submetidos ao protocolo de estresse desenvolveram comportamento anedônico, diminuindo o consumo de sacarose, e, além disso, apresentaram alterações fisiológicas, como diminuição no peso, aumento na glândula adrenal e nos níveis do hormônio ACTH e corticosterona, e ainda, esses efeitos puderam ser revertidos pelo antagonista do receptor NMDA, cetamina (dados submetidos a publicação), mostrando que este modelo animal possui critérios para a validade de um modelo experimental de depressão.

2. HARMINA

Harmina (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole) (**Figura 1**) é uma β-carbolina de distribuição bastante diversificada. Pode ser encontrada em plantas da família Zygophyllaceae, Malphighiaceae, Passifloraceae, Leguminosae, Myristicaceae e Eleganaceae. Ocorre também, embora em menor número, na fumaça do cigarro, nos alimentos excessivamente cozidos ricos em proteínas, em alguns animais, inclusive mamíferos e fungos (HASHIMOTO *et al.*, 1988; TOTSUKA *et al.*, 1999).

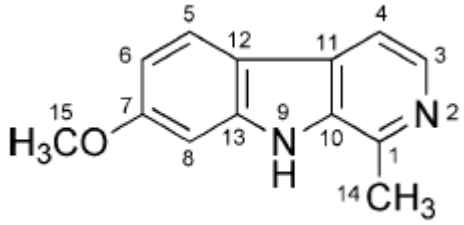

Figura 1: Fórmula estrutural do alcalóide β-carbolínicos harmina (ASTULLA *et al.*, 2008).

A casca e a folha de *Banisteriopsis caapi* (Malpighiaceae), uma planta da América do Sul que contém harmina, são utilizadas na preparação de uma bebida conhecida como ayahuasca (yagé, hoasca, daime ou caapi). Esta bebida é obtida a partir da fervura desta planta junto com folhas de *Psycotria viridis*, que contém triptaminas, resultando em um potente alucinógeno utilizado em rituais religiosos no Brasil, Bolívia, Equador e Peru (CALLAWAY *et al.*, 1996; 1999; FREEDLAND e MANSBACH, 1999).

2.1 Harmina como antidepressivo

A harmina, assim como as demais β-carbolinas, atua sobre o SNC, inibindo a enzima monoamino-oxidase tipo MAO A (KIM *et al.*, 1997; FARZIN e MANSOURI, 2006). A MAO encontra-se na membrana externa das mitocôndrias, localização essa que normalmente protege as monoaminas endógenas contra sua ação oxidativa, mantendo os respectivos teores teciduais dentro dos limites fisiológicos. Devido a isto, a administração de inibidores da MAO (IMAO) acarreta aumento dos depósitos destas aminas, principalmente da dopamina, noradrenalina e serotonina no cérebro. A consequência deste efeito inibitório é um estado de excitação, euforia, aumento da atividade psicomotora (efeito antidepressivo), entre outros (FARZIN e MANSOURI, 2006).

Acredita-se que o efeito alucinógeno das β-carbolinas, entre elas a harmina, além de ocorrer devido a inibição da MAO, aconteça também devido à similaridade estrutural com aminas indólicas, como a triptamina e serotonina. Alguns estudos demonstraram que a harmina interage com moderada afinidade com receptores do SNC. Esse alcalóide liga-se com relativa afinidade a receptores cerebrais de serotonina, subtipos 5-HT_{2C} e 5-HT_{2A} e receptores imidazólicos (I_1 e I_2) (GLENNON *et al.*, 2000; HUSBANDS *et al.*, 2001). Em relação aos receptores de serotonina 5-HT_{1A}, receptores de dopamina D_1 e D_2 e receptores benzodiazepínicos, a harmina interage com pouca afinidade (GLENNON *et al.*, 2000).

Farzin e Mansouri (2006), demonstraram em seus estudos que a harmina diminuiu o tempo de imobilidade no TNF em camundongos, exercendo um efeito antidepressivo, possivelmente mediado pelos receptores I_2. Em uma pesquisa realizada por Santos *et al.* (2007), com voluntários saudáveis, os quais ingeriram uma solução contendo alguns compostos, entre eles, a harmina, foi encontrado

mudanças em sintomas de desespero e de pânico, sugerindo-se mais pesquisas para o uso desses compostos para o tratamento desses sintomas.

2.2 Harmina e ação antioxidante

Precursores indólicos das β-carbolinas, como tripfano e triptaminas, são conhecidos por possuírem atividade antioxidante (UEMURA *et al.*, 1988; CHRISTEN *et al.*, 1990), possivelmente por uma ação de detoxificação de espécies reativas de oxigênio (ERO). As β-carbolinas, por sua vez, possuem propriedades semelhantes (TSE *et al.*, 1991).

Em estudo realizado por Tse *et al*. (1991), a harmina inibiu a peroxidação lipídica em preparações microssomais hepáticas em ratos, agindo como seqüestradora de radicais livres, esta ação antioxidante e sua relativa eficácia são altamente dependente das modificações estruturais do anel β-carbolínico. Estes autores demonstram que a substituição do grupo hidroxil pelo grupo metoxil (harmol para harmina) e a desidrogenação do anel piridínico (harmalina para harmina) reduzem drasticamente a eficácia antioxidante *in vitro* das β-carbolinas.

II OBJETIVOS

1. Objetivo Geral

Avaliar os efeitos comportamentais e neuroquímicos do tratamento agudo e crônico de harmina em modelos animais de depressão.

2. Objetivos específicos

- Verificar se a harmina administrada agudamente, em diferentes doses, exerce efeito antidepressivo no TNF.
- Verificar se a harmina administrada cronicamente, em diferentes doses, exerce efeito antidepressivo no TNF.
- Avaliar os efeitos da administração aguda e crônica de harmina nos níveis de BDNF após o TNF.
- Investigar o efeito da harmina, na dose de 15 mg/kg, sobre as alterações comportamentais induzidas pelo modelo de ECM.
- Avaliar os efeitos da administração crônica de harmina, na dose de 15 mg/kg, nos níveis de BDNF após o ECM.
- Avaliar os efeitos da harmina sobre os níveis de ACTH de ratos submetidos ao protocolo de ECM.
- Avaliar os efeitos harmina no peso da glândula adrenal de ratos submetidos ao protocolo de ECM.

PARTE II

II – CAPÍTULO I

Acute harmine administration induces antidepressive-like effects and increases BDNF levels in the rat hippocampus

Fortunato JJ, Réus GZ, Kirsch TR, Stringari RB, Stertz L, Kapczinski F, Pinto JP, Hallak JE, Zuardi AW, Crippa JA, Quevedo J.

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II – CAPÍTULO II

Chronic administration of harmine elicits antidepressant-like effects and increases BDNF levels in the rat hippocampus

(Neurotoxicity Research – submitted)

CHRONIC ADMINISTRATION OF HARMINE ELICITS ANTIDEPRESSANT-LIKE EFFECTS AND INCREASES BDNF LEVELS IN THE RAT HIPPOCAMPUS

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Abstract

A growing body of evidence has pointed to the β-carboline harmine as a potential therapeutic target for the treatment of major depression. The present study was aimed to evaluate behavioural and molecular effects of the chronic treatment with harmine and imipramine in rats. To this aim, rats were treated for 14 days once a day with harmine (5, 10 and 15 mg/kg) and imipramine (10, 20 and 30 mg/kg) and then subjected to the forced swimming and open-field tests. Harmine and imipramine, at all doses tested, reduced immobility time of rats compared to the saline group, imipramine increase the swimming time at 20 e 30 and harmine increase at all doses, the climbing time increase in the rats treated with imipramine (10 and 30 mg/kg) and harmine (5 and 10 mg/kg), without affecting spontaneous locomotor activity. Brain-derived neurotrophic factor (BDNF) hippocampal levels were assessed in imipramine- and harmine-treated rats by ELISA sandwich assay. Interesting enough, chronic administration of harmine at the higher doses (10 and 15 mg/kg), but not imipramine, increased BDNF protein levels in the rat hippocampus. Finally, these findings further support the hypothesis that harmine could be a new drug target for the treatment of depression.

Keywords: harmine, imipramine, forced swimming test, monoamine oxidase, depression

1. Introduction

Depression is a serious mental illness that affects approximately 17% of the population and is a major cause of disability worldwide. Findings from World Health Organization predict that depression will be the leading cause of disability and premature death in the industrial world by the year 2020 (Mathers and Loncar, 2006).

Major depression encompasses a range of features that strongly suggest a neurobiological substrate. These include symptoms such as include sleep and appetite disturbances (both up and down), loss of interest and pleasure, negative rumination, fatigue, and poor concentration, but also apparent abnormalities of the hypothalamic-pituitary-adrenal axis or of neuroplasticity (Richard and Shelton, 2007; Garcia *et al.,* 2008a,b; Lucca *et al.,* 2008; Lucca *et al.,* 2009; Garcia *et al.,* 2009).

Basically all the clinically-used antidepressants increase the extracellular concentrations of monoamines serotonin or norepinephrine either by inhibiting their reuptake from the synapse or by blocking their degradation by inhibiting monoamine oxidase (Duman *et al.*, 1997; Nestler *et al.*, 2002; Castrén, 2005). Preclinical findings suggest that beta-carboline harmine present antidepressant-like actions in rodents subjected to an animal model of depression (Farzin and Mansouri, 2005; Fortunato *et al*., 2009a;b). In fact, studies have demonstrated that harmine interact with monoamine oxidase A (MAO-A) (Kim *et al*., 1997) and several cell-surface receptors, including serotonin receptor 2A (5-HT2A) (Glennon *et al*., 2000) of which were involved in antidepressant pharmacotherapy (Preskorn *et al*., 2008). Moreover we recently demonstrated that acute treatment with harmine at dose of 15 mg/kg increased BDNF protein levels in hippocampus of rats (Fortunato *et al.,* 2009).

A growing body of evidence has pointed to the role of brain-derived-neurotrophic factor (BDNF) in major depression. Alterations of hippocampal structure and function in response to stress provided the rationale for analysis of neurotrophic factors (Duman and Monteggia, 2006). Reduced brain BDNF levels have been found in *post-mortem* samples from depressed patients (Karege *et al*., 2002), whereas brain infusion of BDNF produces antidepressant-like action in rats (Siuciak *et al*., 1997; Shirayama *et al*., 2002). In addition, exposure to stress decreases levels of BDNF in brain regions associated with depression, while antidepressant treatment produces opposite actions and blocks the effects of stress on BDNF (for a review see: Duman and Monteggia, 2006; Kozisek *et al*., 2008). Interestingly, chronic, but not acute, antidepressant treatment induces increasing of BDNF expression and BDNF immunoreactive fibers in the hippocampus of rodents (Nibuya *et al*., 1996; De Foubert *et al*., 2004). Thus, agents capable of enhancing BDNF levels may lead aid the development of innovative antidepressant drugs (Zarate *et al.,* 2006; Garcia *et al.,* 2008a).

Thus, the main objective of the present study was to compare behavioral and molecular effects induced by chronic administration of harmine and imipramine in rats. The behavioral effects of both drugs were evaluated in the forced swimming test, which is a well valid behavioral despair assay widely used for screening antidepressant drugs (McArthur and Borsini, 2006). The BDNF protein levels were measured using an ELISA kit in the hippocampus of rats chronically treated with harmine and imipramine.

2. Materials and methods

2.1. Animals

Male Adult Wistar rats (60 days old) were obtained from UNESC (Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil) breeding colony. They were housed five per cage with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 7:00 AM). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care and with approval by local Ethics Committee under protocol number 325/2008.

2.2. Drugs and treatments

Harmine was obtained from THC-Pharm/STI-Pharm (Frankfurt, Germany) and imipramine, the standard antidepressant, from Novartis Pharmaceutical Industry (Criciúma, Brazil). Different groups of rats (n=15 each) were administered intraperitoneally (IP) with saline or different doses of harmine (5, 10 and 15 mg/kg) or imipramine (10, 20 and 30 mg/kg) during 14 days (Garcia *et al*., 2008b; Fortunato *et al*., 2009). Imipramine and harmine were dissolved in saline immediately before the intraperitoneal injections. All treatments were administered in a volume of 1 mL/kg. Rats were tested in the open field and forced swim test following chronic imipramine and harmine treatments. Beginning on day 12 of chronic treatment, rats were tested in the open field in order to assess the spontaneous locomotor activity. On day 13 and 14 of chronic treatment, rats were then tested in the forced swimming test. From day 12 to 14 of chronic treatment, drug administration was done 60 min. before the

assessment of animal behaviour in the open field (day 12) and forced swimming test (days 13 and 14).

2.3. Forced swimming test

The forced swimming test was conducted according to previous reports (Porsolt *et al*., 1977; Detke *et al*., 1995; Garcia *et* al., 2008a,b). The test involves two individual exposures to a cylindrical tank with water in which rats cannot touch the bottom of the tank or escape. The tank is made of transparent Plexiglas, 80 cm tall, 30 cm in diameter, and filled with water (22–23 ℃) to a depth of 40 cm. On day 13 of chronic treatment, 1 hr after drug treatment rats were individually placed in the cylinder containing water for 15 min. (pre-test session). On the 14th day, rats received the last intraperitoneal drug treatment, and after 1 hr, they were subjected again to the forced swimming test for a 5-min. session (test session). During the test session some behavioural parameters were recorded in seconds, such as immobility time (i.e. no additional activity is observed other than that required to keep the rat's head above the water), climbing time, which is defined as upward-directed movements of the forepaws along the side of the swim chamber, and swimming time (i.e. movement usually horizontal throughout the swim chamber).

2.4. Open-field test

This apparatus consists of a brown plywood arena 45 × 60 cm surrounded by wood 50 cm high walls and containing a frontal glass wall. The floor of the open field was divided into nine rectangles (15 × 20 cm each) by black lines. Animals were gently placed on the left rear quadrant, and left to explore the arena for 5 min. After 12 days of treatment, rats were exposed to the open-field apparatus, and the number

of horizontal (crossings) and vertical (rearings) activity performed by each rat during the 5-min. observation period was counted by an expert observer.

2.4. BDNF analysis

Immediately after the forced swimming test saline, imipramine and harmine-treated rats were sacrificed and the skulls were removed and hippocampus was dissected and stored at −80 ℃ for biochemical analysis. BDNF levels in hippocampus were measured by anti-BDNF sandwich-ELISA, according to the manufacturer instructions (Chemicon, USA). Briefly, rat hippocampus was homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM (EGTA). Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent and standard curve ranged from 7.8 to 500 pg/ml of BNDF. The plates were then washed four times with sample diluent and a monoclonal anti-BNDF rabbit antibody diluted 1:1000 in sample diluent was added to each well and incubated for 3 h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temperature for 1 h. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF was determined by absorbance in 450 nm. The standard curve demonstrates a direct relationship between Optical Density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard, as previously described by Frey *et al*. (2006).

2.5. Statistical analysis

All data are presented as mean±S.E.M. Differences among experimental groups in the forced swimming, open field test and in the assessment of BDNF levels were determined by one-way ANOVA, followed by Tukey *post-hoc* test when ANOVA was significant; p values less than 0.05 were considered to be statistical significant.

3. Results

As depicted in Fig. 1, the chronic administration of the standard antidepressant imipramine reduced, in a significant manner, the immobility time of rats at 10, 20 and 30 mg/kg compared to saline ($F_{(6-58)}$ = 3,66; P <0.05; Fig. 1A) imipramine increase the swimming time at 20 and 30 mg/kg ($F_{(6-64}$ = 9,79; P <0.05; Fig.1A) and imipramine also increased the climbing time at 10 and 30 mg/kg ($F_{(6-54)}$ = 4,68; P <0.05; Fig. 1A) . The intraperitoneal treatment with harmine at the doses of 5, 10 and 15 mg/kg also decreased significantly the immobility time of rats compared to saline group (P <0.05; Fig. 1B). Moreover harmine also increased the swimming time at all doses ($F_{(6-64)}$ = 9,79; P <0.05; Fig. 1B) and increase the climbing at 5 and 15 mg/kg ($F_{(6-54)}$ = 4,68; P <0.05; Fig. 1B). In the open-field test, the treatment with harmine and imiprimine at all doses tested did not modify the number of crossing and rearing compared to saline treated-rats (Fig. 2A and B).

Fig. 3 illustrated the effects of the chronic treatment with imipramine (10, 20 and 30 mg/kg), harmine (5, 10 and 15 mg/kg) and saline in BDNF protein hippocampus levels of rats. A statistical significant increase in BDNF protein levels in the hippocampus was observed in rats treated with harmine only at the higher doses (10 and 15 mg/kg; $F_{(6-64)}$ = 3.15; P < 0.05), but not with imipramine, compared to saline group.

4. Discussion

The present study demonstrates that (i) the chronic treatment with all doses of harmine and imipramine decreased the immobility time of rats in the forced swimming test; (ii) the swimming time increase with harmine at all doses and imipramine at 20 and 30 mg/kg; (iii) the climbing time increase with harmine at 5 and 15 mg/kg and imipramine at 20 and 30 mg/kg; (iv) harmine and imipramine did not affect spontaneous locomotor activity in the open-field test; and (v) chronic treatment with harmine, but not imipramine, increased BDNF protein levels in the rat hippocampus.

In 1995, Detke *et al.* (1995) reported that despite the anti-immobility effects, antidepressant drugs that enhance noradrenergic neurotransmission increase climbing behaviour, whereas the enhancement of serotonergic neurotransmission increases swimming time in the rat forced swimming test.

In fact, findings from our group have demonstrated that a single injection of imipramine (10 and 20 mg/kg) and chronic administration of imipramine (10, 20 and 30 mg/kg) decreased the immobility time of rats in the forced swimming test, without modifying the locomotor activity (Garcia *et al.,* 2008a,b). Moreover chronic treatment with imipramine increased swimming time at doses of 20 and 30 mg/kg and increased climbing time at doses of 5 and 15 mg/kg in rats (Garcia et al., 2008b).

Farzin and Mansouri (2005) demonstrated that acute treatment with harmane, norharmane and harmine dose-dependently reduced the immobility time in the mouse forced swimming test. In same study they demonstrated that flumazenil at a dose ineffective per se on the duration of immobility, antagonized the antidepressant-like effects of harmane, norharmane and harmine, provide evidence that β-carbolines harmane, norharmane and harmine induce an antidepressant-like effect via

stimulation of the benzodiazepine receptor in an inverse manner (Farzin and Mansouri, 2005). Moreover our group demonstrated recently that acute treatment with harmine at doses of 10 and 15 mg/kg decreased the immobility time and increased the swimming and the climbing time of rats (Fortunato *et al*., 2009a). In this study also were demonstrated that harmine at 15 mg/kg, but did not imipramine increased BDNF protein levels in the rat hippocampus.

Taken together, these findings support rapid effects to harmine on behavioural tests used for screening antidepressant drugs. The present study demonstrates that 14 days of imipramine treatment, at doses of 10, 20 and 30 mg/kg reduced immobility time of rats subjected to the forced swimming test. These findings are in agreement with other authors that support reduction of immobility time in the forced swimming test after repeated administration of imipramine, especially at low doses (such as 10 mg/kg), which were acutely inactive (Gorka and Janus, 1985; Kawashima *et al.,* 1986; Garcia *et al.,* 2008a,b).

Interestingly enough, harmine also reduced immobility time in the forced swimming test at all doses tested, These findings show that chronic administration of harmine at low doses (i.e. 5 mg/kg) induces behavioural responses that were not elicited acutely, while at higher doses (i.e. 10 and 15 mg/kg) no signs of tolerance were observed after the chronic exposure (Fortunato et al., 2009a).

Our findings also showed that chronically administration of harmine (10 and 15 mg/kg), but not imipramine, significantly increased BDNF protein levels in the rat hippocampus compared with saline group. The hippocampus is one of several limbic structures that have been implicated in mood disorders. Included in the functions of hippocampal circuitry are control of learning and memory and regulation of the hypothalamic-pituitary-adrenal (HPA) axis, both of which are altered in depression.

Alterations of hippocampal structure and function in response to stress provided the rationale for analysis of neurotrophic factors (Duman and Monteggia, 2006). Neurotrophic factors are critical regulators of the formation and plasticity of neural networks (Huang and Reichardt, 2001). Moreover, a growing body of evidence supports an important role of neurotrophic factors in mood disorders. Several studies have suggested that normal BDNF-TrkB receptor signaling is both necessary and sufficient for antidepressant drug action (for a review see: Castrén *et al*., 2007; Kozisek *et al*., 2008). BDNF-mediated signaling is involved in neuroplastic responses to stress and antidepressants (Krishnan and Nestler, 2008). In fact, reduced brain BDNF levels might to be correlated to depression (Karege *et al*., 2002), whereas increases in brain BDNF levels is suggested to produce an antidepressant action (Siuciak *et al*., 1997; Shirayama *et al*., 2002). Moreover, analysis of *postmortem* hippocampus demonstrates that the expression of BDNF is decreased in depressed suicide patients and increased in patients receiving antidepressant medication at the time of death (Chen *et al.,* 2001; Dwivedi *et al*., 2003; Karege *et al.,* 2005).

Our present findings revealed that chronically administration of β-carboline harmine causes an increase of BDNF hippocampal levels detected immediately after the forced swimming test, which suggests that the antidepressant-like effects of harmine might be due to the increase of hippocampal BDNF protein levels. Our data also imipramine, did not alter BDNF protein levels in the hippocampus of rats subjected to the forced swimming test. Previous studies of our group also demonstrated that chronically administration of imipramine decreased the immobility time of rats in the forced swimming test, but did not alter BDNF protein levels in the hippocampus (Garcia *et al.,* 2008b).

However the antidepressant-like effects of harmine observed in the present study could be due to interactions of harmine with monoamine oxidase A (MAO-A) (Kim et al., 1997), and several cell-surface receptors, serotonin receptor 2A (5-HT2A) (Glennon et al., 2000), imidazoline receptors (I1 and I2 sites) (Husbands *et al*., 2001), cyclindependent kinases (CDK1, 2, and 5) (Song *et al*., 2004) and benzodiazepine receptor in an inverse manner (Farzin and Mansouri, 2005) involved in the modulation of behavioral and molecular actions of antidepressants. Future double-blind, placebo-controlled studies would be necessary and opportune to further confirm these observations in patients with major depression and to evaluate whether harmine could be a new option for this impairment disorder.

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FIGURES

Figure 1A

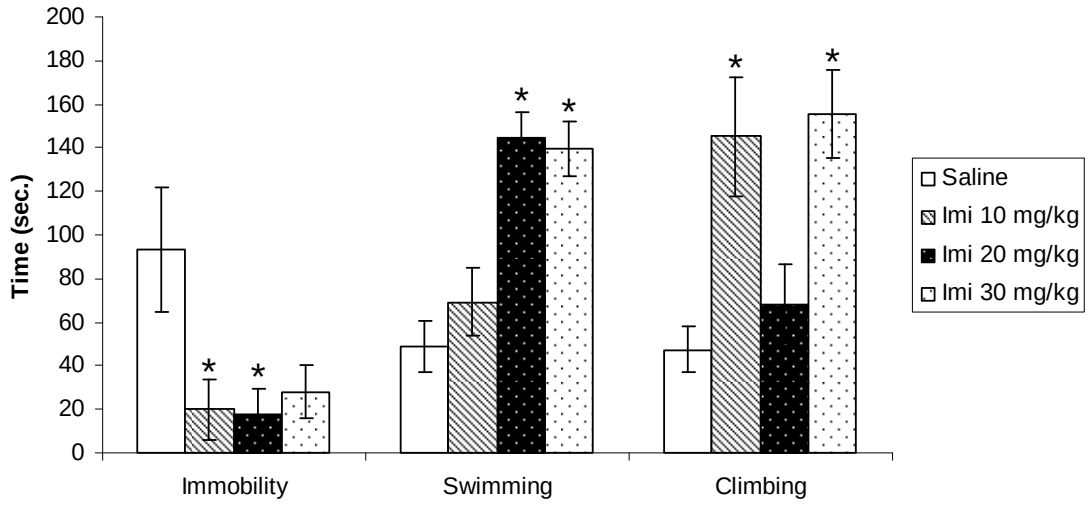


Figure 1B

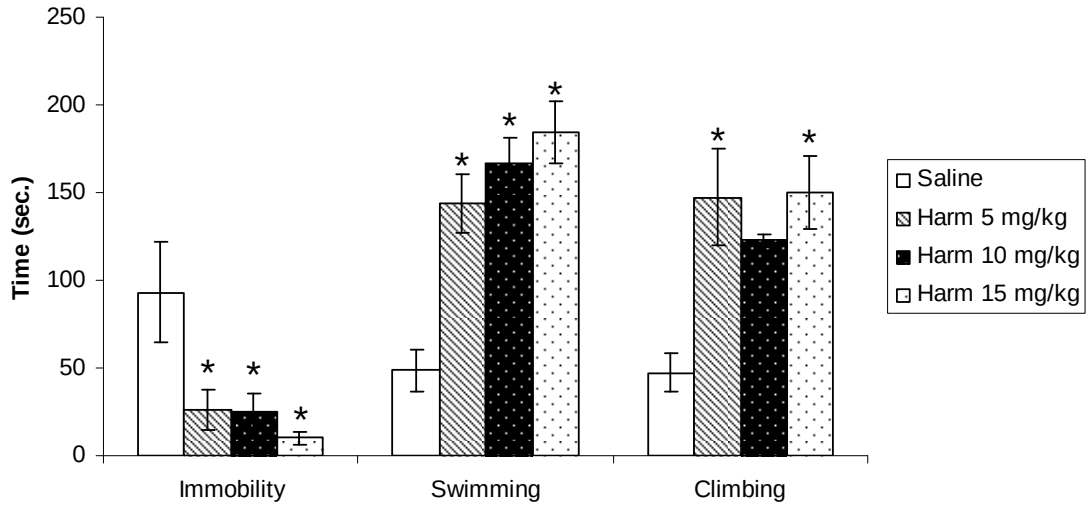


Figure 2A

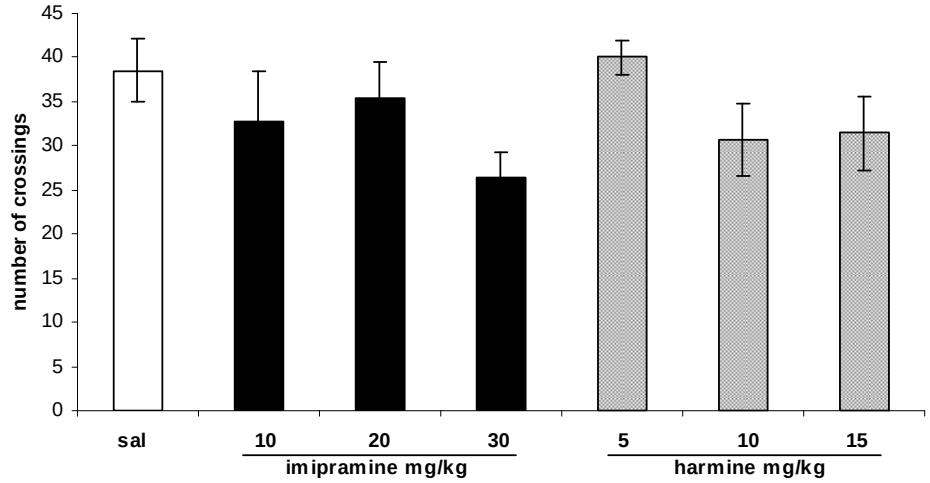


Figure 2B

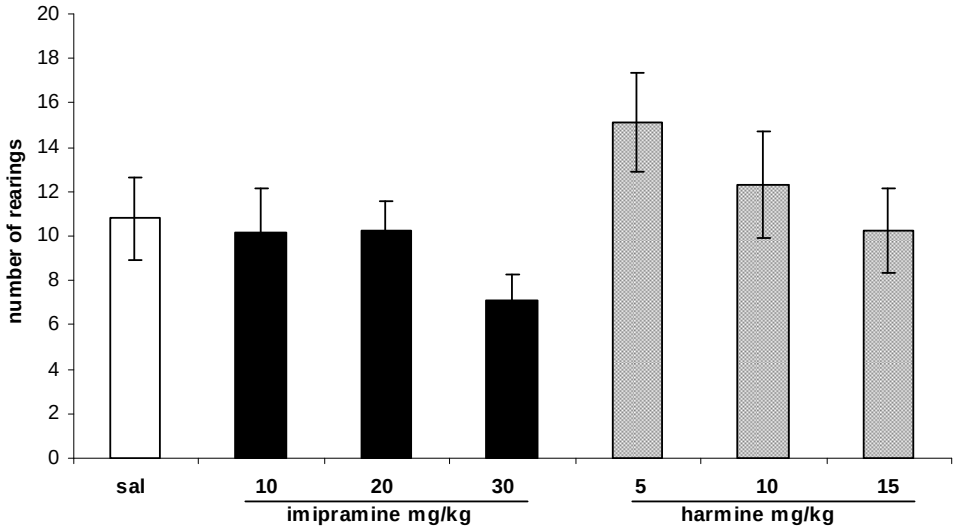
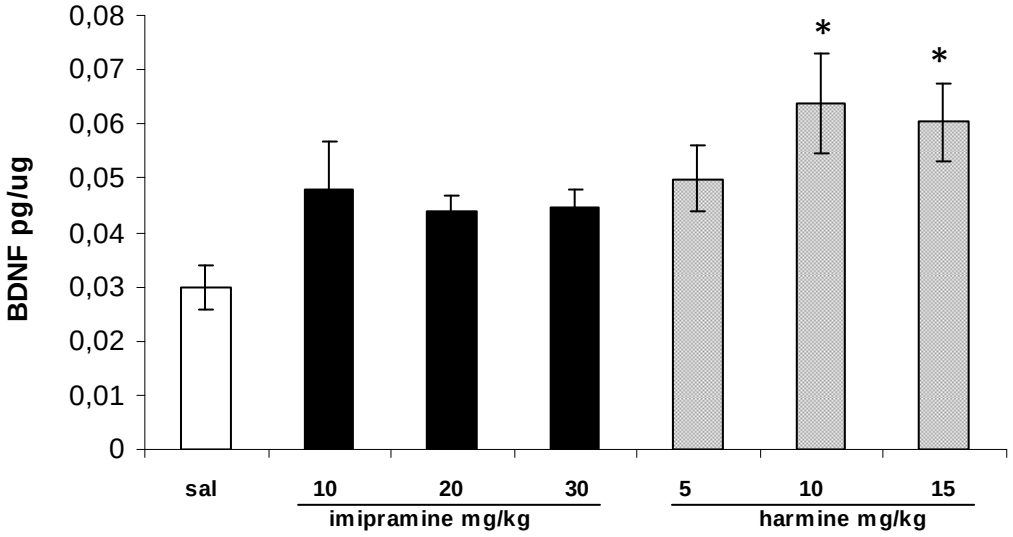


Figure 3


BDNF pg/ug
0,08
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*
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sal
10
20
30
5
10
15
imipramine mg/kg
harmine mg/kg

LEGENDS OF FIGURES

Figure 1 - Effects of the chronic administration of imipramine (10, 20 and 30 mg/kg, i.p.) (A) and harmine (5, 10 and 15 mg/kg, i.p.) (B) on the immobility, swimming and climbing time of rats subjected to the forced swimming test. Bars represent means±S.E.M. of 15 rats. * p <0.05 *vs.* saline according to ANOVA followed by Tukey *post-hoc* test.

Figure 2 - Effects of the chronic administration of harmine (5, 10 and 15 mg/kg, i.p.) and imipramine (10, 20 and 30 mg/kg, i.p.) on the number of crossings (A) and rearings (B) of rats subjected to the open field test. Bars represent means±S.E.M. of 15 rats. * p <0.05 *vs.* saline according to ANOVA followed by Tukey *post-hoc* test.

Figure 3 - Effects of the chronic administration of harmine (5, 10 and 15 mg/kg, i.p.) and imipramine (10, 20 and 30 mg/k, i.p.) on the BDNF levels in the rat hippocampus. Bars represent means±S.E.M. of 10 rats. * p <0.05 *vs*. saline according to ANOVA followed by Tukey post-hoc test.

II – CAPÍTULO III

Effects of beta-carboline harmine on behavioral and physiological parameters observed in the chronic mild stress model: further evidence of antidepressant properties

Fortunato JJ, Réus GZ, Kirsch TR, Stringari RB, Fries G, Kapczinski F, Hallak JE, Zuardi AW, Crippa JA, Quevedo J.

Brain Res Bull. In Press, 2009.

PARTE III

III DISCUSSÃO

O objetivo geral deste estudo foi avaliar os efeitos comportamentais e neuroquímicos da administração aguda e crônica (i.p.) de harmina em modelos animais de depressão. Os resultados mais relevantes deste trabalho mostraram que o tratamento agudo (10 e 15 mg/kg) e crônico (5, 10 e 15 mg/kg) de harmina diminuiu o tempo de imobilidade dos animais no TNF, mas não afetou a atividade locomotora espontânea avaliada no teste de exploração ao campo aberto (TCA); a administração crônica (15 mg/kg) de harmina reverteu o comportamento anedônico, diminuiu os níveis de BDNF, o peso da glândula adrenal e os níveis de ACTH circulante induzidos pelo ECM; os níveis de BNDF foram aumentados no hipocampo dos animais tratados agudamente (15 mg/kg) e cronicamente (10 e 15 mg/kg) com harmina. Desta forma, os resultados mostraram que a administração aguda e crônica de harmina produziu um efeito antidepressivo nos modelos animais utilizados neste estudo.

A harmina atua no SNC inibindo a enzima monoanino-oxidase tipo MAO A (KIM *et al.*, 1997; FARZIN e MANSOURI, 2006). A distribuição da MAO no cérebro apresenta pequenas variações entre as espécies. MAO-A é, predominantemente, encontrada em regiões com alta densidade de neurônios catecolaminérgicos como *locus coeruleus*, substância negra e regiões periventriculares do hipotálamo (WECKER *et al*., 2001). Além de seu efeito inibitório sobre a MAO-A, alguns trabalhos tem demonstrado diferentes atividades biológicas para as β-carbolinas: imunossupressora, mutagênica (BOEIRA *et al*., 2001; DEVEAU *et al*., 2001), antioxidante, possivelmente por sua ação de detoxificação de espécies reativas e

oxigênio (TSE *et al*., 1991) e neuroprotetora (LEE *et al*., 2000; KIM *et al*., 2001; PARK *et al*., 2003).

A depressão é uma condição médica amplamente desabilitadora e prevalente na população mundial associada com morbidade e mortalidade (NESTLER e CARLEZON, 2006; NEMEROFF *et al*., 2007). Embora a terapia para a depressão com fármacos, psicoterapia e terapia eletroconvulsiva seja efetiva, um número significativo de pacientes não respondem bem a estes tratamentos (ANDERSON, 2000; BERTON e NESTLER, 2006). Em virtude disto, há uma grande necessidade de buscar novos alvos terapêuticos, a fim de tratar pacientes resistentes a tratamentos e diminuir os efeitos colaterais que ocorrem com o uso de antidepressivos.

No *Capítulo I*, observamos que os parâmetros utilizados para avaliar os possíveis efeitos indicadores de depressão a partir da administração aguda de harmina foram representados pela diminuição no tempo de imobilidade e aumento nos tempos de nado e *climbings* nas doses de 10 e 15 mg/kg durante o TNF (FORTUNATO *et al*., 2009b). Resultados semelhantes foram descritos por FARZIN e colaboradores (2006).

O TNF foi desenvolvido por Porsolt *et al*. (1977), para auxiliar na pesquisa de substâncias antidepressivas. O objetivo foi criar um modelo animal que reproduzisse um comportamento semelhante à depressão e que fosse sensível aos fármacos utilizados clinicamente no tratamento desta patologia. No TNF, os animais são submetidos a um período de nado forçado em um espaço restrito, uma situação inescapável de estresse. Os animais executam inicialmente movimentos vigorosos na tentativa de escapar e depois de alguns minutos apresentam apenas pequenos movimentos que o impeçam de submergir, adotando uma postura de imobilidade

(PORSOLT *et al*., 1977). Embora este modelo não reproduza adequadamente a sintomatologia da depressão em humanos, ele parece ter um alto valor preditivo na investigação de substâncias antidepressivas (WILLNER, 1984; 1997), uma vez que antidepressivos clássicos reduzem o tempo de imobilidade neste teste (PORSOLT *et al*., 1977; CRYAN *et al*., 2002), por isso, foi escolhido como um dos testes para avaliar o mecanismo de ação da harmina.

Os dados obtidos em camundongos (FARZIN e MANSOURI, 2006) e em ratos submetidos ao TNF (FORTUNATO *et al*., 2009a,b) indicam claramente que o efeito antidepressivo induzido por administração de β-carbolina é um fenômeno consistente que se mantém em diferentes espécies e em diferentes testes experimentais utilizados para avaliar atividade antidepressiva (TNF e ECM).

A administração de imipramina (20 e 30 mg/kg), usada como controle positivo, diminuiu o tempo de imobilidade dos ratos tratados agudamente. Porsolt *et al*. (1978), já haviam demonstrado que o tratamento agudo com imipramina reduzia a variável do TNF de forma dose-dependente e que efeitos mais consistentes ainda poderiam ser obtidos através de tratamento crônico, como nos dados apresentados no Capítulo II, no qual o tratamento repetido diminuiu significativamente o tempo de imobilidade dos ratos.

Fazin e Mansouri (2006), sugerem que o efeito antidepressivo produzido pela harmina no TNF possa estar envolvido com as vias de estimulação do receptor benzodiazepínico (BZD) de maneira inversa. As β-carbolinas originam-se do aminoácido triptofano através da condensação entre a triptamina (produto da descarboxilação do triptofano) com o aldeído ou α-cetoácido (HUSSON, 1985; DUCROT *et al*., 2000). A partir dessa condensação formam-se alcalóides endógenos

que interagem com alta afinidade aos sítios BZD dos receptores $GABA_A$ como agonistas inversos (FAZIN e MANSOURI, 2006).

Atualmente, sabe-se que parte dos receptores para BZD, que seria responsável pela maioria de seus efeitos farmacológicos, localiza-se em membranas neuronais, como parte de um complexo oligomérico com receptores do tipo $GABA_A$ e seu canal de cloro associado. Os BZD modulariam alostéricamente os receptores do tipo $GABA_A$ (BOWERY, 1984; MARTIN, 1984; WILLIAMS, 1984; STEPHENSON, 1986). Entretanto, esta modulação pode determinar diferentes conseqüências funcionais, uma vez que estes receptores contêm sítios de reconhecimento para ligantes exógenos de alta afinidade. Entre eles estão os agonistas inversos, que dificultam o efeito do GABA, possuindo atividade intrínsica negativa, tal como as β-carbolinas (BOWERY, 1984; MARTIN, 1983, 1984).

Na década de 90, foi identificado um provável sítio regulatório da MAO, chamado sítio de ligação imidazolínico tipo I_2. Muitos estudos vêm mostrando ações funcionais das β-carbolinas e sua alta afinidade para sítios de ligação imidazolínicos I_2 (HUDSON et al., 1999; HUBBANDS et al., 2001; FARZIN e MANSOURI, 2006). Os sítios I_2 foram identificados em muitos órgãos, tecidos e tipos celulares, como córtex cerebral (WIKBERG e UHLEN, 1990), astrócitos (REGUNATHAN et al., 1993a), medula adrenal (REGUNATHAN et al., 1993b) e plaquetas (MICHEL et al., 1989). Embora não esteja clara a função fisiológica destes sítios, alguns efeitos dos ligantes seletivos I_2 são relatados. Entre estes, destacam-se: indução de hiperplasia astrocítica em cérebro de ratos adultos (ALEMANY et al., 1995), atenuação da tolerância a antinocicepção induzida por opióides (BORONAT et al., 1998); papel neuroprotetor (BORONAT et al., 1998), aumento do consumo de alimento (BROWN et al., 1995); entre outros.

Nesse sentido, considerando que a harmina interage com sítios específicos I_2 (FARZIN e MANSOURI, 2006) e que seja capaz de alterar a atividade da MAO (KIM *et al.*, 1997) em determinados tecidos, o entendimento dos mecanismos de interação que envolve essas duas proteínas (sítios I_2 e MAO) pode explicar sua ação antidepressiva no TNF.

O TNF foi o teste escolhido para avaliar o mecanismo de ação da harmina, neste estudo, utilizando imipramina como controle positivo. O TCA foi utilizado neste trabalho para verificar se a harmina administrada aguda ou cronicamente provocava alteração na atividade locomotora.

As substâncias que aumentam a atividade locomotora podem produzir resultados positivos no TNF e seriam rejeitados como antidepressivos. No entanto, alguns fármacos como o bupropion, nomifensina e amineptina são antidepressivos utilizados na clínica, e aumentam a atividade locomotora dos animais (BORSINI e MELI, 1988). Neste estudo, o efeito antidepressivo da harmina não está associado a nenhum efeito motor, pois as doses em que a harmina demonstrou atividade antidepressiva não afetaram significativamente a locomoção no TCA. Este resultado indica que o efeito antidepressivo induzido pela harmina é específico.

Várias evidências indicam que os transtornos de humor estão associados com reduções regionais no volume encefálico, bem como no número, tamanho e densidade da glia e neurônios em discretas áreas do encéfalo (ZARATE *et al*., 2003). Embora a fisiopatologia envolvida nessas mudanças morfométricas precise ser elucidada, os dados sugerem que as desordens de humor severas estão associadas com a redução da neuroplasticidade.

Embora os antidepressivos venham sendo utilizados há várias décadas, as bases neurobiológicas para explicar sua eficácia ainda são pouco compreendidas.

Mais recentemente, tem sido proposto que a necessidade de longo tratamento para que os antidepressivos possam exercer seus efeitos terapêuticos é por ativar mecanismos celulares que promovam a plasticidade neuronal. Por outro lado, a neuroplasticidade e a sobrevivência celular são reguladas por diferentes vias de sinalização, que podem ser alteradas na depressão (MANJI *et al*., 2001; MANJI *et al*., 2003). Vários estudos básicos e clínicos observaram reduções regionais do número (morte celular) ou do tamanho de glias e neurônios no hipocampo (atrofia neuronal) de pacientes deprimidos (MANJI *et al*., 2001). Os antidepressivos bloqueiam ou revertem estes comprometimentos celulares através da ativação de vias de sinalização que regulam fatores envolvidos na sobrevivência celular (neuroproteção), como o BDNF (DUMAN *et al*., 2000; MANJI *et al*., 2000; D'AS e DUMAN, 2002).

O aumento da expressão de BDNF no hipocampo de ratos tratados agudamente com harmina, pode estar relacionado com a ação neuroprotetora, provavelmente devido à sua capacidade de bloquear receptores de NMDA e canais de cálcio.

O BDNF exerce sua ação neurotrófica e neuroprotetora através de uma cascata composta do receptor Trk (tirosina quinase), a via de sinalização celular MAPK/ERK e a ativação da expressão da proteína antiapoptótica Bcl2 (MANJI *et al*., 2001; D'AS e DUMAN, 2002; HASHIMOTO *et al*., 2004). A morte celular por apoptose parece estar envolvida em várias doenças neurodegenerativas crônicas (ADAMS e CORY, 1998). Desta maneira, o BDNF possui um efeito trófico para a sobrevivência celular, mas seu efeito neuroprotetor é principalmente devido à inibição da cascata de morte celular pela expressão de Bcl2.

As doses de imipramina administradas agudamente não provocaram efeito, inclusive reduzindo o conteúdo de BDNF no hipocampo. Parece que o tipo de antidepressivo e os distintos tratamentos podem influenciar os diferentes padrões de resposta da expressão de BDNF.

Os níveis de BDNF são também considerados como parâmetros relacionados à depressão (CASTREN *et al*., 2007). Vários estudos suportam a hipótese do envolvimento de BDNF na depressão e sugerem um decréscimo dos níveis plasmáticos em pacientes não tratados com depressão maior (CASTREN *et al*., 2007, LEE *et al*., 2007). Em animais, baixos níveis de BDNF foram descritos quando submetidos a protocolos de estresse crônico (XU *et al*., 2002). Além disso, estudos mostram que a expressão de BDNF também é modificada pelo estresse (TAPIA-ARANCIBIA *et al*., 2004), e está envolvido na regulação da atividade do eixo HHA em resposta ao estresse no hipocampo, hipotálamo e hipófise (GIVALOIS *et al*., 2001; RAGE *et al*., 2002; MARMIGERE *et al*., 2003; GIVALOIS *et al*., 2004). Mais recentemente, um estudo mostrou que a administração intracerebroventricular, aguda ou crônica, de BDNF modifica a síntese e a liberação do hormônio liberador de corticotrofina e/ou a arginina vasopressina, modificando a liberação de hormônios como cortisol e adrenocorticotrofina (GIVALOIS *et al*., 2004).

No *Capítulo II*, observamos que a administração crônica de harmina (5, 10 e 15 mg/kg) confirmou os resultados encontrados com a administração aguda deste composto, evidenciando diminuição no tempo de imobilidade observado no TNF e aumento nos níveis de BDNF no hipocampo dos animais experimentais. O conjunto destes efeitos poderia resultar na neuroproteção induzida pela harmina.

A administração crônica de imipramina também apresentou diminuição do tempo de imobilidade no TNF. Entretanto, a dose mais alta (30 mg/kg) não

apresentou resultados significativos para este parâmetro. Estes resultados sugerem que a dose de imipramina administrada pode influenciar na resposta comportamental deste modelo. Bai e colaboradores (2001), administraram diferentes doses de imipramina e observaram que em doses acima de 15 mg/kg, a duração do tempo de imobilidade retornava a resultados não significativamente diferentes de animais tratados com o veículo.

No *Capítulo IIII*, estudamos os efeitos do protocolo de ECM seguido da administração crônica de harmina (15 mg/kg). Uma das alterações encontradas neste modelo foi a anedonia (diminuição na ingestão de sacarose), apresentada pelos animais submetidos ao ECM. Os dados comportamentais gerados por esse estudo confirmam e expandem os achados prévios de que a exposição de ratos a estressores crônicos leves e variados gradualmente induz um estado anedônico acessado pela redução do consumo de comida doce – sacarose (WILLNER, 1997; STOUT *et al*., 2000; GAMARO *et al*., 2003b). Willner e colaboradores (1987, 1998), tem relatado que exposições crônicas seqüenciais a uma situação de estresse provoca uma diminuição da sensibilidade a recompensa, o que, geralmente, é relatado como uma resposta anedônica de ratos ou camundongos.

Episódios de depressão podem alterar os sistemas noradrenérgico, dopaminérgico e serotoninérgico e juntamente com a alteração do eixo HHA, estar ocasionando alterações comportamentais e bioquímicas relacionadas à este transtorno (MÁXIME, 2007).

Encontramos um aumento do peso médio da glândula adrenal dos ratos estressados em comparação com o grupo controle. O estímulo prolongado e intensificado do ACTH nas células do córtex da adrenal promove uma hipertrofia da glândula com aumento do seu peso (HARRO *et al*., 2001; GAMARO *et al*., 2003b).

Este é um parâmetro indireto da avaliação de hiperativação do eixo HHA. A hiperativação desse eixo promove consequências deletérias aos neurônios, podendo representar uma das causas de redução numérica e volumétrica de distintas regiões encefálicas encontradas em estudos de neuroimagem de pacientes com transtorno depressivo grave recorrente (RAJKOWSKA, 2000; SHELINE *et al*., 2003; GONÇALVES *et al*., 2006).

Animais estressados e pacientes com depressão mostraram volume hipocampal reduzido (FUCHS *et al*., 2004; TSANKOVA *et al*., 2006) ocasionado em parte, pela desregulação do eixo HHA (RAJKOWSKA, 2000). O volume hipocampal reduzido pode ter contribuído para uma liberação menor de BDNF, bem como sua síntese. A diminuição da liberação de BDNF modifica os receptores para esta neurotrofina e, consequentemente, não revertendo a redução do volume hipocampal e ainda interferindo na síntese de neurotransmissores (NETSLER, 2002b).

Ao contrário do que se esperava, embora utilizando um modelo de estresse baseado em modelos de depressão, os animais submetidos ao protocolo de ECM tiveram um aumento significativo nos níveis de BDNF no hipocampo, que foram revertidos com a administração crônica de harmina (FORTUNATO *et al*., 2009a). A dor e o estresse são conhecidos como ativadores do eixo HHA e a estimulação deste sistema pode contribuir para a plasticidade do hipocampo (DURIC e McCARSON, 2006). É possível que os efeitos do ECM tenham produzido uma resposta adaptativa que tenha evidenciado o aumento nos níveis de BDNF. Adicionalmente, a administração crônica de antidepressivos pode regular a neurogênese e, consequentemente, reverter muitos dos efeitos causados pelo estresse no hipocampo (TSAI, 2003; DURIC e McCARSON, 2006).

A reversão do efeito antidepressivo da harmina no ECM parece não estar associada a nenhum efeito motor, pois a administração do antidepressivo isoladamente, não alterou a atividade locomotora no TCA (FORTUNATO *et al*., 2009a), indicando que o efeito observado é específico.

A administração crônica de harmina reverteu o comportamento anedônico, o aumento de peso da glândula adrenal e, consequentemente, os níveis de ACTH circulante, o que demonstra que este modelo tem uma boa validade preditiva.

Por fim, em conjunto à crescente evidência de que a indução da via BDNF/trkB é um dos mecanismos responsáveis pelos efeitos terapêuticos dos antidepressivos (modelo animal e humanos) (COYLE e DUMAN, 2003), este estudo é a primeira evidência de que efeitos indicadores de depressão a partir da administração da harmina estão associados ao aumento nos níveis de BDNF no hipocampo de animais experimentais. Em conclusão, os resultados obtidos neste trabalho auxiliam a compreensão do mecanismo de ação antidepressivo e neuroprotetor da harmina e de seu possível papel no tratamento da depressão.

IV CONCLUSÕES

1. Conclusão Geral

Os resultados apresentados neste estudo revelam que os tratamentos agudo e crônico com a β-carbolina harmina produziram efeito antidepressivo nos modelos animais utilizados.

2. Conclusões Específicas

- A administração aguda e crônica de harmina diminuiu o tempo de imobilidade no TNF.
- O efeito antidepressivo da harmina não está associado a nenhum efeito motor, já que as diferentes doses administradas não afetaram significativamente a atividade locomotora avaliada no TCA.
- A administração de imipramina (20 e 30 mg/kg), usada como controle positivo, diminuiu o tempo de imobilidade dos ratos tratados agudamente. No entanto, quando administrada cronicamente, na dose de 30 mg/kg, não apresentou resultados significativos para este parâmetro.
- Os níveis de BDNF foram aumentados no hipocampo dos ratos tratados aguda e cronicamente com harmina.
- O comportamento anedônico foi observado em animais submetidos ao protocolo de ECM e revertidos com a administração de harmina (15 mg/kg), durante sete dias consecutivos.
- Os animais submetidos ao protocolo de ECM também apresentaram aumento do peso médio da glândula adrenal e aumento nos níveis de ACTH

e de BDNF. Esses resultados também foram revertidos pela administração crônica de harmina (15 mg/kg).

V PERSPECTIVAS

Apesar de novas descobertas e avanços no estudo das bases neurobiológicas e abordagens terapêuticas na depressão, elevadas taxas de recorrência, sintomas subsindrômicos persistentes e refratariedade terapêutica são aspectos clínicos desafiadores nesta doença.

Sendo assim, a busca por substâncias capazes de induzirem uma rápida e sustentável melhora do quadro clínico deste transtorno se faz necessário. Se estudos futuros replicarem nossos achados, indicando o efeito antidepressivo da harmina, este composto poderá representar um promissor alvo farmacológico para o tratamento da depressão.

Nossa perspectiva futura é conduzir paralelamente estudos em modelos animais e em humanos. Em modelos animais, pretendemos complementar os dados mostrados nesse trabalho, para uma melhor compreensão do mecanismo de ação da harmina. Em humanos, nosso desafio é realizar ensaios clínicos de fase I e II que servirão como estrutura para que o efeito terapêutico da harmina possa ser avaliado de forma metodologicamente adequada.

No presente, estamos finalizando uma série de experimentos com esse modelo avaliando o efeito neuroprotetor da harmina através de propriedades antioxidantes. Nosso objetivo é testar a hipótese de que o efeito neuroprotetor possa estar correlacionado com a ação antidepressiva deste composto.

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