UNIVERSIDADE FEDERAL DO PARANÁ

INARA FERNANDA MISIUTA RAUPP BARCARO

EFEITO TIPO ANTIDEPRESSIVO DA AMANTADINA EM MODELOS ANIMAIS

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INARA FERNANDA MISIUTA RAUPP BARCARO

EFEITO TIPO ANTIDEPRESSIVO DA AMANTADINA EM MODELOS ANIMAIS

Tese apresentada como requisito parcial à obtenção do grau de Doutor em Farmacologia, no Curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas da Universidade Federal do Paraná.

Orientador: Prof. Dr. Roberto Andreatini

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A Deus, que é minha força e meu escudo, em quem confio meu coração e por quem sou sempre socorrida na hora da angústia...

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JAMES TOUR, Nanocientista

RESUMO

Segundo a Organização Mundial da Saúde pelo menos 350 milhões de pessoas vivem com depressão. Embora existam vários medicamentos antidepressivos clinicamente eficazes, apenas cerca de 50% dos pacientes responde à primeira droga prescrita. No intuito de minimizar o sofrimento desses pacientes tem se buscado novas estratégias para o tratamento. O sistema glutamatérgico tem sido alvo de estudos, uma vez que a quetamina, antagonista dos receptores N-metil-D-aspartato (NMDA), apresenta efeito antidepressivo mais rápido que os antidepressivos mais usados na clínica, sendo eficaz em pacientes refratários aos antidepressivos atuais. Contudo, dois inconvenientes do seu uso são seus efeitos colaterais (alucinações, mudanças de humor e afeto, delírio e confusão), e sua administração endovenosa. A amantadina, outra droga que bloqueia os receptores NMDA, bem como aumenta indiretamente os níveis de dopamina, tem também apresentado efeitos tipo antidepressivos em modelos animais de depressão e antidepressivo em estudos clínicos abertos com pequeno número de pacientes, conforme observado em revisão sistemática realizada na primeira parte do presente trabalho. Os resultados da segunda parte (parte experimental) do presente estudo, utilizando o teste de natação forçada modificado (ratos) e o teste de suspensão pela cauda (camundongos), sugerem que o efeito do tipo antidepressivo da amantadina ocorre provavelmente devido à participação dos receptores NMDA do glutamato e D_2 da dopamina. Além disso, assim como os antidepressivos monoaminérgicos e a quetamina, observou-se que a amantadina também aumenta a neurogênese hipocampal, que tem sido proposta como importante na melhora dos quadros depressivos. Portanto, a amantadina apresenta-se como um medicamento com potencial antidepressivo, assim como ponto de partida para novos fármacos antidepressivos.

Palavras-chave: amantadina, antidepressivo, glutamato, dopamina, neurogênese

ABSTRACT

According to the World Health Organization at least 350 million people live with depression. Although there are several clinically effective antidepressant medications, only about 50% of patients respond to the first prescribed drug. In order to minimize the suffering of these patients, new treatment strategies have been sought. The glutamatergic system has been studied, since ketamine, an antagonist of N-methyl-D-aspartate (NMDA) receptors, has an antidepressant effect faster than the most commonly used antidepressants in the clinic and is effective in patients refractory to current antidepressants. However, two drawbacks of its use are its side effects (which include hallucinations, changes in mood and affect, delirium and confusion), and its intravenous administration. Amantadine, another drug that blocks NMDA receptors as well as indirectly increases dopamine levels, has also been shown to have antidepressant-like effects in animal models of depression and antidepressant in open clinical studies with a small number of patients. The results of the present study, using the modified forced swimming test (rats) and the tail suspension test (mice), suggest that the anti-depressant effect of amantadine probably occurs due to the participation of NMDA receptors of glutamate and D_2 of dopamine. In addition, as well as the monoaminergic antidepressants and ketamine, it has been observed that amantadine also increases hippocampal neurogenesis, which has been proposed as important in improving depressive disorders. Therefore, amantadine is a drug with antidepressant potential as well as a starting point for new antidepressant drugs.

Key-words: amantadine, antidepressive, glutamate, dopamine, neurogenesis

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LISTA DE ABREVIATURAS E SIGLAS

$5HT_{1A}$ – receptor tipo 1A da serotonina

8-OHDPAT – 8-hidroxi-2-(di-n-propilamina)-tetralina

AA – *automated locomotor activity box*

AMPA – receptor alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico

AMPc – monofosfato cíclico de adenosina

AMPT – α-metil-p-tirosina

BDNF – *brain-derived neurotrophic factor*

BrdU – 5-bromo-2-deoxiuridina

CA – teste do campo aberto

Ca^{2+} – íon cálcio

CGI – *Clinical Global Impression*

CGZ – subgranular zone

CNS – *central nervous system*

CREB – *cAMP response element-binding protein*

CUMS – *chronic umpredictable mild stress*

DB – *double-blind*

DCX – doublecortina

DCX-IR cells – células imunoreativas à doublecortina

DG – dentate gyrus

DSM-IV – *Diagnostic and Statistical Manual of Mental Disorder IV*

FST – *forced swimming test*

GABA – ácido gama-amino-butírico

GABA – ácido gama-amino-butírico

GCL – granular cell layer

HDRS – *Hamilton Depression Rating Scale*

Il-2; Il-10 – Interleucinas 2 e 10

ISRS – inibidor seletivo da recaptação da serotonina

L-DOPA – levodopa

LTP – *long term potentiation*

MADRS – *Montgomery-Asberg Depression Rating Scale*

MD – *major depression*

Mg^{2+} – íon magnésio

mGLU – receptor metabotrópico do glutamato

MK-801 – dizolcipina

MWM – *Morris water maze*

Na^{+}– íon sódio

NMDA – receptor N-metil-D-aspartato

OFT – *open field test*

POMS – *Profile of Mood States*

Receptor D_2 – receptor tipo 2 da dopamina

SNC – sistema nervoso central

SP – *sucrose preference test*

TrkB – receptor da tropomiosina kinase B

TSC – teste da suspensão pela cauda

TST – *tail suspension test*

WHO – *World Health Organization*

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1 INTRODUÇÃO

Segundo a World Health Organization (Organização Mundial da Saúde) (2015), pelo menos 350 milhões de pessoas, adultos, jovens e crianças, vivem com depressão. Trata-se de um transtorno mental comum caracterizado por tristeza, perda de interesse ou do prazer, sentimento de culpa ou diminuição da auto-estima, distúrbios do sono e do apetite, cansaço sem esforço e diminuição da capacidade de concentração (WHO, 2012; APA, 2013). É de difícil diagnóstico e tratamento, resultando em diminuição da qualidade de vida e perdas social e econômica. Um dos maiores desafios da doença é conseguir um tratamento cujo efeito terapêutico seja rápido e efetivo (Vásquez, 2014).

Atualmente a maioria dos fármacos classificados como antidepressivos atua inicialmente aumentando a disponibilidade sináptica de monoaminas, sendo classificados em: inibidores seletivos de serotonina (5-HT) como a fluoxetina, a paroxetina e o escitalopram; inibidores seletivos de noradrenalina (NA) como a reboxetina, a desipramina e a nortriptilina; inibidores seletivos de NA e 5-HT como a duloxetina, a venlafaxina e a imipramina; antagonistas alfa-2 como a mirtazapina; ou os inibidores da monoaminoxidase (MAO) como a tranilcipromina e a moclobemida. Adicionalmente, alguns fármacos apresentam como mecanismo principal, aparentemente, ações não monoaminérgicas, como a agomelatina (agonista de receptores melotoninérgicos). Estes fármacos antidepressivos são a primeira escolha para o tratamento da depressão, porém menos de 50% dos pacientes respondem favoravelmente à primeira droga prescrita. Como estratégia de tratamento, se a primeira droga (geralmente um inibidor seletivo da recaptação da serotonina – ISRS) não é efetiva na dose escolhida e pelo tempo determinado, pode-se aumentar a dose da primeira ou uma segunda droga, com mecanismo de

ação diferente, é administrada (Souery, 2011). Essa dificuldade em se obter resposta antidepressiva ou não ao tratamento é multifatorial e de caráter individual. Questões como acerto da dose, adesão ao tratamento pelo paciente, tolerância aos efeitos colaterais, presença de comorbidades que diminuam a chance de remissão, episódios depressivos anteriores, fatores genéticos e até histórico de vida do paciente podem interferir na obtenção de resultados satisfatórios. Contudo, mudanças estratégicas de conduta terapêutica têm sido tomadas buscando-se outros sistemas neurotransmissores, vias de transdução de sinais que possam estar ativadas ou desativadas e até mesmo o estudo da microbiota intestinal têm sido foco de estudos pré-clínicos e clínicos, no intuito de minimizar o sofrimento dos pacientes e também os gastos com intervençoes psiquiátricas. Quase metade do dinheiro investido anualmente no tratamento de transtornos do humor nos Estados Unidos é utilizado nos casos de depressão resistente ao tratamento (Yang, 2017; Fabbri, 2017; Preskorn, 2013).

Nesse contexto surge tanto o glutamato, principal neurotransmissor excitatório do sistema nervoso central (SNC), cujas vias de sinalização estão envolvidas no processamento das informações, memória e plasticidade neuronal; quanto a quetamina, droga anestésica capaz de reverter os sintomas depressivos mais rapidamente que os antidepressivos de uso comum. Fisiologicamente o glutamato liga-se a vários tipos de receptores, entre eles: os ionotrópicos NMDA (N-metil-D-aspartato), AMPA (alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico) e cainato; e os metabotrópicos, mGLU1 a mGLU8. Os primeiros são permeáveis tanto ao Na^{+} , quanto ao Ca^{2+} e têm participação principalmente na plasticidade sináptica através da potenciação de longa duração – LTP (do inglês *long term potentiation*). Nesse processo, os receptores AMPA levam à rápida despolarização da membrana,

retirando o Mg^{2+} que bloqueia o canal dos receptores NMDA, permitindo assim a entrada de íons Ca^{2+}. O influxo de Ca^{2+} ativa a proteínaquinase dependente de Ca^{2+}/calmodulina (CaMKII) e outras vias de segundos mensageiros que promovem o tráfego e a incorporação de receptores AMPA na membrana. Já os receptores NMDA, podem promover sobrevivência celular e funções neurotróficas ou podem ativar vias de morte celular, o que depende do tempo de ligação e também do local onde se encontra o receptor. Por exemplo, níveis moderados de atividade promovem vias de sinalização neuroprotetoras como a via de ativação do CREB – proteína de ligação ao AMPc (do inglês *cAMP response element-binding protein)*. O CREB induz a expressão de BDNF (fator neurotrófico derivado do encéfalo, do inglês *brain-derived neurotrophic factor*), importante nos processos neuroprotetores e neurotróficos relevantes no estresse e nos transtornos de humor. Por outro lado, uma superativação da via, leva a efeitos deletérios associados à excitotoxicidade e morte neuronal. Estudos de neuroimagem e *post-mortem* mostram que os níveis de glutamato parecem estar elevados no plasma, líquido céfaloraquidiano e cérebro de pacientes com depressão. Além disso, foram relatadas alterações na expressão ou função de subunidades de receptores NMDA em pacientes com transtornos depressivo maior e bipolar, bem como em vítimas de suicídio (tabela 1) (Szakács et al., 2012; Murrough et al., 2017).

Tabela 1 – Resltados de estudos humanos post-mortem que relacionam as subunidades do receptor NMDA na depressão

SUBUNIDADE	AMOSTRA	REGIÃO DO CÉREBRO	MÉTODO	RESULTADOS
NR1	Transtorno bipolar e depressivo	Córtex temporal	Western blot	↓ na densidade de NR1 em ambos
NR2C	Depressão maior	Locus coeruleus	Western blot	↑NR2C
NR1 e NR2A	Depressão maior	Amígdala lateral	Western blot	↑NR2A, nenhuma diferença em NR1
NR1, NR2A e NR2B	Depressão maior	Córtex pre-frontal	Western blot	↓NR2A e NR2B, nenhuma diferença em NR1
NR2B e NR2C	Depressão maior (maioria morte por suicídio)	Locus coeruleus	Expressão gênica	↑NR2B e NR2C
Múltiplos genes relacionados ao glutamato e receptores NMDA	Depressão maior	Córtex pre-frontal dorsolateral	Expressão gênica	↑da expressão da maioria dos genes relacionados ao glutamato

NR1 – subunidade do receptor NMDA onde se liga a glicina

NR2(A,B,C) – subunidades do receptor NMDA onde se liga o glutamato

Fonte: Murrough et al., 2017.

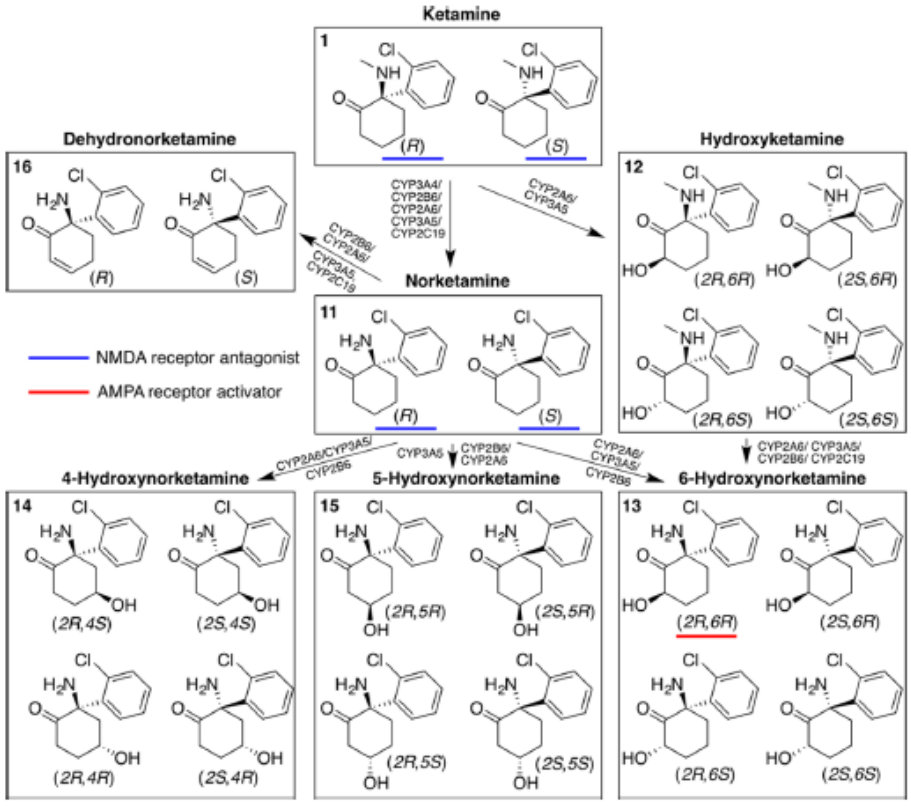

Figura 1 – Estruturas químicas da quetamina e seus metabólitos (Tyler et al., 2017)

Seu principal mecanismo de ação é o antagonismo não-competitivo dos receptores NMDA, o que pode facilitar a ação do glutamato e inibir seus efeitos tóxicos. Esse mecanismo se dá de várias maneiras, dentre as quais destacam-se a inibição de receptores do tipo NMDA em interneurônios, levando à diminuição da atividade de receptores GABA pré-sinápticos, o que acarreta num aumento da liberação de glutamato na fenda sináptica e a ativação de receptores AMPA

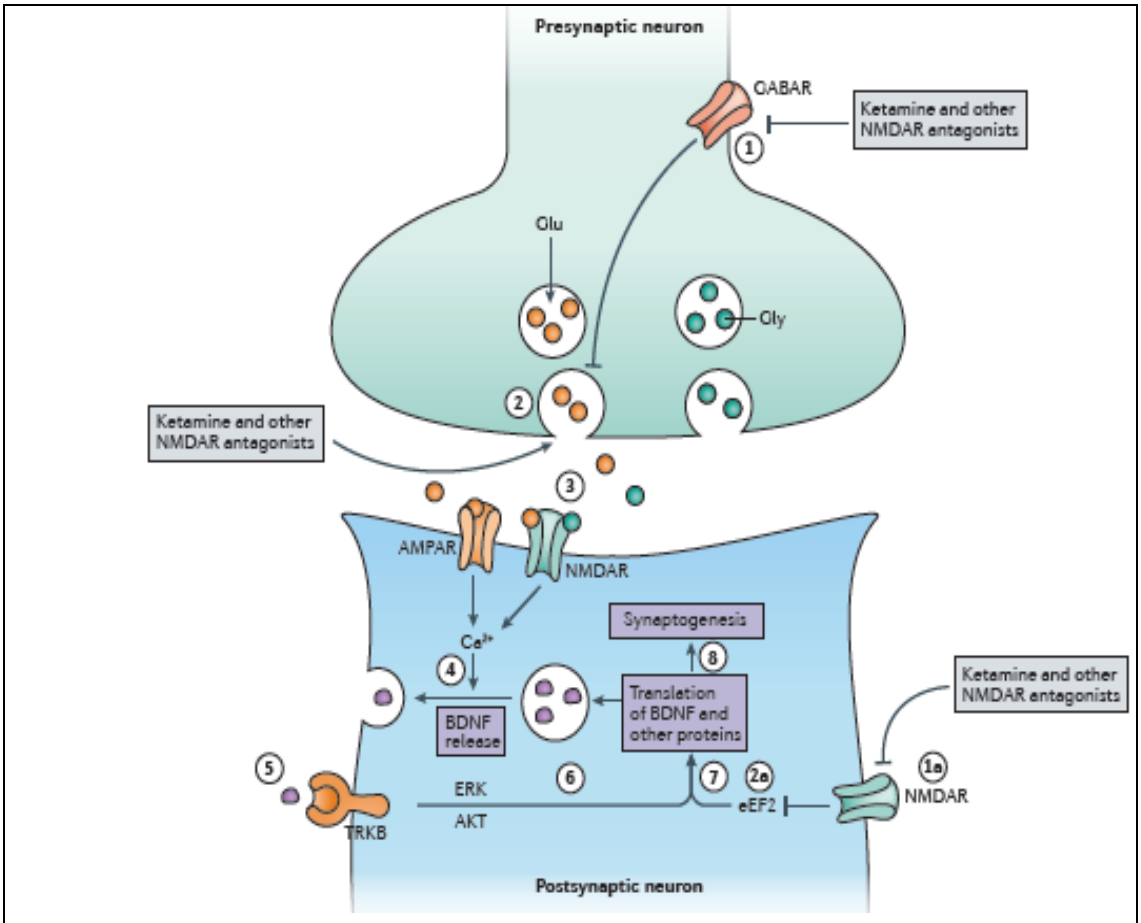

Figura 2 – Quetamina – mecanismo de ação (Murrough et al., 2017). Resumidamente a quetamina bloquearia os receptores N-metil-D-asparatato (NMDAR) no interneurônio GABAérgico (não mostrado), levando à uma diminuição da inibição GABA no neurônio glutamatérgico (1), acarretanto um aumento da liberação de glutamato, que atuaria no receptor alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico (AMPAR), estimulando a liberação de BNDF; o BDNF ligaria-se ao seu receptor TrkB (5) estimulando mais a produção de BNDF e a sinaptogênese (7 e 8). O bloqueio do receptor NMDA extrasinápticos (1a) pela quetamina poderia também frear a inibição do EF2 (7), estimulando a produção de BDNF e a sinaptogênese.

intrasinápticos, os quais levam a uma série de reações intracelulares como aumento do influxo de cálcio, liberação de BDNF, ativação do receptor TrkB (tropomiosina kinase B), translação de proteínas, sinaptogênese e neurogênese (Krystal et al.,2013; Deutschenbaur et al., 2016; Murrough et al., 2017). Além disso, a quetamina apresenta um tempo para ação clínica menor (em torno de 4 horas) em relação aos outros antidepressivos utilizados na clínica (Zanos et al. 2016). A despeito das vantagens da quetamina em relação aos outros antidepressivos, as limitações da quetamina são seus efeitos psicotomiméticos e dissociativos, incluindo *dream-like states*, alucinações, mudanças no humor e afeto, delirio e confusão e potencial de abuso (Tyler et al., 2017).

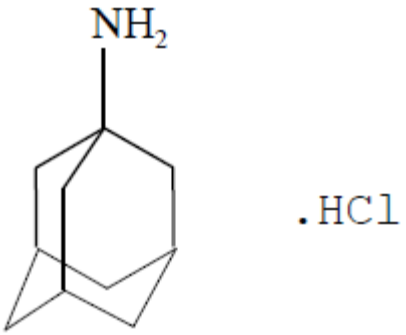

Figura 3 – Estrutura química da amantadina

Ainda seguindo a linha de estratégias alternativas de tratamento atual, outra droga antiglutamatérgica já utilizada na clínica, mas no tratamento da Doença de Parkinson, é a amantadina. A amantadina é uma amina tricíclica sintética altamente permeável no SNC e que após a administração oral é completamente absorvida pelo trato gastro-intestinal. Cerca de 67% da droga se liga a proteínas plasmáticas e é metabolizada por N-acetilação (Novartis Pharmaceutical Australia, 2014). Fora primeiramente utilizada, na década de 60, como agente virostático, tanto na profilaxia, quanto no tratamento de infecções pelo vírus *Influenza* A (H1N1, H1N2,

H2N2 e H3N2). O mecanismo de ação antiviral é a inibição da replicação do vírus através do acoplamento à proteína M_2 de sua membrana (Huber et al., 1999; Endo Pharmaceuticals, 2009). E depois como agente antiparkinsoniano provavelmente atuando indiretamente como agonista dos receptores D_2, uma vez que aumenta a síntese e liberação da dopamina no SNC, bem como retarda sua recaptação pelas vesículas sinápticas (Novartis Pharmaceutical Australia, 2014). Além desse mecanismo de ação, amantadina parece atuar nos sistemas dopaminérgico, noradrenérgico e serotoninérgico, bloquear a enzima monoaminaoxidase e aumentar os níveis de beta-endorfina/beta-lipotropina (Hosenbocus & Cachal, 2013; Owen & Whitton, 2005; Huber et al., 1999; Mizoguchi et al, 1994; para detalhes destas ações, ver box 1 do artigo 1). Assim como os inibidores seletivos da recaptação da serotonina (ISRS), amantadina também é capaz de ativar fatores de transcrição para a produção de neurotrofinas (família de proteínas que promovem a diferenciação e sobrevivência dos neurônios) no hipocampo (Berton & Nestler, 2006), fato importante quando se pensa em tratamento da depressão. Entre as principais neurotrofinas destaca-se o BDNF que, ao se ligar ao seu receptor TrkB, ativa cascatas de crescimento, sobrevivência, plasticidade e apoptose neuroniais (Warner-Schmidt et al, 2010; Rogóz et al., 2007). Este aumento de BDNF, frequentemente tem sido relacionado a um aumento da neurogênese e ao efeito antidepressivo (Björkholm & Monteggia, 2016). Resumidamente, a neurogênese é um efeito comum de vários tratamentos antidepressivos com ação inicial diversa (Eliwa et al., 2017). Também parece haver um paralelismo temporal entre o efeito tipo antidepressivo em modelos animais que requerem tratamento repetido e o aumento da neurogênese(Eliwa et al., 2017). Mais ainda, em estudos pré-clinicos em que a neurogênese é suprimida, há a supressão simultânea do efeito tipo

antidepressivo em certos modelos animais (Eliwa et al., 2017). Entretanto, resultados contraditórios também tem sido obervados (p.ex. David et al., 2009).

Vários modelos animais têm sido propostos para avaliar drogas com possível efeito antidepressivo. Esses modelos podem basear-se nas consequências comportamentais ao estresse, tipo de droga, lesão ou manipulação genética (Cryan et al., 2002; Nestler et al.,2002).

O teste da natação forçada, utilizado a mais de 30 anos para avaliar possíveis drogas antidepressivas agudamente, é o mais conhecido. Nele, os animais são colocados em um tanque com água e por mais que se esforcem nao têm como fugir. O teste é feito em dois dias, sendo o primeiro durante 15 minutos (treino) e o segundo, por 5 minutos (teste). Durante esse periodo o tempo de imobilidade é o parametro analisado. Uma variação do mesmo modelo é o teste da natação forçada modificado onde são analisados, além da imobilidade, os comportamentos de escalada e de natação. Uma vantagem deste teste é que se pode diferenciar, através do comportamento predominante, o papel das monoaminas na neurotransmissão, uma vez que agentes catecolaminérgicos diminuem a imobilidade, tendo como consequência o aumento da escalada e os serotoninérgicos, além de diminuir a imobilidade, também aumentam a natação (Slattery e Cryan, 2012; Cryan et al, 2002). Este teste apresenta uma boa validade preditiva para drogas com ação tipo antidepressiva (Borsini & Meli, 1988; Cryan et al, 2002).

Outro modelo já validado e muito utilizado no estudo de drogas com efeito do tipo-antidepressivo é o teste da suspensão pela cauda (TSC). O TSC baseia-se no fato de que, quando submetidos ao estresse inescapável, os animais apresentam um comportamento inato de imobilidade. Nele, os animais são individualmente

pendurados em um arame posicionado a 30 cm do chão, pelo terço final da cauda, com fita adesiva. Durante os últimos quatro minutos de seis, o tempo de imobilidade é registrado. Animais tratados com antidepressivos tendem a apresentar a diminuição do tempo de imobilidade (Steru et al., 1985; Cryan et al., 2005).

Considerando que amantadina tem demonstrado possíveis efeitos neuroprotetores e que a neuroproteção está intimamente ligada à melhora do quadro depressivo, o presente estudo buscou avaliar sua eficácia em modelos animais de depressão, assim como confirmar se esse mecanismo ocorre através da neurotransmissão monoaminérgica e se a amantadina também induz aumento da neurogênese hipocampal.

1.1 HIPÓTESE

A hipótese deste trabalho é que a amantadina apresentará um efeito tipo antidepressivo nos testes da natação forçada modificado e da suspensão pela cauda, provavelmente mediado pelos sistemas monoaminérgicos e glutamatérgicos. Mais ainda, espera-se, de modo similar a outros tratamentos antidepressivos, que a amantadina aumente a neurogênese hipocampal

1.2 OBJETIVOS

1.2.1 Geral

Avaliar o efeito da amantadina em modelos animais de deteção de drogas tipo antidepressivas, bem como tentar delinear por qual mecanismo essa ação tipo antidepressiva ocorre.

1.2.2 Específicos

Avaliar o efeito da administração aguda e repetida (14 dias) de amantadina no comportamento de ratos testados no TNF modificado e no campo aberto – CA

Avaliar o efeito depletor de catecolaminas pela AMPT administrada antes do tratamento com amantadina em camundongos testados no TSC

Avaliar o possível mecanismo de ação dopaminérgico após prétratamento com antagonistas D_1 (SCH23390) e D_2 (haloperidol) em camundongos tratados com amantadina e testados no TSC

Avaliar o possível mecanismo de ação noradrenérgico após prétratamento com antagonistas beta (propranolol), alfa$_1$(prasozin) e alfa$_2$ (ioimbina) e agonista alfa$_2$ (clonidina) em camundongos tratados com amantadina e testados no TSC

Avaliar o possível mecanismo de ação glutamatérgico após prétratamento com o agonista NMDA em camundongos tratados com amantadina e testados no TSC

Avaliar indução de neurogênese hipocampal através do marcador doublecortina - DCX

2 REVISÃO DE LITERATURA – ARTIGO 1 PUBLICADO NA REVISTA BRASILEIRA DE PSIQUIATRIA

Potential antidepressant effect of amantadine: from preclinical studies to clinical trials

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Running title: Amantadine in depression

Abstract

Objective: Amantadine blocks *N*-methyl-D-aspartate receptors and has dopaminergic and noradrenergic actions, a neurochemical profile that suggests its potential as an antidepressant drug. We conducted a systematic review of preclinical and clinical studies that reported the effects of amantadine in animal models of depression or in patients with depression.

Methods: The PubMed, Science Direct, and Web of Science databases were searched up to September 1 2017 to identify clinical and preclinical studies.

Results: Amantadine had antidepressant-like effects in animal models and appeared to potentiate the antidepressant effects of other antidepressants. These preclinical findings have received some support from the results of small open-label clinical trials, suggesting that amantadine can reduce depressive symptomatology and potentiate the antidepressant effects of monoaminergic drugs. In addition to its glutamatergic and dopaminergic effects, the potential antidepressant-like effects of amantadine have been linked to molecular and cellular actions, such as an increase

in the expression of neurotrophic factors (e.g., brain-derived neurotrophic factor), the activation of σ_1 receptors, a decrease in corticosterone levels, and a decrease in the inflammatory response to stress.

Conclusion: Amantadine appears to be an interesting candidate as a new antidepressant drug for the treatment of depression.

Keywords: Amantadine, animal models, antidepressant, clinical trial, glutamate

Introduction

According to the World Health Organization[1] at least 350 million people live with depression, a mental disorder that is characterized by sadness, loss of interest or pleasure (i.e., anhedonia), inappropriate feelings of guilt, low self-esteem, sleep and appetite disorders, psychomotor retardation, and lower concentration.[1,2] Major depression is difficult to diagnose and treat, resulting in lower quality of life and social and economic losses. A major challenge is to discover a treatment for this disease that is both quick and effective.[1,3] In addition to the delayed onset of treatment efficacy, some patients do not respond at all or have only a partial response to antidepressant treatment.[4]

Glutamate is the main excitatory neurotransmitter in the central nervous system. Alterations in glutamatergic transmission have been associated with the pathophysiology of mood disorders and the mechanism of action of antidepressant treatments.[5,6] Drugs that reduce *N*-methyl-D-aspartate (NMDA) receptor activity have been proposed to treat depression. Both ketamine (a noncompetitive NMDA receptor antagonist) and lamotrigine (which reduces glutamate release) have been shown to

have clinical antidepressant effects.[5] Ketamine has a very interesting clinical profile. Its antidepressant effect can be detected 24-48 h after administration, and it is effective in depressed patients who are refractory to monoaminergic antidepressants.[4,5] However, the clinical use of ketamine has some drawbacks, such as its mode of administration (i.e., intravenous), psychotic-like side effects, and potential drug abuse.[4,7] Amantadine hydrochloride is a synthetic tricyclic amine that is well absorbed orally and excreted largely unchanged in the urine.[8] Amantadine acts as a weak noncompetitive NMDA receptor antagonist and indirectly increases dopamine release.[8,9,10] Amantadine can also increase norepinephrine and serotonin (5-hydroxytryptamine [5-HT]) neurotransmission pre- and postsynaptically[8] (Box 1). However, some of these effects have been observed with doses that are greater than those that are used clinically, leading some authors to question their clinical relevance.[8,9] Thus, amantadine-induced NMDA receptor blockade has been proposed as a relevant mechanism of action of therapeutically relevant doses.[8,9] Amantadine was first used in the 1960s as a prophylactic antiviral agent and for the treatment of Influenza A virus infection. Its mechanism of action involves the blockade of M2 ion channel, inhibition of virus entry into the cell, and inhibition of virus replication[8,11,12] Amantadine's main clinical indication is for the treatment of Parkinson's disease, both as a monotherapy and combined with levodopa or dopaminergic agonists.[13] Other indications of amantadine for neuropsychiatric disorders are drug-induced extrapyramidal effects, motor fluctuations during l-dopa treatment, attention deficit hyperactive disorder, traumatic brain injury, autistic spectrum disorders[12]. After oral administration, amantadine maximal plasma level is observed approximately after 3.3h and its half-life is around 16h; approximately 90%

of amantadine is excreted unchanged in urine The usual dose of amantadine used in clinic is 100 mg twice a day which can be increase up to 400 mg/ day [12]

Considering the pharmacological effects of amantadine and its current clinical use, we reviewed its potential for the treatment of depression. We performed a systematic review of clinical and preclinical studies that focused on the antidepressant and antidepressant-like effects of amantadine.

Methods

The review of preclinical and clinical studies was performed by searching the following databases: PubMed, Science Direct, Web of Science, and Medline. We selected articles that were published in English up to September 1, 2017 that reported the use of amantadine for the treatment of mood disorders or in animal models of depression. The following search terms were used: “amantadine AND depress*,” “amantadine AND mood,” “amantadine AND animal models AND antidepres*,” and “amantadine AND (forced swim, learned helplessness, reserpine, chronic mild stress, anhedonia, etc.).” All results were reviewed by their title and abstract and those articles that did not fill the inclusion criteria or fill the exclusion criteria were withdrawn from further analysis. *Criteria for inclusion of pre-clinical studies*: studies with validated animal models of depression or with antidepressant-like drug screening tests. *Criteria for inclusion of clinical studies*: controlled studies, open studies and case series that evaluated depression (or depressive symptoms) were included in analysis of clinical studies. We manually reviewed the bibliographic references of the articles. Exclusion criteria were: non-English language, review articles, pre-clinical articles that did not used models of depression or antidepressant-like effect screening tests, clinical articles that did not evaluate depression/

depressive symptoms. Pre-clinical studies were reviewed by IFMR-B and RA and clinical studies were reviewed by JCFG and RA.

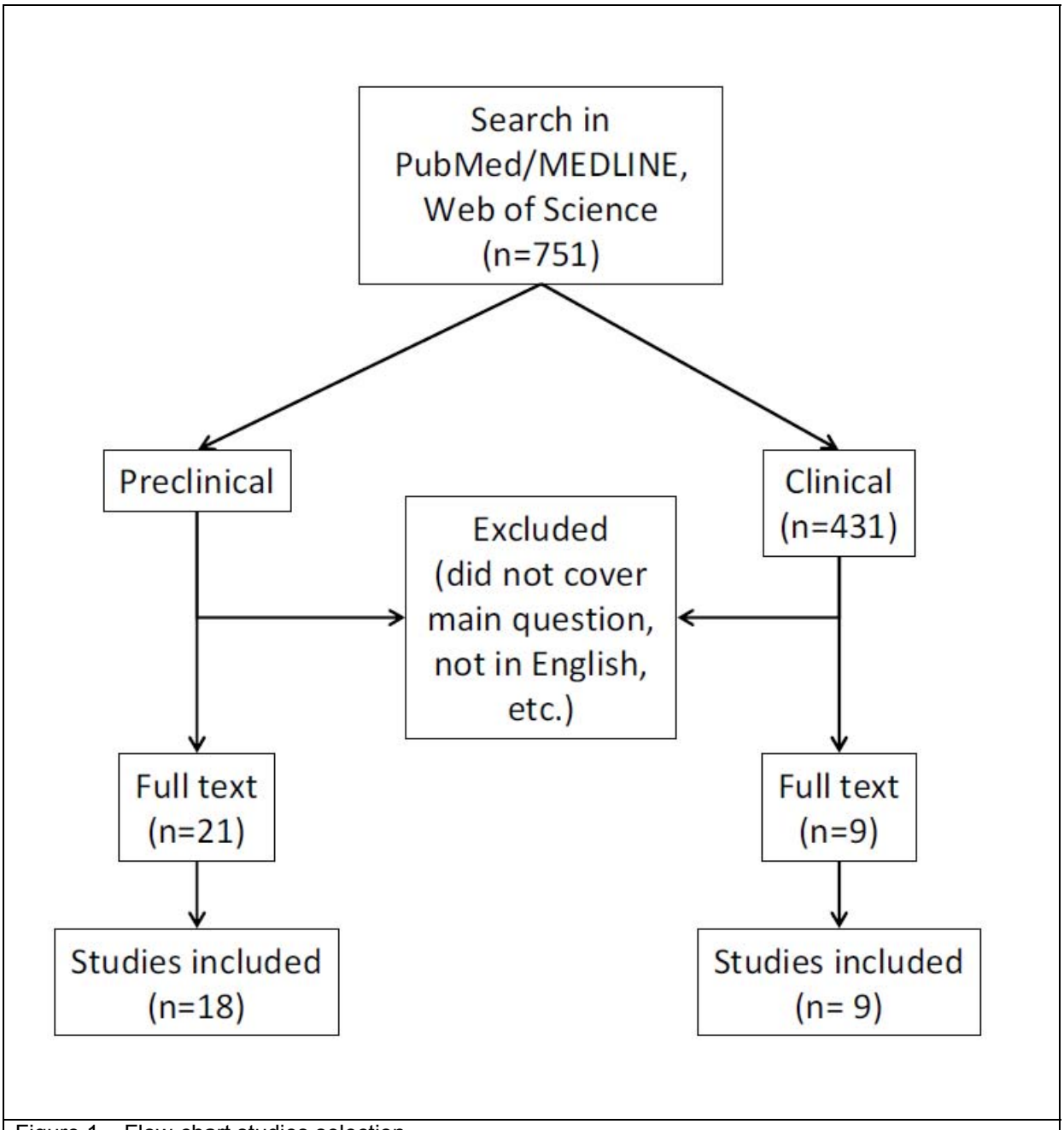

Figure 1 – Flow-chart studies selection

Results

Using the strategy described above 751 articles (Figure 1) were found electronically ("amantadine AND depress*": 366 articles; "amantadine AND mood":

293 articles; “amantadine AND animal models AND antidepres*: 10 articles,” and “amantadine AND (forced swim, learned helplessness, reserpine, chronic mild stress, anhedonia, etc.)”: 112 articles (some articles were included in more than one group). When the articles were restricted to human, 431 articles were retrieved. After reviewing the title and the abstract of all studies, the number of articles were reduced for 21 pre-clinical studies and 9 clinical studies Finally, reading the original articles, 3 articles were withdrawal from pre-clinical analysis and none from clinical analysis. Thus, the numbers of articles remaining for the final analysis were: 18 for pre-clinical and 9 for clinical.

Antidepressant-like activity in preclinical studies

Preclinical studies that have evaluated amantadine in animal models of depression can be divided into three categories: (*i*) studies that evaluated the antidepressant-like effect of amantadine alone in animal models of depression, (*ii*) studies that evaluated the potential of amantadine to potentiate the antidepressant effects of monoaminergic antidepressants, and (*iii*) studies that evaluated the antidepressant-like effects of amantadine on depressive-like behaviors associated with other comorbidities.

Studies that evaluated the antidepressant-like effect of amantadine alone in animal models of depression

These studies evaluated the effects of amantadine in animal models of depression and they used the forced swim test (FST), models of chronic mild stress that induced anhedonia, and the reserpine syndrome model (Table 1).

The forced swim test is well validated and the most frequently used animal model to screen antidepressant drugs. [14,15] In this model, a rodent (rat or mouse) is placed in a cylinder that is filled with water from which it cannot escape. After initial escape-directed behavior, the animal acquires an immobile posture. Antidepressant drugs reduce immobility at doses that do not increase spontaneous locomotor activity. [14,15] In the mouse FST, Moryl et al.[16] found that amantadine (20-80 mg/kg) exerted an anti-immobility effect. In this study, amantadine decreased locomotor activity in the open field test. In the FST, false-positive results are related to an increase locomotor activity. Rogóz and co-workers repeatedly showed that amantadine (20 mg/kg) decreased immobility time in rats in the FST.[17,18] In another series of studies, amantadine was co-administered with antidepressant drugs. Rogóz and colleagues found that amantadine administration alone at a lower dose (10 mg/kg) was ineffective in the FST, although it potentiated the effects of antidepressant drugs, such as fluoxetine and imipramine.[19,20,21,22] This group also found that amantadine did not affect locomotor activity in the open field[17,18] , indicating that locomotor activity did not influence behavior in the FST.

Chronic unpredictable mild stress (CUMS) is an interesting model of depression that induces several depressive-like changes (e.g., anhedonia, sleep disturbances, and cognitive impairment) that can be reversed by repeated but not acute administration of standard monoaminergic antidepressant, although some NMDA antagonists (e.g. ketamine) can revert CUMS-induced anhedonia faster.[23,24] Thus, the CUMS model is particularly interesting to evaluate the time required for antidepressant-like effect of drugs. Yu et al.[25] studied the effects of amantadine on anhedonia and cognitive deficits that were induced by CUMS. They found that rats that were subjected to CUMS exhibited a decrease in sucrose preference (i.e., an

index of anhedonia) and an increase in the latency to find a hidden platform in the Morris water maze (indicative of memory impairment). Amantadine (25 mg/kg, orally, for 20 days) reversed anhedonia and impairments in the Morris water maze in stressed rats. Moreover, rats that were subjected to CUMS exhibited lower weight gain than non-stressed rats and stressed rats that were treated with amantadine. Amantadine also reversed the stress-induced reduction of hippocampal postsynaptic density 95 protein expression, which is related to synaptic plasticity.[25] Unfortunately, in this study, amantadine administration was started at beginning of stress procedure (3rd day) which preclude the evaluation of the time to reach antidepressant-like effect since amantadine prevented depressant-like effect of CUMS instead of reversed it.

Reserpine-induced behavioral alterations (e.g., lower mobility) and physiological changes (e.g., lower body temperature) have been used as a model to screen antidepressant drugs.[26] Reserpine blocks the reuptake and storage of monoamines (e.g., serotonin, norepinephrine, and dopamine) in synaptic vesicles, resulting in monoamine depletion. Antidepressant drugs that affect monoamines are able to reverse these reserpine-induced effects. [26] Maj et al.[27] found that amantadine (40-80 mg/kg) reversed catalepsy and hypoactivity that were induced by reserpine in rats. Similarly, Jurna et al.[28] employed electromyography and found that amantadine (50 mg/kg) blocked muscle rigidity that was induced by reserpine. Lassen et al.[29] found that amantadine reversed reserpine-induced hypomotility but did not alter ptosis. Colpaert[30] also found that amantadine prevented reserpine-induced hypokinesia in female rats. Goldstein et al.[31] found that amantadine (30-60 mg/kg) reversed hindlimb rigidity that was induced by reserpine (5 mg/kg). Furthermore, amantadine at 40 and 80 mg/kg but not 20 mg/kg attenuated reserpine-induced hypothermia.[16] Cox and Tha[32] found that amantadine (25 mg/kg) failed to affect

hypothermia that was induced by reserpine (5 mg/kg), although amphetamine (a multitarget drug that increases monoaminergic transmission) and the dopamine D_2 receptor agonist apomorphine reversed it. Moreover, Messiha[33] found that amantadine (100 mg/kg) enhanced reserpine-induced suppression of locomotor activity.

These animal studies indicate that amantadine has a behavioral profile that is indicative of an antidepressant-like drug. Several of these antidepressant-like effects were seen at doses (e.g., 20-50 mg/kg) that correspond to clinically relevant doses.[9]

Studies that evaluated the potential of amantadine to potentiate the antidepressant effects of monoaminergic antidepressants

These studies tested the hypothesis that the co-administration of amantadine with established antidepressant drugs increases their effectiveness. Rogóz and colleagues[17,18,21,34] evaluated the effects of acute amantadine (10 or 20 mg/kg) alone and combined with imipramine (5 and 10 mg/kg; serotonin and norepinephrine reuptake inhibitor), venlafaxine (10 and 20 mg/kg; serotonin and norepinephrine reuptake inhibitor), and fluoxetine (5 and 10 mg/kg; selective serotonin reuptake inhibitor) in the FST. All of these drugs, when administered alone, with the exception of fluoxetine, decreased immobility time in rats at the highest dose tested. When administered together with amantadine, all of these antidepressants at both lower and higher doses decreased immobility time, suggesting that amantadine had a synergistic effect with these monoaminergic-based antidepressants. To assess whether the mechanism of synergism between amantadine and imipramine involves pharmacokinetic interactions, Rogóz et al.[18] measured plasma and brain concentrations of imipramine (5 and 10 mg/kg) and its active metabolite desipramine

(selective norepinephrine reuptake inhibitor) after the administration of imipramine alone or combined with amantadine (20 mg/kg) in rats that were tested in the FST. No significant changes in plasma or brain imipramine or desipramine concentrations were found, suggesting that no pharmacokinetic interaction occurred.

Using the same approach, Skuza and co-workers[19,20] found that the co-administration of ineffective dose of amantadine and σ receptor agonists (i.e., σ_1 receptor agonist SA4503 and σ_2 receptor agonist siramesine) reduced immobility time in the FST. On the other hand, co-administration of amantadine with PB190, another σ_1 receptor agonist, had no effect.[35]

Studies that evaluated the antidepressant-like effects of amantadine on depressive-like behaviors associated with other comorbidities

Depression is a common psychiatric disorder after traumatic brain injury. Tan et al.[36] evaluated the effects of amantadine on depression that was induced by traumatic brain injury in rats. Lesioned rats presented depressive-like behaviors (i.e., lower sucrose preference and an increase in immobility time in the FST) that were reversed by amantadine treatment (45 and 135 mg/kg for 28 days).

Preclinical studies that evaluated the molecular and cellular effects of amantadine related to its antidepressant-like effect

Studies have evaluated the effects of amantadine alone and combined with antidepressant drugs on molecular and cellular mechanisms that are related to depression (e.g., neurotrophins and immune responses to stress). Brain-derived neurotrophic factor (BDNF) is a neurotrophin that is associated with the pathophysiology of depression and its treatment.[37] Lower BDNF expression has been related to depression, and antidepressant treatments increase activity of the BDNF

signaling pathway (e.g., BDNF activates TrkB receptors). Interestingly, ketamine increases BDNF translation.[38] Amantadine treatment alone increased BDNF mRNA expression in the cerebral cortex. [40] When amantadine was co-administered with fluoxetine, BDNF mRNA expression increased in the cerebral cortex. When amantadine was co-administered with imipramine, BDNF mRNA expression increased in the hippocampus.[21,40]

Another approach that has been used to study the role of specific receptors in the antidepressant-like effects of amantadine involves the induction of a behavioral syndrome by drugs that target specific receptors. For example, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OHDPAT), a 5-HT_{1A} receptor agonist, induces characteristic behaviors (e.g., forepaw treading and flat posture) that can be influenced by drugs that either directly or indirectly act on these receptors (e.g., alter 5-HT release). A low dose of amantadine (10 mg/kg) did not alter the behavioral syndrome that was induced by 8-OHDPAT in rats. It also did not influence head twitches that were induced by the 5-HT_{2A} receptor agonist (±)DOI.[40] The co-administration of amantadine with fluoxetine attenuated the behavioral syndromes that were induced by 8-OHDPAT and (±)DOI.[21] The co-administration of amantadine with imipramine reduced head twitches that were induced by (±) DOI, but this combined treatment also slightly increased the behavioral effects of 8-OHDPAT.[40] A low dose of amantadine (10 mg/kg) that was administered repeatedly for 7 days did not alter hyperlocomotion that was induced by amphetamine and the dopamine $D_{2/3}$ receptor agonist quinpirole.[41] Repeated administration of amantadine combined with fluoxetine enhanced hyperlocomotion that was induced by quinpirole and amphetamine.[41] This latter behavioral effect is consistent with the finding that repeated amantadine administration increases $D_{2/3}$ (quinpirole) binding and D_2 mRNA expression in the

nucleus accumbens.[42] Tan et al.[36] employed a model of depression that was induced by traumatic brain injury. Amantadine reversed dopamine cell loss in the substantia nigra and restored the reduction of dopamine levels in the striatum.

Another behavioral approach is the use of specific receptor antagonists to study the involvement of these receptors in a given effect. The dopamine D_2 receptor antagonist sulpiride blocked the antidepressant-like effect of amantadine alone or combined with imipramine in the FST. The α_1 adrenergic receptor antagonist prazosin blocked the effects of both imipramine treatment alone and combined with amantadine.[18] Sulpiride, prazosin, and the σ receptor antagonists progesterone and PBD1047 blocked the anti-immobility effect of co-administration of amantadine with the σ receptor agonists SA4503, and siramesine in the FST.[20,35]

Pretreatment with α-methyl-*p*-tyrosine, which blocks dopamine and norepinephrine synthesis, attenuated the effect of amantadine on hypoactivity but did not alter its anticataleptic effect.[27] These results suggest that some of the effects of amantadine do not totally depend on monoamine synthesis or release.

An increase in plasma glucocorticoid levels is frequently found in patients with depression. Amantadine attenuated the increase in plasma corticosterone levels that was induced by the FST in rats.[34] This effect was also seen with co-administration of amantadine with fluoxetine.[21]

A series of studies evaluated the effects of co-administration of amantadine (10 mg/kg) with imipramine and fluoxetine on immunological parameters.[17,21,34,43] The co-administration of amantadine with antidepressants was also shown to increase the production of interleukin-10 (IL-10; a cytokine with antiinflammatory actions) and decrease the proinflammatory activity of macrophages. Amantadine administration

alone also increased IL-10.[21,34] These effects on the inflammatory response may be involved in the improvement of depressive symptoms that is induced by amantadine.

Discussion of Pre-Clinical Studies

The studies reviewed suggested an antidepressant-like effect of amantadine. However, some subjects must be considered to translate this conclusion to clinical setting. First, locomotor activity is an important confound factor for behavioral test, such as FST. The open-field and automated activity meter (e.g. chamber with photobeam sensors) are the procedures most frequently used to evaluate locomotor activity in the studies reviewed here (table 1). In general, an antidepressant-like effect was considered when there is no drug effect in locomotor activity, Increased locomotor activity can lead to false positive results in the FST, such was seen with psychostimulant drugs.[14,44], On the other hand, impairment in motor activity can be seen with drugs that induce muscle relaxing or muscle rigidity, increase emotionality (e.g. fear), induce sedation. (e.g.[45,46,47,48).] At doses employed, amantadine did change or decreased locomotor activity in most studies reviewed (table 1), although one showed increased locomotor activity. In theory, sedative or motor impairing drugs could lead to false-negative results (e.g. sedation or motor impairment may reduce activity in forced swim test). However, it is interesting to note that some antidepressant drugs (e.g. tryciclics and ketamine) can reduce immobility in the forced swim test despite they decreased open-field activity (e.g.[44).]

Other points that have been related to translational value of pre-clinical studies with potential antidepressant drugs are: the parenteral drug administration, the response to acute administration and the dose employed for the observed effect. It is important to note that several pre-clinical studies employ intraperitoneal drug administration, which is different from their intended clinical use (generally oral). This

issue is particularly important for new drugs since little is known about their pharmacokinetics (e.g. drug stability in gastric juice, first pass metabolism, bioavailability, etc.). However, the amantadine pharmacokinetics after oral administration and its CNS activity (e.g. antiparkinsonian effect) are already known. Moreover, one study evaluating antidepressant-like effect of amantadine used the oral route of administration[25].

Another concern is of the study of neurobiology of depression or mechanism of action of antidepressant drugs using animal models or test (e.g. forced swim test) that respond to acute drug administration since antidepressant drugs took several days to reach clinical improvement in patients with major depression. On the other hand, the predictive validity of the forced swim test had been well demonstrated even with acute drug administration (e.g.[14,15]). Thus, the results with acute administration of amantadine in the FST are also good indication of its potential clinical effect. However, an issue not covered by this approach is the occurrence (or not) of tolerance of antidepressant-like effect observed in acute experiments. Again, some studies evaluate amantadine repeated administration and they did not observe signal of tolerance of its antidepressant-like effect[25,26], although these studies were not designed to evaluate this subject. Another limitation of the present models for screening antidepressant-like drugs is its initial validation with monoaminergic drugs, which may reduce their capacity to detect non-monoaminergic drugs. However, these models had detected the effects of ketamine and tianeptine (e.g. [49,50]), which suggest its utility to detect drugs beyond classical monoaminergic antidepressant drugs.

The dose used in pre-clinical studies is also an important aspect for pre-clinical results translation. Amantadine showed a dose-response relationship: for example, in rats, in the studies reviewed generally 10 mg/kg are ineffective and

doses above 40 mg/kg were effective (20 mg/kg showed mixed results, table 1). Using the allometric calculation[51] a rat dose of 40 mg/kg would correspond to a human dose of 3.24 mg/kg, which is near to the amantadine clinical dose (e.g. around 190 mg for a 60 kg person).

Thus, at whole, the pre-clinical studies with amantadine in animal models of depression or for antidepressant-like drugs screening suggest a good potential for amantadine. However, the present review also identifies some important gaps in these pre-clinical studies, such as potential sex-specific effects, the role of NMDA receptor antagonism in antidepressant-like effect of amantadine, the effect of chronic amantadine administration, and the use different animal models of depression to detect the time required for antidepressant-like effect.

Clinical trials

Studies of amantadine for the treatment of depressive states began in the 1970s, but we identified only eight studies to date. Each of these studies had a small sample size, and most of them used an open-label design, which prevents reliable conclusions (Table 2).

Vale et al.[52] evaluated 40 patients. The patients continued their usual antidepressant medications and were randomized into three groups. In one group, 10 patients initially received 100 mg amantadine in the first week, followed by 200 mg in the second week until the study ended at 4 weeks. In the second group, 10 patients initially received 200 mg amantadine in the first week, followed by 100 mg in the second week until the study ended at 4 weeks. The remaining 20 patients did not receive amantadine. The authors observed an improvement of depressive symptoms

compared with placebo, but this beneficial effect did not remain after amantadine was discontinued.

Two studies evaluated the effectiveness of amantadine in resistant depression. Stryjer et al.[53] evaluated eight patients with resistant major depression, keeping their current antidepressant treatment (e.g. fluoxetine, venlafaxine, or paroxetine), for 4 weeks. The dose of amantadine reached 300 mg/day over the study. No significant improvement of depression or anxiety was observed.

Rogóz et al.[39] evaluated 50 patients with resistant unipolar depression. After a 2-week washout period, the patients were randomized into two groups: 25 patients received only imipramine (100 mg/day) for 12 weeks, and 25 patients received imipramine (100 mg/day) and amantadine (150 mg/day, twice daily). The patients were evaluated using the Hamilton Depression Rating Scale (HDRS). Imipramine treatment alone did not alter HDRS scores. In the group that was treated with imipramine plus amantadine, HDRS scores decreased at 6 weeks. Additionally, amantadine did not alter the pharmacokinetics of imipramine and its active metabolite desipramine in plasma. A previous report from this group [54] described 12 patients who are also included in this latter study.

These were the only clinical studies we found on the effects of amantadine for the treatment of depression. Additional studies were identified that evaluated the treatment of secondary depression in patients with hepatitis C who were receiving interferon treatment.[55,56] Three studies investigated the treatment of secondary depression in cocaine addiction [57] and depression in patients with Borna disease virus infection. [58,59] In all of these studies, amantadine combined with the usual treatment significantly improved depressive symptomatology compared with the

control groups (Table 2). The dose of amantadine in these studies ranged from 100 to 300 mg/day.

None of these studies reported significant adverse reactions. However, Rizzo et al.[60] planned to evaluate amantadine in an open study with depressive patients, but amantadine caused aggressive behavior in the first patients with depression who were included in this study; this adverse effect quickly disappeared with the discontinuation of treatment. These reactions led these researchers to interrupt the study. Other adverse effects that have been associated with amantadine are anticholinergic-like effects (e.g., blurred vision, nausea, dry mouth, urinary retention, and constipation), livedo reticularis, confusion, nervousness, drowsiness, difficulty concentrating, and insomnia. [8,12,13] Interestingly, some case reports suggest that amantadine can be useful for the treatment of sexual dysfunction that is induced by serotonergic antidepressants. [61,62] A case series with three patients suggested that high-dose amantadine can induce a switch to mania in bipolar patients [63], a side effect that has also been reported for several antidepressant drugs.

Discussion of Clinical Studies

Although these very preliminary studies might suggest a potential antidepressant effect of amantadine, well-controlled clinical trials with large samples are needed to evaluate the possible beneficial effects of amantadine alone and combined with traditional antidepressants for the treatment of depressive disorders.

Antagonism of NMDA receptor are a promising target for new antidepressants and ketamine showed faster efficacy in refractory depressed patients. In this line, amantadine can has potential advantages such as the oral route of administration, compared to intravenous administration of ketamine, and a well known adverse

effects profile and pharmacokinetics interactions, compared to new NMDA antagonists.

Surprisingly, although the clinical efficacy of amantadine in Parkinson's disease, which is frequently associated with depression, no study evaluated the effect of amantadine on patients with depression and Parkinson's disease. Moreover, as observed in pre-clinical studies, none study tried to evaluate sex difference in amantadine's effect on depression. Other gaps are the influence of several clinical characteristics, such as depression severity, depression subtype diagnosis, presence of psychotic symptoms, etc.

Conclusion

Considering the pharmacological profile of amantadine, some studies have evaluated its effects in animal models, which consistently found antidepressant-like effects in different models (e.g., FST, chronic mild stress paradigm, and reserpine syndrome test) and species (e.g., rats and mice). These beneficial effects have received some support from small open-label studies. Moreover, preliminary data suggest that amantadine can potentiate the clinical effects of other antidepressants, suggesting its possible use in refractory depression.

Pharmacologically, amantadine appears to exert antidepressant effects through multiple systems, such as the dopaminergic system (e.g., D_2 receptors), σ receptor transmission (e.g., σ_1 receptors), the noradrenergic system, possibly glutamatergic neurotransmission (although this has not been evaluated), and BDNF mediation or modulation. In conclusion, amantadine is a drug that is already in clinical use and may be promising for the treatment of major depression, but further controlled studies are necessary.

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The authors declare no conflict of interest

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Table 1. Pharmacological effects of amantadine in animal models of depression.

REFERENCE	ANIMALS	AMANTADINE EFFECTIVE DOSE (mg/kg)[1]	TREATMENT (route)	DEPRESSION INDUCTION	ANIMAL MODEL/	RESULTS	CONCLUSION
Forced Swim Test							
Moryl et al.[16]	Rats (male)	20, 40, 80	Acute (ip)		FST	FST:↓ immobility time Locomotor activity (OFT): ↓ ambulation, peeping, rearing, and walking time	Antidepressant -like effects
Rogoz et al.[17]	Rats (male)	(10),20	Acute (ip)		FST	FST:↓ immobility time Locomotor activity (OFT): no effect	Antidepressant -like effects
Rogoz, et al.[18]	Rat (male)	(10), 20	Acute (ip)		FST	FST- ↓ immobility time Locomotor activity (OFT): no effect	Antidepressant -like effects
Chronic Unpredictable Mild Stress							
Yu et al.[25]	Rats (male)	25	Chronic (oral)	CUMS	SP MWM Body weight loss	SP: ↑sucrose consumption MWM: ↓ escape latencies Body weight loss: prevented Locomotor activity (swim velocity in MWM): no effect	Antidepressant -like effects
Reserpine Syndrome							

Maj et al.[27]	Rats (male and female)	(20), 40, 80	Acute (ip)	Reserpine 5 mg/kg	Locomotor activity Catalepsy	↓ reserpine-induced hypolocomotion ↓ catalepsy Locomotor activity (AA): no effect	Antidepressant -like effects
Jurna et al.[28]	Rats (sex not specified)	50	Acute (iv)	Reserpine 10 mg/kg	Electromyo-graphy	↓ muscle rigidity Locomotor activity: NE	Antidepressant -like effects
Lassen[29]	Rats (female)	25, 50	Acute (sc)	Reserpine 7.5 mg/kg	Locomotor activity	Did not affected reserpine-induced hypolocomotion AA: ↑ locomotor activity	Ineffective
Colpaert et al.[30]	Rats (female)	24-110 (ED_{50})	Acute (oral)	Reserpine 40 mg/kg	Hypokinesia Catalepsy	Prevented reserpine-induced hypokinesia ↓ catalepsy Locomotor activity: NE	Antidepressant -like effects
Goldstein et al.[31]	Rats (male)	21.8 (ED_{50})	Acute (sc)	Reserpine 5 mg/kg	Reserpine-induced hind limb rigidity	Prevented reserpine-induced rigidity Locomotor activity: NE	Antidepressant -like effects
Cox and Tha[32]	Mice (male and female)	(25)	Acute (ip)	Reserpine 5 mg/kg	Reserpine-induced hypothermia	No effect Locomotor activity: NE	Ineffective
Messiha[33]	Mice (male)	100	Acute (ip)	Reserpine 0.2mg/kg	Locomotor Activity	↑ reserpine-induced hypolocomotion Locomotor activity (AA): ↓	Ineffective
Moryl et al.[16]	Mice	(20), 40, 80	Acute	Reserpine	Hypothermia	↓ reserpine-induced hypothermia	Antidepressant -like effects

	(male)		(ip)	2.5 mg/kg		Locomotor activity: NE (for mice)	
Comorbidity							
Tan et al.[36]	Rats (male)	45-135	Chronic-28 days (ip)	Depressive-like behavior induced by traumatic-brain injury	SP FST	SP:↑ sucrose preference FST:↓ immobility time Locomotor activity: NE	Antidepressant -like effects
Co-administration							
Skuza and Rogóz[19]	Rats (male)	(10)	Acute (ip)	+ σ_1 receptor agonist SA4503 or σ_2 receptor agonist siramesine	FST	FST:↓ immobility time Locomotor activity (AA): no effect	Potentiates antidepressant -like effects of σ receptor agonists
Rogoz et al.[17]	Rats (male)	(10)	Acute (ip)	+ venlafaxine, imipramine, or fluoxetine	FST	FST: ↓ immobility time Locomotor activity (OFT): no effect or ↓ locomotor activity	Potentiates antidepressant -like effects
Rogoz et al.[18]	Rats (male)	20	Acute (ip)	+ imipramine	FST	FST: ↓ immobility time Locomotor activity (OFT): ↓ locomotor activity	Potentiates antidepressant -like effects
Kubera et al.[34]	Rats (male)	(10)	Acute (ip)	+ imipramine	FST	FST:↓ immobility time Locomotor activity: NE	Potentiates antidepressant -like effects
Skuza and Rogóz[20]	Rats	(10)	Acute	+ σ_1 receptor agonist SA4503	FST	FST: ↓ immobility time Locomotor activity (AA): no	Potentiates antidepressant

	(male)		(ip)			effect	-like effects of σ_1 receptor agonist
Rogóz et al.[21]	Rats (male)	(10)	Acute (ip)	+ fluoxetine	FST	FST: ↓ immobility time Locomotor activity: NE	Potentiates antidepressant -like effects of fluoxetine
Skuza et al.[35]	Mice (male)	(10)	Acute (ip)	+ σ_1 receptor agonist PB190	FST TST	FST: ↓ immobility time TST: no effect Locomotor activity: NE	σ_1 receptor agonist had no effect on antidepressant -like effects of amantadine

[1]Doses in parentheses were ineffective.

CUMS,chronic unpredictable mild stress; SP, sucrose preference test; MWM, Morris water maze; FST, forced swim test; TST, tail suspension test;

OFT, open field test; AA: automated locomotor activity meter (e.g. chamber with photobeams);

NE: not evaluated

Table 2. Clinical studies of potential antidepressant effect of amantadine.

REFERENCE	DISORDER	DESIGN	NUMBER OF PATIENTS	EVALUATION SCALE	AMANTADINE DOSE (mg/day)	TREATMENT DURATION	RESULT	COMMENTS
Antidepressant Studies								
Vale et al.[52]	Chronic depressive syndrome	DB, R, C (placebo)	16-18/group	Zung Self-Rating Scale	100-200	4 weeks	67% of patients improved (*vs.* 25% in placebo group)	Diagnostic criteria not used Response: score below median
Stryjer et al.[53]	MD (refractory)	Open Add-on	8	HDRS CGI	100-300	4 weeks	50% of patients responded (reduction ≥50%)	Pre *vs.*post comparison
Antidepressant Potentiation Studies								
Rogóz et al.[39]	MD (refractory)	Open + imipramine	25/group	HDRS	100	6 weeks	Potentiatedeffect of imipramine	Comparison between imipramine and imipramine + amantadine
Comorbidity/Secondary Depression								
Ferszt et al.[57]	MD, bipolar depression, dysthymia + Borna diseasevir	Open Add-on	30	MADRS	200-350	8-12 weeks	63% of patients improved (reduction ≥40%)	Better response associated with Ag2 BV antigen

	us infection							
Dietrich et al.[58]	MD, bipolar depression + Borna disease virus infection	Open Add-on	25	HDRS Operationalized diagnostic criteria system (OCPCRIT)	100-300	11 weeks (mean)	68% of patients improved (reduction ≥50% or 2 steps on OCPCRIT)	
Ziedonis and Kosten[56]	Depression secondary to cocaine addiction	DB, R, C (placebo/ desipramine)	5-9/group	Beck Depression Inventory	300	12 weeks	Prevented increase in depression score	Reduced cocaine craving and consumption
Quarentini et al.[54]	Depression induced by INF-α	Single-blind, add-on	6-8/group	HospitalAnxiety and Depression Scale	200	24 weeks	Prevented depression	Exclusion criteria: history of depression
Kronenberger et al.[55]	Depression induced by INF-α	DB, R, C (placebo)	131-136/group	POMS	200	48 weeks	No effect on POMS depression factor	Prevented depressive symptoms in a subset of patients

MD, major depression; DB, double-blind; R, randomized; C,comparative (drug comparator); HDRS, Hamilton Depression Rating Scale;

MADRS, Montgomery-Asberg Rating Scale; CGI, Clinical Global Impressions; POMS, Profile of Mood States.

Box 1. General pharmacodynamics of amantadine.

1) *Dopaminergic system*: Amantadine acts on the presynaptic membrane, enhancing the release of dopamine.[64,65] and inhibiting its reuptake in brain homogenates at higher doses.[66,67] Several of these dopaminergic effects are seen at concentrations higher than that is achieved clinically in humans.[68,69,70] The effects of amantadine on dopaminergic transmission are still debatable. Some results show that amantadine has a direct action on D_2 receptors.[27,70,71], but other studies have reported no significant actions on catecholaminergic receptors. [72,73,74] Despite this controversy, Moresco et al.[75] evaluated patients who received amantadine (200 mg/day, 10-14 days) and were being treated with L-DOPA, which was suspended the night before positron emission tomography scans were performed. The patients were free from dopaminergic agonists, anticholinergics, and antidepressants. The patients presented an increase in [^{11}C]raclopride binding in the caudate and putamen. This suggests that amantadine increases the neosynthesis of D_2 receptors, which may represent one mechanism of the drug's actions. Amantadine is also related to the inhibition of dopamine reuptake. Its antiparkinsonian activity may be attributable to the inhibition of dopamine reuptake into presynaptic neurons or an increase in dopamine release from presynaptic fibers.[76]
2) *Noradrenergic system*: Amantadine appears to have pharmacological actions that are similar to tricyclic antidepressants.[8] A significant increase in norepinephrine levels was observed after an oral dose of amantadine in healthy subjects.[77] Noradrenergic mechanisms may also be involved in the actions of amantadine.[16,78]
3) *Glutamatergic system*: Amantadine is an NMDA receptor antagonist that is commonly used for the treatment of Parkinson's disease. It is thought to inhibit NMDA receptor activation through the stabilization of ion channels and more rapid channel closure.[78,79]
4) *Immunomodulation*: Amantadine may also have immunomodulatory properties. It restored the production of IL-2, which is dysfunctional in Parkinson's disease patients.[80,81] IL-2 levels did not correspond to clinical improvement, so the significance of these findings is unclear. Other

studies.[81,82] showed that amantadine treatment was an independent predictor of improved survival in Parkinson's disease.

3 APRESENTAÇÃO DOS RESULTADOS - ARTIGO 2 SUBMETIDO AO JOURNAL OF PSYCHOPHARMACOLOGY

The envolvement of D_2 and NMDA receptors in the antidepressant-like effect of amantadine

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Abstract

Considering that drugs that target N-methyl-D-aspartate receptor has been shown antidepressant-like activity, it was evaluated the potential antidepressant-like effect of amantadine, a non-competitive N-methyl-D-aspartate (NMDA) antagonist, in animal models and its putative mechanism of action. Amantadine showed antidepressant-like effects on modified forced swim test – FST (reducing immobility and increasing climbing) in rats and in the tail suspension test – TST (decreasing immobility time) in mice. Repeated amantadine treatment also increased hippocampal neurogenesis. The antidepressant-like effect of amantadine was blocked by haloperidol, a dopamine-2 (D_2) receptor antagonist and by NMDA, a glutamatergic receptor agonist in the TST. Clonidine (alpha-2 receptor agonist), yohimbine (alpha-2 receptor antagonist), prazosin (alpha-1 receptor antagonist), propranolol (beta receptor antagonist), and AMPT (tyrosine-hydroxilase inhibitor) did not change the anti-immobility effect of amantadine in the TST. These effects were seen at doses that did not changed locomotor activity. The present results show antidepressant-like effects for amantadine which may be mediated by its action on D2 and NMDA receptors and probably involving hippocampal neurogenesis.

Keywords: amantadine, antidepressant, glutamate, dopamine, neurogenesis

Introduction

Depression is a highly prevalent, disabling and costly disorder for patients, their families and the public health system (WHO, 2015; APA, 2013). Despite new antidepressant drugs had been launched in the clinic in the last years, there are still some important limitations in the treatment of major depression episodes, such as a

significant number of patients with unsatisfactory or no-response and intolerable side-effects (Souery, 2014; Murrough et al., 2017). New therapeutic drugs have been searched involving other neurotransmitter systems than the monoamines, including those acting on the glutamatergic system (Fabbri, 2017; Murrough et al., 2017; Yang, 2017).

N-methyl-D-aspartate receptor (NMDAR) antagonists has been recognized as effective antidepressants. Recent evidence suggests that NMDAR antagonists relieve depressive symptoms by increasing neuronal plasticity (Duman et al., 2016; Murrough et al., 2017). Ketamine, an uncompetitive antagonist of glutamatergic NMDA receptors, has shown faster antidepressant effect in depressive patients unresponsive or intolerant to monoaminergic-based antidepressants (Newport et al., 2015; Murrough et al., 2017; Ghasemi et al., 2017). However, ketamine treatment presents some drawbacks such its psychotomimetic and dissociative effects including dream-like states, hallucinations, delirium and confusion, abuse potential and the need of parenteral administration (Murrough et al., 2017; Sanacora et al., 2017; Tyler et al., 2017).

Another antiglutamatergic drug already used in the clinic is amantadine, which acts as a weak non-competitive NMDA receptor antagonist and has been used used in the treatment of Parkinson's disease (Connoly and Lang, 2014). Amantadine appears to act also on dopaminergic, noradrenergic and serotonergic systems, block the monoamine oxidase enzyme, and increase beta-endorphin / beta-lipotropin levels (Huber et al., 1999; Raupp-Barcaro et al., *in press*). Amantadine is a synthetic tricyclic amine that was first used in the 1960s as an antiviral agent for both prophylaxis and treatment of *Influenza* A virus infections. Previous studies found antidepresant-like effects of amantadine in the FST and in stressed rats subjected to

unpredictable chronic mild stress (Moryl et al., 1993; Rogóz et al., 2002; Yu et al., 2016). The antidepressant-like effects of amantadine were related with enhancing monoaminergic neurotransmission and with an increase in the brain derived neurotrophic factor (BDNF) levels (for review see Raupp-Barcaro et al., *in press*). Curiously, none of these studies evaluated the glutamatergic mediation in the antidepressant-like effect of amantadine.

Brain-derived neurotrophic factor has been considered an important mediator of antidepressant effect of drugs (Björkholm and Monteggia, 2016). Its level is increased by acute ketamine administration while it takes several days to increase to respond to repeated monoaminergic antidepressant administration (Björkholm and Monteggia, 2016; Duman et al., 2016; Ghasemi et al., 2017). Besides ketamine, other NMDA antagonists also increase BNDF levels, such as MK-801 and amantadine (Rogóz et al., 2007; Garcia et al., 2008; Autry et al., 2011; Ghasemi et al., 2017). Moreover, increase BDNF, which binds to tropomyosin kinase B (TrkB) receptor, is also linked to an increase in synaptogenesis and neurogenesis, which also may be induced by monoaminergic antidepressants and ketamine (Björkholm and Monteggia, 2016; Duman et al., 2016; Eliwa et al., 2017).

In the recent years, an important issue raised for translational validity of pre-clinical results is their reproducibility by other laboratory researches and their replication in other animal models and species (Curtis and Abernethy, 2015; Steckler et al., 2015). Thus, it is important to replicate initial studies with a putative antidepressant drug by independent laboratories (Steckler et al., 2015).

The present study aimed to: a) replicate the antidepressant-like effect of amantadine using the modified FST in rats and the TST in mice; b) evaluate the antidepressant-like effects of amantadine in mice using the TST; c) investigate the

involvement of cathecolaminergic neurotransmission on antidepressant-like effect of amantadine; d) evaluate the role of NMDA receptors on antidepressant-like effect of amantadine; e) evaluate whether amantadine is able of inducing neurogenesis.

Material and Methods

Animals

Male adult Webster Swiss mice (25-35g) and male Wistar rats (250-300g) were used. The animals were kept in groups in polypropylene cages (41 cm × 34 cm × 16 cm; rats: <5 rats/ cage; mice: <10 mice/cage) in a controlled environmental room (constant temperature at 22±1^{o}C, 12/12h light/ dark cycle with lights on at 7:00 AM), with food (standard commercial chow) and water available *ad libitum*. All of the experiments were approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO-UFPR, protocol no. 733) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and the Brazilian Law (Arouca's Act). All efforts were made to reduce the number and suffering of the animals. The animals were randomly allocated in the experimental groups. All experiments were performed during the light phase.

Drugs

The drugs and doses use were: amantadine (10, 20 and 50 mg/kg, Mantidan®, Eurofarma, São Paulo, Brazil), alpha-methyl-p-tyrosine (AMPT, 80 mg/kg for mice and 200 mg/kg for rats, Sigma-Aldrich, St. Louis, MO, USA), clonidine (0.06 mg/kg; Sigma-Aldrich, St. Louis, MO, USA), haloperidol (0.2 mg/kg, Sigma-Aldrich,

St. Louis, MO, USA), N-methyl-D-aspartate (NMDA, 75 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), prazosin (1.0 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), propranolol (2.0 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), SCH 23390 (0.1 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), and yohimbine (1.0 mg/kg, Sigma-Aldrich, St. Louis, MO, USA). All drugs were prepared immediately before their use and dissolved in distilled water, except: amantadine, which was dissolved in tween 80 and distilled water (1% v/v), AMPT, which was dissolved in 10% tween 80 solution. All drugs (and their vehicles) were given intraperitoneally (ip) at constant volume of 1.0 ml/kg (rats) or 10.0 ml/kg (mice). The drugs doses were selected based on previous experiments in our laboratory and literature data (Kaster et al., 2007; Zeidan et al., 2007; Brocardo et al., 2008; Autry et al., 2011; Kawaura et al., 2012; Sheng et al., 2015; Yan et al., 2016).

Drug challenges procedures

Monoamine depletion studies

Rat: AMPT (200 mg/kg) was given 6 h before the first injection (24h before FST) and with the last injection (1h before FST) of vehicle or amantadine administration (Kawaura et al., 2012).

Mice: AMPT (80 mg/kg) was injected 4 h before vehicle or amantadine administration (Kaster et al., 2007).

Agonist/antagonist studies

Different cohorts of mice were pretreated with haloperidol (D_2 antagonist), SCH23390 (D_1 antagonist), prazosin (α_1 antagonist), propranolol (β antagonist), yohimbine (α_2 antagonist) or clonidine (α_2 agonist), NMDA (NMDA agonist) or their

vehicles 10 min before treatment with amantadine 50 mg/kg and, 30 min later, submitted to TST.

Modified Forced swim test for rats

The FST was developed by Porsolt et al (1978) and modified by Lucki and co-workers (Detke et al., 1995). Briefly, in the training session rats were placed in an opaque plastic cylinder (diameter: 20 cm, height: 50 cm) containing 30 cm of water (24 ± 1°C) for 15 min. The test session was performed 24h later, using the same procedure, and the rat's behavior was recorded during 5 min by a camera positioned above the cylinder for posterior analysis. The behaviors analyzed during the test session were: immobility (when the rat stopped all active behaviors and floated in the water with minimal movements and its head just above the water), swimming (horizontal movements throughout the cylinder) and climbing (upward directed movements of the forepaw along the cylinder walls). At 5-sec intervals of the test session, a blind rater recorded the predominant behavior (immobility, climbing or swimming). The water was changed and the cylinder rinsed with clean water after each rat. In acute experiment, the rat were treated with amantadine (or its vehicle) 24, 5, and 1hr before the test session; in the repeated administration experiment amantadine or its vehicle were administered daily for 14 days, with last administration occurring 1h before the test session.

Tail suspension test for mice

The mice were suspended 30 cm above the floor by fixing its tail with an adhesive tape (placed approximately 1cm from the tip of the tail) during 6 min (Steru et al., 1985; Cryan et al., 2005a). In the last 4 min of the test the time of immobility

(when the mouse hanging passively and did not show any active movement) was recorded.

Locomotor activity

Open-field for rats

The locomotor activity of rats was measured in the open-field, which consists of a wooden rectangular arena (30 x 40 x 63 cm) with a glass front panel (50 x 52 cm). The floor is divided by lines in 20 small squares (10 x 10 cm). The rat was placed individually in the center of the open-field and the number of squares crossed was recorded over 5 min. A crossed square was considered when the rat put its two forepaws in the next square and moved forward (Consoni et al., 2006). The open-field was cleaned with a 10% water-alcohol solution before behavioral testing to avoid bias due to residues and/or odors left by rats tested earlier.

Automated locomotor activity box for mice

Thirty minutes after drug or vehicle administration, the mice were individually placed in an automated activity box that consists of a rectangular wood arena (40 cm $\times$ 20 cm $\times$ 26 cm) with a wire mesh floor. The box has three photoelectric sensors (10 cm apart) on the two longer lateral walls. The locomotor activity was measured by the number of crossings cumulatively recorded by the photoelectric sensors over a 5 min period (Chioca et al., 2013).

Immunohistochemistry

After repeated treatment with amantadine, 5 to 6 rats of each experimental group were randomly selected to immunohistochemistry experiments. The animals

were deeply anesthetized with sodium thiopental (50 mg/kg; Thiopentax®, Cristália, SP, Brazil) and then transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.2 M phosphate buffer. The brains were removed, postfixed in the same fixative for 2 h, and cryoprotected by immersion in 30% sucrose. Frozen tissue was serially sectioned on a cryostat (Cryocut 1800, Reichert-Jung, Heidelberg, Germany) into 30 µm coronal sections that were collected in replicates in Eppendorf tubes that contained antifreeze solution.

Every seventh composite section was processed for the quantitative analysis of DCX by immunohistochemistry to identify neuroblasts. Sections were quenched in 1% H_2O_2 for 30 m in and then blocked with 2% BSA in PBS for 60 min. The sections were incubated with goat polyclonal anti-DCX antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS that contained 0.3% Triton X-100 for 48 h at 4 ◦C. The sections were then incubated with the respective biotinylated secondary antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h. The signals were visualized using avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA), 3,3 -diaminonezidine (DAB) as the chromogen, 0.05% H_2O_2, and nickel sulfate. The sections were then mounted on gelatin-coated slides, dehydrated in ethanol, and coverslipped with Permount® mounting medium.

Quantitative Analysis

Doublecortin-positive cells were quantified in the subgranular zone (SGZ) and inner granular cell layer (GCL) of the dentate gyrus (DG) of the hippocampus. All of the lighting conditions and magnifications were maintained constant during the capture process. The quantification of immunoreactive (IR) cells was conducted in 5-6 sections from 1-in-7 series of hippocampal sections, spaced 180 µm apart and corresponding to the hippocampal extension according to the following coronal

coordinates from bregma: −0.94 to −2.70 mm (Franklin and Paxinos, 1997). Cells were qualified as being in the SGZ if they were touching or were within the SGZ. The number of IR cells were normalized to the DG area determined with X10 objective. The absolute number of positive cells was calculated considering the total hippocampal volume as determined by the sum of the areas of the sampled sections multiplied by the distances between them (Meyer et al., 2017).

2.4 Statistical analysis

The results from forced swimming test were analyzed by one-way Analysis of Variance (ANOVA) followed by Newman-Keuls test, when appropriated. Data from mice (tail suspension test and automotor activity box) were analyzed by two-way ANOVA (pretreatment with challenge drug and treatment with amantadine as factors), followed by Newman-Keuls test when appropriated, or by Unpaired Student t test (TST after repeated amantadine administration. When the raw data did not fit the parametric assumptions, a data transformation [e.g. log (raw data)] or non-parametric tests (Kruskal-Wallis ANOVA followed by multiple comparison test) was used. These data were analyzed using the Statistica 7.0 software (StatSoft Inc, Tulsa, USA). A generalized linear model for data with a Poisson distribution was used for counting the number of DCX-IR cells (Mori et al., 2017). A value $p<0.05$ was considered to be significant.

Results

Forced Swimming Test

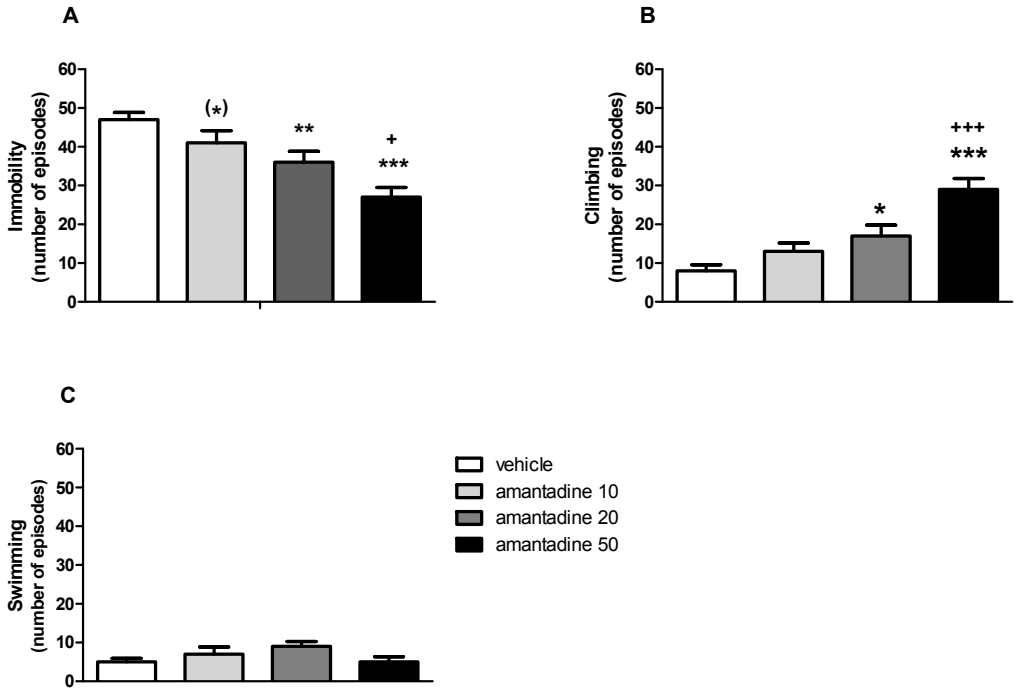


Figure 1 - Effect of acute treatment with amantadine (10, 20, and 50 mg/kg, ip) on behavior of rats in the modified forced swimming test. (A) Immobility, (B) Climbing, (C) Swimming. Treatment: 24, 5 and 1h before the test. Data represent mean ± SEM of the number of episodes (n=5-6 rats/ group). $^{(*)}$ 0.05< p< 0.10; *p<0.05 and ***p< 0.001 vs vehicle; $^{+}$ p< 0.05 and $^{+++}$ p< 0.001 vs amantadine 10 and 20.

Acute amantadine administration significantly reduced the frequency of immobility episodes in the FST in rats ($F(3,35)= 12.66$, $p< 0.001$; Figure 1). Amantadine 20 and 50 mg/kg showed significantly lower immobility than vehicle and amantadine 10 mg/kg (all $p<0.05$). Moreover, this reduction was associated with increasing of the frequency of climbing behavior ($F(3,35)=16.69$, $p< 0.001$). Again, amantadine 20 and 50 mg/kg were significantly different from vehicle (both $p<0.05$); amantadine 50 mg/kg also showed a higher climbing behavior than amantadine 10 mg/kg ($p<0.001$). There is no effect of drug treatment in the frequency of swimming episodes ($F(3,35)= 1.62$, NS).

Amantadine administered for 14 days also significantly reduced the frequency of immobility episodes in the FST in rats ($F(4,23)= 3.59$, $p< 0.05$; Figure 2). Again, this reduction was associated with increasing frequency (log transformed) of climbing behavior ($F(4,23)= 6.82$, $p< 0.001$). There is no effect of treatment in the frequency (log transformed) of swimming episodes ($F(4,23)= 0.30$, NS).

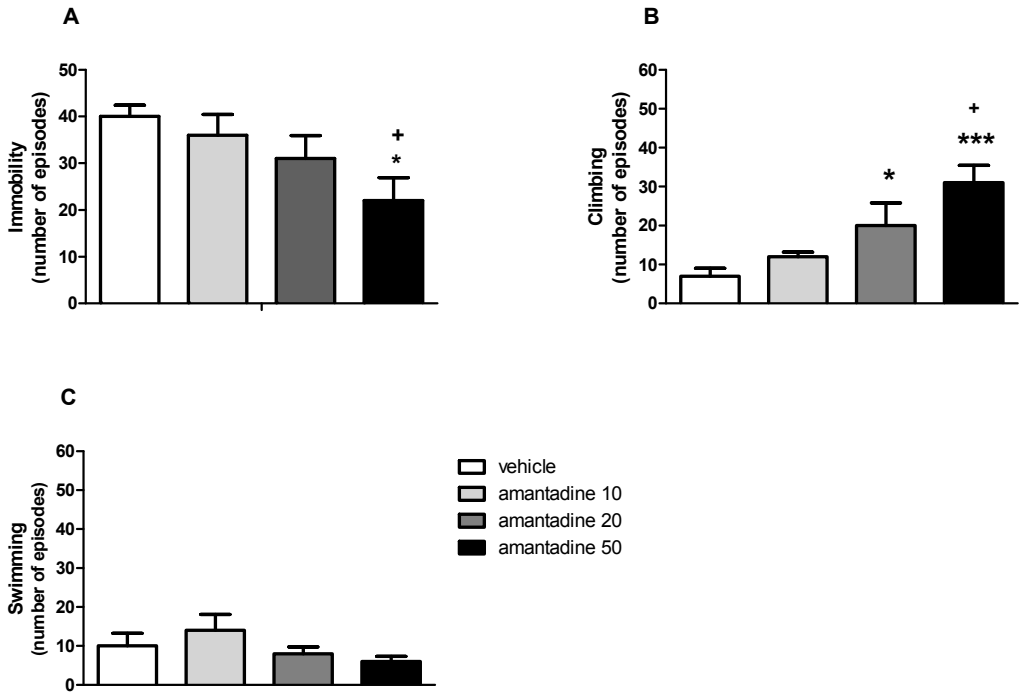


Figure 2 - Effect of repeated treatment with amantadine (10, 20, and 50 mg/kg, ip,) on behavior of rats in the modified forced swimming test. (A) Immobility, (B) Climbing, (C) Swimming. Treatment: daily, 14 days, last administration 1h before the test. Data represent mean ± SEM of the number of episodes (n=5-6 rats/ group). *p<0.05 and *** p< 0.001 vs vehicle, $^{+}$ p<0.05 vs amantadine 10.

AMPT pretreatment effect in the anti-immobility effect of amantadine in the modified FST is showed in figure 3. The anti-immobility effect of amantadine was not affected by AMPT pretreatment (Fig 3A). There was a significant effect of treatment (vehicle or amantadine: $F(1,16)= 20.49$, $p< 0.001$) but not of pretreatment (vehicle or AMPT: $F(1,16)= 0.12$, NS) or pretreatment x treatment interaction ($F(1,16)= 0.30$,

NS) on immobility counts. Treatment with amantadine reduced immobility compared with vehicle treated rats independently from pretreatment with AMPT (p<0.001).

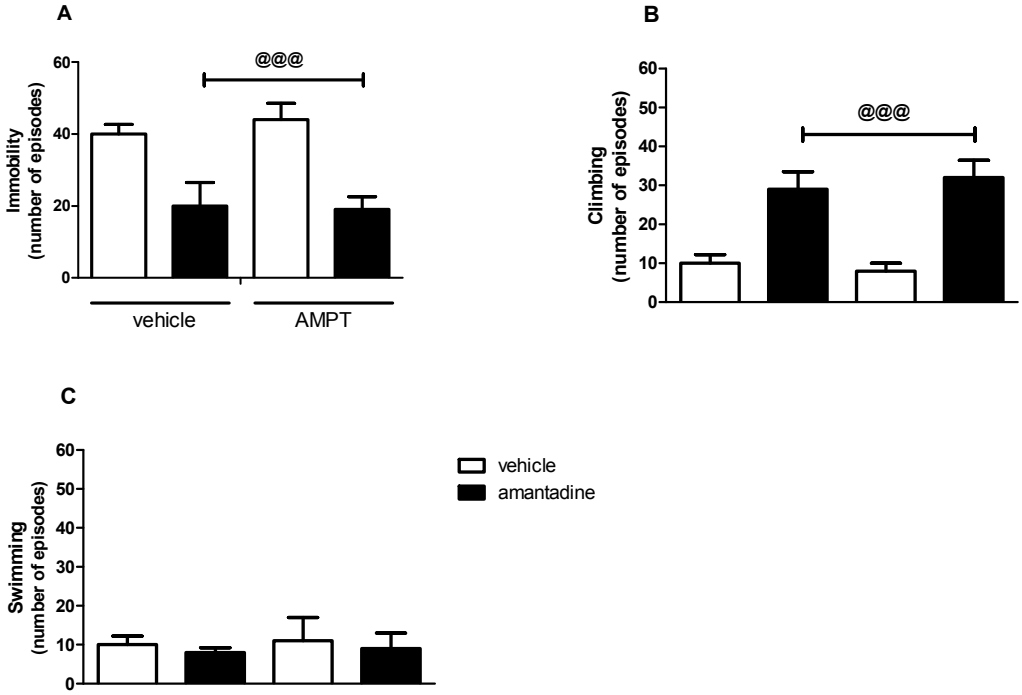


Figure 3 - Effect of pretreatment with AMPT (100 ou 200? mg/kg, ip), a catecholamine-depleting drug, on amantadine (50 mg/kg, ip) anti-immobility action of modified forced swimming test. A) immobility, B) climbing, C) swimming. Data represent mean ± SEM. N=4-6 rats/group. $^{@@@}$p<0.001 vs non-amantadine treated groups (treatment factor)

Climbing behavior was showed in Figure 3B. There was a significant effect of treatment (vehicle or amantadine: $F(1,16)= 39.73$, $p< 0.001$) but not of pretreatment (vehicle or AMPT: $F(1,16)= 0.14$, NS) or pretreatment x treatment interaction ($F(1,16)= 1.06$, NS) on climbing frequency. There was no significant difference between groups in swimming behavior (pretreatment AMPT x vehicle: $F(1,16)= 0.17$, NS; treatment amantadine x vehicle: $F(1,16)= 0.05$, NS; pretreatment x treatment interaction: $F(1,16)= 0.01$, NS).

Tail Suspension Test

The effect of pretreatment with AMPT on the anti-immobility effect induced by amantadine in the TST is showed in Figure 4. There was a significant effect of treatment (vehicle or amantadine: $F(2,31)= 38.41$, $p< 0.001$) but not of pretreatment (vehicle or AMPT: $F(1,31)= 1.44$, NS) or pretreatment x treatment interaction ($F(2,31)= 0.90$, NS) on immobility time (log transformed). Treatment with both doses of amantadine reduced immobility time compared with vehicle treated mice independently from treatment with AMPT (all $p<0.001$).

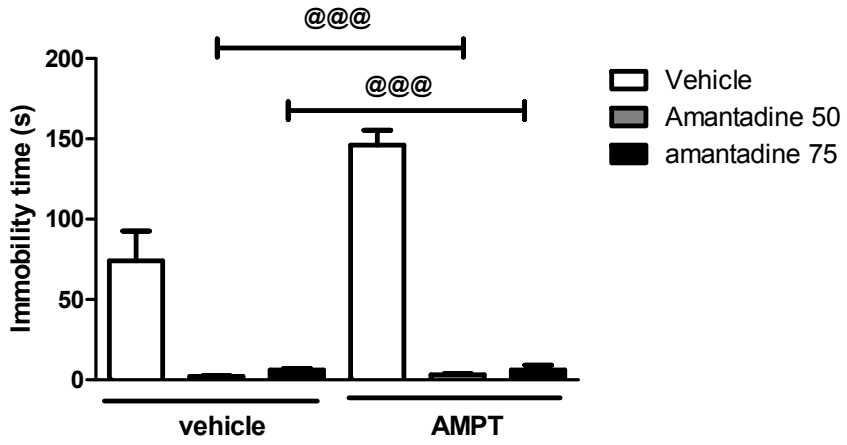


Figure 4 - Effect of pretreatment with AMPT (80 mg/kg, ip), a catecholamine-depleting drug, on amantadine (50 and 75mg/kg, ip)-induced reduction in immobility time in the tail suspension test in mice. Data represent mean ± EPM of immobility time (s). N=6 mice/group. $^{@@@}p<0.001$ vs non-amantadine treated groups (treatment factor)

The effect of pretreatment with dopamine antagonists on the anti-immobility time effect of amantadine is showed in Fig. 5. There was a significant difference among groups (Kruskal-Wallis ANOVA: $H(5,40)= 29.93$, $p<0.001$). Multiple comparison test indicated that amantadine reduced immobility time ($p<0.05$) and haloperidol pretreatment prevented this effect. SCH23390 had mixed results, since there was a significant difference between SCH23390+amantadine and

SCH23390+vehicle (p<0.01) but not between SCH23390+amantadine and vehicle+vehicle (p=.10).

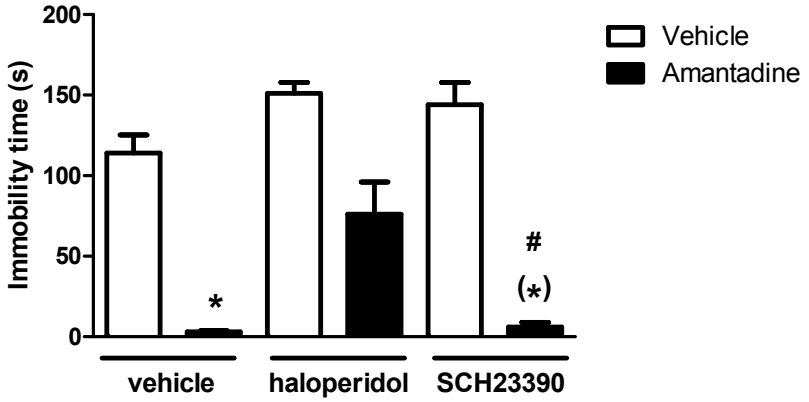


Figure 5 - Effect of pretreatment with haloperidol (0.2 mg/kg, ip), an D_2 antagonist, and SCH 23390 (0.1 mg/kg, ip), an D_1 antagonist, on the anti-immobility effect of amantadine (50 mg/kg, ip) in the tail suspension test in mice. Data represent median + SEM of immobility time (s) (n=6-8 mice/group). $^{(*)}$ 0.05<p< 0.10 and *p<0.05 vs vehicle, $^{\#}$ p< 0.5 vs SCH23390+vehicle.

The effect of pretreatment with prazosin (alpha-1 antagonist) or propranolol (beta-1/beta-2 antagonist) on the anti-immobility effect of amantadine in TST was showed in Fig.6A. There was a significant effect of treatment (vehicle or amantadine: F(1,30)= 51.82, p< 0.001) but not of pretreatment (vehicle, prazosin or propranolol: F(2,30)= 0.32, NS) or pretreatment x treatment interaction (F(2,30)= 1.10, NS) on immobility time (log transformed) in the TST. Treatment with amantadine reduced immobility time compared with vehicle treated mice independently from treatment with prazosin or propranolol.

The effect of pretreatment with clonidine (alpha-2 agonist) or yohimbine (alpha-2 antagonist) on the anti-immobility effect of amantadine in TST was showed in Fig. 6B. There was a significant difference among groups (Kruskal-Wallis ANOVA:

H(5,36)= 25.57, p<0.001). Multiple comparison test indicated that amantadine reduced immobility time (p<0.05) and yohimbine pretreatment did not prevented this effect. Clonidine had a mixed result, since there is a significant difference between clonidine+amantadine and vehicle+vehicle (p<0.05) but not between clonidine+amantadine and clonidine+vehicle.

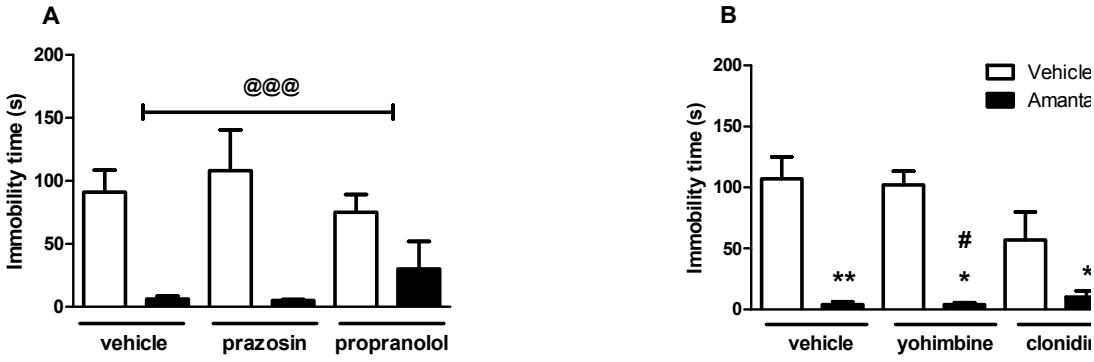


Figure 6 - Effect of pretreatment with prazosin (1.0 mg/kg, ip), an alpha-1 antagonist, propranolol (2.0 mg/kg, ip), an beta antagonist, yohimbine (1.0 mg/kg, ip), an alpha-2 antagonist, and clonidine (0.06 mg/kg, ip), an alpha-2 agonist, on anti-immobility effect of amantadine (50 mg/kg, ip) in the tail suspension test in mice. Data represent mean + SEM (n=6-8 mice/group). *p<0.05 and ** p< 0.01 vs vehicle, # p< 0.5 vs drug challenge+vehicle; @@@ p<0.001 vs non-amantadine treated groups (factor pretreatment)

The effect of pretreatment with NMDA on the reduction of immobility time induced by amantadine is showed in Figure 7. There were a significant effect of treatment (vehicle or amantadine: F(1,35)= 19.40, p< 0.001) and pretreatment x treatment interaction (F(1,35)= 4.38, p< 0.05) but not of pretreatment (vehicle or NMDA: F(1,35)=2.29, NS) on immobility time in the TST. Amantadine reduced the immobility time compared to vehicle and the pretreatment with NMDA prevented this anti-immobility effect of amantadine (p<0.02). NMDA per se had no effect.

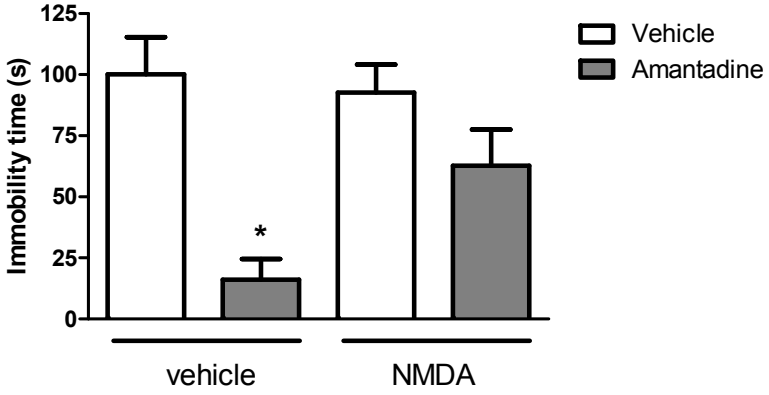


Figure 7 - Effect of pretreatment with NMDA (75 mg/kg, ip) on anti-immobility time of amantadine (50 mg/kg, ip) in the tail suspension test in mice. Data represent mean + SEM of immobility time (s) (n=6-8 mice/group). *p<0.05 vs. vehicle.

Repeated amantadine administration (14 days) reduced immobility time (log transformed) in the TST (t=4.22, p< 0.001; Fig 8).

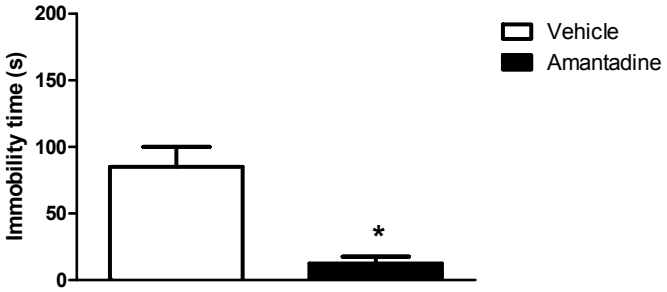


Figure 8 - Effect of amantadine (50 mg/kg, ip) repeated treatment (14 days) on immobility time (s) in the tail suspension test in mice. Data represent mean + SEM (n=8 mice/ group). *p<0.05 vs vehicle.

Locomotor Activity

There was no effect of amantadine on locomotor activity of rats (number of squares crossed in the open-field) or mice (number of beam interruptions in the locomotor acitivity box; table 1).

Table 1 – Locomotor activity of drug treatments on rats (open-field) and mice (automated activity box).

Open-field (rats)					
	Vehicle	Amantadine 10	Amantadine 20	Amantadine 50	
Acute treatment	85 ± 5 (4)	84 ± 4 (4)	78 ± 5 (4)	90 ± 11 (4)	F(3,12)=0.426
Chronic Treatment	90 ± 21 (6)	110 ± 28 (6)	54 ± 20 (5)	115 ± 26 (6)	F(3,19)=1.201
Locomotor Activity Box (mice)					
	Vehicle	Amantadine 50			
Acute Treatment	101 ± 5 (10)	86 ± 10 (9)			t= 1.230
Repeated Treatment	115 ± 9 (8)	119 ± 17 (8)			t= -0.206

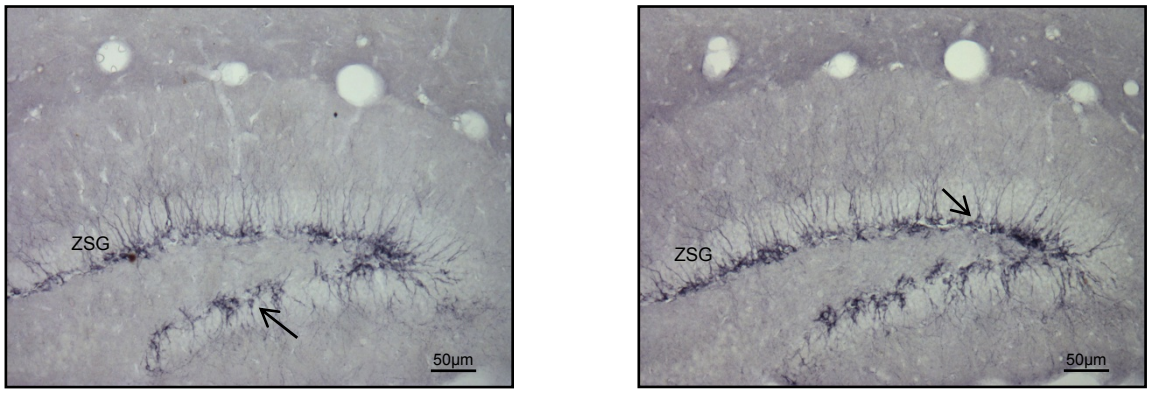
Neurogenesis

DCX-IR neurons were identified in the SGZ of the hippocampus (Fig. 9). Mice that received repeated i.p. amantadine injections presented a significant increase in the number of DCX-IR neurons in the DG when compared to controls (χ^2 = 271.47, p < 0.001).

Discussion

The present study was conducted in order to replicate and expand previous studies indicating the antidepressant-like effects of amantadine in different animal species and to evaluate the role of monoaminergic neurotransmission and NMDA receptors in this effect. To this end, we tested rats in the modified FST and mice in the TST, both tests widely used and well validated to screen antidepressant compounds (Steru et al., 1985; Borsini and Meli, 1988; Detke et al.,1997; Cryan et al., 2005a; Cryan et al., 2005b). In both tests the readout of antidepressant-like effect was immobility, which decreases after administration of drugs with clinically effective antidepressant effect. The modified FST also gives a behavioral indication whether the monoaminergic system is affected by the drug treatment (Detke et al., 1997; Cryan et al., 2005b).

A



vehicle **Amantadine**

B

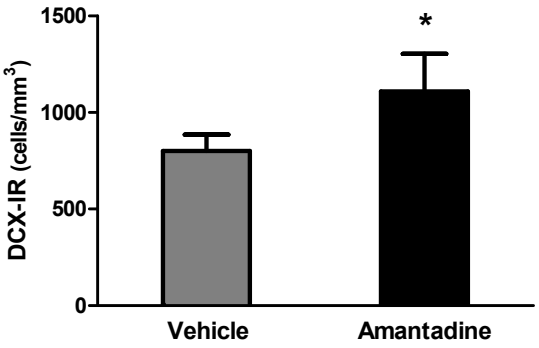


Figure 9 - Effect of repeated amantadine administration (50 mg/kg, 14 days) on double-cortin (DCX) immunoreactivity (A) Representative photomicrographs of DCX-IR neurons in the subgranular zone (SGZ) of hippocampus (B) Graph of the number of DCX cells/ mm^3 on SGZ of hippocampus. * $p < 0.05$ compared to vehicle.

Amantadine reduced the immobility behavior in the modified FST with rats and in the TST with mice, corroborating previous results showing antidepressant-like effects of amantadine (Moryl et al., 1993; Rogoz et al., 2002; Yu et al., 2016). The antidepressant-like effects observed in the present study were seen at doses that did not change locomotor activity in the open-field (rats) aor activity box (mice), indicating that motor activity did not influenced the results.

Furthermore, the present results suggest that the anti-immobility effect of amantadine in the FST is related to cathecolamines, because amantadine increased climbing behavior but did not changed swimming behavior in the FST (Detke et al., 1997; Cryan et al., 2005b). Based in this result, the next step was to evaluate of the influence of noradrenaline and dopamine in the antidepressant-like effect of amantadine using the TST. The blockade of dopamine 2 receptor by haloperidol prevented the effect of amantadine, indicating that D2 receptors participated in the antidepressant-like effect of amantadine. This is similar to observed in the FST, in which sulpiride pretreatment blocked the anti-immobility effect of amantadine (Rogoz et al., 2008). Similarly, haloperidol, but not SCH23390, prevented the anti-immobility effect of ketamine in the FST (Li et al., 2015). This is consistent with the hypothesis that D2 receptors sensitivity has an important role in the antidepressant effect (Gershon et al., 2007). On the other hand, alpha-2 (yohimbine and clonidine) and beta- receptors (propranolol) drugs did not change the antidepressant-like effect of amantadine. Considering that pretreatment with AMPT, a selective inhibitor of tyrosine-hidroxylase (rate-limiting enzyme in the synthesis of noradrenaline and dopamine), did not prevent the antidepressant-like effect of amantadine, indicating that this effect was not dependent from pre-synaptic dopamine release, the above results suggest a direct effect of amantadine in dopamine receptors. This is consistent with previous studies showing that amantadine reversed the hypolocomotion induced by reserpine, which also depleted monoamine content (Maj et al., 1972; Colpaert et al., 1987; Moryl et al., 1993). This is also in line with data showing that amantadine can increase D_2 receptor expression or sensitivity (Moresco et al., 2002; Rogoz et al., 2003; Rogoz et al., 2004). This is in line with data showing

that the anti-immobility effect of ketamine and MK-801 (dizolcipine, another NMDA antagonist) in the FST is dependent of D_2/D_3 receptors (Li et al., 2015).

Glutamatergic NMDA receptors also appear to be related to antidepressant-like effect of amantadine, as suggested by the preventive effect of NMDA administration. NMDA has been proposed as an important target for antidepressant action (Skolnick et al., 2009; Murrough et al., 2017). In accordance, several NMDA antagonists showed antidepressant-like effect in animal models (Skolnick et al., 2009; Murrough et al., 2017). Following this proposal, ketamine was tested in refractory patients and exhibit a rapid antidepressant response (Berman et al., 2000; Newport et al., 2015; Ghasemi et al., 2017; Murrough et al., 2017). Other antagonists or modulator of NMDA receptors have being tested in clinical trials with mixed results (Skolnick et al., 2009; Newport et al., 2015; Ghasemi et al., 2017; Murrough et al., 2017). Amantadine was studied in small open clinical trials with interesting positive results (for review, see Raupp-Barcaro, *in press*).

Several studies have shown a relationship between hippocampal neurogenesis and antidepressant efficacy and that ablation of neurogenesis diminished the behavioral effects of a variety of antidepressants (Malberg et al., 2000; Santarelli et al., 2003; Surget et al., 2008). In the present work, amantadine repeated administration showed an increase in hippocampal neurogenesis which paralleled with its anti-immobility effect in the TST. Clarke et al. (2017) observed that also ketamine increased hippocampal neurogenesis and prevented the decreasing in DCX staining in the middle portion of dentate gyrus of hippocampus of stressed rats.

In conclusion, the present study suggests that amantadine induced behavioral (anti-immobility effect) and molecular (increase neurogenesis) antidepressant-like

activity and this behavioral effect appear to be related to dopamine and NMDA receptors.

Conflict of Interest

The authors declare no conflict of interest.

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4 CONSIDERAÇÕES FINAIS

Como podemos observar na tabela 2 (pg 89), os resultados do presente estudo indicam que:

1. Amantadina administrada tanto aguda, quanto cronicamente apresenta efeito do tipo antidepressivo nos testes da natação forçada em ratos e no teste da suspensão pela cauda em camundongos.
2. O efeito do tipo antidepressivo da amantadina está relacionado provavelmente às catecolaminas, uma vez que houve o aumento do comportamento de escalada no TNF.
3. O pré-tratamento com o inibidor da síntese de tirosina-hidroxilase, AMPT, não afetou o efeito anti-imobilidade da amantadina nem em ratos, nem em camundongos.
4. O efeito da amantadina provavelmente não é dependente da liberação pré-sináptica de dopamina, uma vez que o inibidor da enzima passo-limitante da síntese de catecolaminas – AMPT, não foi capaz de reverter o efeito anti-imobilidade da amantadina.
5. O aumento da liberação de dopamina após administração da amantadina deve ocorrer provavelmente via D_2, uma vez que o antagonista D_2 – haloperidol foi capaz de bloquear o efeito anti-imobilidade da amantadina.
6. Outro receptor que parece estar envolvido na ação da amantadina é o NMDA do glutamato, uma vez que a administração do agonista NMDA preveniu o efeito tipo antidepressivo da amantadina.
7. A administração repetida de amantadina foi capaz de aumentar a neurogênese hipocampal concomitantemente com a diminuição do tempo de imobilidade no TST.

Tabela 2 – Resumo dos resultados comportamentais da administração aguda e repetida de amantadina e influência dos desafios farmacológicos

	Atividade	Veículo	Amantadina
Ratos (TNF)			
Tratamento Agudo		0	↓ imobilidade ↑ escalada
Tratamento Repetido		0	↓ imobilidade ↑ escalada
Camundongos (TSC)			
Tratamento Agudo			
Veículo		0	↓
AMPT	Depleção NA/DA	0	↓
Veículo		0	
Haloperidol	Antagonista D2	0	0
SCH23390	Antagonista D1	0	↓?
Veículo		0	↓
Prazosin	Antagonista Alfa-1	0	↓
Propranolol	Antagonista Beta	0	↓
Veículo		0	↓
Ioimbina	Antagonista Alfa-2	0	↓
Clonidina	Agonista Alfa-2	0	↓
Veículo		0	↓
NMDA	Agonista NMDA	0	0
Tratamento Repetido		0	↓

TSC: ↓ = redução da imobilidade

TSC: 0 = igual ao veículo

Uma hipótese para o efeito tipo antidepressivo da amantadina, articulando os resultados do presente estudo, seria que a amantadina, através do bloqueio dos receptores NMDA (presente estudo), acarretaria um aumento da sensibilidade dos receptores D_2 pós-sinápticos (Rogóz et al., 2003; presente estudo) e, de modo similar à quetamina, aumento da liberação/ síntese de BNDF (Rogóz et al., 2007) e aumento da neurogênese hipocampal (presente estudo), que também pode ser incrementada pelo aumento da atividade dopaminérgica (Gershon et al., 2006).

Portanto, a amantadina é um fármaco promissor para o tratamento da depressão. Similarmente à quetamina, a amantadina atuaria através do sistema glutamatérgico, mas apresentaria como vantagem a sua disponibilidade para administração oral. Em relação a novos fármacos glutamatérgicos, a amatadina teria como vantagens a grande experiência clínica com seu uso e conseqüente conhecimento de seus efeitos adversos, farmacocinética e interações. Entretanto, assim como a quetamina, como é um fármaco antigo, há um menor interesse comercial no seu estudo (p.ex. estudos clínicos em larga escala) e desenvolvimento (e.g. Sanacora et al., 2017). Por outro lado, além de seu emprego clínico, a amantadina pode ser um ponto de partida interessante para a síntese e conseqüente desenvolvimento de novos fármacos antidepressivos.

CONCLUSÂO

O presente estudo sugere que a amantadina é capaz de induzir efeitos do tipo antidepressivos tanto comportamentais, quanto moleculares, mostrando que a droga pode ser promissora no tratamento da depressão ou como ponto de partida para desenvolvimento de novos fármacos.

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