administration of fenproporex treated with litium and valproate. Adult and young rats were given single or repeated intraperitoneal injection of vehicle or Fenproporex. Two hours after the last injection, young rats were killed by decapitation and the brain was removed for evaluation of biochemical parameters. After the last injection the adult animals were submitted to open field task and then rats were killed to evaluate of brain energy metabolism parameters. After chronic administration of fenproporex, adults rats were submitted to reversal and prevention experiments of effect caused by fenproporex. In the reversal experiment, rats were first given fenproporex or vehicle, and then, lithium, valproate or vehicle. In the prevention experiment, rats received lithium, valproate or vehicle, and then, fenproporex or vehicle. Behavior activity and energy metabolism were evaluated. Our results showed that the activities of enzymes of the Krebs cycle, mitochondrial respiratory chain and creatine kinase were also increased after acute and chronic administration of fenproporex in young animals. Our results also showed that acute and chronic administration of fenproporex in adults animals increased the number of crossings, rearings and visits to center. At high doses, repeated injections of fenproporex increased stereotyped behaviors and fecal boli. Additionally, animal body weight was measured continuously during chronic treatment and no significant difference was found between groups in day 1 of experiment. There was a weight gain of control animals on days 7 and 14 of experiment. However, as expected, the administration of fenproporex dose-dependent decreased the weight of animals on days 7 and 14 of experiment. Fenproporex also significantly decreased the activity of: Krebs cycle enzymes, mitochondrial respiratory chain complexes and creatine kinase in the majority of the brain structures evaluated. Finally, our results also showed that fenproporex effects on locomotor activity were prevented and reverted by mood stabilizers. Moreover, while body weight was not altered until the seventh day of the reversion experiment, fenproporex decreased body weight in the second week treatment and this effect was also reversed by lithium and valproate. There was no alteration in the body weight during the prevention experiment. Besides, in the reversion and prevention experiment there was inhibited of enzymes of energy metabolism in the hippocampus. However, mood stabilizers reverted and prevented this inhibition, except lithium and valproate prevented the inhibition in the complex IV activity. Our results are consistent with the literature that demonstrates behavioral changes and mitochondrial dysfunction caused by psychostimulants.

Key-words: Fenproporex; Behavior; Krebs cycle; Mitochondrial respiratory chain; Creatine kinase.

LISTA DE FIGURAS

LISTA DE ABREVIAÇÕES

ADP – Adenosina difosfato

ATP – Adenosina trisfosfato

DNA – Ácido desoxirribonucléico

$FADH_2$ – Flavina adenina dinucleotidio

GABA – Ácido Gama-Aminobutirico

NADH – Nicotinamida adenina dinucleotidio

RNA – Ácido ribonucleico

SUMÁRIO

1. INTRODUÇÃO

1.1. Transtorno bipolar

Todo ser humano apresenta flutuações de humor em resposta a eventos cotidianos de sua vida. No entanto, em alguns indivíduos essas respostas assumem um caráter inadequado em termos de gravidade, persistência ou circunstâncias desencadeadoras, podendo caracterizar a ocorrência de um Transtorno de Humor. O transtorno bipolar é caracterizado por episódios de humor - mania, depressão e mistos - separados por períodos de humor normal - eutimia. Esse caráter cíclico garante a maior gravidade da doença, sendo considerada uma das 10 condições mais debilitantes no mundo ao incapacitar indivíduos afetados por sua significante comorbidade associada, risco de suicídio, prejuízo funcional e diminuição da qualidade de vida (Keck, 2003; Kupfer, 2005).

A prevalência estimada do transtorno bipolar é de aproximadamente 1% ao longo da vida, podendo chegar a 3% considerando as formas mais brandas do transtorno (Merikangas, 2007), sem predileção por nação, raça ou status socioeconômico. A taxa de suicídio no transtorno bipolar foi estimada em 19% igualando a do transtorno depressivo maior (Goodwin & Jamison, 2003). Estudos populacionais sugerem que a prevalência do transtorno bipolar é semelhante entre homens e mulheres (Robins & Regier, 1991). Este transtorno se desenvolve tipicamente em jovens, sendo que o início dos sintomas ocorre geralmente entre o final da adolescência e o início da vida adulta, porém algumas pessoas apresentam os primeiros sintomas durante a infância ou em idades mais avançadas, além de

muitas pessoas sofrerem por anos sem serem corretamente diagnosticadas e tratadas (Baldessarini et al., 1999; Judd et al., 2002).

O transtorno bipolar é um transtorno de humor que envolve uma gama de sintomas maníacos tipicamente precedidos e intercedidos por episódios depressivos (Manning et al., 1997). O termo bipolar expressa dois pólos de humor ou de estados afetivos que se alternam: a depressão e seu oposto, a hipomania ou a mania (Calabrese et al., 2003). No episódio maníaco ocorre uma alteração do humor, deixando-o elevado, expansivo ou irritável, diminui a necessidade de sono, aumenta a energia, a libido, além de inquietação, agitação psicomotora e fuga de idéias (Frey et al., 2006a; Moreno et al., 2005). Segundo Del Porto & Del Porto (2005), impulsividade, agressividade, hipersexualidade e gastos excessivos estão entre os correlatos do comportamento maníaco, mais preocupantes e associados aos riscos para o doente e para terceiros (DSM-IV-TR, 2003). A hipomania é um episódio similar à mania, porém mais leve e com menor duração, em média, menos de uma semana (Moreno et al., 2005). No episódio depressivo o paciente apresenta uma inibição das atividades e pode chegar até a inércia total. Em geral, é uma pessoa triste, pessimista, desesperançada, humor deprimido, perda de interesse, alterações do apetite, sono, atividade psicomotora, diminuição da energia e sentimentos de culpa (DSM-IV-TR, 2003; Quilici, 2006).

O diagnóstico inicial e tratamento podem ajudar a reduzir a gravidade associada a esta condição. Entretanto, pacientes com transtorno bipolar geralmente se apresentam no estágio depressivo e assim são frequentemente diagnosticados apenas como depressão maior (Manning, 1997; Hirschfeld et al., 2003). Mania ou hipomania são tratadas com fármacos antipsicóticos, anticonvulsivantes ou sais de

lítio. Os sais de lítio e certos anticonvulsivantes, como o valproato, têm propriedades de estabilizadores do humor, e são usados para prevenção em longo prazo de recorrências; entretanto, a polifarmácia é regra geral (Baldessarini, 2001).

O valproato atua elevando os níveis de ácido Gama-Aminobutírico (GABA) através da intensificação de sua síntese e liberação ao atuar nos receptores $GABA_A$, por meio da ativação da enzima glutâmica descarboxilase e da inibição das enzimas que o degradam (por exemplo, GABA-amino transferase e succinato semi-aldeído desidrogenase) (Taberner et al., 1980, Loscher, 1981a,b; Gram et al., 1988). Além disso, neurônios GABAérgicos diminuem a atividade do sistema dopaminérgico, modulando receptores D1 de dopamina (Kalivas & Stewart, 1991). O lítio é um inibidor não competitivo de inositol monofosfato, o qual converte inositol trifosfato à mioinositol. Neste sentido, o valproato e o lítio diminuem o transporte de mioinositol para dentro da célula. Essas ações resultam na diminuição de inositol trifosfato e de diacilglicerol, o qual é responsável pela ativação da proteína quinase C (Sherman et al., 1986; Manji & Lenox, 1999). A proteína quinase C regula a liberação de neurotransmissores (Manji & Lenox, 1999).

O entendimento da fisiopatologia do transtorno bipolar se expandiu muito nos últimos anos, porém ainda sabe-se pouco sobre os mecanismos dessa doença (Frey et al., 2004; Cunha et al., 2005; Zarate et al., 2006; Sadock & Sadock, 2007). As bases biológicas do transtorno bipolar incluem estudos relacionados às alterações neuroquímicas por meio da avaliação de diversos hormônios, neurotransmissores e seus metabólitos, assim como avaliação de segundos mensageiros, fatores neurotróficos e gênicos em plasma, líquor, plaquetas e porções do cérebro (Machado-Vieira et al., 2003).

Algumas teorias atuais relacionam possível envolvimento das enzimas do ciclo de Krebs (Corrêa et al., 2007), da cadeia respiratória mitocondrial (Calabrese et al., 2001; Konradi et al., 2004) e da creatina quinase (Dager et al., 2004; Streck et al., 2008) com a fisiopatologia do transtorno bipolar, desta forma demonstrando alteração no metabolismo energético. Existem indícios de que a dopamina interage com a cadeia respiratória mitocondrial, visto que a monoamina oxidase – enzima responsável pelo metabolismo da dopamina – está localizada na membrana externa da mitocôndria (Ben-Schachar, 2002). Consequentemente, as mitocôndrias são alvo preferencial da dopamina e de seus metabólitos. Elevadas concentrações de dopamina no cérebro de ratos, após a administração crônica de levodopa e metanfetamina, resultam em uma redução na produção de adenosina trifosfato (ATP) (Przedborski et al., 1993; Chan et al., 1994)

Vários estudos *in vivo* demonstraram diminuição de fosfocreatina e ATP no lobo frontal em portadores do transtorno bipolar (Kato et al., 1993; Deicken et al., 1995; Kato et al., 1998; Dager et al., 2004). MacDonald e colaboradores (2006) mostraram que os níveis de ácido ribonucleico (RNA) mensageiro para a creatina quinase, uma importante enzima responsável pela homeostase energética celular, estão diminuídos em pacientes com transtorno bipolar, especialmente no hipocampo. Além disso, estudos demonstraram uma diminuição na expressão dos genes nucleares que codificam as enzimas da cadeia respiratória mitocondrial, responsáveis pela fosforilação oxidativa, em hipocampo e córtex de pacientes com transtorno bipolar (Konradi et al., 2004; Sun et al., 2006).

1.2. Metabolismo energético

O ATP é o principal combustível da célula na maioria dos processos que necessitam de energia. A energia é liberada pela hidrólise de ATP e serve para impulsionar uma série de reações (Lehninger et al., 2007). O cérebro desenvolve uma intensa atividade metabólica, porém possui uma pequena reserva energética em relação ao grande consumo de glicose, existindo assim uma necessidade contínua de substratos energéticos (Dickinson, 1996). Estudos mostram que a disfunção mitocondrial resulta de uma falha no funcionamento da cascata bioquímica, sugerindo ser um importante fator na patogênese de muitas doenças, tais como transtorno bipolar, depressão maior e esquizofrenia (Fattal et al., 2006; Horn & Barrientos, 2008; Rezin et al., 2008). Além disso, uma anormalidade no metabolismo energético pode desencadear lesão e morte celular (Heales et al., 1999; Blass, 2001; Calabrese et al., 2001; Schurr, 2002).

A mitocôndria é a organela responsável pelos processos de respiração celular e obtenção de energia na forma de ATP. Em sua matriz, existem as enzimas envolvidas no ciclo de Krebs, onde se destaca a citrato sintase. Esta enzima dá início ao ciclo e é considerada como marcadora de metabolismo mitocondrial (Marco et al., 1974). A citrato sintase também catalisa a condensação do oxaloacetato ao grupo acetil da acetil coenzima-A (Shepherd & Garland, 1969). Além disso, a malato desidrogenase e a succinato desidrogenase, também fazem parte do ciclo de Krebs. A malato desidrogenase catalisa a desidrogenação de L-malato em oxaloacetato na etapa final do ciclo de Krebs (Kelly et al., 1989) e a succinato desidrogenase é um dos marcadores mais confiáveis de capacidade mitocondrial para fornecer uma

quantidade adequada de ATP, uma vez que faz parte tanto o ciclo de Krebs quanto da cadeia respiratória (complexo II) (Tyler, 1992).

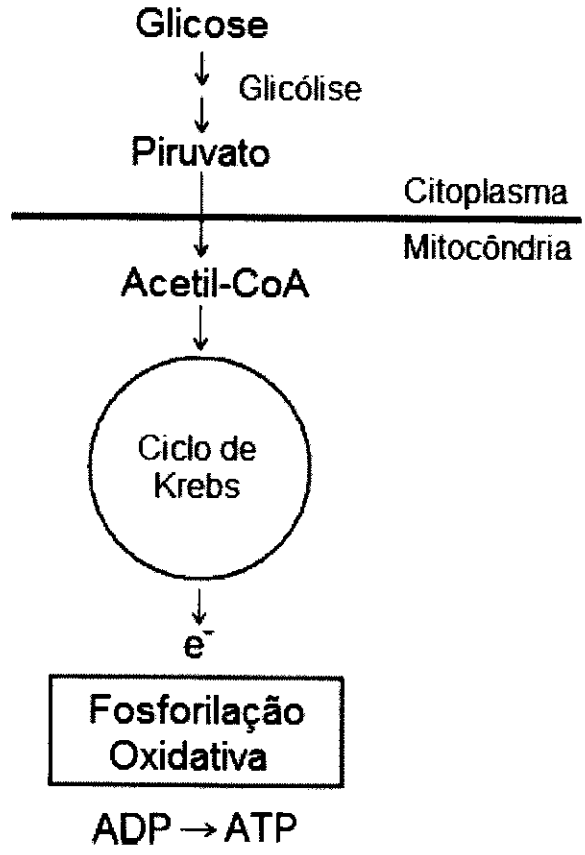

Figura 1: Metabolismo energético (Rezin et al., 2008)

Na membrana interna da mitocôndria estão os complexos enzimáticos envolvidos no transporte de elétrons e na fosforilação oxidativa (Devlin & Michelacci, 2003). A ligação íntima entre o ciclo de Krebs e a fosforilação oxidativa é responsável pela maior parte da produção de ATP gerada pelos seres humanos, sendo que a cadeia de transporte de elétrons é composta por quatro complexos enzimáticos (complexos I, II, III e IV) e dois componentes que não fazem parte dos

complexos, a coenzima Q, que transporta elétrons dos complexos I e II ao complexo III, e o citocromo c, que transporta elétrons do complexo III ao complexo IV. Os elétrons presentes nas coenzimas nicotinamida adenina dinucleotidio (NADH) e flavina adenina dinucleotidio ($FADH_2$) provenientes do ciclo de Krebs são transferidos para os complexos I e II, respectivamente, do complexo I e II para coenzima Q, depois para o complexo III, citocromo c, complexo IV e finalmente para o oxigênio (Navarro & Boveris, 2007).

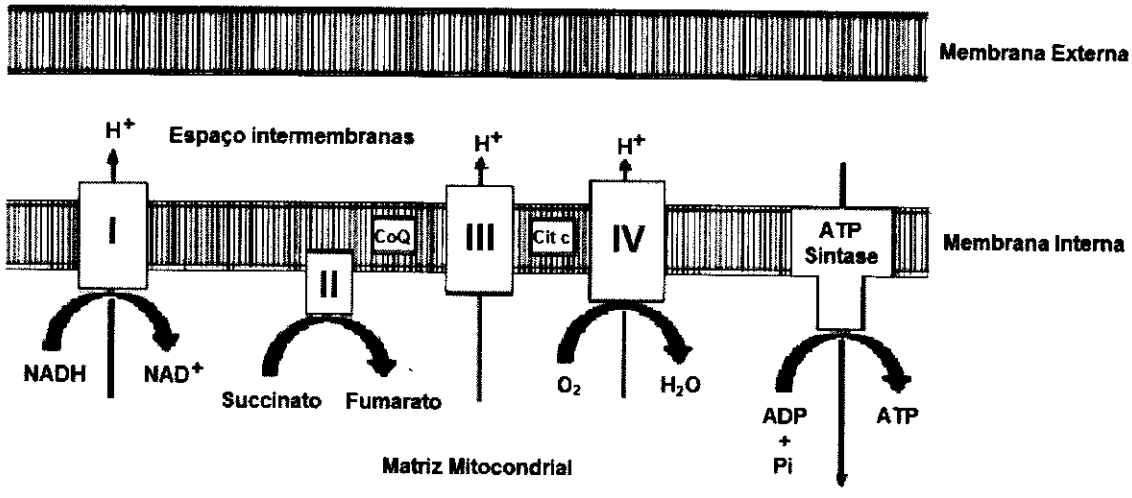

Figura 2: Cadeia respiratória mitocondrial (Rezin et al., 2008)

A creatina quinase também desempenha um papel central no metabolismo dos tecidos de alta demanda energética, tais como cérebro, músculo esquelético e coração, onde a creatina quinase funciona como um eficaz sistema de tamponamento dos níveis de ATP celular (Bessman & Carpenter, 1985; Schnyder et al., 1991; Wallimann et al., 1992). A síntese do ATP na mitocôndria é resultado da fosforilação oxidativa, na qual o adenosina difosfato (ADP) é fosforilado, originando o ATP. A produção de ATP pela fosforilação oxidativa ocorre separadamente do

transporte de elétrons até o oxigênio, no entanto a cadeia transportadora de elétrons está acoplada à síntese de ATP pela fosforilação do ADP. A operação da cadeia de transporte de elétrons leva a um bombeamento de prótons através da membrana interna da mitocôndria, criando um gradiente eletroquímico. A produção de ATP ocorre quando esses prótons migram de volta para o interior da matriz mitocondrial (Devlin & Michelacci, 2003).

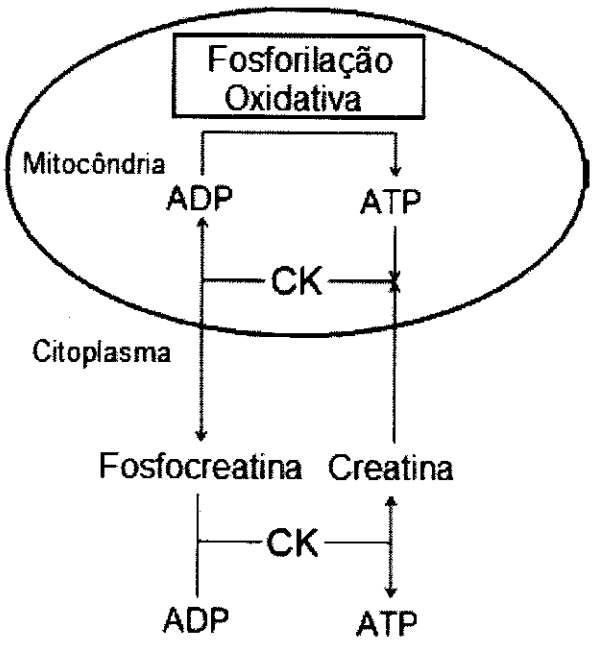

Figura 3: O sistema creatina quinase/fosfocreatina/creatina (Rezin et al., 2008)

Estudo de Streck e colaboradores (2008) demonstraram uma inibição na atividade da creatina quinase em cérebro de ratos submetidos ao modelo de mania induzido pela administração de anfetamina. Outro estudo apresentou inibição da atividade da citrato sintase em cérebro de ratos submetidos ao mesmo modelo animal de mania (Corrêa et al., 2007). Estudo de Valvassori e colaboradores (2010)

mostrou uma inibição na atividade dos complexos da cadeia respiratória mitocondrial em animal submetidos ao modelo de mania por anfetamina. Estudos com modelo animal de mania induzido por ouabaína de Freitas e colaboradores (2010a,b; 2011) mostraram inibição da citrato sintase, creatina quinase e ativação dos complexos enzimáticos da cadeia respiratória mitocondrial.

1.3. Uso de psicoestimulantes para induzir modelo animal de mania

O desenvolvimento de um modelo animal de transtorno bipolar é um desafio, pois apresenta uma complexa evolução que altera o curso clínico, que varia entre episódios de mania e depressão (Machado-Vieira et al., 2004). Um modelo animal deve demonstrar três características principais: mimetizar os sintomas da doença determinada (validade de face); habilidade do modelo em reproduzir alguns aspectos fisiopatológicos da doença (validade de constructo) e, finalmente, os agentes terapêuticos usados no tratamento devem reverter os sintomas induzidos no modelo animal (validade preditiva) (Ellenbroek & Cools, 1990). O desenvolvimento de modelos animais é uma ferramenta importante para o estudo dos sistemas intracelulares envolvidos no transtorno bipolar (Manji & Chen, 2002; Einat et al., 2003). O marco clínico para o diagnóstico de transtorno bipolar é a presença de sintomas maníacos (Belmaker, 2004); assim, um modelo animal adequado de transtorno bipolar deve assemelhar-se a algumas características de um episódio maníaco (Frey et al., 2006b).

Através de observações clínicas sugeriu-se uma provável relação entre a dopamina e a mania. Além disso, dados de neuroimagem em pacientes com

transtorno bipolar (Anand et al., 2000) e estudos em modelos animais de mania (Frey et al., 2006a,b,c) fornecem fortes evidências de que a dopamina tem uma importante participação na fisiopatologia do transtorno bipolar. Neste sentido, níveis elevados de dopamina na urina foram associados às emergências de episódios maníacos (Joyce et al., 1995) e outros estudos mostram mudanças em receptores de dopamina em pacientes com transtorno bipolar (Pantazopoulos et al., 2004; Vogel et al., 2004).

Em roedores, a administração aguda de fármacos psicoestimulantes, como a anfetamina, metanfetamina e a cocaína, é empregada como modelo animal de mania (Frey et al., 2006b; Valvassori et al., 2007; Rodvelt et al., 2011). Este modelo apresenta algumas limitações, mas, que devido aos grandes esforços feitos nos últimos anos, houve um constante incremento na validade preditiva e de constructo deste modelo (Sena et al., 2011). Estudos de Frey e colaboradores (2006a,b) caracterizam bem este modelo e mostram que administrações repetidas por 14 dias desenvolvem nos animais alterações comportamentais e neuroquímicas semelhantes as apresentados em pacientes com transtorno bipolar. Além disso, os estabilizadores de humor, lítio e valproato, reverteram e preveniram estas alterações. A anfetamina, além de induzir comportamentos semelhantes aos de mania em animais de laboratório, também induz sintomas maníacos em pacientes com transtorno bipolar (Joyce et al., 1995), bem como em voluntários saudáveis (Anand et al., 2000).

1.4. Femproporex

O femproporex foi sintetizado a partir da anfetamina, em laboratórios franceses em 1965, com modificações moleculares no sentido de aumentar o efeito anorexígeno e reduzir o efeito estimulante central (Fogliatto, 1998; Balíková, 2005). Sendo assim, o femproporex contém o núcleo básico da beta-fenetilamina e a ação farmacológica de aminas simpatomiméticas, agindo como inibidor do centro da fome hipotalâmico, com ação lipofílica. Entretanto, por se transformar em anfetamina no organismo, espera-se que o femproporex, ao menos em parte, possa trazer os efeitos da anfetamina, como efeitos psicoestimulantes (Nappo et al.,1994; Mariz, 2004). A redução da ingestão de alimentos, causada pelo femproporex, ocorre por alteração no controle químico da transmissão do impulso nervoso. Além disso, o efeito do femproporex torna-se semelhante ao da anfetamina, incluindo estimulação do sistema nervoso central, onde o femproporex age bloqueando a recaptação de noradrenalina e dopamina, com isso eleva os níveis destes neurotransmissores na fenda sináptica (Coutts et al., 1986; Mattei & Carlini, 1996). Vários estudos demonstram os riscos à saúde, decorrentes da utilização irracional deste grupo de fármacos, especialmente no Brasil (Coutts et al., 1986; Blackburn & Kanders, 1994; Hardman et al., 1996). Essa substância também é muito utilizada por caminhoneiros para diminuir o sono e aumentar o estado de vigília e atenção (Bell et al., 2001; Teixeira et al., 2001; Mariz & Silva, 2003).

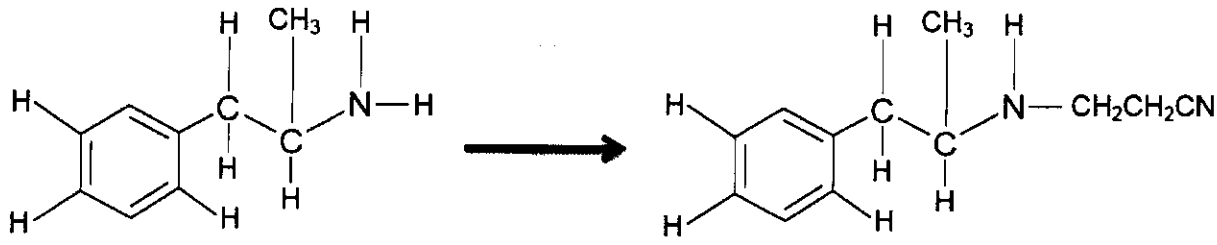

Figura 4: Estrutura química da anfetamina e do femproporex (Oga, 2003)

A dose usual de femproporex varia de 20 a 25 mg/dia, sendo preconizada a administração antes da refeição (Bell et al., 2001). Cerca de 30% da dose ingerida de femproporex sofre biotransformação pré-sistêmica devido ao efeito de primeira passagem e à ação enzimática da flora intestinal. Um dos principais metabólitos produzidos é a anfetamina (Cody & Valtier, 1996; Larini, 1999; Bell et al., 2001; Mariz & Silva, 2003), o que pode ser detectado na urina por até 60 horas após a ingestão (Kraemer et al., 2000). Testes toxicológicos revelam que uma concentração excedente de 1.0 ng/mL de anfetamina na urina é considerada tóxica (Cohen, 2009). Neste sentido, como o femproporex é convertido em anfetamina, o consumo de 10 mg/dia de femproporex leva a uma detecção na urina de 4.0 ng/mL de anfetamina após 3 horas da ingestão (Cody et al., 1999).

Todos os fármacos simpatomiméticos, incluindo o femproporex, imitam os efeitos da adrenalina, noradrenalina e dopamina. Essas substâncias promovem o aumento da pressão sanguínea e batimentos cardíacos, dilatação brônquica, constrição periférica dos vasos sangüíneos, dilatação das pupilas, esvaziamento da bexiga e intestinos, aumento da atenção, sensibilidade aos estímulos, perda de apetite e aumento da autoconfiança. O resultado do consumo de anfetaminas é a

falta de fome e de sono e uma forte necessidade de movimento. Em caso de aparecimento de tolerância, torna-se necessário o aumento da dose, o que implica maior probabilidade de ocorrência de efeitos secundários de natureza estimulante sobre o sistema nervoso central, como agressividade, ansiedade, angústia, inquietude e pânico (Utrilla, 2000). Além disso, sintomas como euforia, irritabilidade, agressividade, hiperatividade, insônia, ou atividade sexual aumentada caracterizam o episódio de mania em pacientes com transtorno bipolar (Ellenbroek & Cools, 1990). Estudos de neuroimagem em pacientes com transtorno bipolar sugerem que a neurotransmissão dopaminérgica está fortemente ligada aos sintomas do episódio maníaco (Anand et al., 2000). Assim, estudos mostram que administrações repetidas de anfetamina induzem, em animais, sintomas similares aos apresentados em pacientes com transtorno bipolar devido ao aumento na neurotransmissão dopaminérgica (Frey et al., 2006a,b,c).

2. OBJETIVOS

2.1. Objetivo Geral:

Avaliar os efeitos da administração aguda e crônica de femproporex sobre a atividade locomotora e metabolismo energético cerebral de ratos, além de avaliar o efeito dos estabilizadores do humor sobre estes parâmetros em ratos submetidos à administração crônica de femproporex.

2.2. Objetivos Específicos:

Avaliar atividade das enzimas do ciclo de Krebs, cadeia respiratória mitocondrial e creatina quinase em cérebro de ratos jovens submetidos à administração aguda e crônica de femproporex.

Avaliar parâmetros comportamentais (atividade locomotora, estereotipia e quantidade de bolo fecal), peso corporal e atividade das enzimas do ciclo de Krebs, cadeia respiratória mitocondrial e creatina quinase em cérebro de ratos adultos submetidos à administração aguda e crônica de femproporex.

Avaliar parâmetros comportamentais (atividade locomotora, estereotipia e quantidade de bolo fecal), peso corporal e atividade das enzimas do ciclo de Krebs, cadeia respiratória mitocondrial e creatina quinase em cérebro de ratos submetidos

à administração crônica de femproporex, lítio e valproato, no experimento de prevenção e reversão.

3. RESULTADOS

3.1. ARTIGO 1

Brain energy metabolism is activated after acute and chronic administration of fenproporex in young rats

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Brain energy metabolism is activated after acute and chronic administration of fenproporex in young rats

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Abstract

Obesity is a chronic disease of multiple etiologies, including genetic, metabolic, environmental, social, and other factors. Pharmaceutical strategies in the treatment of obesity include drugs that regulate food intake, thermo genesis, fat absorption, and fat metabolism. Fenproporex is the second most commonly consumed amphetamine-based anorectic worldwide; this drug is rapidly converted *in vivo* into amphetamine. Studies suggest that amphetamine induces neurotoxicity through generation of free radicals and mitochondrial apoptotic pathway by cytochrome *c* release, accompanied by a decrease of mitochondrial membrane potential. Mitochondria are intracellular organelles that play a crucial role in ATP production. Thus, in the present study we evaluated the activities of some enzymes of Krebs cycle, mitochondrial respiratory chain complexes and creatine kinase in the brain of young rats submitted to acute and chronic administration of fenproporex. In the acute administration, the animals received a single injection of fenproporex (6.25, 12.5 or 25mg/kg i.p.) or tween. In the chronic administration, the animals received a single injection daily for 14 days of fenproporex (6.25, 12.5 or 25mg/Kg i.p.). Two hours after the last injection, the rats were killed by decapitation and the brain was removed for evaluation of biochemical parameters. Our results showed that the activities of citrate synthase, malate dehydrogenase and succinate dehydrogenase were increased by acute and chronic administration of fenproporex. Complexes I, II, II-III and IV and creatine kinase activities were also increased after acute and chronic administration of the drug. Our results are consistent with others reports that showed that some psychostimulant drugs increased brain energy metabolism in young rats.

Keywords: fenproporex; obesity; mitochondrial dysfunction.

1. Introduction

Obesity is a chronic disease of multiple etiologies, including genetic, metabolic, environmental, social, and other factors (Bray and Champagne, 2005). Moreover, the obesity presents increased oxidative stress (Quigley et al., 2009), decreased mitochondrial gene expression (Crowe et al., 2008), reduced mitochondrial density (Ritov et al., 2005) and decreased ATP production (Petersen et al., 2004). It has been estimated that worldwide over 22 million children under the age of 5 years are obese, and one in ten children is overweight (Han et al., 2010). Among children aged 6 through 19 years, around 30% were overweight and 16% were obese (Raj and Kumar, 2010).

Pharmaceutical strategies in the treatment of obesity include drugs that regulate food intake, thermo genesis, fat absorption, and fat metabolism (Bray and Tartaglia, 2000). Very few drugs of this group are approved for clinical use due to their potential for abuse and addiction (Rodrigues et al., 2002).

Fenproporex is the second most commonly consumed amphetamine-based anorectic worldwide (Cohen, 2009). Although developed to provide appetite suppression without stimulant effects, it has been shown that fenproporex has addictive potential (Pélisser-Alicot et al., 2006). It acts by releasing or blocking neuronal reuptake of noradrenaline and dopamine (Coutts et al., 1986; Mattei and Carlini, 1996). In addition, fenproporex is rapidly converted *in vivo* into amphetamine (Cody et al., 1999), which can be detected in urine for up to 60 hours after ingestion (Kraemer et al., 2000). Moreover, studies suggest that amphetamine induces neurotoxicity through production of free radicals (Valvassori et al., 2008) and mitochondrial apoptotic pathway through cytochrome *c* release (Jimenez et al., 2004; Oliveira et al., 2003), accompanied by a decrease in mitochondrial potential (Cunha-Oliveira et al., 2006).

Mitochondria are intracellular organelles which play a crucial role in adenosine triphosphate (ATP) production (Calabrese et al., 2001). Most cell energy is obtained through

oxidative phosphorylation, a process requiring the action of various enzyme complexes located in a special structure of the inner mitochondrial membrane, the mitochondrial respiratory chain (Horn and Barrientos, 2008). Citrate synthase is localized within cells in the mitochondrial matrix and catalyzes the condensation of oxaloacetate and the acetyl group of acetyl coenzyme-A, the first step of Krebs cycle (Shepherd and Garland, 1969). Already malate dehydrogenase catalyzes the dehydrogenation of L-malate to oxaloacetate in the final step of TCA cycle (Kelly et al., 1989). And still succinate dehydrogenase is one of the most reliable markers of the mitochondrial capability to supply an adequate amount of ATP, as it is part of both the Krebs cycle and the respiratory chain (complex II) (Tyler, 1992).

The mitochondrial respiratory chain is composed of four complexes, in most organisms, where the electron transport couples with translocation of protons from the mitochondrial matrix to the intermembrane space. The generated proton gradient is used by ATP synthase to catalyze the formation of ATP by the ADP (Fattal et al., 2006; Madrigal et al., 2001). Another enzyme that is involved in energy production is the creatine kinase, it catalyzes the reversible transphosphorylation of creatine by ATP, playing a key role in cellular energy buffering and energy transport, particularly in cells with high and fluctuating energy requirements, including neurons (Andres et al., 2008).

Considering that data supporting the use of pharmacological therapy for pediatric obesity are still limited, and based on the hypothesis that fenproporex is converted in amphetamine, and that amphetamine has been involved in the production of free radicals and mitochondrial dysfunction, in the present study we evaluated the activities of enzymes of Krebs cycle, mitochondrial respiratory chain complexes and creatine kinase in the brain of young rats submitted to acute and chronic administration of fenproporex.

2. Material and methods

2.1. Animals: Young male Wistar rats (100-150 g) were obtained from Central Animal House of Universidade do Extremo Sul Catarinense. The animals were caged in group of 5 with free access to food and water, maintained on a 12-h light-dark cycle (lights on 7:00 am), at a temperature of 23°C ± 1°C. All experimental procedures were carried out in accordance with the "Principles of Laboratory Animal Care" (NIH publication no. 80-23, revised 1996) and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of UNESC Ethics Committee (protocols number 43/2009). Moreover, all efforts were made to minimize animal suffering as well as to reduce the number of animals.

2.2. Acute administration of drugs: Animals received a single injection of fenproporex (6.25; 12.5 or 25 mg/kg, intraperitoneal) or tween 2% intraperitoneal. Two hours after the injection the rats were killed by decapitation.

2.3. Chronic administration of drugs: Animals received a single injection daily of fenproporex (6.25; 12.5 or 25 mg/kg, intraperitoneal) or tween 2% intraperitoneal for 14 days. Two hours after the last injection the rats were killed by decapitation.

2.4. Tissue and homogenate preparation: Prefrontal cortex, posterior cortex, hippocampus, striatum and cerebellum were removed and homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin). The homogenates were centrifuged at 800 × *g* for 10 min at 4°C and the supernatants kept at −70°C until being used for enzymes activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. Protein content

was determined by the method described by Lowry and colleagues (1951) using bovine serum albumin as standard.

2.5. Activities of enzymes of Krebs cycle

2.5.1. Citrate synthase activity: Citrate synthase activity was assayed according to the method described by Shepherd and Garland (1969). The reaction mixture contained 100 mM Tris, pH 8.0, 100 mM acetyl CoA, 100 mM 5,5'-di-thiobis-(2- nitrobenzoic acid), 0.1% triton X-100, and 2–4 µg supernatant protein and was initiated with 100 µM oxaloacetate and monitored at 412 nm for 3 min at 25 °C.

2.5.2. Malate dehydrogenase activity: Malate dehydrogenase was measured as described by Kitto (1969). Aliquots (20 mg protein) were transferred into a medium containing 10 mM rotenone, 0.2% Triton X-100, 0.15 mM NADH, and 100 mM potassium phosphate buffer, pH 7.4, at 37°C. The reaction was started by addition of 0.33 mM oxaloacetate. Absorbance was monitored as described above.

2.5.3. Succinate dehydrogenase activity: Succinate dehydrogenase activity was determined according to the method of Fischer and colleagues (1985),measured by following the decrease in absorbance due to the reduction of 2,6-di-chloro-indophenol (2,6-DCIP) at 600nm with 700nm as reference wavelength (ε = 19.1 mM^{-1} cm^{-1}) in the presence of phenazine methasulphate (PMS). The reaction mixture consisting of 40mM potassium phosphate, pH 7.4, 16mM succinate and 8µM 2,6-DCIP was preincubated with 40–80µg homogenate protein at 30°C for 20 min. Subsequently, 4mM sodium azide, 7µM rotenone and 40µM 2,6-DCIP were added and the reaction was initiated by addition of 1mM PMS and was monitored for 5 min.

2.6. Activities of mitochondrial respiratory chain enzymes

2.6.1. Complex I activity: NADH dehydrogenase (complex I) was evaluated according to Cassina and Radi (1996) by the determination of the rate of NADH-dependent ferricyanide reduction at λ = 420 nm.

2.6.2. Complex II activity: The activities of succinate-2,6- dichloroindophenol (DCIP)-oxidoreductase (complex II) was determined by the method described by Fischer and colleagues (1985). Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at λ = 600 nm.

2.6.3. Complex II-III activity: The activity of succinate:cytochrome *c* oxidoreductase (complex III) was determined by the method described by Fischer and colleagues (1985). Complex II-III activity was measured by cytochrome *c* reduction using succinate as substrate at λ = 550 nm.

2.6.4. Complex IV activity: The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin and colleagues (1994), measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* (prepared by reduction of cytochrome with $NaBH_4$ and HCl) at λ = 550 nm with 580 nm as the reference wavelength (μL= 19.1 mM^{-1} cm^{-1}).

2.7. Activity of creatine kinase: Creatine kinase activity was measured in brain homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 µg protein in a final volume of 100 µL. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 3.2 mmol of ADP plus 0.8 mmol of reduced

glutathione. 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). 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 after 20 min at 540 nm.

2.8. Statistical analysis: Data were analyzed by one-way analysis of variance followed by the Tukey test when F was significant and are expressed as mean ± standard deviation. All analyses were performed using the Statistical Package for the Social Science (SPSS; version 16.0) software.

3. Results

We evaluated the effect of acute and chronic administration of fenproporex at three different doses on energy metabolism parameters in different brain regions of young rats. Acute administration of fenproporex increased the activity of citrate synthase (Figure 1) in hippocampus (12.5 and 25 mg/kg) and posterior cortex (25 mg/kg). Moreover, malate dehydrogenase activity (Figure 2) was increased in the following areas: prefrontal cortex (12.5 mg/kg), hippocampus (6.25 and 12.5 mg/kg), striatum (12.5 and 25 mg/kg) and posterior cortex (6.25 mg/kg). We also observed increased activity of succinate dehydrogenase (Figure 3) in striatum (25 mg/kg) and posterior cortex (12.5 and 25 mg/kg).

We also investigated the effect of acute fenproporex administration on activities of mitochondrial respiratory chain enzymes (Figure 4) and verified that acute fenproporex administration provoked an increase on complex I activity in hippocampus (6.25, 12.5 and 25 mg/kg), as well as in posterior cortex (12.5 mg/kg) and cerebellum (12.5 and 25 mg/kg). Complex II activity was also increased in hippocampus (6.25 mg/kg) and cerebellum (25

mg/kg). The activity of complex II-III was increased in hippocampus (6.25 mg/kg) and posterior cortex (12.5 and 25 mg/kg). Complex IV activity was also increased (25 mg/Kg) in striatum and posterior cortex. In addition, creatine kinase activity (Figure 5) was increased in hippocampus (12.5 mg/kg) and posterior cortex (12.5 and 25 mg/Kg).

We also demonstrated the effect of chronic fenproporex administration on the activities of the same enzymes. Citrate synthase activity (Figure 6) was increased in cerebellum (25mg/kg); malate dehydrogenase (Figure 7) was increased in hippocampus (12.5 and 25mg/kg), striatum (12.5 and 25mg/kg), posterior cortex (6.25; 12.5 and 25mg/kg) and cerebellum (12.5 and 25mg/kg). We also observed an increase in the activity of enzyme succinate dehydrogenase (Figure 8) in prefrontal cortex (12.5 and 25mg/kg), hippocampus (6.25; 12.5 and 25mg/kg) and posterior cortex (6.25 and 12.5mg/kg).

Finally, we showed that chronic fenproporex administration increased the activity of complex I (Figure 9) in hippocampus (6.25mg/kg) and posterior cortex (6.25 and 12.5 mg/kg). Complex II activity was also increased in prefrontal cortex (12.5 and 25mg/kg), posterior cortex (12.5 and 25mg/kg) and cerebellum (25 mg/kg). The activity of complex II-III was increased in posterior cortex (6.25 mg/kg) and cerebellum (25 mg/kg). Complex IV activity was also increased in prefrontal cortex and cerebellum (25 mg/kg). Creatine kinase activity (Figure 10) was increased in striatum (25 mg/kg).

4. Discussion

Fenproporex is an amphetamine-derived anorectic used in the treatment of obesity as an appetite suppressor and acts by increasing dopamine levels in the synaptic cleft (Coutts et al., 1986; Mattei and Carlini, 1996). In addition to its adverse effects, the use of fenproporex may lead to negative economic and legal consequences. In addition, fenproporex is rapidly converted *in vivo* into amphetamine (Cody et al., 1999), which can be detected in urine for up to 60 hours after ingestion (Kraemer et al., 2000). Urine toxicology

screening is used in many occupational settings to detect amphetamine abuse. Amphetamine screening tests are commonly considered positive if the concentration of this drug in urine exceeds 1.0 ng/mL (Cohen, 2009). Moreover, fenproporex is not detected by commonly used clinical urine tests. However, since fenproporex is rapidly converted *in vivo* into amphetamine, its metabolite is readily detected by commercial urine toxicology tests. Consumption of a low daily dose of 10 mg of fenproporex led to the detection of amphetamine within 3 hours in urine with peak urinary levels above 4.0 ng/mL (Cody et al., 1999).

Moreover, amphetamine and derived substances induce neurotoxicity through the production of free radicals and mitochondrial dysfunction (Yeh, 1997). Oxidative stress may occur by excessive formation of free radicals, which may cause oxidative damage to proteins, lipids, and deoxyribonucleic acid (Arnaiz et al., 1999; Halliwell, 1996). It is established that the impairment of the respiratory chain in mitochondria leads to an increase of free radicals generation, especially superoxide, and that these reactive oxygen species inhibit the mitochondrial respiratory chain, resulting in the generation of more reactive species, forming a cyclic phenomenon (Adam-Vizi, 2005; Navarro and Boveris, 2007).

In the present study, our results showed an increase in the activities of some enzymes of Krebs cycle, mitochondrial respiratory chain complexes and creatine kinase in the brain of young rats submitted to acute and chronic administration of fenproporex. Several reports present variate effects of psychostimulant drugs in young rats. Kolb and colleagues (2003) showed that psychostimulant drugs (amphetamine and cocaine) administration promotes neuronal growth in some brain regions of young rats. Some studies evaluated the effects of amphetamine in the central nervous system and its relationship with the age and time of exposure to the drug (Husson et al., 2004). The effect of amphetamine may be attributed to alterations in the dopaminergic circuits function (Brandon and Steiner, 2003; Federici et al., 2005; Mague et al., 2005), gene expression (Brandon and Steiner, 2003;

Chase et al., 2003; Penner et al., 2002) and others molecular changes related to neuronal metabolism (Fukui et al., 2003).

We have also recently reported that amphetamine administration inhibited citrate synthase activity in the brain of adult rats (Correa et al., 2007). Complexes I, II, II-III and IV activities were also decreased after the administration of amphetamine in adult rats (Valvassori et al., 2010). Finally, we showed that amphetamine administration inhibited creatine kinase activity in the brain of adult rats (Streck et al., 2008).

In order to understand the different effects of psychostimulant drugs in young and adult rats, especially in mitochondrial metabolism, Andreu and colleagues (1998) compared mitochondrial DNA transcription products in rats. This study reported an age-related reduction in newly-synthesized mitochondrial RNAs that reflects a decrease in the synthesis rate. An age-related increase in protein carbonyl content of the mitochondrial membranes was observed in "older" mitochondria.

5. Conclusion

Our results showed an increase in the activities of some enzymes of Krebs cycle, mitochondrial respiratory chain complexes and creatine kinase in the brain of young rats submitted to acute and chronic administration of fenproporex. Our results are consistent with others reports that showed that some psychostimulant drugs also increased brain energy metabolism in young rats.

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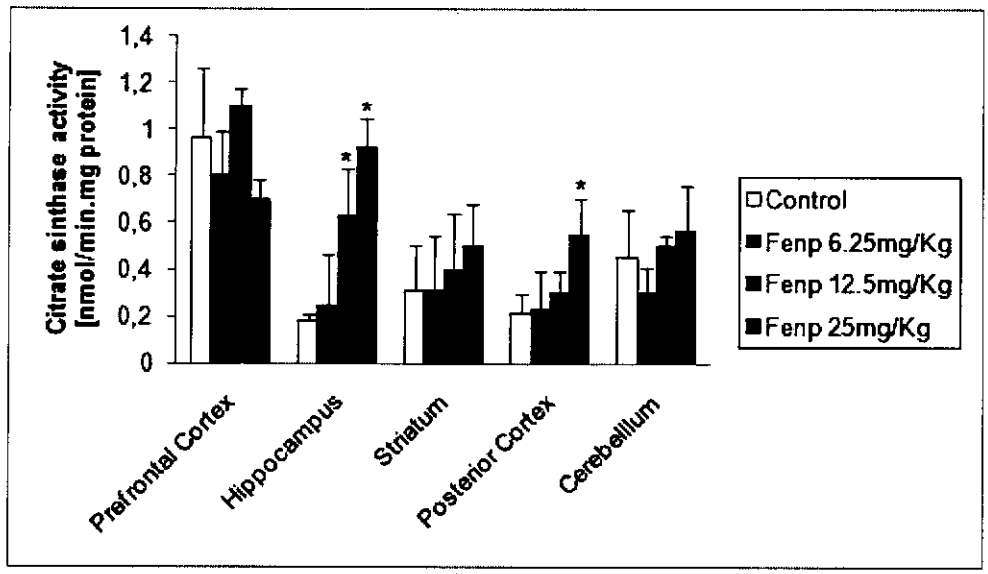

Figure 1 – Citrate synthase activity after acute administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

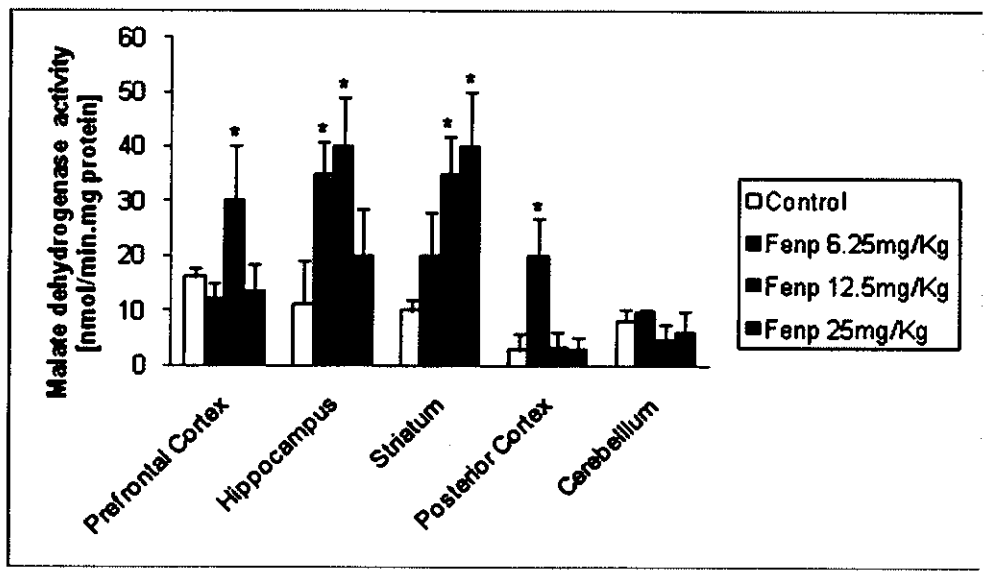

Figure 2 – Malate dehydrogenase activity after acute administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

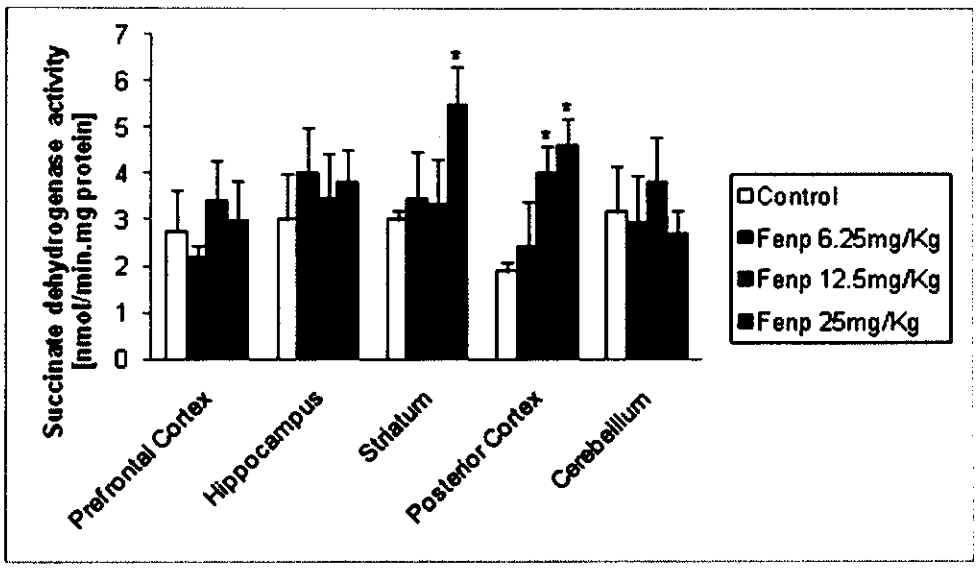

Figure 3 – Succinate dehydrogenase activity after acute administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

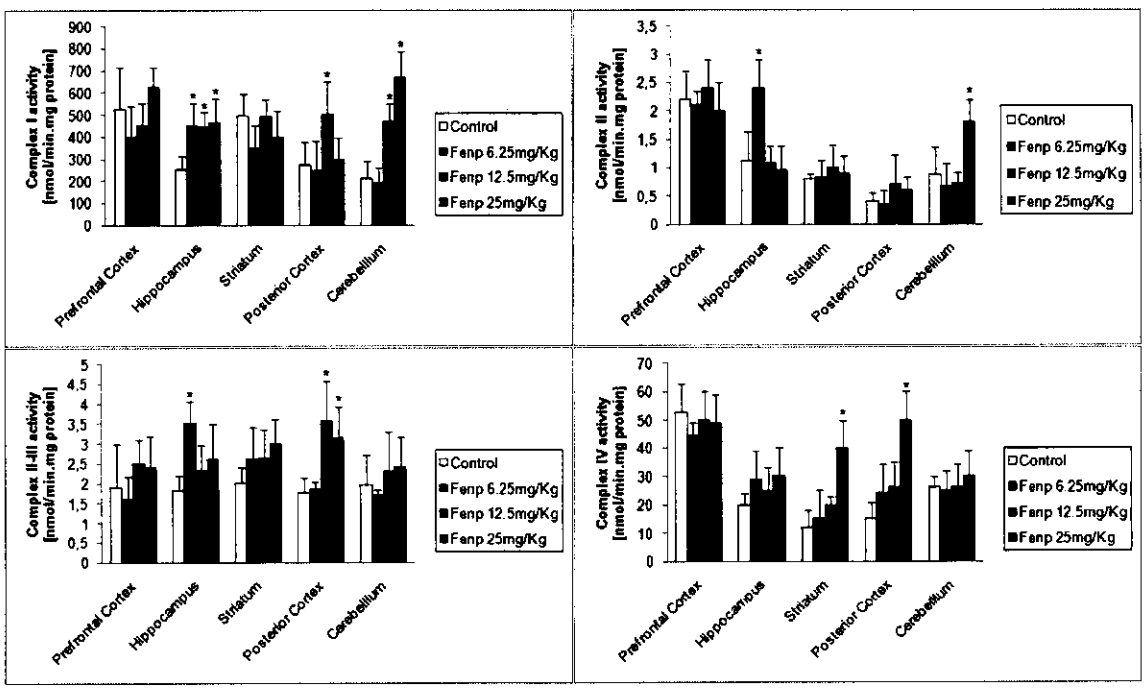

Figure 4 – Complex I, II, II-III and IV activity after acute administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was

significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

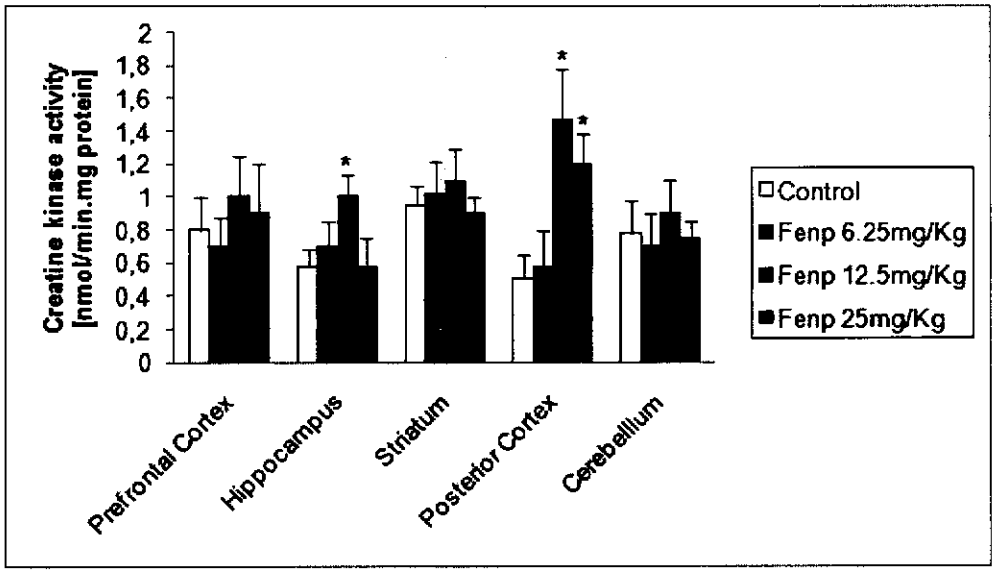

Figure 5 – Creatine kinase activity after acute administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

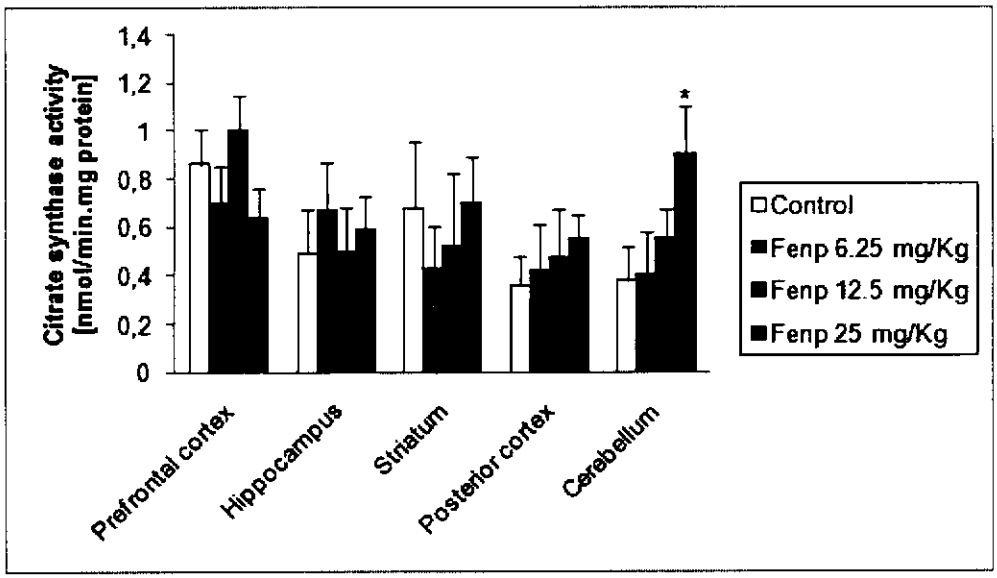

Figure 6 – Citrate synthase activity after chronic administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was

significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

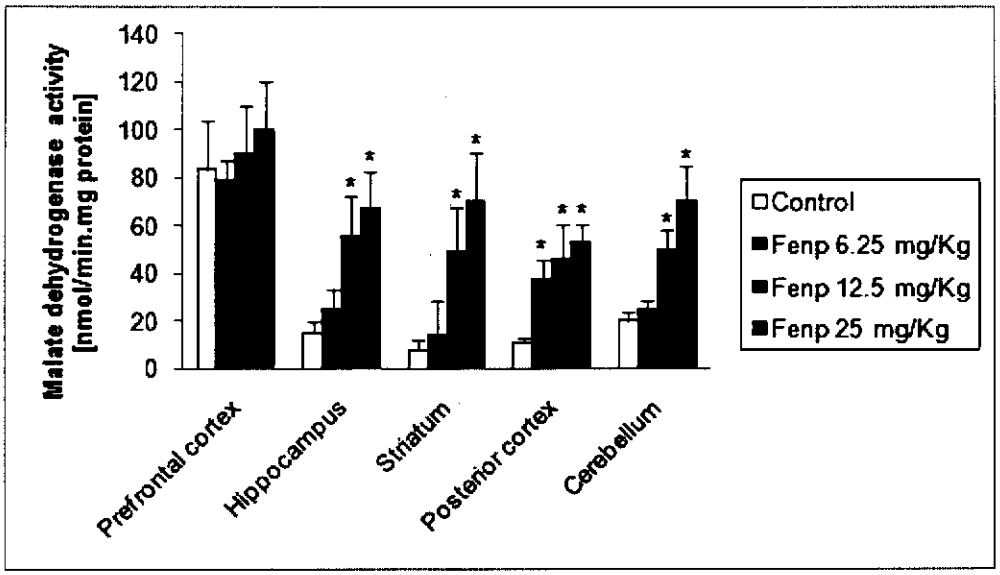

Figure 7 – Malate dehydrogenase activity after chronic administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

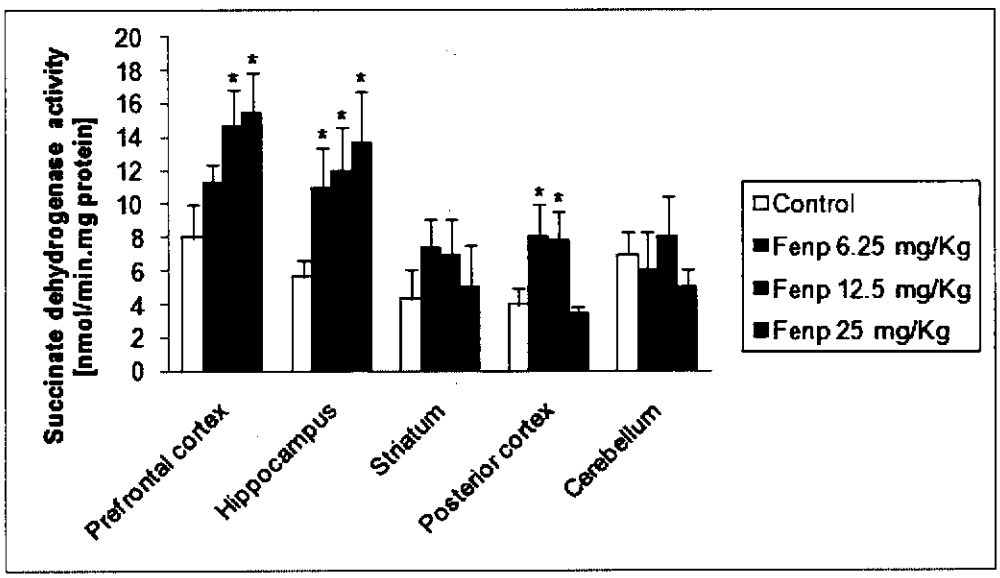

Figure 8 – Succinate dehydrogenase activity after chronic administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test

when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

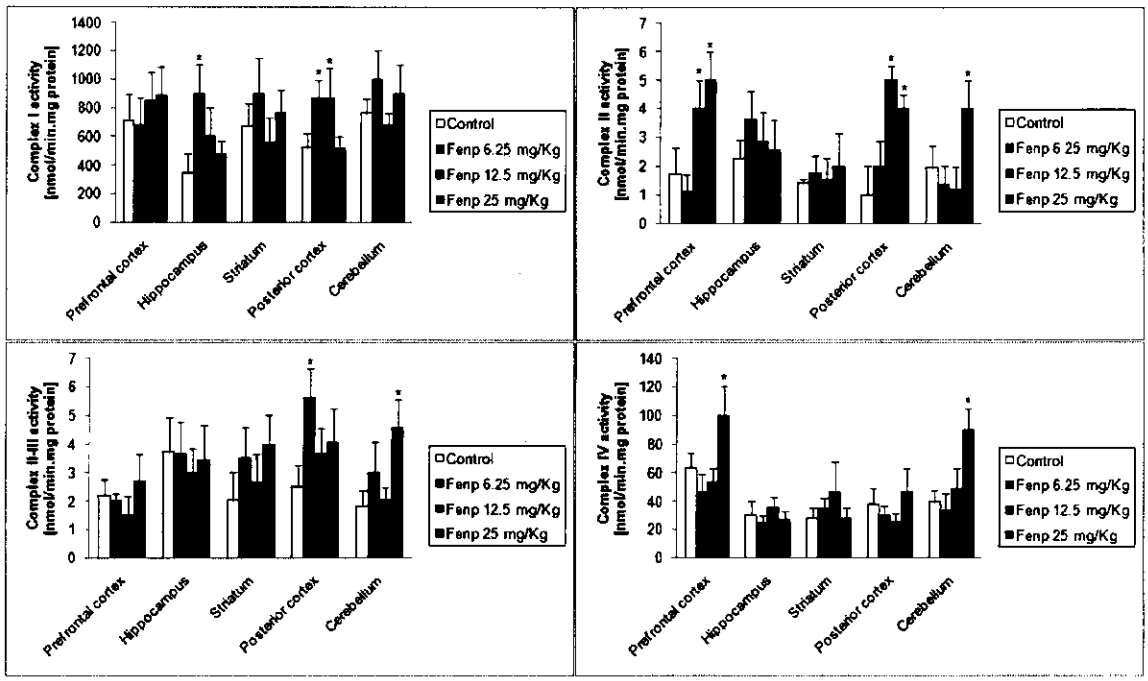

Figure 9 – Complex I, II, II-III and IV activity after chronic administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

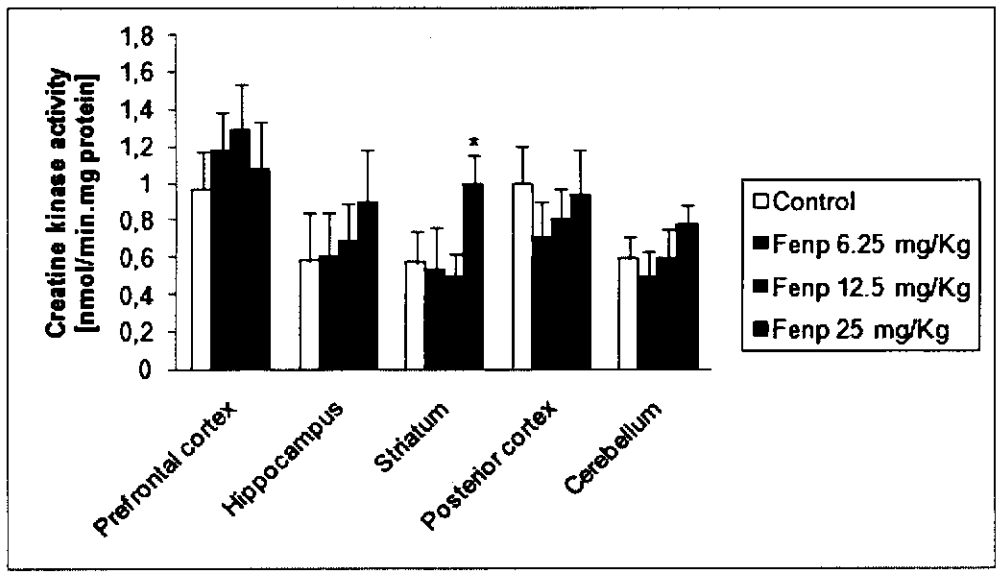

Figure 10 – Creatine kinase activity after chronic administration of fenproporex. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

3.2. ARTIGO 2

Acute and chronic administration of fenproporex alters behavior and brain energy metabolism in adult rats

Artigo submetido ao periódico *Journal of Psychiatric Research*

Acute and chronic administration of fenproporex alters behavior and brain energy metabolism in adult rats

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Abstract

Fenproporex (Fen) is an amphetamine-based anorectic and amphetamine is involved in the production of free radicals and mitochondrial dysfunction. In this work we evaluated the behavioral and energy metabolism parameters. Male adult Wistar rats were given single or repeated (14x/day) intraperitoneal injection of vehicle or Fen (6.25, 12.5 or 25mg/kg i.p.). The animals were submitted to open field task for behavioral assessment. The energy metabolism parameters were measured in the prefrontal cortex, hippocampus and striatum. The administration of Fen (6.25, 12.5 and 25mg/kg) increased the number of crossings and rearings in both experimental protocols. The number of visits to center was increased by Fen at 12.5 and 25 mg/kg, after single or repeated injections. At high doses (12.5 and 25mg/kg) repeated injections of Fen increased stereotyped behaviors and fecal boli. Fen significantly decreased the activity of: Krebs cicle enzymes, mitochondrial respiratory chain complexes and creatine kinase, in most brain structures evaluated. However, the changes in energetic metabolism enzymes activity were more evident in the acute protocol. Our results are consistent with the literature that demonstrates behavioral changes and mitochondrial dysfunction caused by psychostimulants.

Key words: Fenproporex; Behavior; Mitochondrial respiratory chain; Krebs cycle; Creatine kinase.

1. Introduction

Fenproporex (Fen) is the second most commonly used anorect substance in the word (Cohen, 2009). Fen is rapidly converted *in vivo* into amphetamine (AMPH) (Cody et al, 1999), thus acts releasing or blocking neuronal reuptake of noradrenaline and dopamine (Coutts et al, 1986; Mattei & Carlini, 1996).

Previous studies have shown that chronic use of Fen leads to some adverse effects, similar to AMPH, as behavioral alterations, addiction and abstinence syndrome (Pélissier-Alicot et al, 2006). Therefore, Food and Drug Administration has banned most of the AMPH-based anorectics (Colman, 2005). However, the psychoactive anorexigenic drugs AMPH-base are commonly consumed in many parts of the world, mainly in South America (Cohen, 2009).

Previous studies have showed that AMPH induce neurotoxicity through production of free radicals (Frey et al, 2006a; Frey et al, 2006b; Frey et al, 2006c; Valvassori et al, 2008) and mitochondrial apoptotic pathway through cytochrome *c* release (Jiménez et al, 2004; Oliveira et al, 2003), accompanied by a decrease in mitochondrial potential (Cunha-Oliveira et al, 2006).

Mitochondria are intracellular organelles important in adenosine triphosphate (ATP) production (Calabrese et al, 2001). The Krebs cycle occurs within the mitochondrial matrix and is the final common pathway for oxidation of carbohydrates, lipids and some amino acids, which contributes to production of large amounts of adenosine triphosphate (ATP) via mitochondrial oxidative phosphorylation (Kelly et al, 1989). Another form of ATP production is through creatine kinase that acts in the brain and other tissues with high and variable rates of ATP metabolism (Bessman & Carpenter, 1985; Schnyder et al, 1991; Wallimann et al, 1992).

Knowing that Fen is converted in AMPH, and that AMPH has been involved in the dysfunction mitochondrial, in the present study we evaluated behavioral parameters (locomotor activity, stereotypy activity and fecal boli amount), body weight and the activities

of enzymes citrate synthase, malate dehydrogenase, succinate dehydrogenase, complexes I, II, II-III, IV of mitochondrial respiratory chain and creatine kinase in brain of rats submitted to acute and chronic administration of Fen.

2. Material and Methods

2.1. Animals

Adult male Wistar rats (250-300 g) were obtained from Central Animal House of Universidade do Extremo Sul Catarinense. The animals were caged in group of 5 with free access to food and water, maintained on a 12-h light-dark cycle (lights on 7:00 am), at a temperature of 23°C ± 1°C. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of UNESC Ethics Committee (Protocols number 43/2009). Moreover, all efforts were made to minimize animal suffering as well as to reduce the number of animals.

2.2. Acute Fen administration

Animals received a single injection of Fen (6.25; 12.5 or 25 mg/kg, intraperitoneal) or tween 2% intraperitoneal. Locomotor activity was measured 2 h after the injection, and the rats were killed by decapitation right after the open-field task.

2.3. Chronic Fen administration

Fen was administered once a day (6.25, 12.5 and 25 mg/kg, intraperitoneal) during 14 days. Control groups received tween 2% intraperitoneal at the same volume. Locomotor activity was measured two hours after the last injection, and the rats were killed by decapitation right after the open-field task.

Note: The weight of the rats was assessed 3 times: 1) before the first injection of Fen (day 1), 2) on the seventh day of treatment (day 7) and after the last application of Fen (day 14) (Figure 5).

2.4. Behavior patterns of rat in the open field test

The task was performed in a 40×60 cm open field surrounded by 50 cm high walls. The floor of apparatus was constructed with varnished wood and divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear rectangle, in order to explore the arena for 5 min. In the open-field test was assessed the following behavioral parameters:

2.4.1. Crossings: Total number of square crossings in whole test period (Ericson et al, 1991; Prut & Belzung, 2003; Wultz et al, 1990).

2.4.2. Rearings: Total number of erect posture in whole test period (Ericson et al, 1991; Prut & Belzung).

2.4.3. Visits to center: Total number of visit to the center of open-field. A center square of 30x30cm was defined as the "center" area of the field.

2.4.4. Grooming: Total time (in seconds) of grooming behavior in whole test period. Include rat paw licking, nose/face grooming, head washing, body grooming/scratching, leg licking and tail/genitals grooming (Kalueff & Tuohimaa, 2004; Kalueff & Tuohimaa, 2005; Kalueff et al, 2007).

2.4.5. Sniffing: Total time (in seconds) of sniffing behavior in whole test period. Rats sniffs the environment in moving (walking + rearing) (Casarrubea et al, 2008; Casarrubea et al, 2009a; Casarrubea et al, 2009b; Meyerson & Hoglund, 1981).

2.4.6. Fecal boli: Total number of fecal boli produced in whole test period.

2.5. Tissue and homogenate preparation

Prefrontal cortex, hippocampus and striatum were removed and homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin). The homogenates were centrifuged at 800 × g for 10 min at 4°C and the supernatants kept at −70oC until being used for enzymes activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. Protein content was determined by the method described by Lowry and colleagues (1951) using bovine serum albumin as standard.

2.6. Activities of enzymes of Krebs cycle

2.6.1. Citrate synthase activity: Citrate synthase activity was assayed according to the method described by Shepherd and Garland (1969). The reaction mixture contained 100 mM Tris, pH 8.0, 100 mM acetyl CoA, 100 mM 5,5'-di-thiobis-(2- nitrobenzoic acid), 0.1% triton X-100, and 2–4 µg supernatant protein and was initiated with 100 µM oxaloacetate and monitored at 412 nm for 3 min at 25 °C.

2.6.2. Malate dehydrogenase activity: Malate dehydrogenase was measured as described by Kitto (1969). Aliquots (20 mg protein) were transferred into a medium containing 10 mM rotenone, 0.2% Triton X-100, 0.15 mM NADH, and 100 mM potassium phosphate buffer, pH

7.4, at 37°C. The reaction was started by addition of 0.33 mM oxaloacetate. Absorbance was monitored as described above.

2.6.3. Succinate dehydrogenase activity: Succinate dehydrogenase activity was determined according to the method of Fischer and colleagues (1985), measured by following the decrease in absorbance due to the reduction of 2,6-di-chloro-indophenol (2,6-DCIP) at 600nm with 700nm as reference wavelength (ε = 19.1 mM−1 cm−1) in the presence of phenazine methasulphate (PMS). The reaction mixture consisting of 40mM potassium phosphate, pH 7.4, 16mM succinate and 8µM 2,6-DCIP was preincubated with 40–80µg homogenate protein at 30oC for 20 min. Subsequently, 4mM sodium azide, 7µM rotenone and 40µM 2,6-DCIP were added and the reaction was initiated by addition of 1mM PMS and was monitored for 5 min.

2.7. Activities of mitochondrial respiratory chain enzymes

2.7.1. Complex I activity: NADH dehydrogenase (complex I) was evaluated according to Cassina and Radi (1996) by the determination of the rate of NADH-dependent ferricyanide reduction at λ = 420 nm.

2.7.2. Complex II activity: The activities of succinate-2,6- dichloroindophenol (DCIP)-oxidoreductase (complex II) was determined by the method described by Fischer and colleagues (1985). Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at λ = 600 nm.

2.7.3. Complex II-III activity: The activity of succinate:cytochrome c oxidoreductase (complex III) was determined by the method described by Fischer and colleagues (1985).

Complex II-III activity was measured by cytochrome c reduction using succinate as substrate at λ = 550 nm.

2.7.4. Complex IV activity: The activity of cytochrome c oxidase (complex IV) was assayed according to the method described by Rustin and colleagues (1994), measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome c (prepared by reduction of cytochrome with NaBH4 and HCl) at λ = 550 nm with 580 nm as the reference wavelength (ε= 19.1 mM−1 cm−1). The activities of the mitochondrial respiratory chain complexes were calculated as nmol. min-1. mg protein-1.

2.8. Activity of creatine kinase enzyme

Creatine kinase activity was measured in brain homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 μg protein in a final volume of 100 μL. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 3.2 mmol of ADP plus 0.8 mmol of reduced glutathione. 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). 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 after 20 min at 540 nm. Results were expressed as units/min×mg protein.

2.9. Statistical analysis

Data were analyzed by one-way analysis of variance followed by the Tukey test when F was significant and are expressed as mean ± standard deviation. All analyses were performed using the Statistical Package for the Social Science (SPSS; version 16.0) software.

3. Results

3.1. The effects of acute (Fig. 1A) or chronic (Fig. 1B) administration of fenproporex on open field behavior:

The acute administration of Fen significantly increased the number of crossing and rearing, at all doses administered. In addition, a single injection of Fen in high doses (12.5 mg/kg and 25mg/kg) increased the number of visits to center of open-field. No differences were observed for grooming, sniffing and amount of fecal boli, after acute administration of Fen **(Fig. 1A)**. Once more, the repeated administration of Fen increased the motor behaviors of rats – crossings and rearings. The total visits to center of open-field, sniffing and fecal boli were significantly higher after chronic Fen administration at the doses of 12.5 and 25.mg/kg, compared with the control group. Finally, the repeated administration of Fen significantly increased the amount of grooming at the dose of 25mg/kg **(Fig. 1B)**.

3.2. The effects of acute (Fig. 2A) or chronic (Fig. 2B) administration of fenproporex on enzymes of the Krebs cycle activities. Citrate synthase (CS), malate dehydrogenase (MD) and succinate dehydrogenase (SD) activities were measured in prefrontal cortex, hippocampus and striatum of rats:

In the acute protocol, Fen inhibited CS activity in the prefrontal at the dose of 25mg/kg, in the hippocampus at all doses administered (6.25, 12.5 and 25mg/kg) and striatum at 12.5 and 25mg/kg. Acute administration of Fen also inhibited the activity of MD in the prefrontal cortex at higer doses (12.5 and 25mg/kg) and hippocampus and striatum in all administered doses of Fen (6.25, 12.5 and 25mg/kg). SD activity was also measured and Fen inhibited the enzyme activity only in the hippocampus at all doses (6.25, 12.5 and 25mg/kg) administered **(Fig. 2A)**. In contrast, after repeated Fen (6.25, 12.5 and 25mg/kg) administration only SD was inhibited in the hippocampus **(Fig. 2B)**.

3.3. The effects of acute (Fig. 3A) or chronic (Fig. 3B) administration of fenproporex on mitochondrial respiratory chain complexes (I, II-II, III, IV) activity in the prefrontal cortex, hippocampus and striatum of rats:

In acute protocol, administration of Fen at all doses (6.25, 12.5 and 25mg/kg) resulted in a significant decreased of complexes I, II, III and IV of mitochondrial respiratory chain in prefrontal cortex and hippocampus **(Fig. 3A)**. However, administration of Fen in chronic protocol resulted in a significant inhibition of complexes II and IV of mitochondrial respiratory chain only in hippocampus **(Fig. 3B)**.

3.4. The effects of acute (Fig. 4A) or chronic (Fig. 4B) administration of fenproporex on creatine kinase activity in the prefrontal cortex, hippocampus and striatum of rats:

Acute Fen at all doses (6.25, 12.5 and 25mg/kg) administered significantly inhibited CK activity in the rat's hippocampus **(Fig. 4A)**. After repeated Fen (6.25, 12.5 and 25mg/kg) administration the CK activity was also inhibited in the hippocampus **(Fig. 4B)**.

3.5. The effects of chronic (Fig. 5) administration of fenproporex on body weight of rats:

Additionally, the body weight of all rats was measured continuously during chronic treatment on day 1, day 7 and day 14. No significant difference on body weight was found between groups in day 1. There was a weight gain of control animals on days 7 and 14. However, as expected, the administration of Fen dose-dependent decreased the weight of animals on days 7 and 14.

4. Discussion

In the present study, we found that acute and chronic administration of Fen increased locomotor and exploratory behavior. Fen is rapidly converted *in vivo* into AMPH, its

metabolite is readily detected by commercial urine toxicology tests (Cody et al, 1999), then, we suggest that the changes Fen- induced both, behavioral and neurochemical is via dopaminergic system. It is well described in literature that psychostimulants have serious possible side effects and particular behavioral changes and potential for abuse (Zhu & Reith, 2008).

Previous studies have shown that dopaminergic drugs such as amphetamine (Frey et al, 2006b), methylphenidate (Valvassori et al, 2007) and cocaine (Rodvelt et al, 2011) lead to hyperactivity in rats. Frassetto and colleagues (2006) also have demonstrated that rats treated with sibutramine - an anti-obesity drug with the ability to inhibit serotonin (5-HT), noradrenaline, and dopamine re-uptake – increases locomotion in rats.

In our study, we also found that acute and chronic administration of Fen in high doses increases visits to center of open-field - representing risk-taking behavior. In addition, we also found that chronic, but not acute administration, of Fen in high doses induces grooming and sniffing behavior. According to our data, Einat and colleagues (2007) showed that administration of amphetamine increased risk behavior in rats. Besides, methamphetamine, cocaine and nicotine also increased rearing, walking, grooming and sniffing until 80 min after the administration of these psychotropic drugs in rats (Izawa et al, 2006). It is well described in literature that drugs of abuse, such as cocaine and methamphetamine have the ability to stimulate dopamine transmission in the nucleus accumbens, inducing behavioral activation in rats, mice and other laboratory animals (Di Chiara, 2002).

In the present study, we also demonstrated that chronic administration of Fen, in the highest dose, increases the amount of feces produced by rats. This observation is indicative that this drug alters brain-gut axis, through central corticotropin-releasing factor receptors signals – an important mediator of the autonomic nervous system, particularly the parasympathetic, to increase colonic motility (Saito et al, 2005; Taché et al, 1999). It is know that hypersecretion of hypothalamic and/or extrahypothalamic CRF systems is a major factor in the pathogenesis of affective and anxiety disorders (Heinrichs, 1997). Pringle and

colleagues (Pringle et al, 2008) have demonstrated that CRF2 receptors are increased in the dorsal raphe nucleus 20 h following chronic amphetamine administrations and remain elevated up to 6 weeks of withdrawal.

In the present work, we observed that the activities of enzymes of Krebs cycle - CS, MD, SD - mitochondrial respiratory chain – complex I, II, II-II, IV - and creatine kinase were inhibited in some cerebral structures of rats submitted to administration of Fen. According to the data presented in this article, previous studies from our and other laboratories have demonstrated that chronic administration of amphetamines in rats also inhibited enzymes of energetic metabolism (Bachmann et al, 2009; Corrêa et al, 2007; Moretti et al, 2011; Streck et al, 2008; Valenzuela et al, 1987; Valvassori et al, 2010).

The enzymatic degradation or autooxidation of dopamine results in the formation of hydrogen peroxide and superoxide radical. Hydrogen peroxide is susceptible to iron catalyzed formation of hydroxyl free radical via Fenton reaction (Kopin, 1993; Olanow, 1992). It is know that AMPH induces generation of free radicals via the oxidative catabolism of dopamine and the subsequent dysfunction of mitochondrial respiration (Burrows et al, 2000; Cadet et al, 2005; Deng et al, 2002; Valvassori et al, 2010). Several studies have demonstrated that AMPHs induces oxidative damage in protein, lipids and DNA in the brain of rats (Andreazza et al, 2008; Frey et al, 2006a; Frey et al, 2006b, Frey et al, 2006c).

Another important point to consider here is that changes in energy metabolism did not occur similarly in brain structures. This can be explained by the fact that the brain is the most complex biological structures known, formed by several regions that respond differently, and partly with different types of neurons. In addition, inside a homogeneous population of cells, there is heterogeneity in terms of physiological and metabolic characteristics (Lai et al, 1977; Sims, 1991; Sonnewald et al, 2008).

Here we also observed that the acute treatment causes more changes in energy metabolism that in the chronic treatment. The reason for this discrepancy this study is unclear but could be related to desensitization to the effects of repeated Fen or still for

adaptation mechanism. Excessive stimulation of dopamine receptors during exposure to psychostimulants induces various molecular adaptive changes in the mesolimbic dopamine pathway (Nestler, 2005; White & Kalivas, 1998). However the increase in grooming, sniffing and fecal boli was only observed with chronic treatment, suggesting that changes in energy metabolism are not related to these behavioral changes.

In conclusion, the present study showed that acute Fen administration induced manic-like hyperactivity and energetic metabolism alteration in the rat brain. However, longer periods of exposure to Fen induce hyperactivity, stereotyped behaviors – grooming and sniffing – and increased fecal boli, but does not alter energy metabolism in rat brain. Thus, we suggest that administration of Fen induces behavioral and neurochemical changes similar to those observed with administration of psychostimulants such as amphetamine.

Acknowledgements

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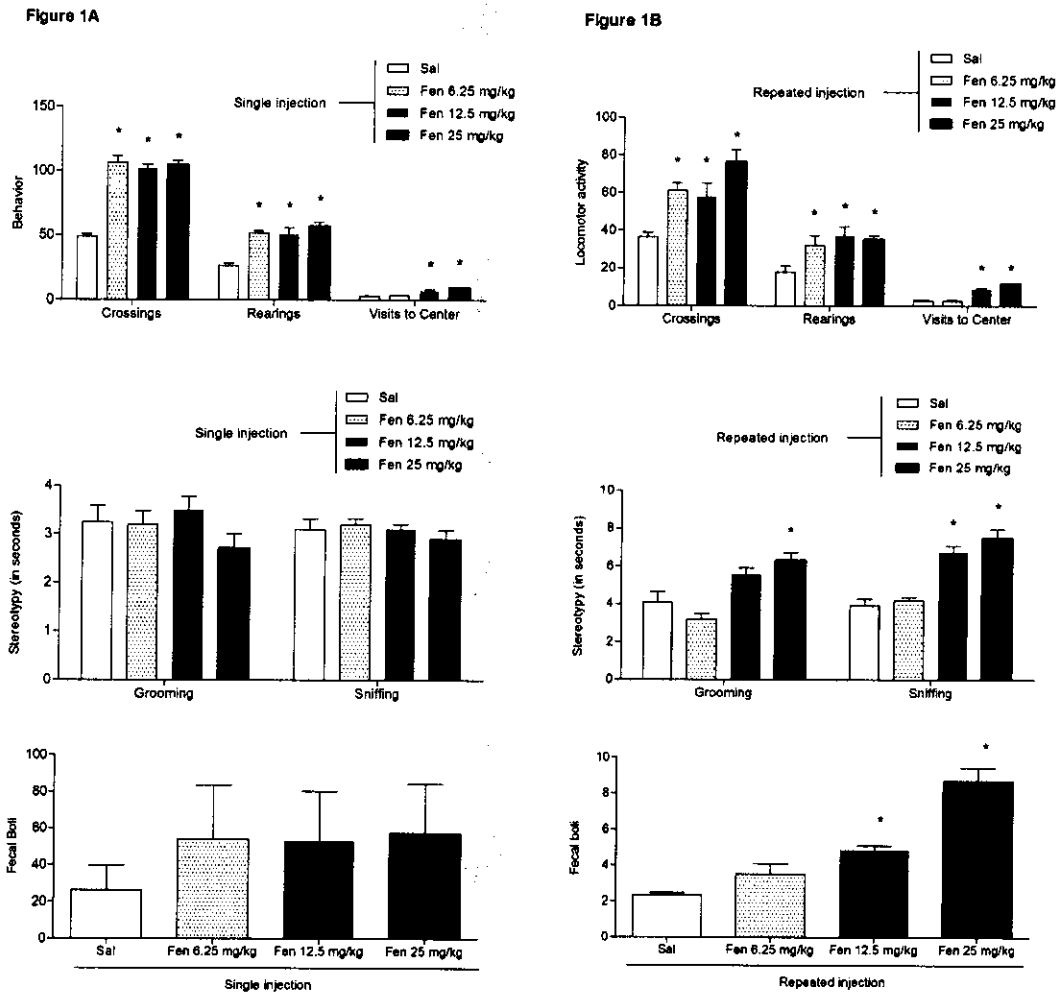

Figure 1 – The effects of acute **(A)** or chronic **(B)** administration of fenproporex on open field behavior. Values are expressed as mean ± S.D., for ten animals in each group. * Different from control; $p<0.05$.

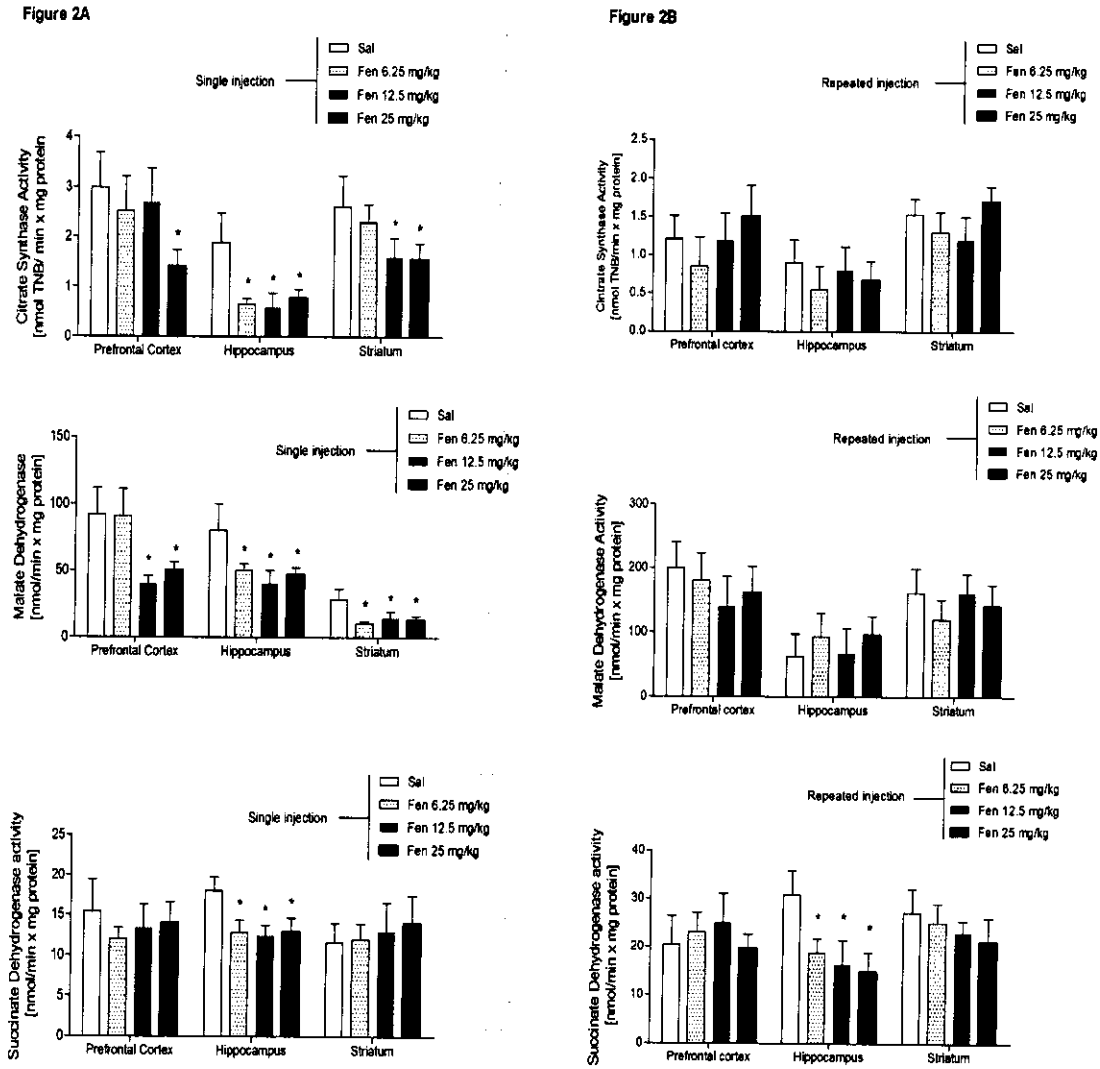

Figure 2 – The effects of acute **(A)** or chronic **(B)** administration of fenproporex on enzymes of the Krebs cycle activities. Citrate synthase (CS), malate dehydrogenase (MD) and succinate dehydrogenase (SD) activities were measured in prefrontal cortex, hippocampus and striatum of rats: Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6), for six independent experiments performed in duplicate. * Different from control; $p<0.05$.

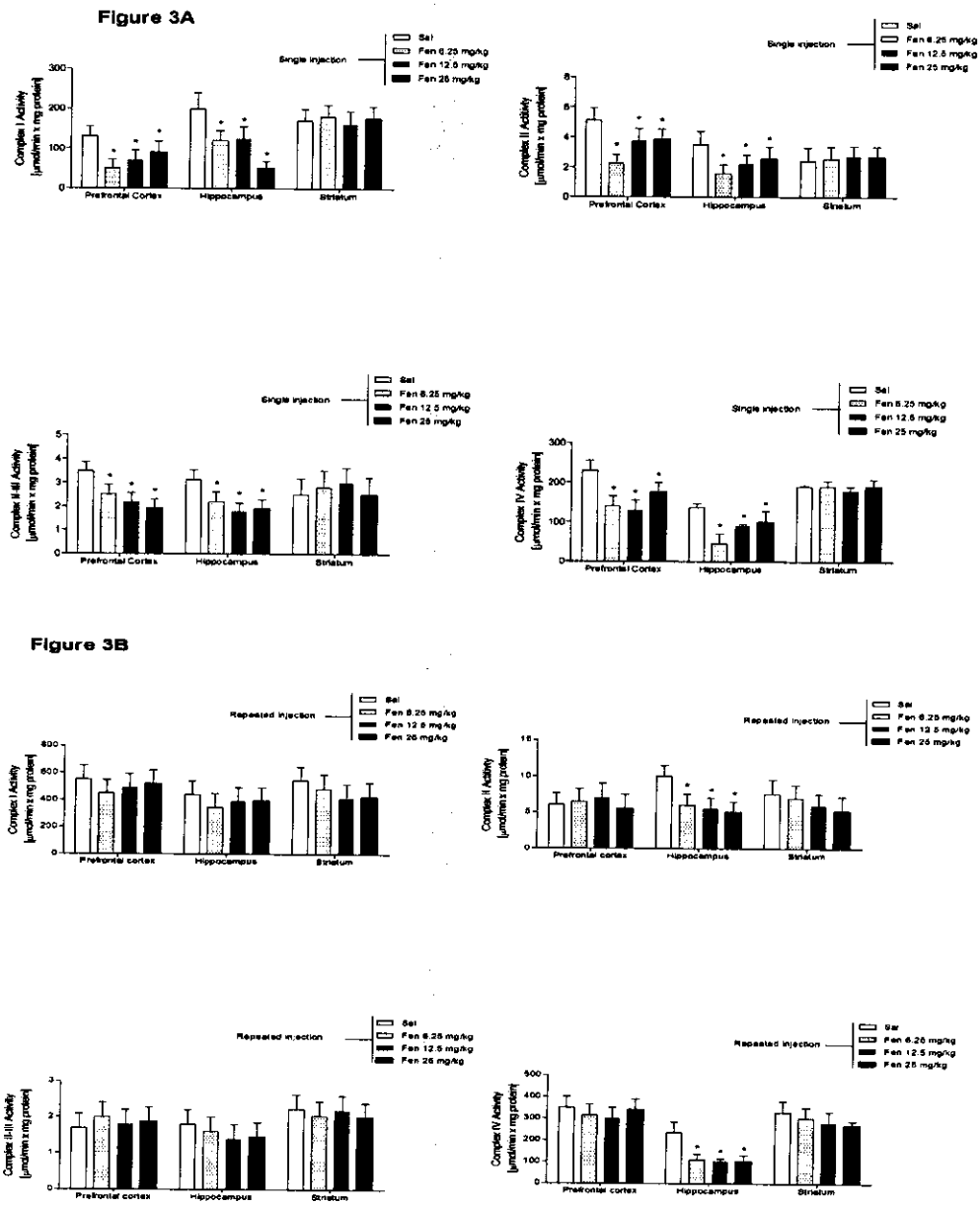

Figure 3 – The effects of acute **(A)** or chronic **(B)** administration of fenproporex on mitochondrial respiratory chain complexes (I, II-II, III, IV) activity in the prefrontal cortex, hippocampus and striatum of rats: Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min.mg protein, mean ± S.D. (n=6), for six independent experiments performed in duplicate. * Different from control; $p<0.05$.

Figure 4A

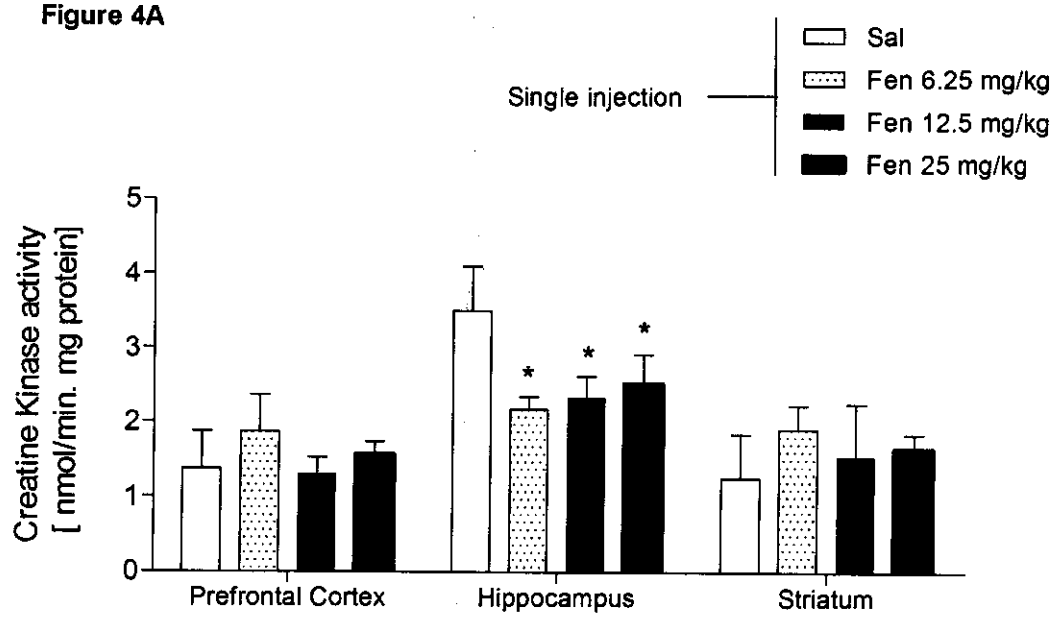

Figure 4B

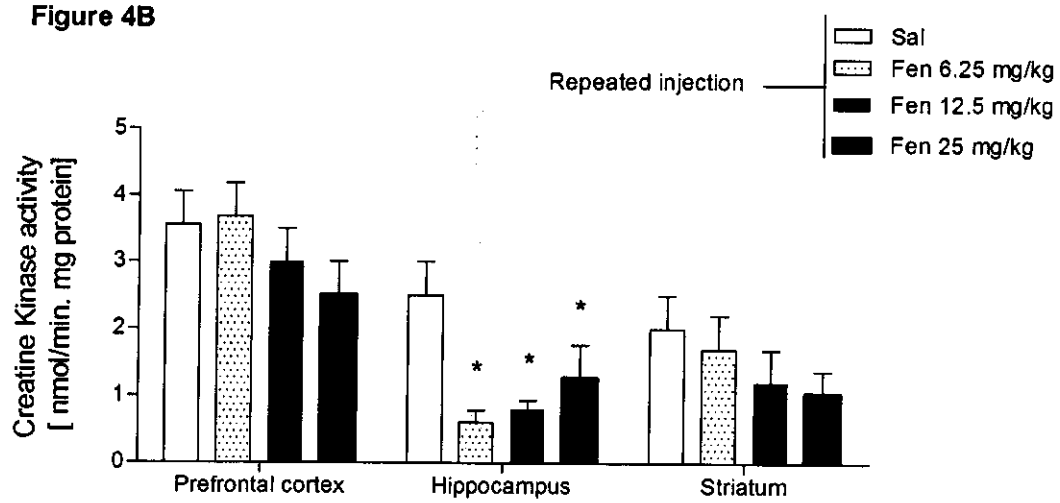

Figure 4 – The effects of acute **(A)** or chronic **(B)** administration of fenproporex on creatine kinase activity in the prefrontal cortex, hippocampus and striatum of rats. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min . mg protein, mean ± S.D. (n=6), for six independent experiments performed in duplicate. * Different from control; $p<0.05$.

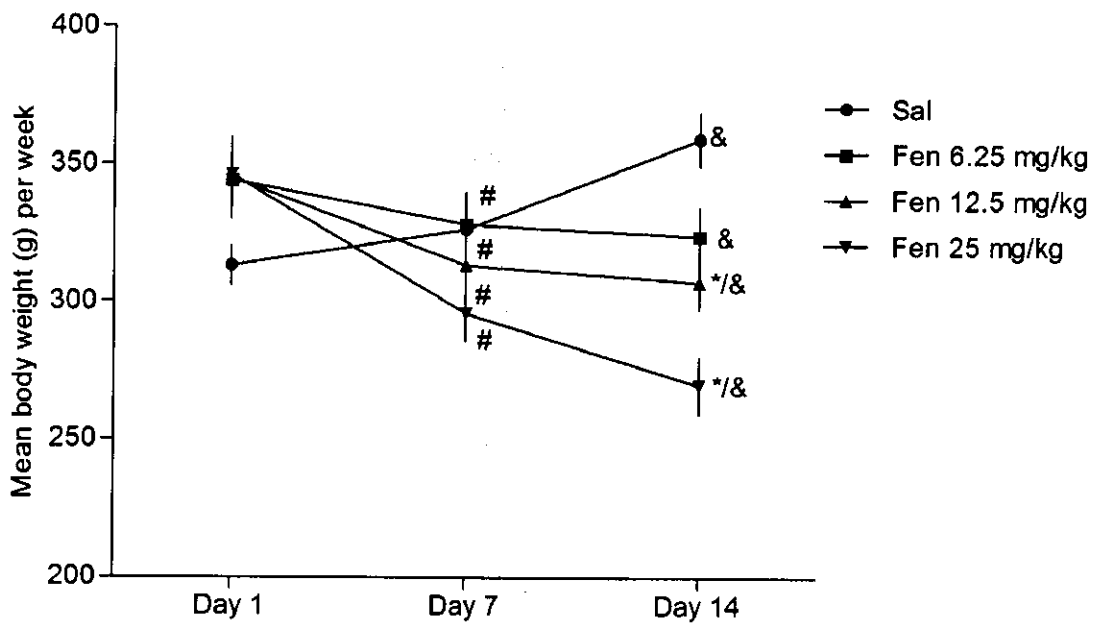

Figure 5 – The effects of chronic administration of fenproporex on body weight of rats. Values are expressed as mean ± S.D., for ten animals in each group. * Different from control; $p<0.05$. #Different from day 1; $p<0.05$. &Different from day 7; $p<0.05$.

3.3. ARTIGO 3

Effects of lithium and valproate on biochemical and behavioral parameters in brain of rats treated with fenproporex

Artigo submetido ao periódico *molecular neurobiology*

Effects of lithium and valproate on biochemical and behavioral parameters in brain of rats treated with fenproporex

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Abstract

Fenproporex is converted *in vivo* into amphetamine, which is used to induce mania-like behaviors in animals. We evaluated the behavioral and biochemical parameters in the brain of rats submitted to chronic administration of fenproporex and treated with lithium (Li) and valproate (VPA). In the reversal experiment, rats were first given fenproporex or vehicle for 14 days, and then, between days 8-14, rats were treated with Li, VPA or vehicle. In the prevention experiment, rats were pretreated with Li, VPA or vehicle, and then, with fenproporex or vehicle. Behavior and brain energy metabolism were evaluated. Results showed that in the reversion and prevention experiments, fenproporex induced hyperlocomotion, but treatment with mood stabilizers prevented and reversed this effect. In the reversal experiment, body weight was not altered in day 1 and 7. However, in the second week of fenproporex administration, body weight decreased. In the prevention experiment, body weight was not altered. Moreover, in the reversion and prevention experiments we verified inhibition of enzymes of energy metabolism in the hippocampus. Moreover, mood stabilizers reversed and prevented these alterations. These findings suggest that chronic administration of fenproporex may be used as an animal model of mania.

Keywords: Bipolar disorder; Fenproporex; Behavior activity; Mitochondrial dysfunction; Mood stabilizers.

Introduction

Bipolar disorder (BD) is a chronic and often severe psychiatric illness characterized by manic and depressive episodes. Among the most effective treatments, mood stabilizers represent the keystone in acute mania, depression, and maintenance treatment of BD. The drugs that are considered first line for BD are lithium (Li) and valproate (VPA). They offer reasonable protection against recurrent mood episodes and have a modest antidepressant property *[1]*. However, treatment response is a highly heterogeneous trait, thus emphasizing the need for a structured informational framework of phenotypic and genetic predictors *[2]*.

Modeling of human neuropsychiatric disorders in animals is extremely challenging given the subjective nature of many symptoms, and lack of biomarkers and objective diagnostic tests. Nonetheless, progress in understanding pathophysiology and in treatment development would benefit greatly from improved animal models *[3-7]*. Animal models should meet three sets of criteria: face, construct, and predictive validities. Face validity represents the ability of mimicking the symptoms of a determinate human disorder, whereas construct validity refers to similarity between the mechanisms of the animal model and of the human disorder. Finally, the predictive validity refers to the efficacy of treatment drugs use for human disease for the phenotype of the model animal *[9]*. An adequate animal model of BD should resemble some features of a manic episode such as euphoria, irritability, aggressiveness, hyperactivity, insomnia or increased sexual drive.

Considering the difficulty of modeling the highly complex mood swinging nature of BD, the psychostimulant-induced hyperactivity model is the best established *[8]*. Repeated injections of amphetamine (AMPH) induce extracellular dopamine increase [10], thus leading to hyperactivity in animals *[8]*. This has been suggested as a relevant animal model of mania *[5, 11-13]* also because studies have reported dopaminergic alterations in BD patients *[14,15]*. Studies with this animal model showed that longer periods of induced manic-like hyperactivity are associated with severe brain damage *[5-7,16]*. Besides, studies also

showed that mood stabilizers reverse and prevent the symptoms induced by AMPH in the animal model of mania *[4,5,17-21]*.

Fenproporex is the second most commonly consumed AMPH-based anorectic worldwide *[22]*. It acts by releasing or blocking neuronal reuptake of noradrenaline and dopamine *[23,24]*. In addition, fenproporex is rapidly converted *in vivo* into AMPH *[25]*, which can be detected in urine for up to 60 hours after ingestion *[26]*. Studies suggest that AMPH induce neurotoxicity through production of free radicals *[27]* and mitochondrial apoptotic pathway through cytochrome *c* release *[28,29]*, accompanied by a decrease in mitochondrial potential *[30]*.

Mitochondria are intracellular organelles which play a crucial role in adenosine triphosphate (ATP) production *[31]*. The tricarboxylic acid (TCA) cycle is the major final common pathway for oxidation of carbohydrates, lipids and some amino acids, which produces reducing equivalents in the form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) that result in production of large amounts of ATP via oxidative phosphorylation *[32]*.

Citrate synthase (CS) (EC 4.1.3.7) is localized within cells in the mitochondrial matrix and catalyzes the condensation of oxaloacetate and the acetyl group of acetyl coenzyme-A (Acetil CoA), an important regulatory step of TCA cycle. Malate dehydrogenase catalyzes the dehydrogenation of L-malate to oxaloacetate in the final step of TCA cycle *[32]*. Succinate dehydrogenase (SDH) is one of the most reliable markers of the mitochondrial capability to supply an adequate amount of ATP, as it is part of both the TCA cycle and the respiratory chain (complex II). The SDH catalyses the oxidation of succinate to fumarate in the TCA cycle, and feeds electrons to the respiratory chain ubiquinone pool *[33,34]*. Most cell energy is obtained through of action of various enzyme complexes located in a special structure of the inner mitochondrial membrane, the mitochondrial respiratory chain *[35]*.

Creatine kinase (CK) (EC 2.7.3.2) is important for normal energy homeostasis by exerting several integrated functions, such as temporary energy buffering, metabolic

capacity, energy transfer and metabolic control. The brain, like other tissues with high and variable rates of ATP metabolism, presents high phosphocreatine concentration and CK activity *[36-38]*.

Based on the hypothesis that fenproporex is converted into AMPH, and that AMPH has been involved in the production of free radicals and mitochondrial dysfunction, in the present study we evaluated the locomotor activity and brain energy metabolism in rats submitted to chronic administration of fenproporex and treated with Li and VPA.

Material and methods

Animals

Adult male Wistar rats (250-300g) were obtained from Central Animal House of Universidade do Extremo Sul Catarinense. The animals were caged in group of 12 with free access to food and water, maintained on a 12-h light-dark cycle (lights on 7:00 am), at a temperature of 23°C ± 1°C. All experimental procedures were carried out in accordance with the "Principles of Laboratory Animal Care" (NIH publication no. 80-23, revised 1996) and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of UNESC Ethics Committee (Protocols number 43/2009). Moreover, all efforts were made to minimize animal suffering as well as to reduce the number of animals.

Treatment protocols

Reversion experiment

In the reversion experiment, we simulated the treatment of acute manic episode. Animals received one daily intraperitoneal (i.p.) injection of fenproporex 12.5 mg/kg or vehicle (Tween 2%; control group) for 14 days (45 animals per group). On the 8th day of

experiment, the animals in the vehicle and fenproporex group were divided in 3 groups (15 animals per group): 1) treatment with Li (47.5 mg/kg i.p.); 2) treatment with VPA (200mg/kg i.p.) and 3) treatment with vehicle for 7 days twice a day for all drugs. On the 15th day of experiment, animals received a single injection of fenproporex or vehicle and behavior was assessed 2h after the last injection. Rats were killed by decapitation immediately after the open-field task and hippocampus, striatum and prefrontal cortex were dissected, rapidly frozen and stored at -70°C until assayed (See scheme 1).

Prevention experiment

In the prevention experiment, we simulated the maintenance phase of BD treatment. Animals were treated with Li (47.5mg/kg i.p.), VPA (200mg/kg i.p.) or vehicle (Tween 2%) twice a day for 14 days. Between 8th and 14th days, Li, VPA and tween 2% treated animals additionally received one daily i.p. injection of either fenproporex 12.5 mg/kg or vehicle (15 animals per group). Locomotor activity was measured 2h after the last injection. Rats were killed by decapitation immediately after the open-field task and hippocampus, striatum and prefrontal were dissected, rapidly frozen and stored -70°C until assayed (See scheme 2).

Body weight

Rat body weight was measured: 1) before the first injection (day 1), 2) on the seventh day of experiment (day 7) and after the last injection (day 14).

Behavior patterns of rat in the open field test

The task was performed in a 40×60 cm open field surrounded by 50 cm high walls. Floor of apparatus was constructed with varnished wood and divided into 12 equal rectangles by black lines. Animals were gently placed on the left rear rectangle, in order to explore the arena for 5 min. In the open-field test was assessed the following behavioral parameters:

Crossings: total number of square crossings in whole test period *[39-41]*.

Rearings: total number of erect posture in whole test period *[39,40]*.

Visits to center: total number of center visits of open-field. A center square of 30x30cm was defined as the "center" area of the field.

Grooming: total time (in seconds) of grooming behavior in whole test period. Include rat paw licking, nose/face grooming, head washing, body grooming/scratching, leg licking and tail/genitals grooming *[42-44]*.

Sniffing: total time (in seconds) of sniffing behavior in whole test period. Rats sniff the environment in moving (walking + rearing) *[45-48]*.

Fecal boli: total number of fecal boli produced in whole test period.

Tissue and homogenate preparation

Prefrontal cortex, hippocampus and striatum were removed and homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin). Homogenates were centrifuged at 800 × g for 10 min at 4°C and the supernatants kept at −70°C until being used for enzymes activity determination. The maximal period between homogenate preparation and enzyme analysis was less than 5 days. Protein content was determined by the Lowry's method *[49]* using bovine serum albumin as standard.

Activities of enzymes of TCA cycle

Citrate synthase activity: CS activity was assayed according to the method described by Shepherd and Garland *[50]*. The reaction mixture contained 100 mM Tris, pH 8.0, 100 mM acetyl CoA, 100 mM 5,5'-di-thiobis-(2- nitrobenzoic acid), 0.1% triton X-100, and 2–4 μg supernatant protein and was initiated with 100 μM oxaloacetate and monitored at 412 nm for 3 min at 25 °C.

Malate dehydrogenase activity: Malate dehydrogenase was measured as described by Kitto *[51]*. Aliquots (20 mg protein) were transferred into a medium containing 10 mM rotenone, 0.2% Triton X-100, 0.15 mM NADH, and 100 mM potassium phosphate buffer, pH 7.4, at 37°C. Reaction was started by addition of 0.33 mM oxaloacetate. Absorbance was monitored as described above.

Succinate dehydrogenase activity: SDH activity was determined according to Fischer's method *[52]*, measured by following the decrease in absorbance due to the reduction of 2,6-di-chloro-indophenol (2,6-DCIP) at 600nm with 700nm as reference wavelength (ε = 19.1 mM−1 cm−1) in the presence of phenazine methasulphate (PMS). Reaction mixture consisting of 40mM potassium phosphate, pH 7.4, 16mM succinate and 8μM 2,6-DCIP was preincubated with 40–80μg homogenate protein at 30oC for 20 min. Subsequently, 4mM sodium azide, 7μM rotenone and 40μM 2,6-DCIP were added and the reaction was initiated by addition of 1mM PMS and was monitored for 5 min.

Activities of mitochondrial respiratory chain enzymes

Complex I activity: NADH dehydrogenase (complex I) was evaluated according to Cassina and Radi *[53]* by the determination of the rate of NADH-dependent ferricyanide reduction at λ = 420 nm.

Complex II activity: succinate-2,6- dichloroindophenol (DCIP)-oxidoreductase (complex II) activity was determined by the method described by Fischer and colleagues *[52]* following absorbance decrease of 2,6-DCIP due to the reduction at λ = 600 nm.

Complex II-III activity: succinate:cytochrome c oxidoreductase (complex III) acivity was determined by the method described by Fischer and colleagues *[52]* measuring cytochrome c reduction using succinate as substrate at λ = 550 nm.

Complex IV activity: cytochrome c oxidase (complex IV) acitivity was assayed according to the method described by Rustin and colleagues *[54]*, measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome c (prepared by reduction of cytochrome with NaBH4 and HCl) at λ = 550 nm with 580 nm as the reference wavelength (ε= 19.1 mM−1 cm−1). Activities of the mitochondrial respiratory chain complexes were calculated as nmol. min-1. mg protein-1.

Activity of creatine kinase enzyme

CK activity was measured in brain homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 μg protein in a final volume of 100 μL. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 3.2 mmol of ADP plus 0.8 mmol of reduced glutathione. Reaction was stopped after 10 min by addition of 1 μmol of p-hydroxymercuribenzoic acid. Creatine formed was estimated according to the colorimetric method of Hughes *[55]*. Color was developed by addition of 100 μL 2%α-naphtol and 100 μL 0.05% diacetyl in a final volume of 1 mL and read spectrophotometrically after 20 min at 540 nm. Results were expressed as units/min×mg protein.

Statistical analysis

Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant and are expressed as mean ± standard error to behavior text and expressed

as mean ± standard deviation to biochemical text. All analysis were performed using the Statistical Package for the Social Science (SPSS; version 16.0) software.

Results

Results showed that there was a significant effect of fenproporex and stabilizers of mood in the behavior, stereotypy and fecal boli. We showed that administration of fenproporex increased the number of crossings, rearings, visits to center, grooming, sniffing and fecal boli, both in reversion (Figure 1A) and prevention (Figure 1B) experiment, and this effect was reverted and prevented by Li and VPA. Li or VPA alone did not alter behavioral parameters, indicating that mood stabilizers effects on fenproporex-treated rats were not associated with sedation. Additionally, rat body weight was measured continuously during the experiment on day 1, day 7 and day 14, during both reversion and prevention experiment. In the reversion experiment (Figure 2A), no significant difference on body weight was found between groups in day 1 and 7, while that in the second week of fenproporex administration there was a decreased in weight body of rats. However, this decrease was reversed by Li and VPA. In the prevention experiment (Figure 2B), no significant difference on body weight was found between groups.

In the present study, we also showed that the fenproporex-induced hyperlocomotion in the reversion experiment inhibited SDH activity (figure 3A), complex II (figure 4A), complex IV (figure 4A) and CK (figure 5A) enzyme in the hippocampus. Li and VPA treatments reversed this inhibition. Moreover, CS (figure 3A), malate dehydrogenase (figure 3A), complex I (figure 4A) and complex II-III (figure 4A) activities were not altered in the brain regions studied. None of the enzymes were altered in the prefrontal cortex and striatum.

Our results also showed that fenproporex, in the prevention experiment, inhibited malate dehydrogenase (figure 3B), SDH (figure 3B), complex II (figure 4B), complex IV (figure 4B) and CK (figure 5B) activities enzyme in the hippocampus and both Li and VPA

treatments were only not able to prevent the complex IV inhibition. CS (figure 3B), complex I (figure 4B) and complex II-III (figure 4B) activities were not altered. None of the enzymes were altered in the prefrontal cortex and striatum.

Discussion

Recent advances regarding underlying neural mechanisms, etiology, genetics and new pharmacological approaches have facilitated the development of an animal model of mania *[3-7]*. Several studies suggested that AMPH may be used to induce mania-like symptoms in animals *[5, 8, 11-13]* such as hyperactivity probably due extracellular dopamine increase *[10]*. In this context, several studies have reported that BD patients represent dopaminergic and locomotor alterations *[14,15]*.

Fenproporex is an AMPH-derived anorectic rapidly converted *in vivo* into AMPH. Urine toxicology screening is used in many occupational settings to detect AMPH abuse. AMPH screening tests are commonly considered positive if the concentration of this drug in urine exceeds 1.0 ng/mL *[22]*. Besides, fenproporex is not detected by commonly used clinical urine tests. However, its metabolite is readily detected by commercial urine toxicology tests. Consumption of a low daily dose of 10 mg of fenproporex led to the detection of AMPH within 3 hours in urine with peak urinary levels greater than 4.0 nanograms per milliliter *[25]*.

Femproporex is classified as dopaminergic agents of indirect action. Thus, represent complexes actions on the dopaminergic presynaptic terminal, but its action most significant is the connection to the transporter protein and the induction of reverse transport of dopamine. The resulting end of the actions of AMPH in terminals of dopamine is the increased in the concentration of dopamine in the synaptic cleft. Dopamine accumulated in the cleft interacts with dopamine D1 and D2 receptors initiating a sequence of events that alters the neural activity and expression of behavior *[56]*.

Studies have shown that psychostimulants such as AMPH *[5]*, methylphenidate *[57]* and cocaine *[58]* have the ability to stimulate dopamine transmission in the nucleus accumbens, inducing behavioral activation in rats, mice and other laboratory animals *[59]*. Besides, Frassetto and colleagues *[60]* have demonstrated that rats treated with sibutramine, an anti-obesity drug, also increases locomotion in rats.

In this scenario, our results for behavior activity of reversion and prevention experiment showed an increase in the number of crossings, rearings, groomings, sniffing and visits to center in rats submitted to chronic administration of fenproporex, and the treatment with Li and VPA reversed and prevented this alteration. Einat and colleagues *[61]* showed that administration of AMPH increased risk behavior in rats. Besides, methamphetamine, cocaine and nicotine also increased rearing, walking, grooming and sniffing until 80 min after the administration of these psychotropic drugs in rats *[62]*.

We have also demonstrated increased amount of feces in both experiments with both Li and VPA treatments reverting and preventing this alteration. This observation is indicative that this drug may alter brain-gut axis, probably through central corticotropin-releasing factor receptors signals to increase colonic motility *[63,64]*. It is know that hypersecretion of hypothalamic and/or extrahypothalamic CRF systems is a major factor in the pathogenesis of affective and anxiety disorders *[65]*. Pringle and colleagues *[66]* have demonstrated that CRF2 receptors are increased in the dorsal raphe nucleus 20 h following chronic AMPH administrations and remain elevated up to 6 weeks of withdrawal.

Finally, body weight was decreased in the reversion experiment only after the second week of femproporex administration. In the prevention experiment, body weight was not altered in any of the times evaluated. Moreover, studies with this animal model showed that longer periods of induced manic-like hyperactivity are associated with severe brain damage *[5-7, 16]*. Thus, AMPH and derived substances induce neurotoxicity through the production of free radicals and mitochondrial dysfunction *[67]*. Oxidative stress may occur by excessive formation of free radicals, which may cause oxidative damage to proteins, lipids, and

deoxyribonucleic acid *[68,69]*. It is established that the impairment of the respiratory chain in mitochondria leads to an increase of free radicals generation, especially superoxide, and that these reactive oxygen species inhibit the mitochondrial respiratory chain, resulting in the generation of more reactive species, forming a cyclic phenomenon *[70,71]*. In this scenario, the oxidative damage induced by AMPH might be responsible for the alterations in the energy metabolism caused by fenproporex administration in the animal model of mania here studied.

The present results also showed that the administration of fenproporex in the prevention experiment inhibited the activity of malate dehydrogenase, SDH, complex II, complex IV and CK enzyme in the hippocampus. However, the treatment with Li and VPA prevented this inhibition, except prevented the inhibited of complex IV. In addition, our results also showed that chronic administration of fenproporex in the reversion experiment inhibited the activity of SDH, complex II, complex IV and CK enzyme in the hippocampus. But the treatment with Li and VPA reversed this inhibition in all enzymes.

The hippocampus is one region in the brain that has received significant attention in mood disorder research, and the highly plastic, stress sensitive hippocampal complex may play a central role in mood disorder *[72]*. Studies have indicated the presence of cellular damage and volumetric changes in the hippocampus of patients with mood disorders and schizophrenia *[73,74]*. Enlarged right hippocampal volume is reportedly associated with poor neuropsychological functioning in bipolar disorder *[75]*, and findings from a neuropathological study have indicated a reduction and dysgenesis of various neuronal cell lines in entorhinal and hippocampal cortex of bipolar subjects *[76]*.

In addition, damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of psychiatric disorders *[77]*. The CS, malate dehydrogenase and SDH enzyme belong to TCA cycle that is responsible for the generation of approximately 67% of all reducing equivalents per molecule of glucose, hence factors that influence TCA cycle flux will be of critical importance for oxidative

phosphorylation *[78]*. In addition, the complexes I, II, III and IV of mitochondrial respiratory chain belong to major ATP-regeneration pathway, which supplies more than 95% of the total energy requirement in the cells *[79]*. CK enzyme is present in tissues with high demand energy, and also contributes to maintain ATP levels *[36-38]*.

Studies have shown deletions and mutations in genes coding for complex I subunits in patients with BD *[80]*. Recent deoxyribonucleic acid (DNA) microarray analyses *[81]*, in postmortem samples from the frontal cortex and hippocampus found that the expression of many messenger ribonucleic acid (mRNA) coding for subunits of complexes I–V was decreased in patients with BD *[81,82]*.

Konradi et al. *[82]* reported decreased expression of nuclear gene coding for enzymatic complexes responsible for oxidative phosphorylation and reduced expression of nuclear genes related to proteasome degradation in the hippocampi of nine subjects affected by BD. A study by Kato et al. *[83]* found a significantly higher ratio of deleted to wild-type mitochondrial deoxyribonucleic acid (mtDNA) in the cerebral cortex of patients with BD, when compared to age-matched controls. More recently, post mortem studies in hippocampus *[83]* and cortex *[81]* of BD patients showed a decrease in the expression of nuclear genes coding for mRNA of the mitochondrial respiratory chain enzymes. MacDonald et al. *[84]* presented very interesting results, showing that levels mRNA of CK are decreased in BD patients, especially in the hippocampus.

Corrêa et al. *[18]* showed that animal model of mania induced by AMPH inhibited CS activity in rat hippocampus. However, VPA administration reversed the CS activity inhibition and the Li prevented the enzyme inhibition. Streck et al. *[19]* showed that AMPH inhibited CK activity in brain rats and that VPA and Li were not able to reverse and/or prevent the enzyme inhibition. Zugno et al. *[21]* showed that AMPH increased Na(+),K(+)-ATPase activity in brain rats and that VPA or Li reversed this effect. Moreover, VPA and Li did not alter Na(+),K(+)-ATPase activity. Valvassori et al. *[20]* showed that AMPH inhibited mitochondrial respiratory chain activity in brain of rats. However, VPA reversed AMPH-induced dysfunction, but the Li

was not able to reverse. Besides, VPA and Li exert protective effects against this AMPH-induced mitochondrial dysfunction. Others studies showed that the CS and CK enzymes were inhibited in animal submitted to model of mania induced by ouabain *[85,86]*. Study of Freitas et al. *[87]* showed that the activity of complexes I, II, II-III and IV was increased in brain of rats after induced of animal model of mania by ouabain.

Finally, the present study shows increased locomotion, decreased body weight and inhibition of energy metabolism in brain of rats submitted to chronic administration fenproporex. Moreover, mood stabilizers, such as Li and VPA, reversed and prevented these alterations. Thus, we suggested that this regimen of fenproporex administration may be used as an animal model of mania.

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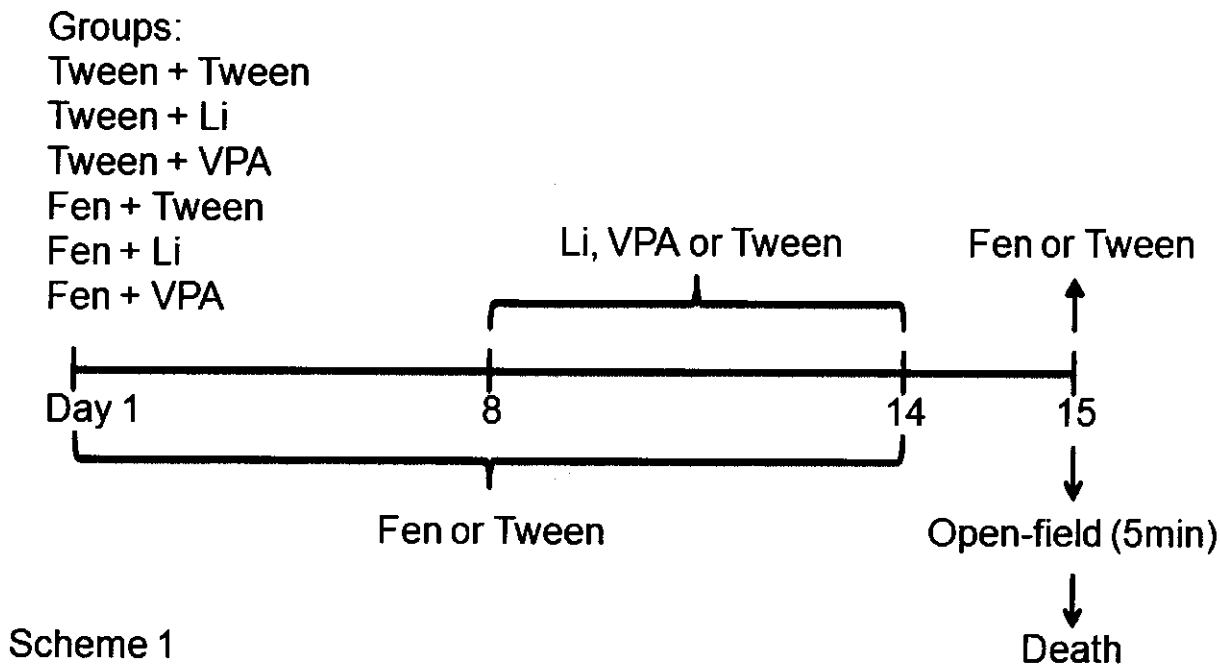
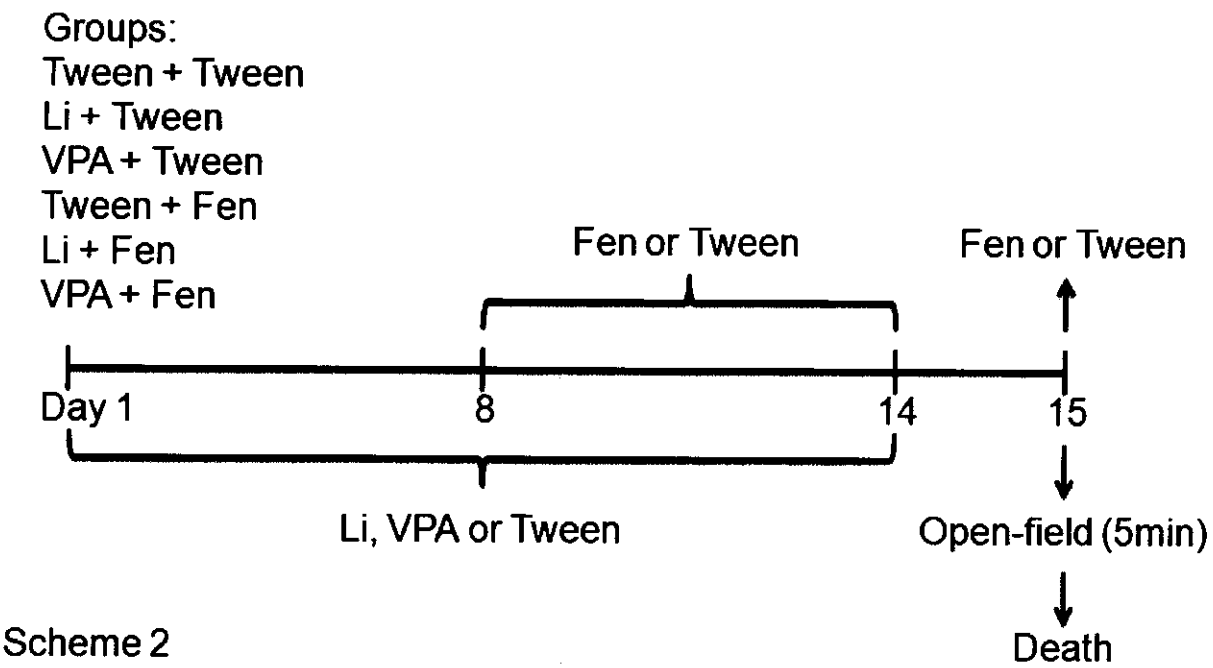


Scheme 1 – Reversion experiment



Scheme 2 – Prevention experiment

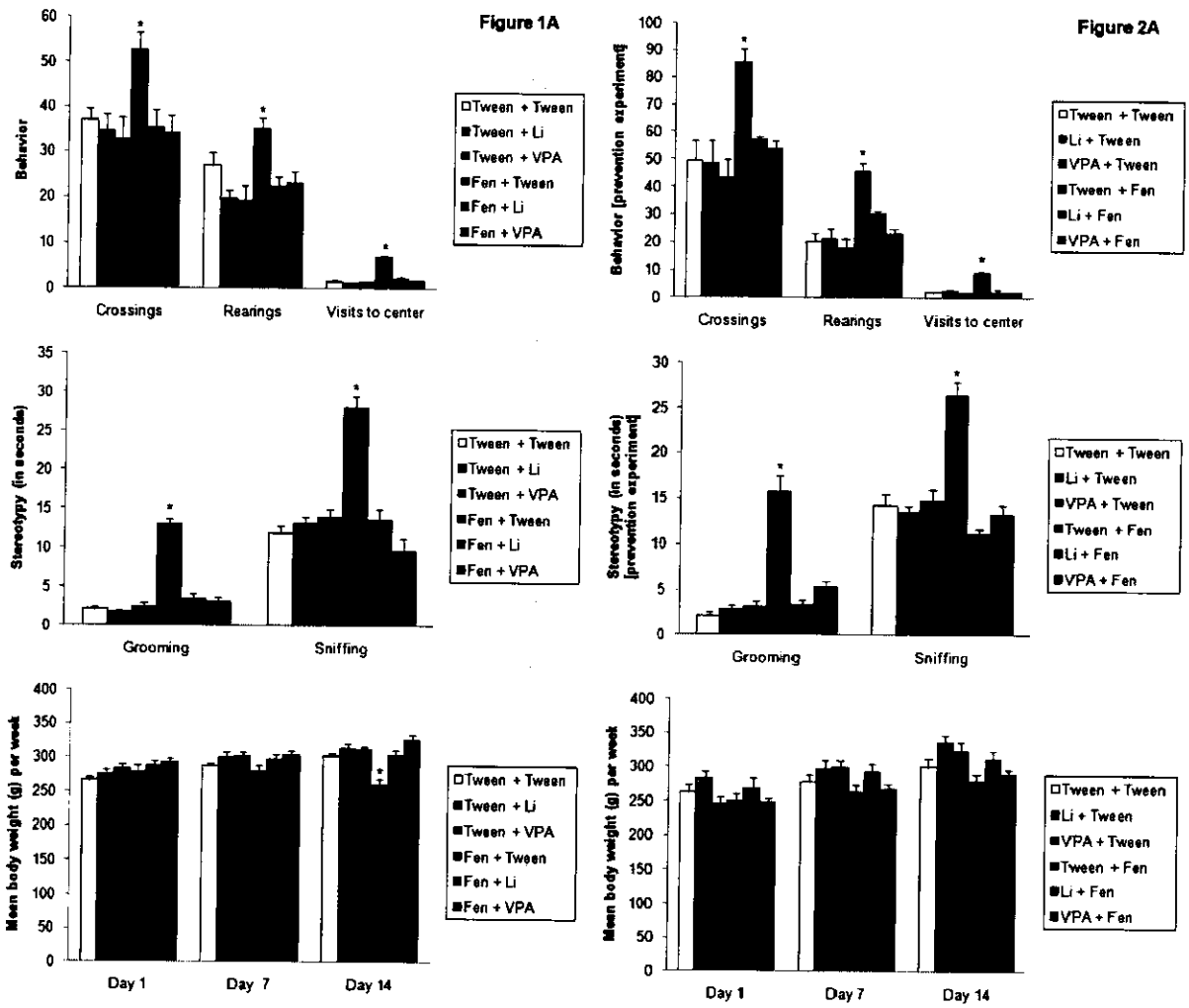
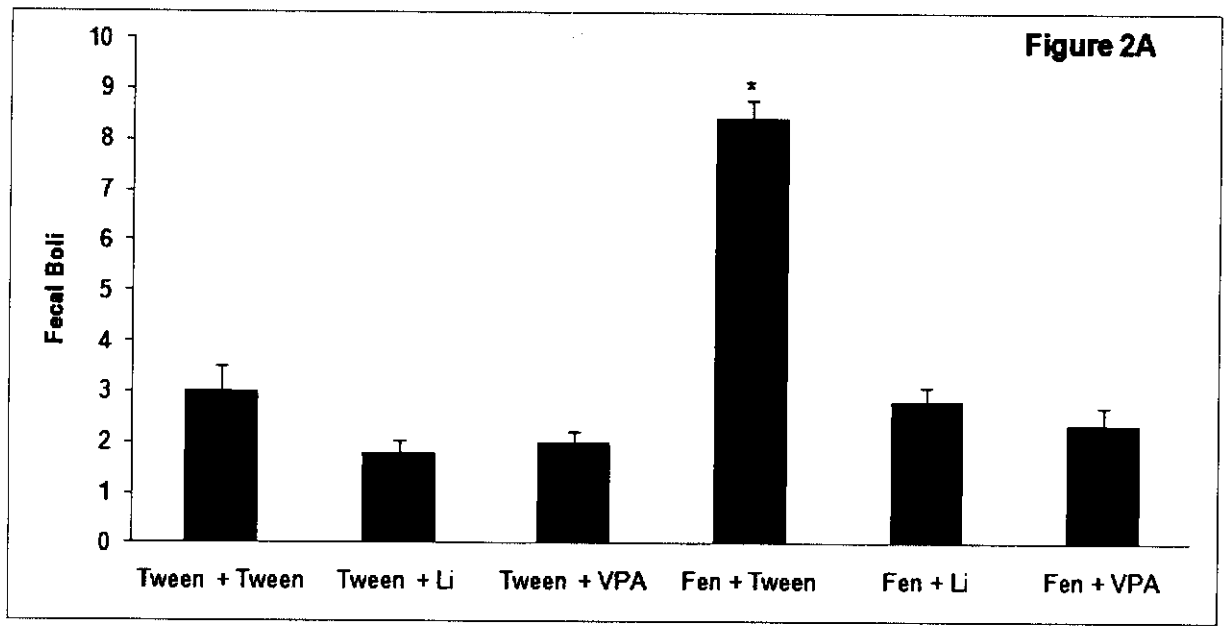


Figure 1 – Open field test in reversion (A) and prevention (B) experiment. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Mean ± Standard Error (n=12). * Different from control; $p<0.05$.



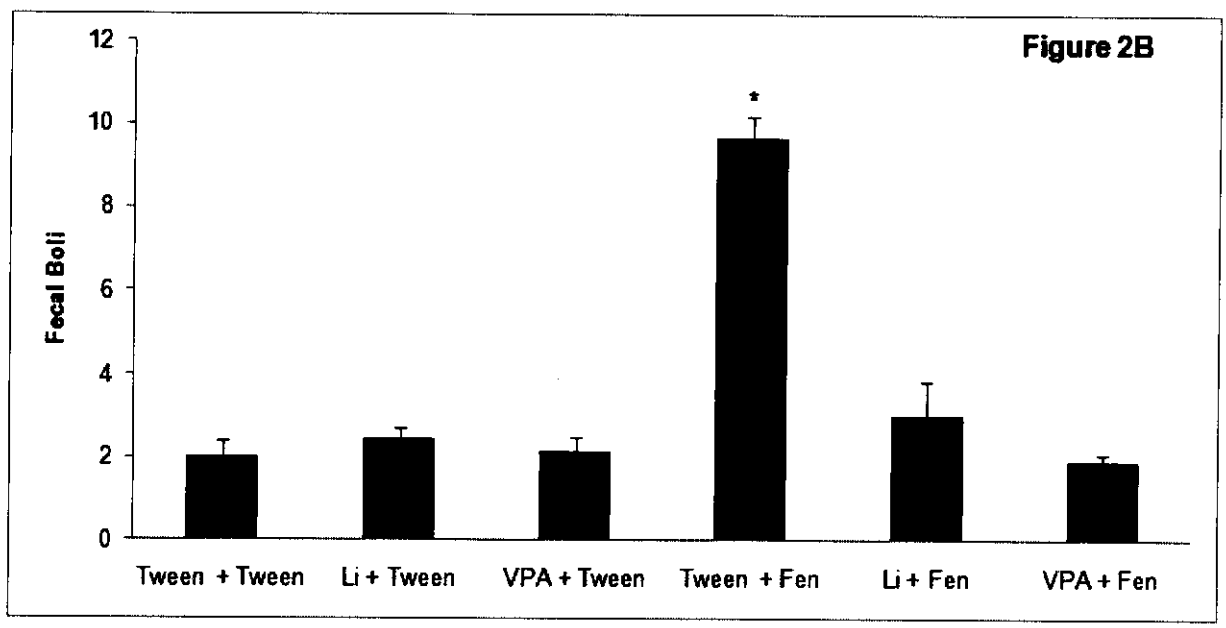


Figure 2 – Body weight in reversion(A) and prevention (B) experiment. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Mean ± Standard Error (n=12). * Different from control; $p<0.05$.

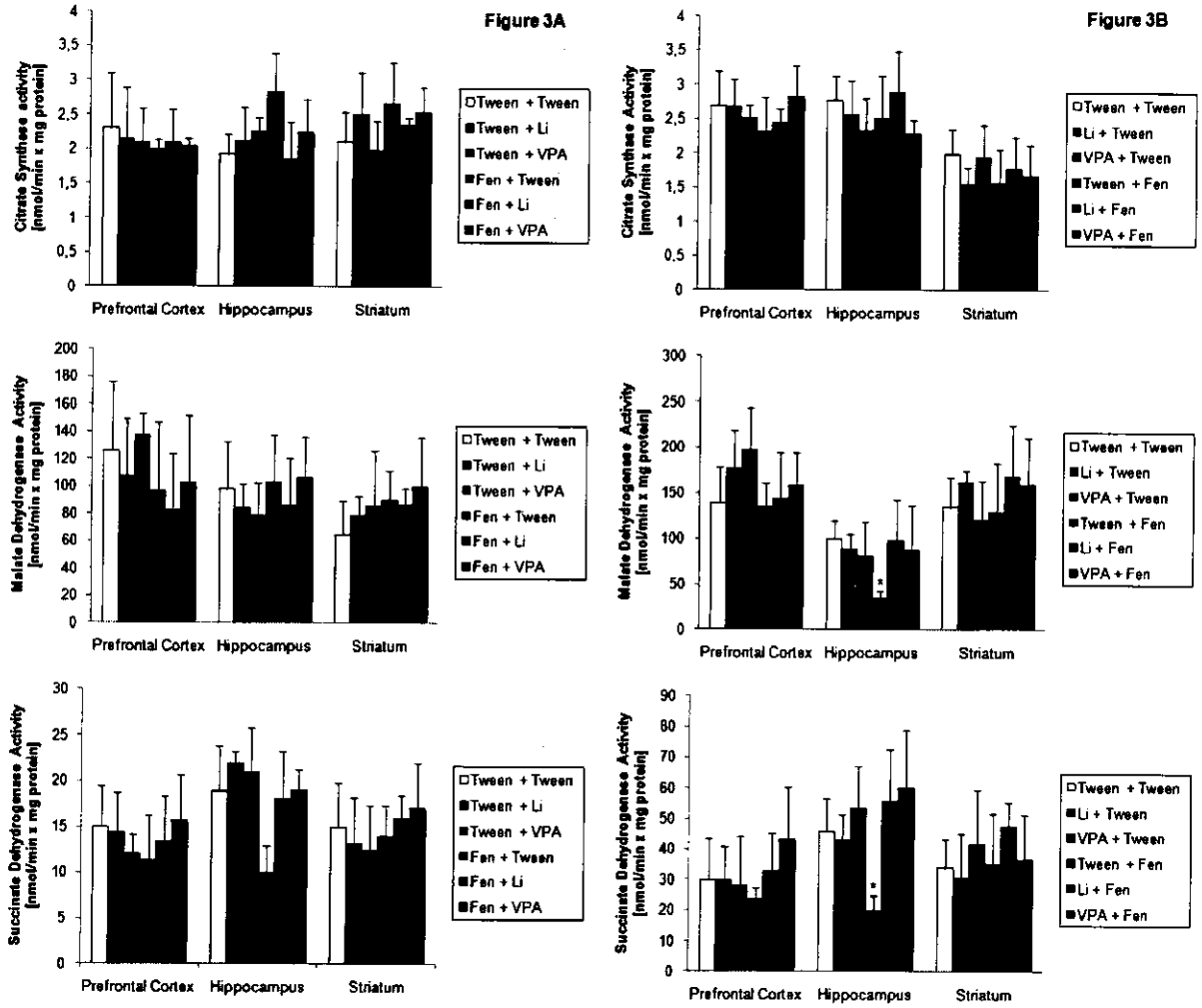


Figure 3 – Citrate synthase, malate dehydrogenase and succinate dehydrogenase activities in reversion(A) and prevention (B) experiment. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min . mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

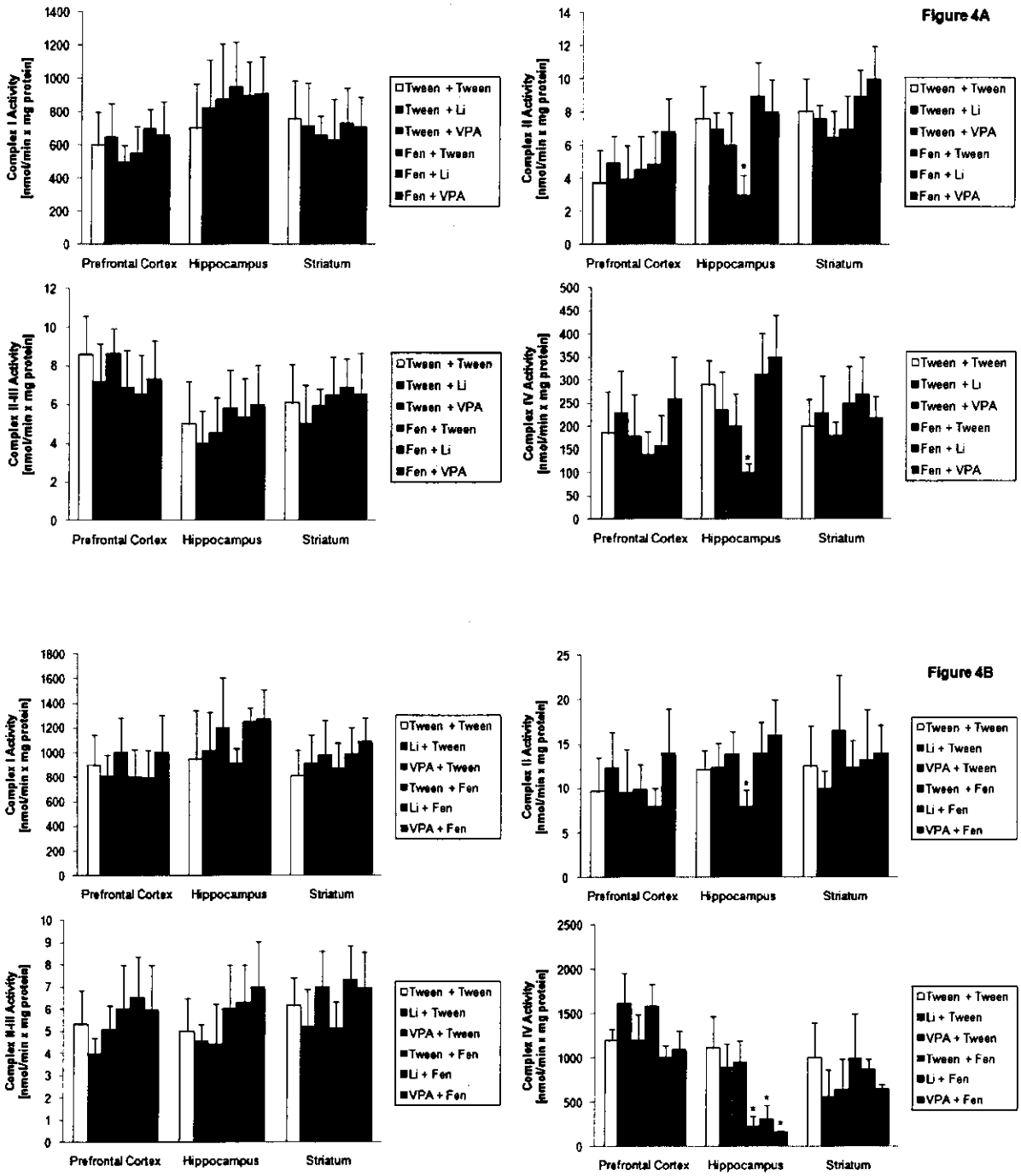
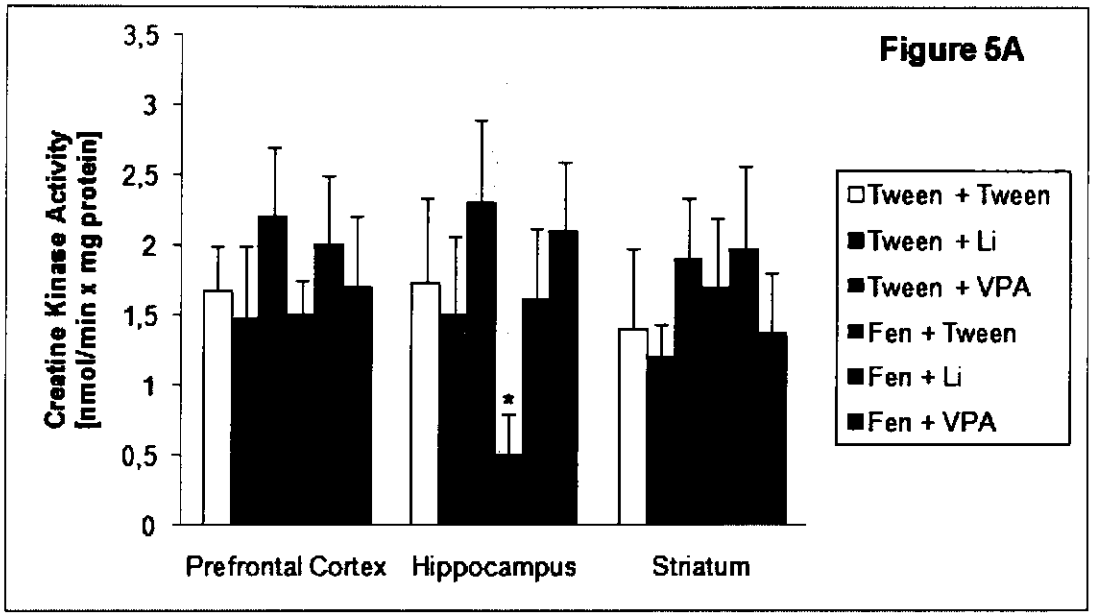


Figure 4 – Complexes of mitochondrial respiratory chain activities in reversion(A) and prevention (B) experiment. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min . mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.



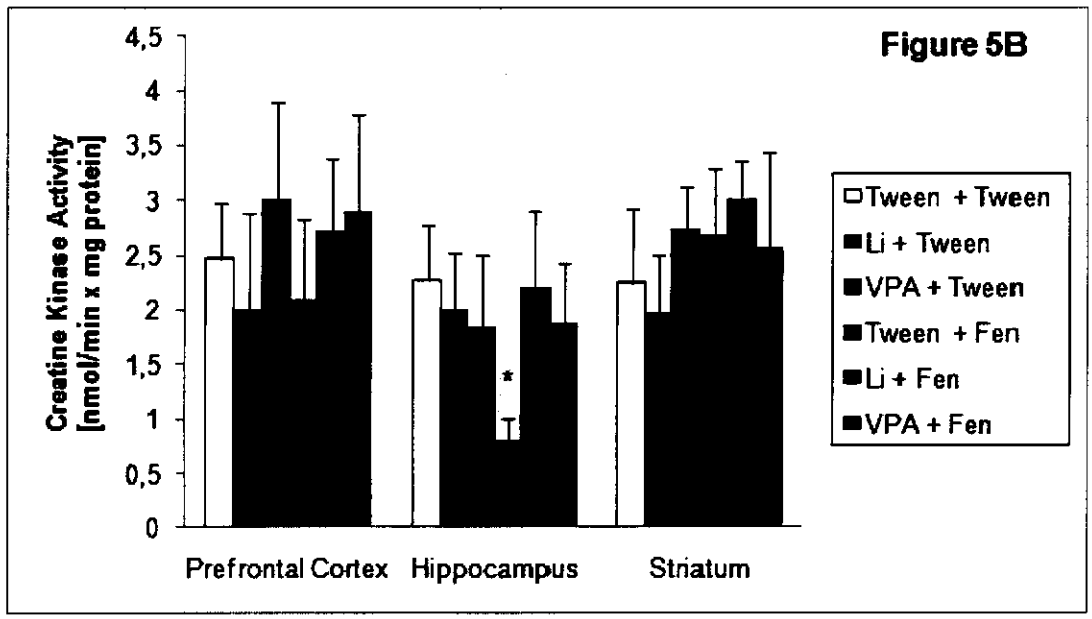


Figure 5 – Creatine Kinase activity in reversion(A) and prevention (B) experiment. Data were analyzed by one-way analysis of variance followed by Tukey test when F was significant. Values are expressed as nmol/min . mg protein, mean ± S.D. (n=6). * Different from control; $p<0.05$.

4. DISCUSSÃO

Primeiramente nós avaliamos a atividade do metabolismo energético cerebral em ratos jovens após administração aguda e crônica de femproporex. No presente trabalho, nossos resultados mostraram um aumento na atividade de algumas enzimas do ciclo de Krebs, cadeia respiratória mitocondrial e creatina quinase em cérebro de ratos jovens submetidos à administração aguda e crônica de femproporex. Outros estudos também mostraram ativação do complexo I e creatina quinase após administração aguda de outro psicoestimulante, o metilfenidato, enquanto sua administração crônica ativa a succinato desidrogenase, complexo II, complexo IV e creatina quinase (Fagundes et al., 2007; Scaini et al., 2008; Fagundes et al., 2010a).

Kolb e colaboradores (2003) mostraram que a administração de psicoestimulantes (anfetamina e cocaína) promove crescimento neural em algumas regiões cerebrais de ratos jovens. Alguns estudos também avaliaram o efeito de anfetaminas no sistema nervoso central e relacionaram com idade e tempo de exposição à droga (Husson et al., 2004). A fim de compreender melhor o efeito diferente dos psicoestimulantes em ratos jovens e adultos, especialmente no metabolismo mitocondrial, Andreu e colaboradores (1998) compararam os produtos da transcrição de ácido desoxirribonucleico (DNA) mitocondrial em ratos. Este estudo relatou uma redução, relacionada à idade dos animais, nos RNAs mitocondriais sintetizados que reflete em uma diminuição na taxa de síntese. O aumento, relacionado à idade, do conteúdo de proteína carbonil da membrana mitocondrial também foi observado em mitocôndrias senescentes.

Os mecanismos pelos quais o metabolismo energético foi ativado em cérebro de ratos jovens são desconhecidos, porém nossos resultados estão de acordo com os dados apresentados por outros autores que também mostram ativação do metabolismo energético cerebral causado por outro psicoestimulante, o metilfenidato. Além disso, outros autores também mostram diferenças funcionais entre animais jovens e adultos, que talvez possam colaborar com os dados apresentados por nós.

Num primeiro momento, nós buscamos avaliar somente a toxicidade do femproporex. Assim nós focamos nossos estudos em animais jovens, e utilizamos todas as estruturas cerebrais (córtex pré-frontal, cerebelo, hipocampo, estriado, córtex posterior) possíveis de isolamento em nosso laboratório e avaliamos a atividade de enzimas envolvidas no metabolismo energético, sem intenção de observar a atividade locomotora de animais jovens.

Na sequência nós avaliamos a atividade locomotora e do metabolismo energético cerebral em ratos adultos após administração aguda e crônica de femproporex. No presente estudo, nós achamos que a administração aguda e crônica de femproporex aumentou o atividade locomotora e exploratória nos ratos adultos. Está descrito na literatura que psicoestimulantes apresentam efeitos colaterias possíveis de causar mudanças comportamentais e potencial de abuso (Zhu & Reith, 2008). Estudos prévios mostraram que fármacos psicoestimulantes tais como anfetamina (Frey et al., 2006b), metilfenidato (Valvassori et al., 2007) e cocaína (Rodvelt et al., 2011) levam à hiperatividade em animais.

Em nosso estudo, nós achamos que a administração aguda e crônica de femproporex nas altas doses aumenta a visita ao centro pelos ratos no teste de

campo aberto, representando um comportamento de risco. Além disso, nós achamos que na administração crônica, mas não na administração aguda, o femproporex nas altas doses induz comportamento *grooming* e *sniffing*. De acordo com nossos dados, Einat e colaboradores (2007) mostraram que a administração de anfetamina aumentou o comportamento de risco nos ratos. Outro estudo mostra que metanfetamina, cocaína e nicotina também aumentam *rearing*, *grooming* e *sniffing* por até 80 minutos após a administração destes psicotrópicos em ratos (Izawa et al., 2006). Está bem descrito na literatura que drogas de abuso, tal como a cocaína e metanfetamina tem a capacidade de estimular a neurotransmissão dopaminérgica no núcleo *accumbens*, induzindo ativação comportamental em ratos, camundongos e outros animais de laboratório (Di Chiara, 2002).

Neste trabalho, nós também demonstramos que a administração crônica de femproporex em doses altas aumenta o número de bolos fecais produzidos pelos ratos. Esta observação é indicativa de que este fármaco altera o eixo cérebro intestino, provavelmente através da sinalização dos receptores do fator liberador de corticotrofina, um importante mediador do sistema nervoso autônomo, particularmente o parassimpático, para aumentar a motilidade do cólon (Saito et al, 2005; Taché et al, 1999). Sabe-se que a hipersecreção do fator liberador de corticotrofina hipotalâmico e/ou extrahipotalâmico é o maior fator na patogênese de transtornos afetivos e de ansiedade (Heinrichs, 1997). Pringle e colaboradores (2008) demonstraram que o receptor do fator liberador de corticotrofina 2 aumentou no núcleo da rafe dorsal 20 horas após a administração crônica de anfetamina e permaneceu elevado por até 6 semanas de abstinência.

Os resultados da atividade comportamental encontrados apóiam outros dados descritos na literatura. O femproporex apresenta efeitos comportamentais semelhantes aos de outros psicoestimulantes, provavelmente por ser convertido à anfetamina após a administração, e assim, apresenta mecanismo de ação similar aos outros psicoestimulantes, tal como, cocaína, metanfetamina e anfetamina.

Neste estudo, nós também observamos que a atividade das enzimas do ciclo de Krebs – citrato sintase, malato desidrogenase, succinato desidrogenase – cadeia respiratória mitocondrial – Complexo I, II, II-III e IV e creatina quinase foram inibidas em algumas estruturas cerebrais de ratos adultos submetidos à administração aguda e crônica de femproporex. De acordo com estes dados apresentados, outros estudos demonstraram que a administração de anfetaminas em ratos também inibe as enzimas do metabolismo energético (Valenzuela et al, 1987; Corrêa et al, 2007; Streck et al, 2008; Bachmann et al, 2009; Fagundes et al., 2010b; Valvassori et al, 2010; Moretti et al, 2011).

A degradação enzimática ou a autooxidação de dopamina resulta na formação de peróxido de hidrogênio e radical superóxido. O peróxido de hidrogênio é suscetível ao ferro para catalisar a formação do radical hidroxil pela reação de fenton (Olanow, 1992; Kopin, 1993). Sabe-se que a geração de radicais livres é induzida por anfetamina via oxidação de dopamina e subsequentemente disfunção da respiração mitocondrial (Burrows et al, 2000; Deng et al, 2002; Cadet et al, 2005; Valvassori et al, 2010). Vários estudos demonstraram que a anfetamina induz dano oxidativo a proteínas, lipídeos e DNA em cérebro de ratos (Frey et al, 2006a; Frey et al, 2006b,c; Andreazza et al, 2008).

Outro importante ponto a considerar é que mudanças no metabolismo energético não ocorrem similarmente nas estruturas cerebrais. Isto pode ser explicado pelo fato que o cérebro é a estrutura biológica mais complexa conhecida, formada por várias regiões que respondem de forma diferente, e em parte com diferentes tipos de neurônios. Além disso, dentro de uma população homogênea de células, há heterogeneidade em termos de características fisiológicas e metabólicas (Lai et al, 1977; Sims, 1991; Sonnewald et al, 2008).

Aqui nós observamos que a administração aguda causa mais mudanças no metabolismo energético do que a administração crônica em animais adultos. A razão para discrepância neste estudo é desconhecida, mas pode estar relacionada à dessensibilização do efeito de repetidas administrações de femproporex ou ainda pelo mecanismo de adaptação. Estimulação excessiva dos receptores de dopamina durante a exposição à psicoestimulantes induz várias mudanças moleculares adaptativas na via dopaminérgica mesolímbica (White & Kalivas, 1998; Nestler, 2005). No entanto, o aumento do *grooming*, *sniffing* e bolos fecais foram observados apenas pela administração crônico de femproporex, sugerindo que mudanças no metabolismo energético não estão relacionadas a essas mudanças de comportamento.

Por último nós avaliamos o efeito do lítio e valproato sobre alterações comportamentais e bioquímicas induzidas por femproporex. Avanços recentes sobre os mecanismos neurais subjacentes, etiologia, genética e de novas abordagens farmacológicas têm facilitado o desenvolvimento de modelos animais de mania (El-Mallakh et al., 2003; Frey et al., 2006a,b,c). Vários estudos sugerem que a anfetamina é usado para induzir mania em animais (Post & Weiss, 1996; Gould et

al., 2001; Shaldivin et al., 2001; Frey et al., 2006b). Além disso, uma revisão de Machado-Vieira e colaboradores (2004) mostraram que repetidas injeções de anfetamina induz hiperatividade em animais, por uma elevação de dopamina extracelular (Martinez et al., 2003). Neste contexto, estudos têm relatado que pacientes com transtorno bipolar apresentam alterações dopaminérgicas e locomotoras (Pantazopoulos et al., 2004; Vogel et al., 2004).

Neste contexto, alguns psicoestimulantes, em especial a anfetamina, são utilizados para induzir modelo animal de mania, e com isso buscar descobrir mais sobre sua fisiopatologia, além de expandir o desenvolvimento para novas terapias. Como visto anteriormente, o femproporex causa alterações comportamentais e bioquímicas semelhantes as apresentadas por psicoestimulantes, inclusive pela anfetamina. Assim, é possível tentar validar um modelo de mania utilizando femproporex, já que no Brasil, a venda de anfetamina (psicoestimulante mais utilizado para estudar este transtorno) é proibida.

Nossos resultados mostraram que a atividade locomotora, em ambos, experimento de reversão e prevenção dos efeitos causados pelo femproporex, ativou o número de *crossing*, *rearing*, *grooming*, *sniffing* e visita ao centro em ratos submetidos à administração crônica de femproporex, mas a administração de lítio e valproato reverteu e preveniu esta alteração. Além disso, nós também demonstramos um aumento na quantidade de bolos fecais produzidos pelos animais em ambos os experimentos, porém a administração de lítio e valproato também reverteu e preveniu esta alteração. O peso corporal diminuiu no experimento de reversão somente após a segunda semana de administração de femproporex, mas o uso de lítio e valproato foram eficazes e reverteu esta inibição. No experimento de

prevenção o peso corporal dos animais não foi alterado em nenhum dos tempos avaliados.

A atividade locomotora e estereotípica são comportamentos mediados por dopamina e vários estudos já mostraram que um aumento na atividade dopaminérgica resulta na hiperatividade e comportamento estereotípico (Feigenbaum et al., 1984; Laviola et al., 1988; Laviola et al., 1994). Porém, estes tipos de comportamento são mediados por diferentes sistemas dopaminérgicos, sendo que a locomoção é mediada pelo sistema límbico e o comportamento estereotípico é mediado pelo sistema Nigro-estriatal (Fink & Smith, 1980; Robinson & Berridge, 1993; Staton & Solomon, 1994).

Todas as alterações comportamentais apresentados estão de acordo com os resultados descritos na literatura, ou seja, a ativação locomotora, comportamento de risco, estereotipia e ansiedade, é apresentada por outros modelos de mania, bem como por pacientes acometidos com transtorno bipolar. Assim, é válido investir na padronização de modelo animal de mania induzido por femproporex, visto que ele apresenta validade de face.

O presente resultado mostrou também que a administração crônica de femproporex no experimento de prevenção inibiu a atividade das enzimas malato desidrogenase, succinato desidrogenase, complexo II, complexo IV e creatina quinase no hipocampo. Porém, a administração lítio e valproato preveniu esta inibição, exceto preveniu a inibição da atividade do complexo IV. Além disso, nossos resultados também mostraram que a administração crônica de femproporex no experimento de reversão inibiu a atividade das enzimas succinato desidrogenase,

complexo II, complexo IV e creatina quinase no hipocampo. Mas a administração de lítio e valproato reverteu esta inibição em todas as enzimas alteradas.

Considerando os resultados recém apresentados, onde nossos dados mostram alterações bioquímicas similares as apresentados por outros psicoestimulantes, bem como, por outros modelos animais de mania, nós podemos observar uma possível validade de constructo, já que o femproporex aumento da neurotransmissão dopaminérgica, bem como alterações metabólicas vistas em pacientes com transtorno bipolar.

O hipocampo é uma região do cérebro que recebeu atenção significativa nas pesquisas sobre transtorno de humor e o complexo hipocampal sensível ao estresse desempenha um papel central no transtorno do humor (Campbell & Macqueen, 2004). Estudos indicaram a presença de dano celular e alterações volumétricas no hipocampo de pacientes com transtornos do humor e esquizofrenia (Beyer & Krishnan, 2002; Harrison, 2004). O Volume hipocampal está declaradamente associadao ao funcionamento neuropsicológico deficiente no transtorno bipolar (Ali et al., 2000), e os resultados de um estudo neuropatológico indicaram uma redução de várias linhagens de células neuronais no córtex e hipocampo de pacientes bipolares (Benes et al., 1998).

Estudos mostraram deleções e mutações nos genes que codificam subunidades do complexo I em pacientes com transtorno bipolar (Munakata et al., 2004). Recentes analises no DNA (Sun et al., 2006), em amostras *post-mortem* do córtex pré-frontal e hipocampo mostraram que a expressão do RNA mensageiro que codifica subunidades dos complexos I-V foi diminuído em pacientes com transtorno bipolar (Munukata et al., 2004; Sun et al., 2006). Konradi e colaboradores (2004)

mostraram uma diminuição da expressão dos genes que codificam os complexos enzimáticos responsáveis pela fosforilação oxidativa.

Um estudo *post-mortem* no hipocampo (Kato et al., 1998) e no córtex (Sun et al., 2006) de pacientes com transtorno bipolar mostraram uma diminuição na expressão de genes nucleares que codificam mRNA das enzimas da cadeia respiratória mitocondrial. MacDonald e colaboradores (2006) apresentaram resultados muito interessantes, mostrando que os níveis de mRNA de creatina quinase estão diminuídos em pacientes com transtorno bipolar, especialmente no hipocampo.

Nossos resultados são semelhantes aos apresentados em outros estudos que envolvem modelo animal de mania induzido por anfetamina. Repetidas administrações de anfetamina (14 dias) induzem em animais alterações comportamentais e neuroquímicas muito similares as apresentadas neste trabalho. Corrêa e colaboradores (2007) mostraram que no modelo animal de mania induzido por anfetamina houve uma inibição na atividade da citrato sintase no hipocampo de ratos. Porém, a administração de valproato reverteu e a administração de lítio preveniu esta inibição. Streck e colaboradores (2008) mostraram também que no modelo animal de mania induzido por anfetamina que a atividade da creatina quinase foi inibida em cérebro de ratos e que a administração de lítio e valproato não reverteram, nem preveniram a inibição desta enzima. No entanto, a administração de lítio e valproato não reverteram nem preveniram esta alteração. Valvassori e colaboradores (2010) mostraram que a anfetamina inibiu a atividade dos complexos da cadeia respiratória mitocondrial em cérebro de ratos. Porém, o valproato reverteu a disfunção induzida pela anfetamina, enquanto que o lítio não foi capaz de reverter.

Além disso, valproato e lítio exercem efeito protetor sobre a disfunção mitocondrial induzida por anfetamina. Outros estudos mostraram que as enzimas citrato sintase e creatina quinase foram inibidas em cérebro de ratos submetidos ao modelo animal de mania induzido por ouabaína (Freitas et al., 2010a,b). Estudo de Freitas e colaboradores (2011) mostraram que a atividade dos complexos I, II, II-III e IV foram aumentados em cérebro de ratos após indução de mania por ouabaína.

Por fim, com as avaliações feitas utilizando lítio e valproato, nós podemos sugerir que alcançamos os três critérios solicitados, visto que o uso de estabilizadores de humor também conseguiram reverter e prevenir os parâmetros avaliados neste trabalho. Assim, nós conseguimos nos aproximar de um modelo animal de mania utilizando o femproporex como indutor.

5. CONSIDERAÇÕES FINAIS

- O presente trabalho mostrou ativação das enzimas do ciclo de Krebs, complexos da cadeia respiratória mitocondrial e creatina quinase em cérebro de ratos jovens, por ambas as administrações aguda e crônica de femproporex.
- Nossos resultados mostram um aumento da atividade locomotora, estereotípica e quantidade de bolo fecal, além de diminuição do peso corporal em ratos adultos submetidos à administração aguda e crônica de femproporex. Além disso, nós também observamos inibição das enzimas do ciclo de Krebs, complexos da cadeia respiratória mitocondrial e creatina quinase no cérebro destes animais, tanto pela administração aguda, quanto pela administração crônica.
- Por fim, nós verificamos que a administração crônica de femproporex pode ser validade como modelo animal de mania, visto que a administração de lítio e valproato revertem e previnem o aumento da atividade locomotora, estereotípica e quantidade de bolo fecal, bem como reverte à diminuição do peso corporal causados pela administração crônica de femproporex. Além disso, o lítio e o valproato também revertem e previnem a inibição das enzimas do ciclo de Krebs, complexos da cadeia respiratória mitocondrial e creatina quinase, observados após administração crônica de femproporex.
- Assim, nós sugerimos uma interação entre dopamina, um fator predominantemente etiológico do transtorno bipolar, comportamento e metabolismo energético, logo o femproporex poderá futuramente ser utilizado

para induzir mania em animais com a finalidade de estudar melhor as vias intracelulares envolvidas neste transtorno.

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