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HIPERLOCOMOÇÃO E PARÂMETROS OXIDATIVOS EM UM MODELO ANIMAL DE MANIA INDUZIDO POR OUABAINA

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RESUMO

O Transtorno Afetivo Bipolar (TAB) é uma patologia multifatorial compreendendo uma diversidade de sintomas clínicos que podem variar grandemente e representar uma fonte significante de sofrimento aos pacientes, atingindo em torno de 1 a 3% da população. Apesar disso, as bases biológicas do TAB ainda são pouco conhecidas. Considerando-se que a mania é um ponto chave do transtorno, o desenvolvimento de modelos animais de mania torna-se crucial para melhorarmos as medidas terapêuticas oferecidas a estes pacientes. Evidências atuais apontam para deterioro cognitivo nestes pacientes, sugerindo que processos de lesão celular acompanham o transtorno. Níveis de oxidação de proteína e lipídeos e atividade das enzimas superóxido dismutase (SOD) e catalase (CAT) foram avaliadas em hipocampo e estriado, bem como a peroxidação lipídica (TBARS) em líquor e estruturas submitocondriais, além de oxidação de proteínas em líquor, hipocampo e estriado. Os parâmetros oxidativos foram avaliados em um modelo animal de mania induzida pela administração de ouabaína, um potente inibidor da bomba de sódio e potássio intracerebroventricular (ICV). Os resultados evidenciaram aumento da atividade locomotora em doses subconvulsivas de ouabaína ICV, bem como uma alteração nos perfis de oxidação e enzimas antioxidantes nas diferentes estruturas avaliadas com diferentes concentrações de ouabaína .

Palavras-Chaves: Ouabaína. SOD. CAT. Transtorno Afetivo Bipolar. Estresse oxidativo. TBARS.

ABSTRACT

The Bipolar disorder (BD) is a complex illness with a diversity of clinical symptoms ranging hugely and causing a significant burden of suffering to patients and their relatives. The BD is estimated to affect 1-3% of the population worldwide. In addition, the pathophysiology and pathogenesis of the illness remains unclear. Considering mania a key factor for de disease, the development of suitable mania animal models is crucial to improve the pharmacological approach offered to patients. Some recent evidences point out to cognitive impairment in bipolar patients, suggesting oxidative cellular damage following the course of the disease. Oxidative stress in proteins and lipids were assessed in the present study, as well as the activity of anti oxidant enzymes CAT e SOD in hippocampus and striatum and also the TBARS levels in liquor and submitocondrial particles. Protein oxidative levels in liquor, hippocampus and striatum were assessed as well. The oxidative parameters were investigated using a animal mania model using ouabain, a potent Na pump inhibitor, injected ICV. The results showed increasing locomotor activity in subconvulsive doses of ouabain ICV, and a change in oxidative stress and antioxidant enzymes in different biological sites using different ouabain doses ICV.

KEYWORDS: Ouabain. SOD. CAT. Bipolar disorder. Oxidative stress. TBARS.

LISTA DE ABREVIATURAS

AMPH – D-Anfetamina

ATP – Adenosina Tri-fosfato

CAT – Catalase

DNA – Ácido Desoxirribonucléico

ERO – Espécies Reativas ao Oxigênio

GPx – Glutationa peroxidase

H2O2 – Peróxido de Hidrogênio

I.C.V. – Intracerebroventricular

K+ - Potássio

Na+ - Sódio

O2__ - Ânion Superóxido

O2 - Oxigênio

OH_ – Radical Hidroxil

SNC – Sistema Nervoso Central

SOD – Superóxido Dismutase

TBARS – Espécies Reativas ao Ácido Tiobarbitúrico

TAB – Transtorno Afetivo Bipolar

SUMÁRIO

1. INTRODUÇÃO

1.1. Transtorno Afetivo Bipolar - TAB

O Transtorno Afetivo Bipolar (TAB) é uma doença psiquiátrica crônica, severa e altamente incapacitante que afeta em torno de 1–3% da população mundial (Belmaker, 2004). Estudos recentes indicam que a maioria dos pacientes com TAB apresentam altas taxas de prejuízo funcional e cognitivo, até mesmo nos períodos de remissão sustentada dos sintomas de humor; o que incrementa a morbidade psicossocial associada ao transtorno (Zarate et al., 2000). O Transtorno Afetivo Bipolar (TAB) é uma patologia multifatorial compreendendo uma diversidade de sintomas clínicos que podem variar grandemente e representar uma fonte significante de sofrimento aos pacientes acometidos e aos seus familiares, devido ao caráter recorrente dos episódios depressivos, maníacos e estados mistos. Varias abordagens terapêuticas, incluindo medidas psicoeducacionais, eletroconvulsoterapia, e medidas farmacológicas, tem sido usadas para aliviar sintomas e diminuir os prejuízos funcionais e psicossociais destes indivíduos portadores do TAB (Machado-Vieira et al., 2004)(a).

Estudos neuropatológicos *postmortem* em pacientes com TAB têm mostrado reduções anormais no volume cortical, número de células gliais e no tamanho neuronal no córtex pré-frontal, córtex dorso-antero-lateral, amígdala e hipocampo (Cotter et al.,2001; Manji & Duman, 2001).

Não se sabe exatamente se estas evidências de déficit neuronal constituem anormalidade de desenvolvimento que pode conferir vulnerabilidade a transtornos de humor, mudanças compensatórias de outros processos patogênicos ou seqüelas de recorrentes

episódios de mania e depressão (Manji et al., 2003). Assim sendo, o papel da morte celular no progressivo deterioro observado nos pacientes bipolares merece ser mais profundamente investigado. Pesquisas na fisiopatologia dos transtornos de humor têm englobado, além dos neurotransmissores e receptores da superfície celular, também as rotas de sinalização intracelulares (Manji et al., 2003). Evidências crescentes apontam para a associação de eventos intracelulares, envolvendo o sistema de segundos mensageiros como parte das alterações neurobiológicas do TAB (Frey, 2004). Entender a pato-fisiologia deste transtorno é crucial para avançarmos no tratamento e prognóstico desta patologia além do estágio atual. Uma teoria significante reside na associação entre mania e retenção e níveis de sódio e cálcio intracelular, assim como decréscimo na atividade da bomba de sódio (Na, K-ATPase) (Goodwin and Jamison,1990; Coppen et al.,1966; Dubovsky et al.,1991).

O glicosídeo ouabaína é usado freqüentemente em pesquisas biomédicas como inibidor específico da Na+,K+-ATPase da membrana plasmática, proteína que cataliza o transporte ativo acoplado de Na+ e K+, estabelecendo um gradiente eletroquímico através da membrana plasmática. Portanto, diferentemente de outros esteróides, a ouabaína liga-se a uma proteína de membrana. Considerando que a Na+,K+-ATPase é o principal sistema de transporte ativo na maioria das células animais, promovendo a extrusão de três íons Na+ e a entrada de dois íons K+, sua inibição gera uma condição que favorece o acúmulo intracelular de Na+ (Birks & Cohen, 1968; Gomez *et al.*, 1975; Aizman *et al.*, 2001; McFadden *et al.*, 2001).

A Ouabaína é um potente derivado esteróide, cardiotônico, obtido a partir de sementes maduras de *Strophantus gratus* e *Acokanthera ouabaio*, plantas de origem africana. Contudo, trabalhos recentes indicam possível síntese endógena e também apontam a existência de esteróides semelhantes à ouabaína em tecidos de mamíferos. Em 1991, um isômero da ouabaína foi identificado como um hormônio endógeno sintetizado pela glândula

adrenal e também pelo hipotálamo, contudo o seu mecanismo de ação e sua significância fisiológica não foram ainda precisamente determinados (Hamlyn *et al*., 1991 & 2003; Boulanger *et al*., 1993; Scheneider *et al*., 1998; Kawamura *et al.*, 2001). A ouabaína inibe especificamente a Na+,K+ATPase, bomba iônica que mantém o equilíbrio eletroquímico entre as faces externa e interna da membrana plasmática, promovendo a extrusão de íons Na+ e a intrusão de íons K+. Portanto, a ouabaína determina um acúmulo intracelular de Na+. A Na^+K^+ATPase é o maior determinante da concentração citoplasmática de sódio. Como tal, possui importante papel na regulação do volume celular e do pH citoplasmático, bem como dos níveis de cálcio, via transportadores Na/H e Na/Ca respectivamente; também dirigindo uma variedade de processos secundários de transporte, tais como o transporte de sódio dependente de glicose e aminoácidos. A Na^+K^+ATPase é um oligômero composto por dois maiores polipeptídeos denominados de subunidades alfa e beta (Mobasheri et al 2000), sendo uma enzima presente na membrana plasmática de todas as células dos mamíferos. A enzima hidrolisa ATP e usa a energia livre para dirigir o transporte de potássio para dentro da célula e sódio para fora da célula, contra seus gradientes eletroquímicos (Blanco and Mercer1998 ; Scheiner-Bobis 2002).

1.2. Modelo animal de mania

Para um modelo animal ser válido em transtornos psiquiátricos, ele deve preencher três critérios principais: validez de face, validez de construção e validez de predição (Ellenbroek & Cools, 1990). O primeiro, validez de face, pode ser avaliado pela similaridade entre a evolução dos sintomas maníacos e o modelo animal proposto. Validez de construção avalia a possível correlação do modelo animal com as mudanças moleculares descritas na patofisiologia da doença. Validez de predição avalia como o modelo responde aos mesmos fármacos utilizados para tratar a doença. Modelos que apresentam validez de construção

usualmente apresentam algum grau de validez de face e de predição e vice-versa (Ellenbroek and Cools, 1990).

A validez de face na maioria dos modelos animais de mania tem priorizado a hiperatividade como sintoma chave (Machado- Vieira et al., 2004 a). Em relação a estes fatores, a administração intracerebroventricular (ICV) do inibidor específico da Na^+K^+ATPase (ouabaína), induz hiperatividade em ratos (validez de face), o qual foi normalizada pelo uso de lítio, carbamazepina e haloperidol (validez de predição) (El- Mallakh et al., 2003, 2006). Também, a bomba de sódio e potássio encontra-se reduzida em pacientes bipolares maníacos e deprimidos (Looney and El-Mallakh, 1997), indicando o potencial de validade de construção do modelo animal de mania induzido por ouabaína. Contudo, os mecanismos bioquímicos envolvidos no desenvolvimento das alterações causadas pelos inibidores da Na^+K^+ATPase permanecem desconhecidos.

Os agentes disponíveis para tratar o TAB incluem lítio, o qual ainda representa o mais comumente utilizado estabilizador de humor (EH), bem como a carbamazepina, ácido valpróico, lamotrigina, topiramato, gabapentina, benzodiazepínicos adjuntos e agentes antipsicóticos atípicos (Keck et al.,1998). Mesmo considerando as diferentes opções de manejo do TAB e as recentes novas descobertas em relação à neurobiologia do transtorno, subtipos da doença bipolar como cicladores rápidos e estados mistos, geralmente não respondem bem aos tratamentos disponíveis, apresentando altos índices de recaídas e recorrências e de refratariedade ao tratamento (Coryell et al., 1992). Por estas razões o desenvolvimento de modelos animais torna-se crucial, tendo em vista que recentemente um grande número de agentes potencialmente estabilizadores de humor tem sido examinado no tratamento da fase maníaca e depressiva, na esperança de que se encontrem tratamentos mais efetivos para esta severa patologia mental(Machado- Vieira et al., 2004)(a). O TAB é particularmente desafiador no se refere às tentativas de se desenvolver um adequado modelo

animal, devido à intrigante e complexa alternância de estados maníacos, depressivos, eutímicos e mistos, vistos nos pacientes. Muitos modelos animais têm proposto mimetizar comportamentos maníacos e depressivos, reproduzindo algumas das suas características principais. Comportamentos maníacos incluem hiperatividade motora (avaliada neste estudo), fala e energia excessivas, grandiosidade, agressividade e insônia. Modelos animais, por serem úteis na compreensão da pato-fisiologia do transtorno, auxiliam no desenvolvimento de novos agentes farmacológicos direcionados ao manejo do TAB (Machado- Vieira et al., 2004)(a).

Apesar disso, pouco é sabido a respeito das precisas circunstâncias neurobiológicas envolvidas no TAB; circunstâncias estas essenciais para o desenvolvimento de terapias mais específicas, efetivas, rápidas e melhor toleradas que as terapias hoje disponíveis (Zarate et al., 2006). O desenvolvimento de modelos animais tem sido um instrumento importante na investigação de novos sistemas intracelulares que possam estar envolvidos no TAB (Einat et al., 2003; Manji and Chen, 2002) e novas abordagens farmacológicas (Lambert et al., 2001; Zarate et al., 2006).

Segundo Belmaker (2004), o único marco deste transtorno é a mania, então um modelo animal de TAB deveria contemplar algumas características do episódio maníaco como euforia, irritabilidade, agressividade, hiperatividade, insônia e aumento da libido.

Estudos prévios tem reportado o papel da Na^+/K^+ ATPase na fisiopatologia do TAB, propondo que a inibição farmacológica da Na^+/K^+ATPase pela administração intracerebroventricular (ICV) de ouabaina em ratos, causando hiperatividade, pode ser usado como um modelo de TAB (Decker et al., 2000; El-Mallakh et al., 2003). Nesta perspectiva de ligar achados fisiopatológicos de modelos animais com observações de estudos clínicos; observa-se que o tratamento a longo prazo com lítio tem resultado em um aumento do acúmulo de lítio e aumento da atividade da Na^+/K^+ATPase nas membranas de eritrócitos, com a concomitante redução de sódio e cálcio nos eritrócitos de pacientes bipolares. Pelo fato de

as concentrações de íon cálcio livre tenderem a ser paralelas as concentrações de íon sódio livre, estes achados contribuem para a hipótese de aumento de cálcio intracelular em pacientes com TAB; bem como é digno de nota que quando pacientes bipolares são tratados com lítio, a atividade da Na^+/K^+ATPase se mostrou aumentada, consistente com as observações de redução de cálcio após tratamento (Dubovsky et al.,1998). Importante também, mostra-se a hipótese de que o acúmulo intracitoplasmático de Na+ poderia promover o vazamento de íons cálcio armazenados na mitocôndria por reversão do trocador Na+/Ca2+ mitocondrial (Zhong *et al.*, 2001 e Yang *et al.*, 2003).

Em relação ao funcionamento da mitocôndria; existem evidências crescentes ligando a disfunção mitocondrial com um papel central na patofisiologia do TAB (Kuloglu et al., 2002; Sun et al., 2006; Andreazza et al., 2007; Machado-Vieira et al., 2007; Frey et al., 2007a). A disfunção mitocondrial resulta em anormalidades no metabolismo energético celular e aumento da produção de Espécies Reativas de Oxigênio (ROS), portanto a neuroproteção contra o estresse oxidativo pode ser um dos mecanismos de ação dos estabilizadores de humor usados no tratamento do TAB (Machado-Vieira et al., 2007).

1.3. Estresse oxidativo

Baseado na relação entre estresse oxidativo e comportamento psicótico mimético, são necessários estudos sobre o efeito da ouabaína, um agente psicótico mimético, sobre o perfil oxidativo em diferentes estruturas e meios biológicos relacionados com alterações comportamentais. Desta forma, o presente estudo investiga a formação de oxidação lipídica e protéica no LCR (Líquido Cérebro Raquidiano), a geração de espécies reativas de oxigênio (ERO) cerebrais e a atividade da catalase (CAT) e da superóxido dismutase (SOD) cerebrais de ratos submetidos ao modelo de mania induzido por administração de ouabaína ICV. Estudos prévios sugerem que concentrações sub-convulsivas de ouabaína produzem um

aumento da atividade locomotora dose dependente, com concentrações variando de 10^{-3} a 10^{-6} M, dependendo das condições experimentais como as dimensões da arena, volume de ouabaína injetada no ventrículo e a linhagem de ratos usados (Machado-Vieira et al., 2004(b); El-Mallakh et al., 2003; Decker et al., 2000; El-Mallakh and Wyatt, 1995). Deste modo, baseado na variabilidade de dose, o presente estudo objetiva também investigar a curva de concentração e resposta do efeito comportamental da ouabaína ICV, nas nossas condições experimentais.

Atualmente sabe-se que a geração de espécies reativas de oxigênio (ERO) exerce um papel fundamental na fisiopatologia de diversos transtornos neuropsiquiátricos como transtorno de humor bipolar e esquizofrenia, (Frey et al., 2006a; Frey et al., 2006b). As ERO são radicais livres capazes de reagir indiscriminadamente com qualquer tipo de molécula orgânica, extraindo elétrons e gerando novos radicais livres em reações em cadeia altamente citotóxicas e com potencial de oxidar moléculas biológicas incluindo proteínas, lipídeos e DNA (Middleton et al., 2000; Coleman 2001; Yen et al., 2003; Aldred et al., 2004). As lesões causadas pelas ERO podem desencadear um processo de morte celular programada denominado apoptose (Halliwell, 2006). Para evitar a formação de ERO, assim como reparar os danos oxidativos em tecidos e macromoléculas, os organismos possuem um sistema de defesa antioxidante (Molina et al., 2003). Algumas destas defesas são enzimas: superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx), mas existem também as defesas antioxidantes não-enzimáticas, como as vitaminas A, C, E, e a glutationa (Ames et al., 1993).

A enzima SOD converte o ânion superóxido (O2-) em peróxido de hidrogênio (H2O2) e oxigênio molecular. Todos os subtipos de SOD apresentam, pelo menos, um metal de transição em seu sítio ativo. A manganês-SOD é localizada na membrana mitocondrial interna, sendo sua expressão regulada por EROs. O cobre, zinco-SOD, por outro lado,

apresenta uma localização citosólica (Halliwell e Gutteridge, 2006). A elevação da atividade da SOD está envolvida com o aumento de estresse oxidativo, provavelmente devido ao aumento da produção de H2O2, que é um dos substratos desta enzima e um dos responsáveis pela modulação da sua atividade (Berg et al., 2004).

A CAT tem o H2O2 como único substrato, sendo sua atividade intimamente relacionada com a concentração das ERO. A catalase tem como função principal dismutar o H2O2, formando H2O e oxigênio molecular. Esta enzima atua complementarmente à GPx, não permitindo a produção de radical hidroxil (OH) a partir do H2O2, que ocorre na presença de Fe^{+2} (Halliwell e Gutteridge, 2006); o que denominamos reação de Fenton. A GPx, uma enzima selênio-dependente, é importante para a proteção contra peróxidos orgânicos e H2O2. Para a sua atividade a GPx necessita da presença de glutationa reduzida (Halliwell e Gutteridge, 2006). A carbonilação de proteínas é muito usada como biomarcador de dano oxidativo em proteínas, pois reflete o dano celular induzido por várias formas de ERO (Dalle-Donne et al., 2005). O grupo carbonil pode ser introduzido nas proteínas por várias sinalizações oxidativas. As ERO podem reagir diretamente com proteínas ou reagir com moléculas como açúcares e lipídeos, gerando produtos, como espécies reativas ao carbonil (ERC), que reagem com proteínas (Stadtman, 1990). Tanto as membranas celulares quanto às organelas, como as mitocôndrias, contêm ácidos graxos que podem sofrer oxidação, podendo haver modificação em suas propriedades, como alteração na estrutura e na permeabilidade (Mursu et al., 2004).

O cérebro é particularmente vulnerável à produção das ERO, porque ele metaboliza 20% do oxigênio corporal total e tem uma capacidade antioxidante limitada (Halliwell, 2006). As enzimas superóxido dismutase (SOD) e catalase (CAT) exercem papel importante na rota de eliminação dos radicais livres gerados no interior das células. A enzima SOD é responsável pela conversão do ânion superóxido em peróxido de hidrogênio, o qual,

através da ação da CAT é convertido em oxigênio e água (Halliwell, 2006). Caso a SOD esteja em baixas concentrações há uma tendência ao acúmulo de superópxido e conseqüentemente desencadeia estresse oxidativo. Se a enzima CAT apresentar-se em baixas concentrações o estresse oxidativo se instala pela conversão do peróxido de hidrogênio em hidroxila, altamente reativo e cititóxico, pois a CAT impede esta reação (Halliwell, 2006). Em situações onde a geração de radicais livres excede a capacidade das defesas antioxidantes, o estresse oxidativo pode levar a degradação da membrana, disfunção celular, dano ao DNA e apoptose (Frey et al. 2006b; Halliwell, 2006). As defesas antioxidantes no cérebro são modestas. Os níveis de catalase, enzima que detoxifica peróxido de hidrogênio, são baixos na maior parte das regiões cerebrais (Halliwell, 2001). Desequilíbrio mitocondrial pode levar a um aumento de Ca++ intracelular, aumentando a produção de espécies reativas de oxigênio (Halliwell, 2006). Desequilíbrio energético mitocondrial pode interromper mecanismos antioxidantes uma vez que a glutationa é sintetizada no citoplasma e seu transporte para o interior mitocondrial é um mecanismo dependente de ATP (Banaclocha, 2001). Trabalhos evidenciando que as disfunções mitocondriais e, conseqüentemente a geração de radicais livres, estão envolvidas em transtornos psiquiátricos como TAB (Kato & Kato, 2000; Kuloglu et al., 2002; Dager et al., 2004; Sun et al., 2006; Andreazza et al., 2007; Machado-Vieira et al., 2007; Frey et al., 2007a). Disfunções mitocondriais podem levar a um decréscimo na fosforilação oxidativa e a um aumento na produção anaeróbica de energia, elevando os níveis de lactato e a geração de ROS. Tem sido reportado que pacientes com TAB possuem aumento dos níveis de lactato na substância cinzenta (Dager et al., 2004).

Coerentemente com esses achados, Sun et al. (2006) encontraram diminuição no pH em córtex cerebral *post-mortem* de indivíduos com TAB. Kuloglu et al. (2002) avaliaram os níveis plasmáticos de TBARS (Espécies Reativas ao Tiobarbitúrico), indicadores de peroxidação lipídica, e os níveis de atividade das enzimas anti-oxidantes: superóxido-

dismutase e glutationa-peroxidase em hemolisado de eritrócitos de pacientes com TAB, utilizando indivíduos saudáveis como controle. Os níveis plasmáticos de TBARS nos pacientes com TAB estavam aumentados significantemente em relação ao grupo controle. Os níveis de atividade da superóxido-dismutase estavam significantemente aumentados em relação ao grupo controle, embora não houvesse diferença significante entre os níveis de atividade da glutationa- peroxidade entre o grupo de pacientes com TAB e o grupo controle.

Andreazza et al. (2007) avaliaram os níveis plasmáticos de TBARS (Espécies Reativas ao Tiobarbitúrico) e os níveis de atividade da superóxido-dismutase, glutationaperoxidase e catalase em pacientes com TAB em diferentes fases da doença: mania, depressão e eutimia, comparando-os com indivíduos saudáveis. Os níveis de atividade da superóxidodismutase, glutationa-peroxidade e catalase estavam significantemente aumentados nos pacientes com TAB nas fases de mania ou depressão. Já os níveis de TBARS estavam aumentados de maneira significante nos indivíduos com TAB independentemente da fase da doença.

Machado-Vieira et al. (2007) avaliaram os níveis plasmáticos de TBARS e os níveis de atividade da superóxido-dismutase e catalase em indivíduos bipolares em fase inicial de mania comparando-os com indivíduos saudáveis. Os níveis de TBARS e os níveis de atividade da superóxido-dismutase e catalase estavam aumentados significantemente nos indivíduos com TAB. Contudo após tratamento agudo com Lítio houve uma diminuição significante nos níveis de TBARS, superóxido-dismutase e catalase, mostrando efeitos antioxidantes do Lítio na mania.

2. OBJETIVOS:

2.1.Objetivo Geral:

O presente estudo tem como objetivo investigar os efeitos da administração intracerebroventricular de ouabaína na atividade locomotora espontânea e em parâmetros de estresse oxidativo avaliados em estruturas cerebrais e liquido cérebro raquidiano de ratos wistar, bem como avaliar a hipótese de metabolismo energético alterado em um modelo animal de mania, pela formação de espécies reativas de oxigênio em partículas submitocondriais.

2.2. Objetivos Específicos:

- Determinar os efeitos da administração aguda de ouabaína ICV em diferentes concentrações na locomoção espontânea de ratos wistar submetidos ao teste de campo aberto;
- Replicar, em nosso laboratório, o modelo animal de mania com a administração ICV de ouabaína;
- Avaliar as concentrações de substâncias reativas ao ácido tiobarbitúrico e de proteínas carboniladas em diversas estruturas cerebrais e líquido cérebro raquidiano de ratos wistar em um modelo animal de mania;
- Verificar a atividade das enzimas superóxido dismutase e catalase em estruturas cerebrais de ratos wistar em um modelo animal de mania;
- Avaliar as concentrações de substâncias reativas ao ácido tiobarbitúrico e a produção de superóxido em partículas de estruturas submitocondriais de ratos wistar em um modelo animal de mania.

3. ARTIGOS

3.1. Artigo1 (Psychiatry Research)

Increase oxidative stress in submitocondrial particles in rat model of mania induced by ouabain

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Abstract

Intracerebroventricular (ICV) administration of ouabain (a specific Na^+K^+ATPase inhibitor) in rats has been suggested to model human bipolar mania. Previous studies have suggested that bipolar disorder (BD) may be associated with mitochondrial dysfunction. We studied the mania induced by ICV administration of ouabain on reactive oxygen species (ROS) production in submitochondrial particles of rat brain. The effects of ICV administration of ouabain (at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M) on locomotion and thiobarbituric acid reactive substances (TBARS) and superoxide production were measured in submitocondrial particles of the prefrontal cortex, hippocampus, striatum and cortex. Our findings demonstrated that ouabain at 10^{-6} and 10^{-5} M induced hyperlocomotion in rats. However, at higher concentrations (10^{-3} and 10^{-2} M) ouabain induced convulsions in rats. ICV administration of ouabain (10^{-5}M) increased TBARS generation in submitocondrial particles in the hippocampus. We also found that ICV administration of ouabain increase superoxide generation in submitochondrial particles in the hippocampus (10-^{5}M) and striatum (10^{-6}M, 10^{-5}M, 10^{-3}M and 10^{-2}M). In conclusion, ouabain-induced mitochondrial ROS generation may provide a useful model to test the hypothesis of altered brain energy metabolism associated to BD.

Key words: Bipolar disorder, ouabain, particles mithochondial, ROS, mania

1 Introduction

Bipolar disorder (BD) is among the 10 most disabling medical conditions in the word (Lopez and Murray, 1998), and has been associated with increased risk of morbidity, mortality (Osby et al., 2001) and psychiatric comorbidity (Baldessarini and Tondo, 2003). The lack of a neurobiological model restrains the development of specific treatment with a rational pathophysiological basis in bipolar disorder (Zarate et al., 2006). The development of animal models has been an important tool in investigating new intracellular systems that may be involved in BD (Einat et al., 2003; Manji and Chen, 2002).

Animal models of human diseases should meet three sets of criteria: face validity, construct validity, and predictive validity (Einat et al., 2003; Machado-Vieira et al., 2004). Face validity represents how similar the model can mimic the symptoms of a determinate human disorder, whereas construct validity refers to commonalties between the mechanism of the 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.

Intracerebroventricular (ICV) injection of ouabain - a specific Na^+K^+ATPase inhibitor - induced hyperactivity in rats (face validity), which was normalized by treatment with lithium, carbamazepine, and haloperidol (predictive validity) (El- Mallakh et al., 2003, 2006). In addiction, the sodium pump activity is reduced in manic and depressed bipolar patients (Looney and El-Mallakh, 1997), pointing to the potential constructs validity of animal of

mania induced by ouabain. However, the biochemical mechanism underling the development of these alterations caused by the Na^+K^+ATPase inhibitors remains to be clarified.

Studies have demonstrated evidence supporting the contention that mitochondrial function is integral to many facets of BD. Mitochondria are intracellular organelles best known for their critical roles in regulating energy production through oxidative phosphorylation, regulation of Ca^+, and as critical mediators of cellular apoptosis (Quiroz et al., 2008).

Based on the possible relationship between mitochondrial function and psychotomimetic behaviors, studies are needed on the effect of ouabain, a psychotomimetic agent, on the mitochondrial function in the brain related to behavioral changes. Therefore, we investigated the generation of reactive oxygen species (ROS) in the brain of rats submitted to the model of bipolar mania caused by ICV administration of ouabain. In addiction, previous studies have suggested that subconvulsive concentrations of ouabain produce a dose-related increase in locomotor activity at distinct concentrations, varying from 10^{-3} to 10^{-6} M, depending on experimental conditions: arena dimensions, volume of ouabain injected into the ventricle and rat strain used (El-Mallakh et al., 2006; Machado-Vieira et al., 2004; El-Mallakh et al., 2003; Decker et al., 2000; El-Mallakh and Wyatt, 1995). Thus, based on the dose variability, the present study also aimed to perform a concentration response curve of ouabain in order to investigate, under our experimental conditions, the behavioral effects of this compound in rats.

2 Methods

2.1 Animals

We conducted the study using adult male Wistar rats obtained from our breeding colony. The animals were housed 5 to a cage, on a 12-hour light/dark cycle (lights on at 7:00 am), with free access to food and water. 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 Behaviour (SBNeC). This study was approved by the local ethics committee (Comitê de Ética em Pesquisa da Universidade do Extremo Sul Catarinense).

2.2 Surgical procedure and treatment

Animals were anesthetized with ketamine (30 mg/kg) and xylasine (4 mg/kg). In a stereotaxic apparatus, the skin of the skull was removed and a 27 gauge 9 mm guide cannula was placed at 0.9 mm posterior to bregma, 1.5 mm right from de midline and 1.0 mm above the lateral brain ventricle. Through a 2 mm hole made at the cranial bone, a cannula was implanted 2.6 mm ventral to the superior surface of the skull, and fixed with jeweler acrylic cement. Animals were tested on the third day following surgery. A 30 gauge cannula was fitted into the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula aiming the right lateral brain ventricle.

Each animal was administered 5 µl of either vehicle (NaCl 0.9%) or ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) over 30 s and after that the animal was immediately placed into the open field.

2.3 Behavioral assessment

We used the open field task to assess locomotor activity. The task was performed in a 40 × 60 cm open field surrounded by 50 cm-high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 equal rectangles by black lines. The

animals were gently placed on the left rear rectangle and were allowed to explore the arena. Crossings of the black lines and rearings were counted for 5 minutes. Animals were observed in the open field three times: (1) before the cannula implantation (0 h); (2) on the third day following surgery (72 h); and (3) on the fourth day following surgery (96 h), immediately after ICV injection of ouabain or saline. After the ICV infusion of ouabain, especially at high doses, some rats displayed behavioral seizures. Aiming to assess the locomotor effects of ouabain, data obtained from animals that displayed seizures after the ICV injection of ouabain were ruled out from the locomotor activity analysis.

2.4 Oxidative stress in submitochondrial particles

Immediately after the open-field task rats were sacrificed by decapitation, the prefrontal cortex, hippocampus, striatum and cortx were dissected, rapidly frozen and stored at −80 °C. As an index of uncoupling of electron transporter chain (ETC), the generation of mitochondrial superoxide ($O2^{-}$) was measured as previously described (Poderoso et al., 1996). In brief, superoxide anion production was determined in washed submitochondrial particles (SMP) using a spectrophotometric assay based on superoxide-dependent oxidation of epinephrine to adrenochrome at 37 °C (E480 nm = 4.0 mM cm). Mitochondria (1 mg/ml) were treated for 10 min, at 37 °C. SMP were obtained by freezing and thawing (three times) the mitochondria solution, washed (twice) with 140 mM KCl, 20 mM Tris–HCl (pH 7.4) and suspended in the same medium. The reaction medium consisted of 230 mM mannitol, 70 mM sucrose, 10m MHEPES–KOH (pH 7.4), 4.2mMsuccinate, 0.5mM KH2PO4, SMP (1.0 mg protein/ml), 0.1 μM catalase and 1 mM epinephrine. Superoxide dismutase (E.C. 1.15.1.1.) was used at 0.1–0.3 μM final concentration as a negative control to confirm assay specificity. As a marker of lipid peroxidation, we measured the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described (Esterbauer and Cheeseman, 1990). Briefly, the samples were mixed with 1ml of trichloroacetic acid 10% and

1ml of thiobarbituric acid 0.67%, and then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm. All the results were normalized by the protein content, using bovine albumin as standard (Lowry et al., 1951).

2.5 Statistical analysis

All data are presented as mean and standard error of the mean, except figure 2 which expresses the percentage of rats displaying convulsions after the ICV injection of ouabain or saline. Differences among the experimental groups evaluating locomotor activity were determined by one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. Seizure behavior was analyzed by Chi-square test. Biochemical data were analyzed by one-way ANOVA, and multiple comparisons were performed with the Newman–Keuls test. In all comparisons, statistical significance was set at $P < 0.05$.

3 Results

In the open-field task (Figure 1), ICV administration of 10^{-6} and 10^{-5} M ouabain increased locomotion of rats compared to control group. Interestingly, the ICV injection of ouabain at higher concentrations (10^{-3} and 10^{-2} M) induced convulsions in 60 and 80% of rats, respectively (Figure 2).

As seen in the figure 3, ICV administration of ouabain (10^{-5}M) increased TBARS generation in submitocondrial particles in the hippocampus, but not in the prefrontal cortex, striatum and cortex. We also found that ICV administration of ouabain increase superoxide generation in submitocondrial particles (Figure 4) in the hippocampus (10-^{5}M) and striatum (10^{-6}M, 10^{-5}M, 10^{-3}M and 10^{-2}M), but not in the prefrontal cortex and cortex.

4 Discussion

In the present study, we demonstrated that the ICV administration of ouabain at 10^{-5} and 10^{-6} M elicited hyperlocomotor effects in rats. Ouabain at 10^{-4} M did not affect locomotion, while at higher doses (10^{-3} and 10^{-2} M), ouabain induced convulsions, but in those non-convulsing rats, no changes in locomotion were observed. According to our model, previous studies demonstrated that ouabain caused a significant increase in open field locomotion at low dose (10^{-5}M) (El-Mallakh et al., 2006, Machado-Vieira et al., 2004). Meanwhile, other studies show the opposite, lower doses of ouabain (10^{-3}M) increased locomotor activity, while 10^{-5}M ouabain significantly reduced activity level (Kim et al., 2008; Decker et al., 2000). Differences in experimental design may account for this discrepancy: arena dimensions, volume of ouabain injected into the ventricle and rat strain used.

We also found that ICV administration of ouabain increased TBARS and superoxide generation in submitocondrial particles in the hippocampus at lower dose (10^{-5}M) and TBARS generation in the striatum at lower and higher doses (10^{-6}, 10^{-5}, 10^{-3} and 10^{-2}M). No effects on ROS production in submitocondrial particles in prefrontal cortex and cortex after ICV administration of ouabain.

Reduced hippocampal volume was observed in a population of older bipolar patients and in bipolar children and adolescents (Strakowski et al., 2005; Frazier et al., 2005). Functional imaging studies showed abnormal brain activation in the hippocampus and its closely related regions during emotional (Kauer-Sant'anna et al., 2008), attentional (Glahn et al., 2007), and memory tasks (Pavuluri et al., 2006). Postmortem studies indicate abnormal glutamate and GABA transmission in the hippocampus of BD patients (Bielau et al., 2007). The basal ganglia, and in particular the striatum, play a significant role in both cortical and emotional regulation. Multiple striatal pathways project to orbitofrontal, medial, and lateral prefrontal regions implicated in the modulation of emotional behavior (Ongur and Prince, 2000; Alexander et al., 1986). Structural and MRS findings in the basal ganglia suggest the

presence of primary functional deficits in these subcortical structures. Putamen volume is increased in bipolar patients as well as in both affected and non-affected bipolar twins, suggesting deficits in pruning that may be related to increased activity in these structures (Strakowski et al., 2005). Increased basal ganglia choline levels in bipolar patients are also consistent with altered neuronal energetics in this brain region (Adleman et al., 2004).

There is an emerging a body of data indicating that impaired energetic metabolism due to mitochondrial dysfunction may play a role in the pathophysiology of BD (Kato and Kato, 2000; Quiroz et al., 2008). Brain magnetic resonance spectroscopy studies have demonstrated decreased N-acetyl-aspartate (a marker of mitochondrial energy production) (Clark, 1998) and lower pH and phosphocreatine levels in BD (Stork and Renshaw, 2005). In addiction, recent postmortem studies have reported changes in mitochondrial-related gene expression in BD (Iwamoto et al., 2005; Munakata et al., 2005).

The central nervous system requires a high-energy supply due to its intense ATP-consuming processes. Thus, abnormal cellular energy metabolism may impair neuronal function and plasticity. Under normal conditions, mitochondria are major source of ROS, which are produced in the complexes of the electron transport chain (ECT) (Mattiasson, 2004). On the other hand, a shift in the antioxidant/pro-oxidant balance towards oxidative stress may inhibit ECT complexes, leading to decrease in ATP production and cellular dysfunction (Calabrese et al., 2001).

Soon after the characterization of the Na^+/K^+-ATPase, some authors investigated its activity in mood disorders. These studies showed that Na^+ pump activity is decreased in acute mania compared to recovered euthymic bipolar individuals (Reddy et al., 1992). Decreases in the activity of Na^+/K^+-ATPase, due to a reduction in ATP synthesis or to an increased production

of inhibitors, could be an important link in the pathological response to deficiencies in energy metabolism.

In conclusion, ouabain-induced mitochondrial ROS generation may provide a useful model to test the hypothesis of altered brain energy metabolism associated to BD. Studies addressing the effect of mood stabilizers and antipsychotics may provide new insights about their mechanism of action.

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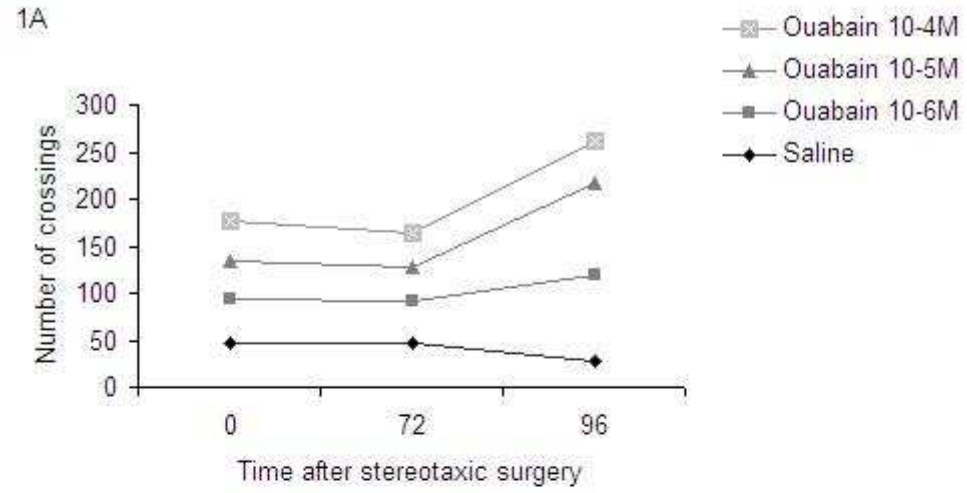
Legends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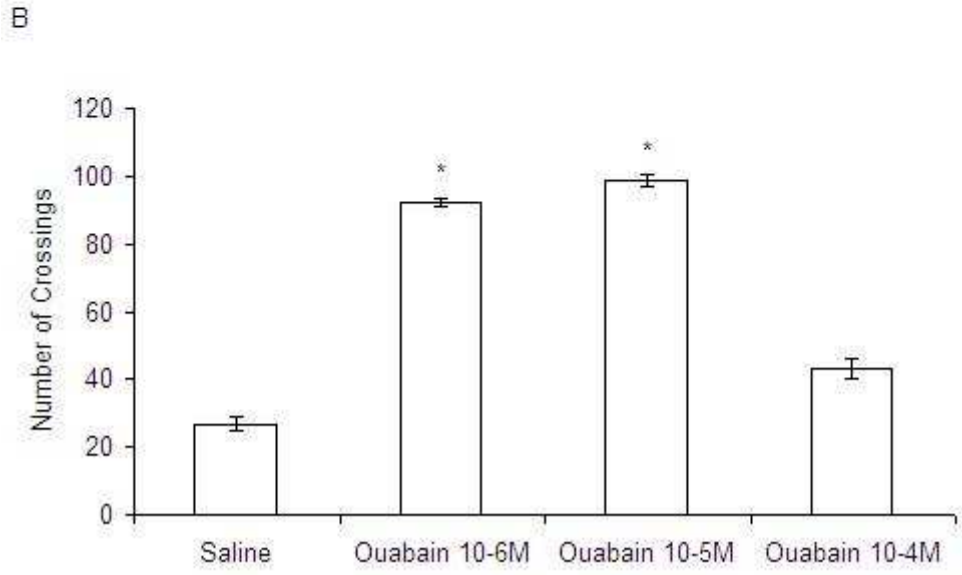
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Saline
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Ouabain 10-5M
Ouabain 10-4M

Figure 1. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on number of crossings in rats subjected to the open field test, for 5 minutes. Locomotor activity was assessed in the open-field test three times: (1) before surgery (0 h); (2) on the third day following surgery (72 h); and (3) immediately after ICV injection of ouabain or saline, on the fourth day following surgery (96 h) (A). Bars represent means ± standard error of means of 15 animals on locomotion immediately after ICV injection of ouabain or saline, on the fourth day following surgery (B). **P*<0.05 vs. saline group, according to ANOVA followed by the Tukey test.

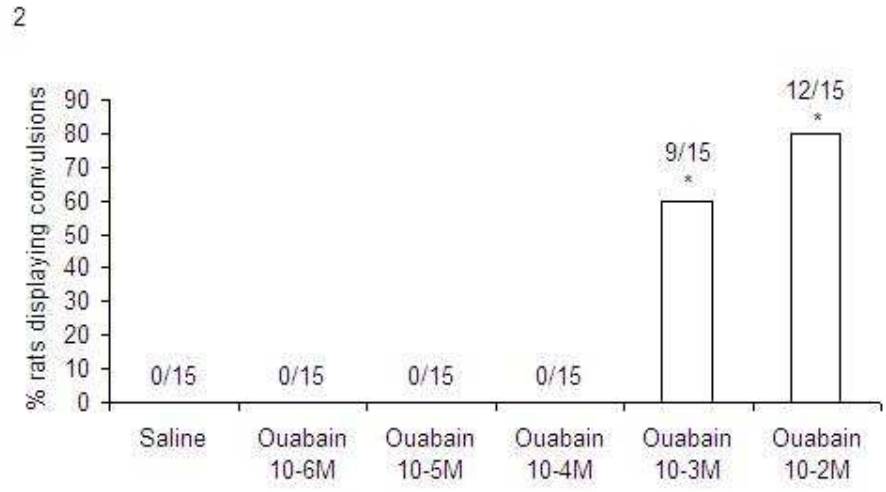

Figure 2. Percentage of rats displaying convulsions in response to the ICV ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline (n=9-10 for each group). * *P*<0.05 compared with saline group, according to Chi-square test.

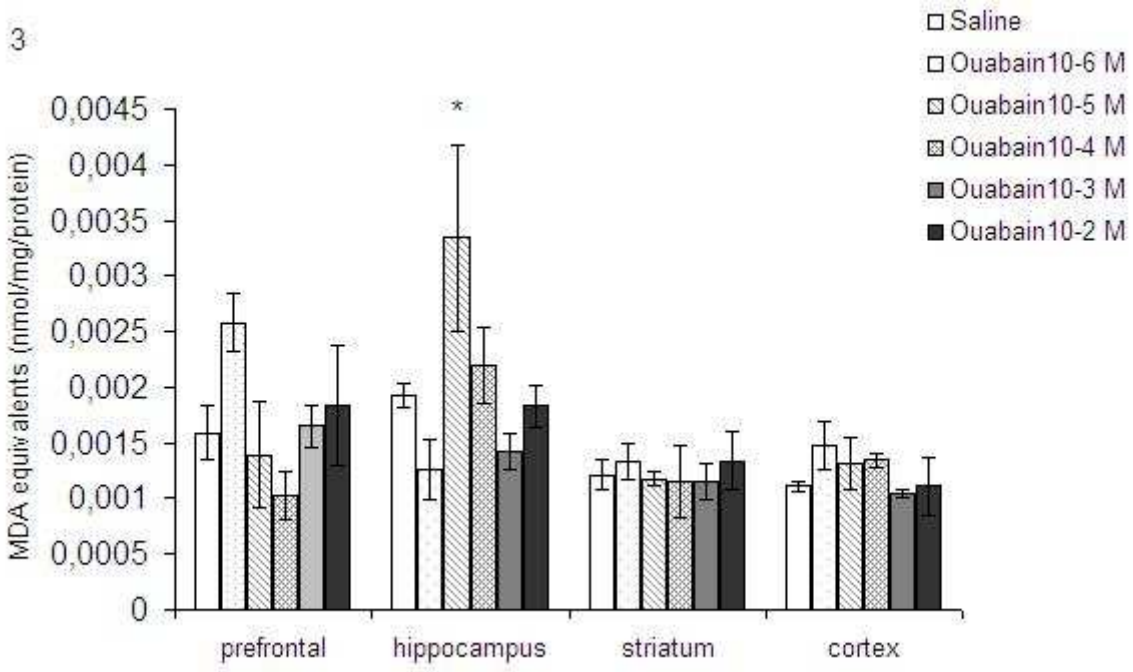

Figure 3. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on TBARS levels in submitochondrial particles in the prefrontal cortex, hippocampus, striatum and cortex of rats. Bars represent means ± standard error of means of 5-6 animals. * P<0.05 vs. saline group, according to ANOVA followed by the Newman–Keuls test.

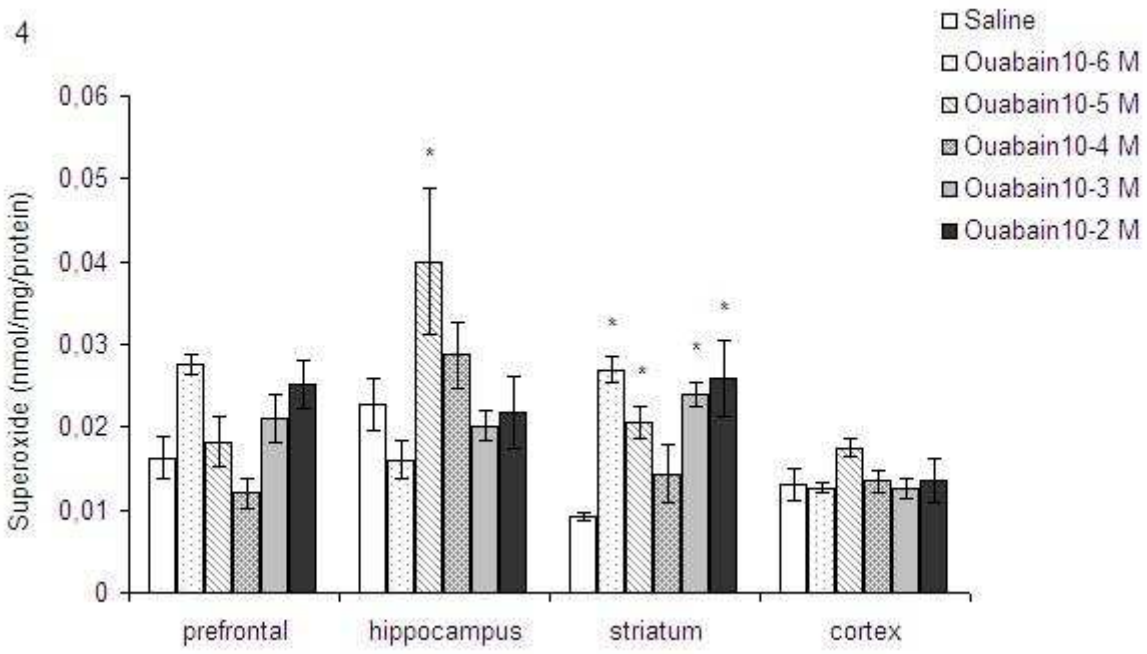

Figure 4. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on superoxide levels in submitochondrial particles in the prefrontal cortex, hippocampus, striatum and cortex of rats. Bars represent means ± standard error of means of 5-6 animals. * P<0.05 vs. saline group, according to ANOVA followed by the Newman–Keuls test.

3.2. Artigo 2 (Neurotoxicity Reserch)

CHANGES IN ANTIOXIDANT DEFENSE ENZYMES IN RAT MODEL OF MANIA INDUCED BY OUABAIN

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Abstract

Previous studies have suggested that reactive oxygen species (ROS) production may play a role in the pathophysiology of bipolar disorder (BD). Intracerebroventricular (ICV) administration of ouabain, a potent and selective sodium pump inhibitor, has been employed as an animal model of mania. In this study we evaluated the effects of the ICV administration of ouabain on ROS production in rat brain. Male adult Wistar rats were treated with 5 µl of vehicle (NaCl 0.9%) or ouabain (at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M) and immediately placed into an open-field to access locomotor activity. As parameters of oxidative stress, we measured thiobarbituric acid reactive substances and protein carbonyl formation. The activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were also measured in the hippocampus and striatum homogenates. Our findings demonstrated that ouabain at 10^{-6} and 10^{-5} M induced hyperlocomotion in rats. By contrast, at higher concentrations (10^{-3} and 10^{-2} M) ouabain induced convulsions in rats. Regarding the oxidative stress parameters, the ICV injection of ouabain at higher concentrations increased lipid peroxidation and protein carbonyl formation in the striatum and hippocampus. Additionally, ouabain injection increased SOD and CAT activity in the hippocampus at all concentrations, except to 10^{-2} M. In the striatum, ouabain increased SOD activity at 10^{-6} and 10^{-2} M concentrations, while CAT activity was decreased at 10^{-4}, 10^{-3} and 10^{-2} M. Taken together present data suggest that 10^{-6} and 10^{-5} M ouabain induced hyperlocomotion in rats, which is not correlated to lipid peroxidation or carbonyl formation in the hippocampus and striatum. In addition, a marked change in antioxidant defense enzymes was observed into the brain of those ouabain-treated rats. In conclusion, our findings reinforce the role of ROS in the pathophysiology of BD.

Key-words: bipolar disorder; hippocampus; ouabain; oxidative stress

Abbreviations: BD bipolar disorder; CAT catalase; DNPH dinitrophenylhidrazine; ICV intracerebroventricular; ROS reactive oxygen species; SOD superoxide dismutase; TBARS thiobarbituric acid reactive species

1. Introduction

Bipolar disorder, also known as manic-depressive illness, is a brain disorder that causes unusual shifts in a person's mood, energy, and ability to function. The symptoms of bipolar disorder are severe and disabling, which can result in damaged relationships, failure of job or school performance, and even suicide (Emilien *et al*., 2007). Bipolar disorder is estimated to affect 1-3% of the population worldwide (Belmaker, 2004). The defining clinical feature of the condition is the manic episodes. Mania is characterized by an elated or irritable mood, reduced need for sleep, psychomotor activation, and excessive involvement in potentially problematic behavior (El-Mallakh *et al*., 2003).

The understanding of pathophysiology and pathogenesis of bipolar disorder remains uncertain and its treatment will likely advance substantially when the underlying processes are elucidated (Emilien *et al*., 2007) . One of the reasons for the lack of successful drug development in bipolar disorder is the absence of an established animal model (Kato *et al*., 2007). It should be noted that an adequate animal model of bipolar disorder is quite particularly challenging because of the intriguing alternation of mania, depression, euthymia, and mixed states that these patients often present (Machado-Vieira *et al*., 2004).

The ouabain model of mania is considered by Herman and colleagues (Machado-Vieira *et al*., 2004) as an adequate model for studying bipolar illness, since it fulfills the three criteria

required to validate a model: face validity, predictive validity and construct validity. Investigations into the pathophysiology of bipolar illness have revealed altered homeostasis of metal ions. These alterations could result from a reduction of sodium-potassium/ATPase (Na^+/K^+-ATPase) activity (Herman *et al*., 2007). The Na^+/K^+-ATPase pump is of paramount importance for the proper functioning of brain tissue (Bignoto & Benedito, 2006). The enzyme is involved in several aspects of cell functioning. In the brain, a function of Na^+/K^+-ATPase is to translocate Na^+ ions out and K^+ ions into the membrane, thus reestablishing the intra/extracellular differences in these two ions concentrations, which are necessary for the neuronal firing (Blanco & Mercer, 1998).

Soon after the characterization of the Na^+/K^+-ATPase, some authors investigated its activity in mood disorders. These studies showed that Na^+ pump activity is decreased in acute mania compared to recovered euthymic bipolar individuals (Reddy *et al*., 1992). In preclinical studies, when the potent sodium pump inhibitor, ouabain, is intracerebroventricularly (ICV) administered in rats, it induces a dose-dependent motor hyperactivity (El-Mallakh & Wyatt, 1995; Decker *et al*., 2000), which may persist for over a week after a single injection (Ruktanonchai *et al*., 1998). Additionally, while each mood stabilizing agent has its own unique actions, activity-dependent inhibition of intracellular sodium accumulation has been proposed as a common mechanism of action of effective mood stabilizers such as lithium, valproic acid, and carbamazepine (El-Mallakh & Huff, 2001). These aspects support the view that the ICV administration of ouabain mimics behavioral and biological aspects of a manic state (Herman *et al*., 1998).

There is an emerging body of data indicating that major neuropsychiatric disorders, such as bipolar disorder and schizophrenia, are associated with increased oxidative stress and changes in antioxidant enzymatic defense (Kuluglu *et al*., 2002; Ranjekar *et al*., 2003; Ozcan *et al*., 2004). It is well known that the brain is particularly prone to oxidative damage due to its

relative high content of peroxidable fatty acids and limited antioxidant capacity (Floyd, 1999). Increased neuronal oxidative stress levels generate deleterious effects on signal transduction, structural plasticity and cellular resilience, mostly by inducing lipid peroxidation in membranes, proteins and genes (Mahadik et al., 2001; Schafer *et al*., 2004).

Given the recent evidence that oxidative stress may play a role in the pathophysiology of bipolar disorder, the present study aims to investigate the behavioral and biochemical alterations induced by the ICV injection of ouabain in rats. We also assessed the effects of ICV ouabain administration on lipid and protein oxidation levels (markers of oxidative stress) and on catalase (CAT) and superoxide dismutase (SOD) activities (the major antioxidant enzymes) in the rat brain. More specifically, we decided to investigate the hippocampus and striatum because alterations in these brain regions are thought to be associated with bipolar disorder (Soares & Mann, 1997; Fossati *et al*., 2004).

2. Methods

2.1. Animals

We conducted the study using adult male Wistar rats obtained from our breeding colony. The animals were housed 5 to a cage, on a 12-hour light/dark cycle (lights on at 7:00 am), with free access to food and water. 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 Behaviour (SBNeC). This study was approved by the local ethics committee (Comitê de Ética em Pesquisa da Universidade do Extremo Sul Catarinense).

2.2. Surgical procedure and treatment

Animals were anesthetized with ketamine (30 mg/kg) and xylasine (4 mg/kg). In a stereotaxic apparatus, the skin of the skull was removed and a 27 gauge 9 mm guide cannula was placed at 0.9 mm posterior to bregma, 1.5 mm right from de midline and 1.0 mm above the lateral brain ventricle. Through a 2 mm hole made at the cranial bone, a cannula was implanted 2.6 mm ventral to the superior surface of the skull, and fixed with jeweler acrylic cement. Animals were tested on the third day following surgery. A 30 gauge cannula was fitted into the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula aiming the right lateral brain ventricle.

Each animal was administered 5 µl of either vehicle (NaCl 0.9%) or ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) over 30 s and after that the animal was immediately placed into the open field.

2.3. Behavioral assessment

We used the open field task to assess locomotor activity. The task was performed in a 40 × 60 cm open field surrounded by 50 cm-high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 equal rectangles by black lines. The animals were gently placed on the left rear rectangle and were allowed to explore the arena. Crossings of the black lines and rearings were counted for 5 minutes.

Animals were observed in the open field three times: (1) before the cannula implantation (0 h); (2) on the third day following surgery (72 h); and (3) on the fourth day following surgery (96 h), immediately after ICV injection of ouabain or saline.

After the ICV infusion of ouabain, especially at high doses, some rats displayed behavioral seizures. Aiming to assess the locomotor effects of ouabain, data obtained from animals that

displayed seizures after the ICV injection of ouabain were ruled out from the locomotor activity analysis.

2.4. Oxidative stress parameters

Immediately after the open-field test, rats were sacrificed and the skulls removed and hippocampus and striatum were dissected and stored at -80ºC for biochemical analyses.

To determine oxidative damage, we measured the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described (Esterbauer & Cheeseman, 1990). The samples were mixed with 1 mL of trichloroacetic acid 10% and 1 mL of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 15 minutes. TBARS were determined by the absorbance at 535 nm.

Oxidative damage to proteins was measured by the quantification of carbonyl groups based on the reaction with dinitrophenylhidrazine (DNPH), as previously described (Levine et al., 1994). Proteins were precipitated by the addition of 20% trichloroacetic acid and were redissolved in DNPH; the absorbance was read at 370 nm.

To determine CAT activity, the brain tissue was sonicated in 50 mmoL/L phosphate buffer (pH 7.0), and the resulting suspension was centrifuged at 3000 *g* for 10 minutes. The supernatant was used for enzyme assay. CAT activity was measured by the rate of decrease in hydrogen peroxide absorbance at 240 nm (Aebi, 1984). SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described (Bannister & Calabresse, 1987). All biochemical measures were normalized to the protein content with bovine albumin as standard (Lowry et al., 1951).

2.5. Statistical analysis

All data are presented as mean and standard error of the mean, except figure 2 which expresses the percentage of rats displaying convulsions after the ICV injection of ouabain or saline. Differences among the experimental groups evaluating locomotor activity were determined by one-way analysis of variance (ANOVA), followed by the Dunnett post-hoc test. Seizure behavior was analyzed by Chi-square test. Biochemical data were analyzed by one-way ANOVA, and multiple comparisons were performed with the Dunnett test. In all comparisons, statistical significance was set at $P < 0.05$.

3. Results

3.1. Behavioral assessment

In the open-field task (Figure 1), ICV administration of 10^{-6} and 10^{-5} M ouabain increased locomotion of rats compared to control group. Despite the hyperlocomotor effect of ouabain at low concentrations, no differences in the number of rearings were observed among groups (Figure 1). Interestingly, the ICV injection of ouabain at higher concentrations (10^{-3} and 10^{-2} M) induced convulsions in 22 and 78 % of rats, respectively (Figure 2).

3.2. Oxidative stress parameters

The ICV injection of ouabain at 10^{-2} M increased lipid peroxidation in the hippocampus and striatum of rats (Figure 3). In addition, as illustrated in Figure 3, the ICV injection of ouabain at higher doses (10^{-3} and 10^{-2} M) also increased protein carbonyl levels in the hippocampus compared to control group. In the striatum, the infusion of ouabain at the doses of 10^{-2} and 10^{-5} M increased protein carbonylation (Figure 3).

Figure 4 illustrates the effects of the ICV injection of ouabain on the activity of SOD enzyme in the hippocampus and striatum of rats. Ouabain at the concentration of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}M increased SOD activity in the hippocampus. In a similar way, the injection of ouabain also increased SOD activity in the rat striatum at 10^{-2}, 10^{-3} and 10^{-5} M.

Figure 4 also illustrates the CAT activity in the hippocampus and striatum of rats treated with ouabain. The ICV injection of ouabain increased CAT activity in the hippocampus at the concentrations of 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M, but not at 10^{-2} M. In the striatum, the central administration of ouabain at 10^{-2} and 10^{-6} M decreased CAT activity in rats (Figure 4).

4. Discussion

In the present study, we demonstrated that the ICV administration of ouabain at 10^{-5} and 10^{-6} M elicited hyperlocomotor effects in rats. Ouabain at 10^{-4} M did not affect locomotion, while at higher doses (10^{-3} and 10^{-2} M), ouabain induced convulsions, but in those non-convulsing rats, no changes in locomotion were observed. Previous studies have suggested that subconvulsive concentrations of ouabain produce a dose-related increase in locomotor activity at distinct concentrations, varying from 10^{-3} to 10^{-6} M, depending on experimental conditions: arena dimensions, volume of ouabain injected into the ventricle, rat strain used, etc (El-Mallakh *et al*., 2006; Machado-Vieira *et al*., 2004; El-Mallakh *et al*., 2003; Decker *et al*., 2000; El-Mallakh & Wyatt, 1995). Thus, based on the dose variability, the present study aimed to perform a concentration response curve of ouabain in order to investigate, under our experimental conditions, the behavioral effects of this compound in rats.

In this study, we demonstrated that the ICV injection of ouabain at higher concentrations increased lipid peroxidation and carbonyl proteins in the rat hippocampus and striatum. It should be noted that at higher concentrations of ouabain, convulsions were observed immediately after the ICV injection, which could explain these suddenly alterations.

Evidence from literature have shown that individuals with bipolar disorder have increased serum TBARS in the initial manic episode (Machado-Vieira *et al*., 2007; Frey *et al*., 2007), and in all phases of the bipolar illness (Andreazza *et al*., 2007). Additionally, the activities of antioxidant enzymes are also altered in bipolar patients. Serum superoxide dismutase (SOD) activity is increased in bipolar patients at manic and depressed states while serum catalase activity was decreased in euthymic and manic states (Andreazza *et al*., 2007; Frey et *al*., 2007). Together, these observations suggest that bipolar disorder is associated with both increased oxidative stress parameters and altered antioxidant defenses.

Interesting enough, the amphetamine model of mania in rats also supports the view that oxidative stress is occurring during a manic-like state. In fact, chronic treatment with amphetamine increases TBARS levels in the hippocampus and prefrontal cortex (Frey *et al*., 2006a). In contrast with serum SOD and CAT activity reported to bipolar patients, the activity of both enzymes was little affected by amphetamine injection in rats (Frey *et al*., 2006b).

Present findings have shown that SOD and CAT activity was consistently increased in the hippocampus at all concentrations tested, except at 10^{-2} M. In contrast, in the striatum, while SOD activity was increased at 10^{-4} M to 10^{-2} M concentrations, CAT activity was reduced at 10^{-2} and 10^{-6} M of ouabain. Here, we demonstrated that ICV injection of ouabain modified SOD and CAT activities in the striatum and hippocampus including at those concentrations which induced hyperlocomotion in rats. It was also observed an increase of SOD/CAT activity ratio in hippocampus including at those concentrations which induced hyperlocomotion in rats. We suppose that administration ICV of ouabain at lower concentrations could increase antioxidant defenses, in this way protecting the brain from oxidative damage. In the striatum there was a decrease in CAT, but the SOD has not changed, demonstrating a marked imbalance between the two enzymes activity. The similarities in the SOD and CAT activity reported in the serum of bipolar patients and in brain structures of rats

injected with ouabain reinforce the view that the ICV injection of ouabain fulfills one more criterion for an adequate animal model of mania.

During physiological states, SOD metabolizes superoxide anion (O^{2-}), producing hydrogen peroxide (H_2O_2), which can react with iron to generate highly reactant hydroxyl radicals via the Fenton reaction. CAT is the most important peroxidase in detoxifying excess hydrogen peroxide to prevent hydroxyl production. Thus, an increase in SOD or CAT levels per se does not necessarily indicate increased oxidative stress, whereas an imbalance between SOD and CAT activities could lead to an excessive generation of free radicals (Frey *et al*., 2006b; Andrades *et al*., 2005) Some brain areas are significant in mood disorders. Striatum and hippocampus are some of the brain regions showed to be abnormal in both depression and mania (Fossati *et al*., 2004). Decreases in the activity of Na^+/K^+-ATPase, due to a reduction in ATP synthesis or to an increased production of inhibitors, could be an important link in the pathological response to deficiencies in energy metabolism. Ouabain has been shown to produce lesions in the hippocampus following intracerebral injection of low concentrations (Bonatto *et al*., 2005; Lees & Leong, 1994). The vulnerability of other regions to low doses of ouabain has not been investigated. This is of potential relevance to disease processes as brain regions differ markedly in their ability to with stand a temporary loss of supply of 'energy rich' metabolites (Lees, 1991).

In conclusion, we found no correlation between ouabain-induced hyperactivity and lipid peroxidation in brain. However, the ICV injection of ouabain at the dose of 10^{-5} M that increased locomotion is associated with increased of protein carbonylation in the striatum of rats. Aditionally, our findings sugest that ICV administration 10^{-6} M ouabain-induced hyperactivity are associated with an increased of SOD and CAT activies in the hippocampus.. The increase in SOD and CAT may have been due to generation of ROS, which did not cause damage because of the action of these enzymes. In the striatum we show an imbalance

between SOD and CAT, this is probably due to changes in the intracelular redox state, in the striatum. Such imbalance may increase the predisposition to the generation of the free radicals and therefore increase the susceptibility to oxidative damage in longs periods of induced manic-like hyperactive Finally, our findings reinforce the role of ROS in the pathophysiology of BD.

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Legends

Figure 1

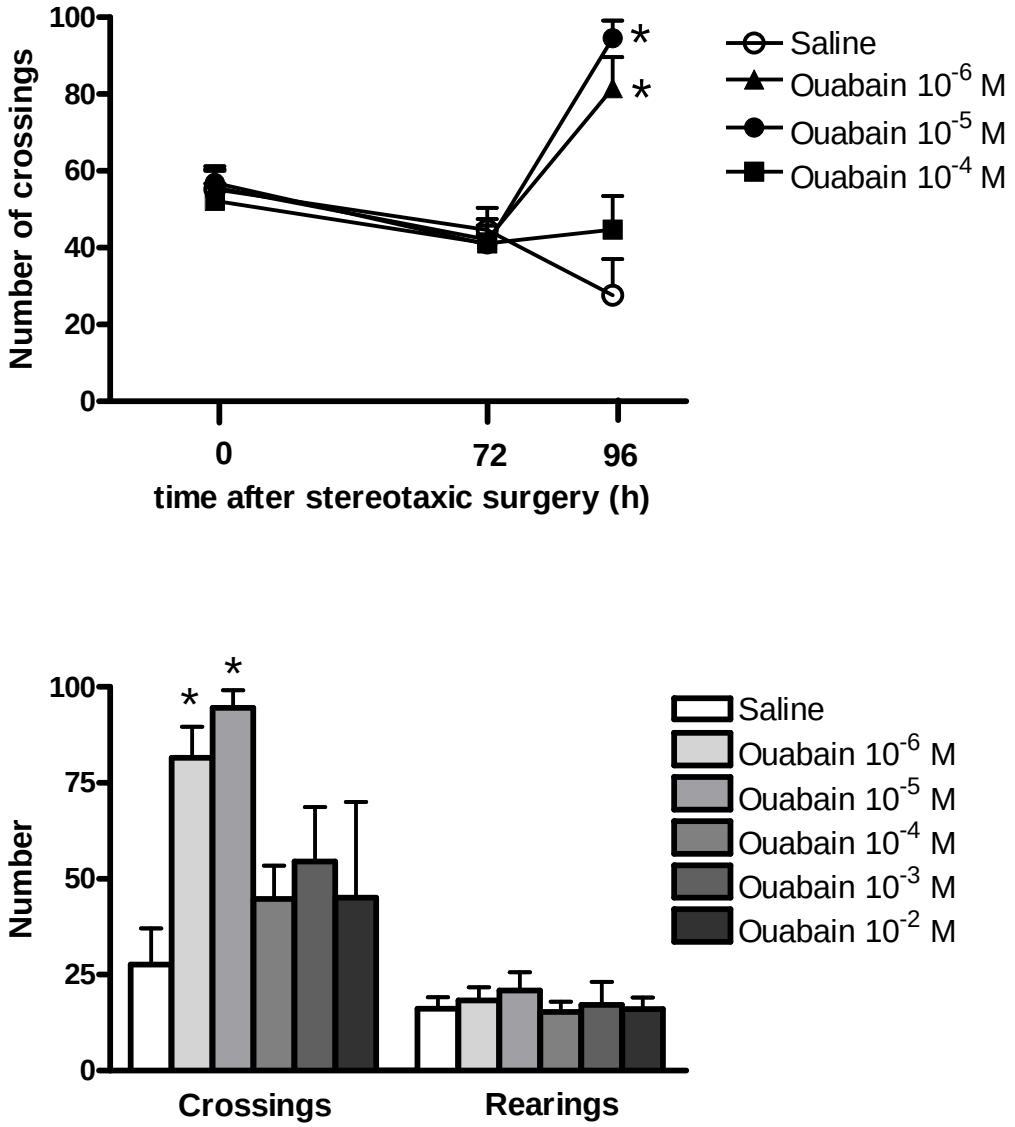


Figure 1. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on number of crossings (top panel) and crossing and rearings (bottom panel) in rats subjected to the open field test, for 5 minutes. Locomotor activity was assessed in the open-field test three times: (1) before surgery (0 h); (2) on the third day following surgery (72 h); and (3) immediately after ICV injection of ouabain or saline, on the fourth day following surgery (96 h). Bars represent means ± standard error of means of 9-10 animals. * P<0.05 vs. saline group, according to ANOVA followed by the Dunnett test.

Figure 2

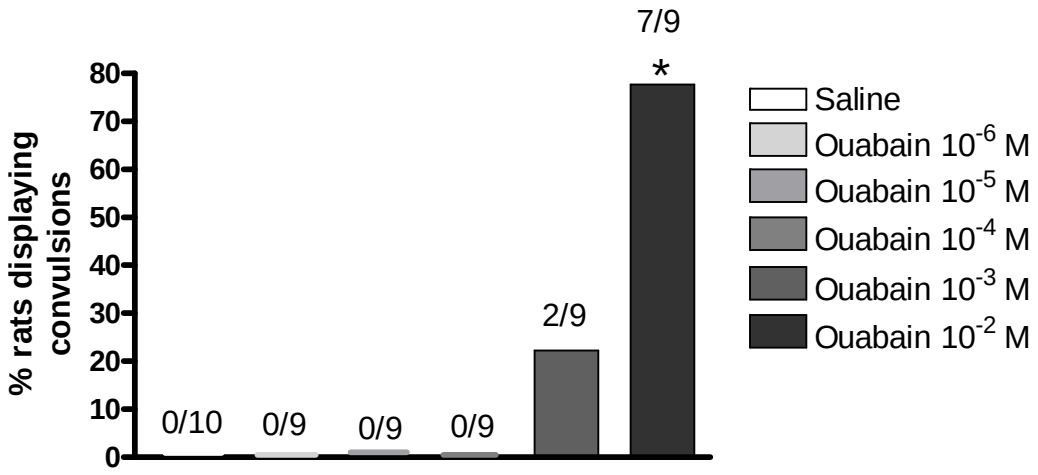
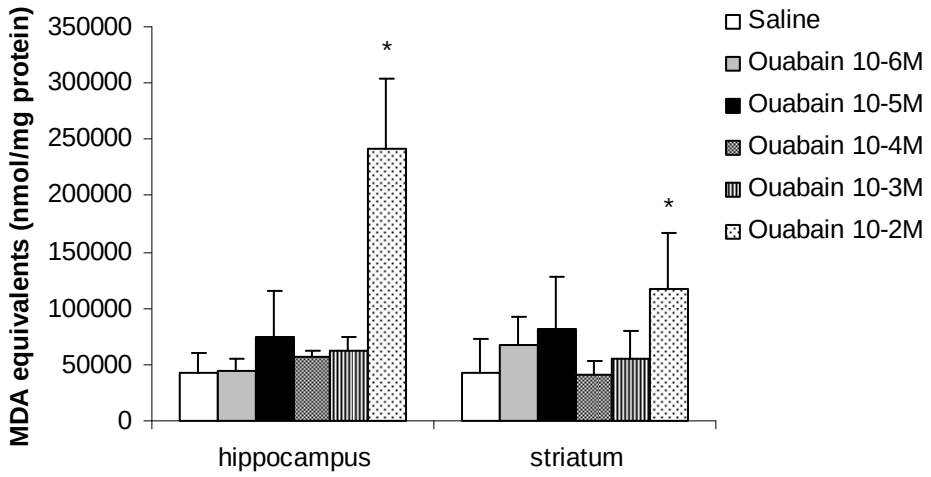


Figure 2. Percentage of rats displaying convulsions in response to the ICV ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline (n=9-10 for each group). * P<0.05 compared with saline group, according to Chi-square test.

Figure 3



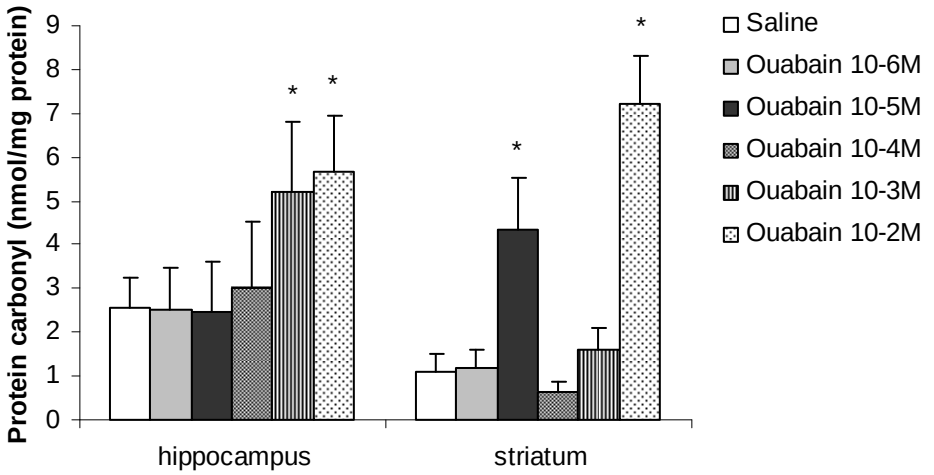
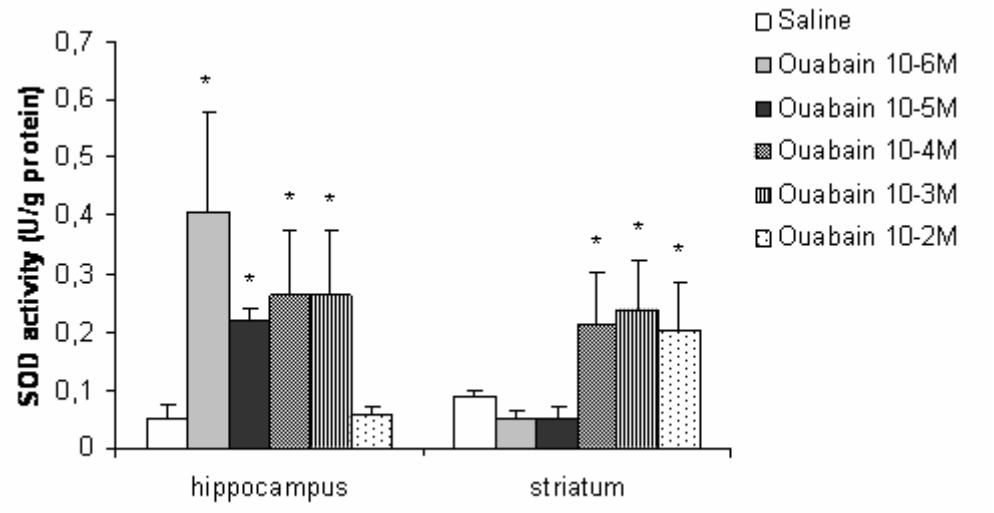


Figure 3. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on TBARS (top panel) and protein carbonyls (bottom panel) levels in the hippocampus and striatum of rats. Bars represent means ± standard error of means of 5-6 animals. * P<0.05 vs. saline group, according to ANOVA followed by the Dunnett test.

Figure 4



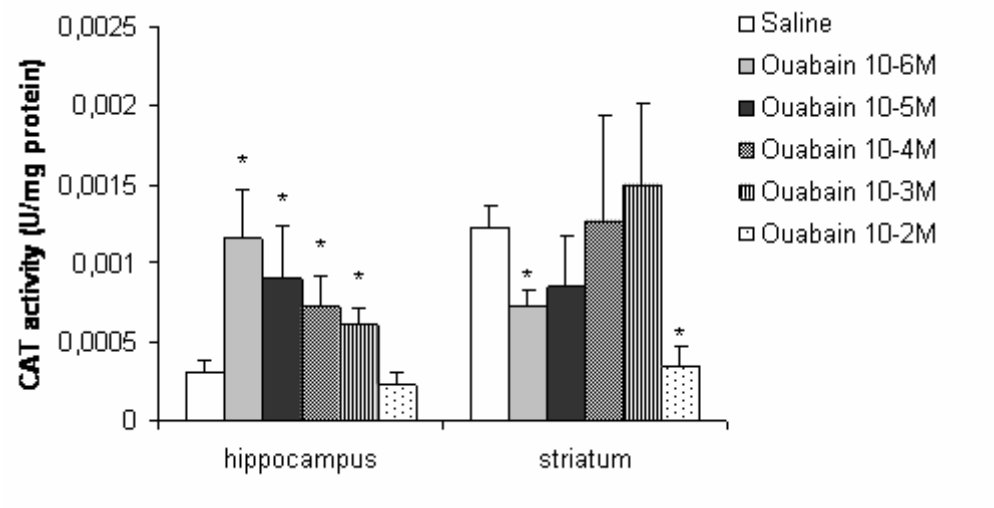


Figure 4. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on the catalase (CAT; top panel) and superoxide dismutase (SOD; bottom panel) enzyme activity in the hippocampus and striatum of rats. Bars represent means ± standard error of means of 5 animals. * $P<0.05$ vs. saline group, according to ANOVA followed by the Dunnett test.

3.3. Artigo 3 (Life Sciences)

INCREASED CEREBROSPINAL FLUID LEVELS OF OXIDATIVE STRESS IN RAT MODEL OF MANIA INDUCED BY OUABAIN

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Abstract

There is an emerging body of data suggesting that oxidative stress may be associated with the pathophysiology of bipolar disorder (BD). Intracerebroventricular (ICV) injection of ouabain, a specific Na^+/K^+ ATPase inhibitor, induced hiperlocomotion in rats, and has been used as an animal model of mania. Therefore, we investigated the formation of lipid and protein oxidation in the cerebrospinal fluid (CSF) of rats submitted to the model of bipolar mania caused by ICV administration of ouabain. The effects of ICV administration of ouabain (at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M) on locomotion and the production of lipid and protein oxidative markers in rat cerebrospinal fluid were assessed. Our findings demonstrated that ouabain at 10^{-6} and 10^{-5} M induced hyperlocomotion in rats. By contrast, at higher concentrations (10^{-3} and 10^{-2} M) ouabain induced convulsions in rats. Regarding the oxidative stress parameters, the ICV injection of ouabain at lower concentrations increased lipid peroxidation and protein carbonyl formation in the CSF. These findings suggest the possible involvement of the oxidative stress in the ouabain rat model of bipolar disorder.

Keywords: Bipolar disorder, Ouabain, Oxidative stress, Mania

Introduction

Bipolar disorder (BD) is a prevalent, chronic, and life-threatening illness characterized by altering episodes of mania and depression (Vieta, 2005). However, little is known about the precise neurobiological underpinnings of BD, which is essential for the development of specific-targeted therapies that are more effective, work rapidly, and are better tolerated than existing therapies (Zarate et al., 2006). The development of animal models has been an important tool in investigating new intracellular systems that may be involved in BD (Einat et al., 2003; Manji and Chen, 2002) and new pharmacological approaches (Lambert et al., 2001; Zarate et al., 2006). According to Belmaker (2004), the unique hallmark of the illness is mania, thus 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.

Previous studies have reported a role of Na^+/K^+ ATPase in the pathophysiology of BD, and proposed that pharmacological inhibition of Na^+/K^+ATPase by intracerebroventricular (ICV) of ouabain in rats, causing hyperactivity, can be used as a model of BD (Decker et al., 2000; El-Mallakh et al., 2003). Thus, it seems to be a promising animal model in the study of energy disturbances related to the pathophysiology of glial and neuronal cells in BD (El-Mallakh et al., 1995, 2003; El-Mallakh and Wyatt, 1995).

Oxidative stress has been implicated in the pathogenesis of diverse states, and may be a common pathogenic mechanism underlying many major psychiatric disorders, as the brain has comparatively greater vulnerability to oxidative damage (Ng et al., 2008). Previous studies have provided evidence for oxidative dysfunction in bipolar disorder. Oxidative disturbances have been demonstrated in both dopaminergic animal models (Frey et al., 2006a,

2006b, 2006c) and human studies (Abdalla et al., 1986; kuloglu et al., 2002; Ranjekar et al., 2003; Andreazza et al., 2007; Frey et al., 2007; Machado-Vieira et al., 2007).

Preclinical studies have shown increased levels of S100β (a sensitive marker of central nervous systems damage) in cerebrospinal fluid (CSF) in rat model of mania induced by ouabain (Machado-Vieira et al., 2004), similar to that seen in patients with affective disorders (Peskind et al., 2001; Herrmann et al., 2000; Grabe et al., 2001; Lara et al., 2001; Rothermundt et al., 2001; Schroeter et al., 2002; Arolt et al., 2003; Dietrich et al., 2004).

Based on the relationship between oxidative stress and psychotomimetic behaviors, studies are need on the effect of ouabain, a psychotomimetic agent, on the oxidative stress in the CSF related to behavior changes. Therefore, we investigated the formation of lipid and protein oxidation in the CSF of rats submitted to the model of bipolar mania caused by ICV administration of ouabain. In addiction, previous studies have suggested that subconvulsive concentrations of ouabain produce a dose-related increase in locomotor activity at distinct concentrations, varying from 10^{-3} to 10^{-6} M, depending on experimental conditions: arena dimensions, volume of ouabain injected into the ventricle and rat strain used (El-Mallakh et al., 2006; Machado-Vieira et al., 2004; El-Mallakh et al., 2003; Decker et al., 2000; El-Mallakh and Wyatt, 1995). Thus, based on the dose variability, the present study also aimed to perform a concentration response curve of ouabain in order to investigate, under our experimental conditions, the behavioral effects of this compound in rats.

Methods

Animals

We conducted the study using adult male Wistar rats obtained from our breeding colony. The animals were housed 5 to a cage, on a 12-hour light/dark cycle (lights on at 7:00 am), with

free access to food and water. 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 Behaviour (SBNeC). This study was approved by the local ethics committee (Comitê de Ética em Pesquisa da Universidade do Extremo Sul Catarinense).

Surgical procedure and treatment

Animals were anesthetized with ketamine (30 mg/kg) and xylasine (4 mg/kg). In a stereotaxic apparatus, the skin of the skull was removed and a 27 gauge 9 mm guide cannula was placed at 0.9 mm posterior to bregma, 1.5 mm right from de midline and 1.0 mm above the lateral brain ventricle. Through a 2 mm hole made at the cranial bone, a cannula was implanted 2.6 mm ventral to the superior surface of the skull, and fixed with jeweler acrylic cement. Animals were tested on the third day following surgery. A 30 gauge cannula was fitted into the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula aiming the right lateral brain ventricle.

Each animal was administered 5 µl of either vehicle (NaCl 0.9%) or ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) over 30 s and after that the animal was immediately placed into the open field.

Behavioral assessment

We used the open field task to assess locomotor activity. The task was performed in a 40 × 60 cm open field surrounded by 50 cm-high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 equal rectangles by black lines. The animals were gently placed on the left rear rectangle and were allowed to explore the arena. Crossings of the black lines and rearings were counted for 5 minutes.

Animals were observed in the open field three times: (1) before the cannula implantation (0 h); (2) on the third day following surgery (72 h); and (3) on the fourth day following surgery (96 h), immediately after ICV injection of ouabain or saline.

After the ICV infusion of ouabain, especially at high doses, some rats displayed behavioral seizures. Aiming to assess the locomotor effects of ouabain, data obtained from animals that displayed seizures after the ICV injection of ouabain were ruled out from the locomotor activity analysis.

Oxidative stress parameters

Immediately after the open-field test, the rats were anesthetized with 40 mg/kg of sodium thiopental (Cristalia, Itapira, SP, Brazil), i.p., and the CSF was drawn (80–100 μl per rat), by direct puncture of the cisterna magna with an insulin syringe (27 gauge 31/20 length). Individual samples that presented visible blood contamination were discarded. After sampling, the samples were centrifuged at 4500 ×g at 5 °C for 5 min, to obtain cell-free supernatants (Cruz Portela et al., 2002). CSF samples were stored at −70 °C and defrosted only when measurements were carried out.

To determine oxidative damage, we measured the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described (Esterbauer and Cheeseman, 1990). The samples were mixed with 1 mL of trichloroacetic acid 10% and 1 mL of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 15 minutes. TBARS were determined by the absorbance at 535 nm.

Oxidative damage to proteins was measured by the quantification of carbonyl groups based on the reaction with dinitrophenylhidrazine (DNPH), as previously described (Levine et al., 1994). Proteins were precipitated by the addition of 20% trichloroacetic acid and were redissolved in DNPH; the absorbance was read at 370 nm.

Statistical analysis

All data are presented as mean and standard error of the mean, except figure 2 which expresses the percentage of rats displaying convulsions after the ICV injection of ouabain or saline. Differences among the experimental groups evaluating locomotor activity were determined by one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. Seizure behavior was analyzed by Chi-square test. Biochemical data were analyzed by one-way ANOVA, and multiple comparisons were performed with the Newman–Keuls test. In all comparisons, statistical significance was set at $P < 0.05$.

Results

In the open-field task (Figure 1), ICV administration of 10^{-6} and 10^{-5} M ouabain increased locomotion of rats compared to control group. Interestingly, the ICV injection of ouabain at higher concentrations (10^{-3} and 10^{-2} M) induced convulsions in 60 and 80% of rats, respectively (Figure 2).

The ICV injection of ouabain at lower doses (10^{-6} and 10^{-5}M) increased lipid peroxidation and protein carbonyl levels in the CSF of rats (Figure 3). In contrast, no changes in lipid peroxidation and protein carbonyl formation were observed with the ICV injection of ouabain at higher doses (10^{-4}, 10^{-3} and 10^{-2}M) when compared to the control group.

Discussion

In the present study, we demonstrated that the ICV administration of ouabain at 10^{-5} and 10^{-6} M elicited hyperlocomotor effects in rats. Ouabain at 10^{-4} M did not affect locomotion, while at higher doses (10^{-3} and 10^{-2} M), ouabain induced convulsions, but in those non-convulsing rats, no changes in locomotion were observed. Lower doses of ouabain stimulated, but higher

doses inhibited the activity of Na+ /K+-ATPase in rat brain homogenates (Lichtstein et al., 1985). Activation of Na^+/K^+-ATPase can induce hyperpolarization of neurons, and inhibition of Na^+/K^+-ATPase induces depolarization-inducing neuronal excitation, which might be related to the increased locomotor activity (McCarren and Alger, 1989; Herman et al., 2007)

Here, we demonstrated that ICV injection of ouabain at concentration which induced hyperlocomotion (10^{-5} and 10^{-6} M) in rats, increased lipid peroxidation and protein carbonyl levels in CSF of rats. The amphetamine model of mania in rats also supports the view that oxidative stress is occurring during a manic-like state. In fact, treatment with amphetamine increases TBARS levels and protein carbonyl formation in the rat brain (Frey et al., 2006a). In addiction, studies have shown that individuals with bipolar disorder have increased serum TBARS in the initial manic episode (Machado-Vieira et al., 2007; Frey et al., 2007), and in all phases of the bipolar illness (Andreazza et al., 2007). These aspects support the view that the ICV administration of ouabain mimics behavioral and biological aspects of a manic state (Herman et al., 1998).

Soon after the characterization of the Na^+/K^+-ATPase, some authors investigated its activity in mood disorders. These studies showed that Na^+ pump activity is decreased in acute mania compared to recovered euthymic bipolar individuals (Reddy et al., 1992). Decreases in the activity of Na^+/K^+-ATPase, due to a reduction in ATP synthesis or to an increased production of inhibitors, could be an important link in the pathological response to deficiencies in energy metabolism. Ouabain has been shown to produce lesions in the brain following ICV injection of low concentrations (Bonatto et al., 2005; Lees and Leong, 1994).

In this study, no changes in protein carbonyl formation and lipid peroxidation were observed with the ICV injection of ouabain at higher doses (10^{-4}, 10^{-3} and 10^{-2}M). We suppose that

administration ICV of ouabain at higher concentrations could increase antioxidant defenses, in this way protecting the CSF from oxidative damage.

In summary, ICV administration of ouabain in lower doses (10^{-5} and 10^{-6}M) induced hiperlocomotor effect, which could be associated with formation of lipid and protein oxidation products in the rat CSF. These findings suggest the possible involvement of the oxidative stress in the ouabain rat model of bipolar disorder. Further studies are warranted to better determine the role of oxidative stress and Na^+/K^+-ATPase in the pathophysiology of BD.

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Legends

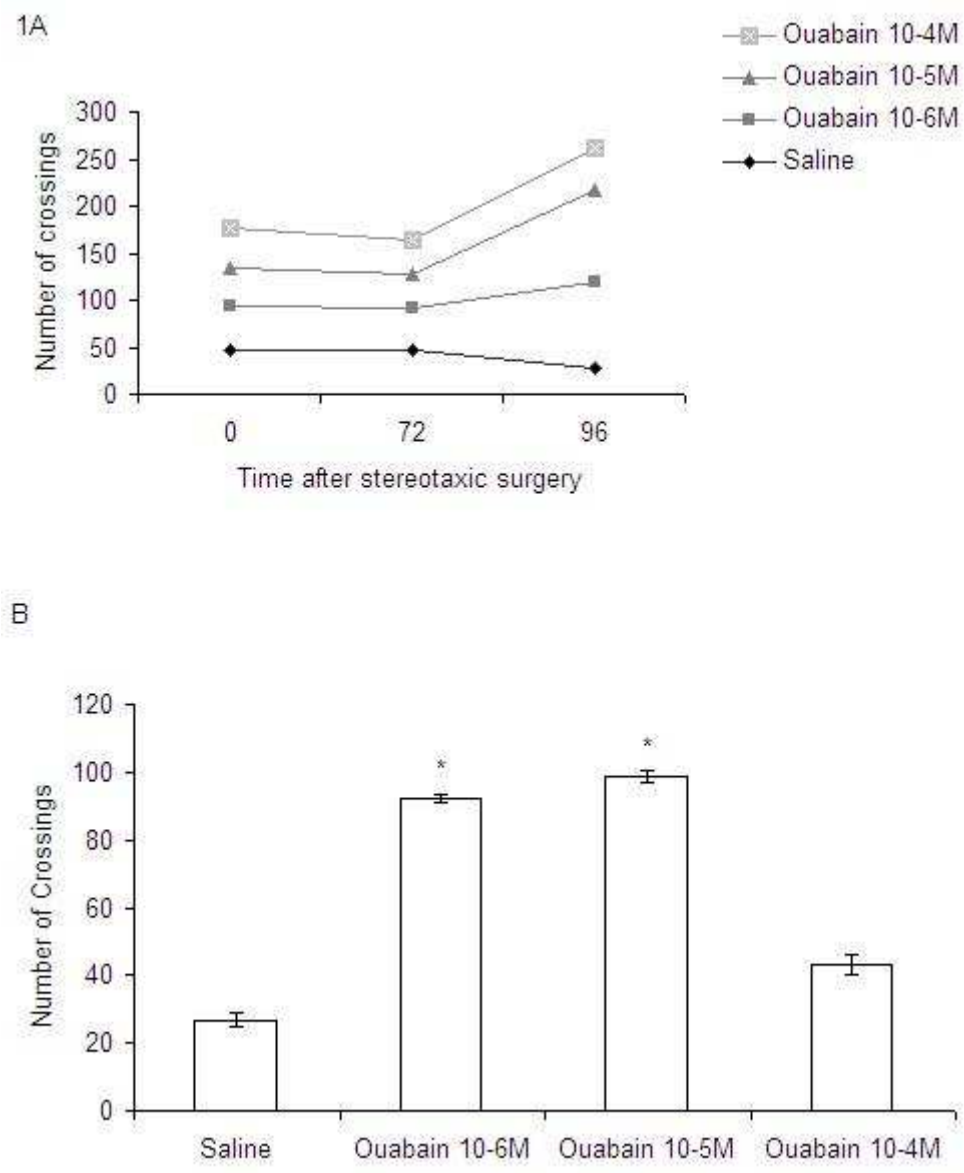


Figure 1. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on number of crossings in rats subjected to the open field test, for 5 minutes. Locomotor activity was assessed in the open-field test three times: (1) before surgery (0 h); (2) on the third day following surgery (72 h); and (3) immediately after ICV injection of ouabain or saline, on the fourth day following surgery (96 h) (A). Bars represent means ± standard error of means of 15 animals on locomotion immediately after ICV injection of ouabain or saline, on the fourth day following surgery (B). *P<0.05 vs. saline group, according to ANOVA followed by the Tukey test.

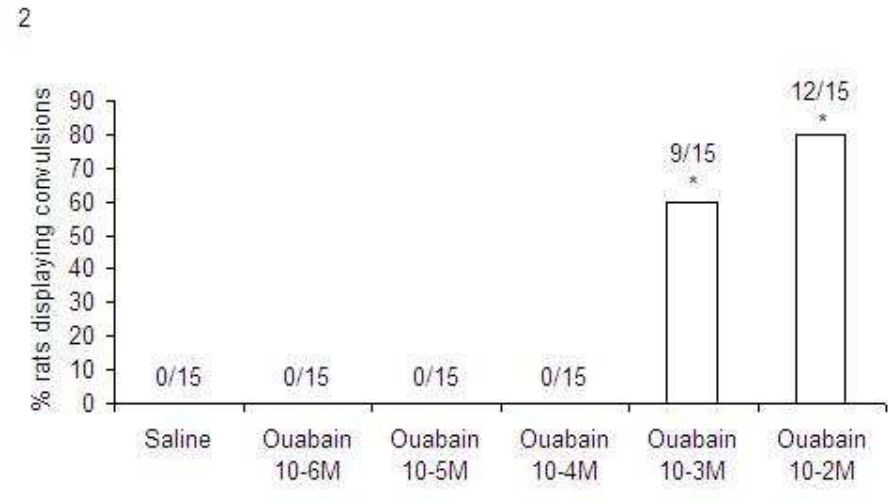
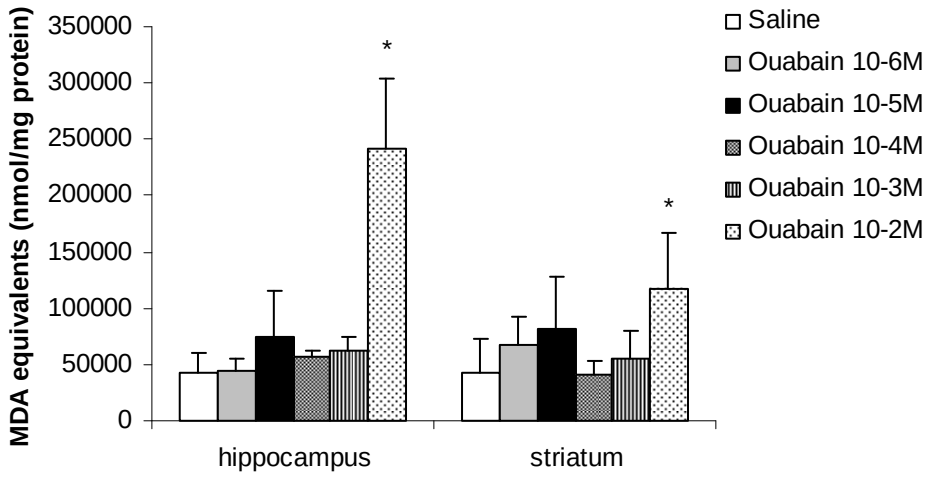


Figure 2. Percentage of rats displaying convulsions in response to the ICV ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline (n=9-10 for each group). * P<0.05 compared with saline group, according to Chi-square test.

Figure 3

A



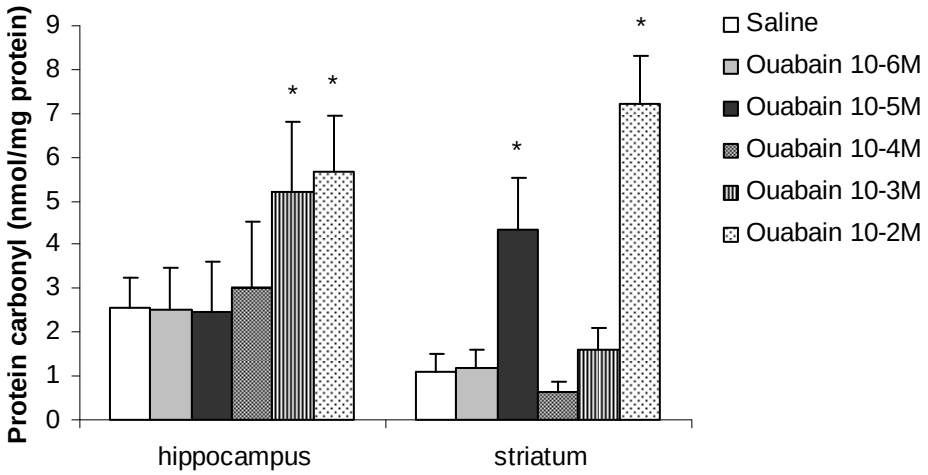


Figure 3. Effects of the ICV injection of ouabain (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} M) or saline on TBARS (A) and protein carbonyls (B) levels in the CSF of rats. Bars represent means ± standard error of means of 5-6 animals. * P<0.05 vs. saline group, according to ANOVA followed by the Dunnett test.

4. DISCUSSÃO

O presente estudo objetivou replicar o modelo animal de mania com a administração ICV de ouabaína em diferentes concentrações, demonstrando atividade hyperlocomotora nas concentrações 10^{-5} e 10^{-6} M de ouabaína. Na concentração 10^{-4} M a ouabaína ICV não provocou alterações na locomoção dos ratos e em doses maiores (10^{-3} e 10^{-2} M) verificamos a indução de convulsões, mas nestas doses maiores não houveram alterações na locomoção dos ratos que não apresentaram convulsões. Estudos anteriores têm sugerido que concentrações subconvulsivas de ouabaína produzem um aumento dose dependente na atividade locomotora em concentrações distintas que variam de 10^{-3} a 10^{-6} M, dependendo das condições experimentais (El-Mallakh *et al*., 2006; Machado-Vieira *et al*., 2004; El-Mallakh *et al*., 2003; Decker *et al*., 2000; El-Mallakh & Wyatt, 1995). Então, considerando-se a variabilidade das doses, o presente estudo conseguiu replicar a curva resposta de concentração de ouabaína observada nos estudos prévios, atingindo os efeitos comportamentais deste composto em ratos, nas nossas condições experimentais.

Também foi demonstrado que ouabaína ICV em concentrações altas aumenta a peroxidação lipídica e carbonilação de proteínas no hipocampo e estriado de ratos wistar. Uma possível explicação disso seria a ocorrência de convulsões em altas doses de ouabaína logo após a injeção ICV, o que poderia explicar possíveis alterações abruptas. Evidências tem demonstrado que pacientes com TAB apresentam TBARS elevado nos episódios maníacos iniciais (Machado-Vieira *et al*., 2007) e em todas as fases do transtorno bipolar (Andreazza *et al*., 2007). Conjuntamente, estas observações sugerem que o TAB está associado tanto com o aumento dos parâmetros de stress oxidativo, como com alterações nas defesas antioxidantes.

O presente estudo demonstra que a atividade da SOD e CAT mostraram-se consistentemente elevadas no hipocampo em todas as concentrações com exceção de 10^{-2} M.

Em estriado enquanto a atividade da SOD aumentou nas concentrações de 10^{-4} M a 10^{-2} M, CAT apresentou atividade reduzida em 10^{-2} e 10^{-6} M de ouabaína. Verificou-se que a administração de ouabaína ICV modificou as atividades da SOD e da CAT em hipocampo e estriado, incluindo nas concentrações que induziram hiperlocomoção nos ratos. A razão na atividade SOD/CAT sofreu um incremeto em hipocampo, mesmo em doses baixas. Uma possível explicação seria que doses baixas de ouabaína poderia aumentar as defesas antioxidantes, protegendo o cérebro de stress oxidativo. Em estriado houve decréscimo na CAT, sem alterações na SOD, demonstrando um marcante desequilíbrio entre a atividade das duas enzimas.

A similaridade na atividade da SOD e da CAT encontradas no sangue de pacientes bipolares e nas estruturas cerebrais dos ratos submetidos a este modelo de mania reforçam a idéia de que a injeção de ouabaína ICV preenche mais um critério em direção a um adequado modelo animal de mania.

Os aumentos na atividade da SOD e da CAT aqui observados podem ter ocorrido pela geração de ERO, sem resultar em danos devido à ação destas enzimas. Foi demonstrado um desequilíbrio entre SOD e CAT em estriado, refeletindo provavelmente mudanças intracelulares causadas pelo modelo de mania. Tais desequilíbrios podem aumentar a predisposição de geração de radicais livres, e conseqüente dano celular. Ao ligarmos estados maníacos com aumento de dano oxidativa, apontamos o papel das ERO na patofisiologia do TAB.

Este estudo demonstrou o aumento na geração de TBARS e superóxido em partículas submitocondriais de hipocampo em concentrações baixas de ouabaína (10^{-5} M) e geração de TBARS em estriado em concentrações altas e baixas(10^{-6}, 10^{-5}, 10^{-3} and 10^{-2}M). Nenhum efeito na produção de ERO em partículas submitocondriais em cortéx pré-frontal após administração de ouabaína ICV. Volume hipocampal diminuido foi observado em

populaçoes de pacientes bipolares idosos (Strakowski et al., 2005). Existe um crescente aparecimento de informações indicando alterações no metabolismo energético por disfunções mitocondriais, e que estas alterações exercem papel importante no transtorno afetivo bipolar (Kato and Kato, 2000; Quiroz et al., 2008). As alterações na geração de ERO mitocondriais vistas neste estudo fornecem um modelo para a verificação da hipótese de alterações no metabolismo energético cerebral associado ao transtorno bipolar.

Neste estudo também foi demonstrado que ouabaína ICV em baixas concentrações, as mesmas que induziram hiperlocomoção nos animais, 10^{-5} e 10^{-6} M, aumentaram a peroxidação lipídica e a carbonilação de proteínas no LCR de ratos submetidos ao modelo de mania em questão. Considerando que alterações similares são observadas em pacientes bipolares (Andreazza et al., 2007) este modelo mimetiza aspectos biológicos e comportamentais dos estados maníacos. Concentrações maiores de ouabaína (10^{-4}, 10^{-3} and 10^{-2}M) não provocaram alteraçoes na formação de peroxidação lipídica e carbonilação de proteínas no LCR. Talvez essas concentrações aumentadas possam aumentar as defesas antioxidantes e evitar o dano.

5. CONCLUSÃO

A partir do presente estudo é possível inferir que a ouabaína mostrou-se um adeqüado modelo animal de mania, potencialmente útil para o desenvolvimento de novas abordagens terapêuticas para os pacientes bipolares.

Os resultados evidenciaram aumento da atividade locomotora em doses de ouabaína ICV subconvulsivas e nenhuma alteração motora ou convulsões em doses maiores. O perfil oxidativo em diferentes estruturas demonstrou alterações na oxidação de proteínas e lipídios, bem como na ação de mecanismos de defesa antioxidantes nos diferentes sítios neuronais avaliados neste modelo animal de mania, ligando as alterações de deterioro cognitivo, observados em pacientes bipolares, com resultados experimentais de um modelo de mania induzido por ouabaína ICV.

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