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GLUCOCORTICÓIDES E DEPRESSÂO: ANEDONIA INDUZIDA POR DEXAMETASONA

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Farmacologia, Programa de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná

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Dedico este trabalho aos meus pais e ao meu irmão, sem dúvida as pessoas mais importantes da minha vida.

Aos ratos que involuntariamente dedicaram suas vidas, literalmente, para realização do trabalho.

“A normalidade é tão somente uma questão de estatística”

(Aldous Huxley)

NOTA EXPLICATIVA

Esta dissertação é apresentada em formato alternativo – artigo para publicação – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná.
Constando de dois artigos abordando os experimentos realizados e a discussão dos resultados. Ambos foram formatados conforme as normas propostas pelas revistas escolhidas.

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RESUMO

O presente estudo foi desenvolvido para avaliar os efeitos da dexametasona sobre o comportamento (anedonia) e os níveis de BDNF hipocampais de ratos. Os animais tratados com dexametasona nas doses de 5 e 10 mg/kg, ip (única administração) apresentaram redução na preferência por sacarose em relação ao grupo controle, enquanto os animais tratados com 1 mg/kg de dexametasona não apresentaram tal comportamento. O tratamento com paroxetina (10mg/kg, ip, 14 dias) ou desipramina (10mg/kg, ip, 14 dias) foi capaz de reverter a anedonia induzida por dexametasona, e mais, o tratamento com paroxetina (10mg/kg, ip, 28 dias) foi capaz de reverter a anedonia induzida por estresse crônico (8 semanas). Além disso, os ratos apresentaram redução dos níveis de BDNF no hipocampo após 48h da administração de dexametasona 5 mg/kg. O tratamento com paroxetina (10mg/kg, ip, 14 dias) foi capaz de elevar os níveis de BDNF no grupo previamente tratado com dexametasona. Concluindo: a administração de uma dose de dexametasona foi capaz de promover tanto alterações bioquímicas quanto comportamentais nos animais, assim como tais alterações foram moduladas pelo tratamento com drogas antidepressivas. Os dados corroboram a hipótese do papel dos glucocorticóides na patogênese da depressão.

1. INTRODUÇÃO

1.1 GLUCOCORTICÓIDES E DEPRESSÃO

Há uma vasta literatura a respeito da relação funcional entre CRH (hormônio liberados de corticotrofina*)*, o eixo HPA (hipotálamo-pituitária-adrenais), glucocorticóides (GC), monoaminas e humor (Wong and Licinio 2004). De fato um aumento da atividade do eixo HPA é a mais comum e consistente alteração endócrina envolvendo a depressão. Os mecanismos sobre a influência dos corticosteróides são complexos e não estão completamente elucidados.

Os glucocorticóides possuem um amplo espectro de ações sobre o sistema nervoso central (SNC), tanto via não-genômica (rápida) quanto genômica (lenta), sendo esta última reflexo também das ações da via não-genômica. A via genômica da ação dos GC é mediada, basicamente, por duas classes de receptores nucleares. Os receptores mineralocorticóides (MR ou tipo I) são amplamente expressos no hipocampo, amigdala e hipófise e apresentam uma alta sensibilidade aos GC: normalmente estão saturados sob "condições de repouso" (níveis basais de GC no SNC). Já os receptores glucocorticóides (GR ou tipo II) são distribuídos por todo o SNC, são recrutados durante o pico do ciclo circadiano de secreção de GC e sob condições de estresse agudo e crônico (De Kloet et al 1994; Millan 2006)..

Uma série de estudos tem demonstrado o papel dos glucocorticóides (GC) na patogênese da depressão, estabelecendo uma relação entre tal patologia, o eixo HPA e o tratamento com antidepressivos. Embora o exato papel dos GC na depressão permaneça obscuro alguns estudos mostram evidências consistentes sobre a ligação entre hipersecreção de corticóides, depressão e os níveis do fator neurotrófico derivado do cérebro (BDNF – *brain derived neurotrophic factor*) (Nestler et al 2002).

A secreção de GC é controlada pelo eixo HPA da seguinte forma: o CRF é secretado pelo núcleo paraventricular do hipotálamo e estimula a liberação da corticotrofina (ACTH) da pituitária anterior, que, por sua vez, estimula a secreção de corticóides das glândulas adrenais. O eixo HPA é um componente essencial para a capacidade do organismo de responder ao estresse. A

excessiva estimulação desse eixo tem sido considerada na patogênese da depressão (Juruena et al 2004).

A hiperatividade do eixo HPA é freqüentemente observada em pacientes deprimidos, sendo manifestada como aumento na expressão de CRF no liquor, e redução do *feedback* inibitório dos GC sobre o eixo HPA e a hipercortisolemia. Além disso a não responsividade de pacientes deprimidos ao teste de supressão pela dexametasona e os níveis elevados de cortisol plasmático em pacientes deprimidos corrobora a hipótese do papel do eixo HPA na patogênese da depressão. Em modelos animais a hipersecreção de corticóides pode potenciar excitoxicidade dos neurônios piramidais do hipocampo, bem como inibir a produção de novas células neuronais no giro denteado. Muitas dessas alterações são prevenidas pelo tratamento antidepressivo (incluindo terapia farmacológica ou eletroconvulsoterapia) (Hoomisen et al 2003; Lamont et al 2001). Excesso de GC pode, ainda, ser um fator causador da atrofia hipocampal observada em pacientes deprimidos ou com PTSD (transtorno de estresse pós-traumático – *post-traumatic stress disorder*) (Dubovsky 2003).

1.2 BDNF E DEPRESSÃO

A hipótese “neurotrófica” da depressão e da ação de antidepressivos foi originalmente baseada em observações sobre o papel do estresse em modelos animais, na diminuição da expressão de BDNF hipocampal e no efeito oposto de terapia antidepressiva. Tais observações guiaram para a sugestão de que tais mudanças nos níveis de BDNF poderiam, pelo menos em parte, remediar os efeitos lesivos do estresse sobre o hipocampo. (Berton and Nestler 2006).

A infusão local de BDNF em regiões cerebrais específicas tem induzido efeitos antidepressivos em modelos animais de depressão. No teste de natação forçada (FST), a infusão de BDNF mostrou efeito de redução do tempo de imobilidade, parâmetro considerado efeito tipo-antidepressivo (Hoshaw et al 2005). No modelo de desamparo aprendido (*Learned Heplessness*) a infusão após o choque inescapável reduziu a latência e as taxas de erros em relação aos animais controle (Siuciak et al 1997). Juntos, esses dados corroboram a hipótese de que o BDNF é necessário para produzir respostas antidepressivas (Duman and Monteggia 2006).

Os modelos animais disponíveis atualmente, tanto para a prospecção de novas drogas antidepressivas quanto para o estudo da neurobiologia da depressão (como natação forçada, desamparo aprendido, bulbectomia olfatória e estresse crônico e imprevisível - *CMS*) baseiam-se, de alguma forma, na exposição do animal a algum evento estressante. No modelo CMS, por exemplo, o animal, após exposição prolongada (2-4 semanas) a situações ou eventos estressantes imprevisíveis, apresenta decréscimo na preferência por soluções adocicadas ou diminuição da auto-estimulação de áreas de recompensa no sistema nervoso central, o que se correlaciona com a anedonia (diminuição ou ausência da capacidade de sentir prazer) apresentada por paciente deprimidos (Willner 1997). Tal modelo guarda uma grande similaridade com a depressão clínica uma vez que o episódio depressivo de pacientes frequentemente é precedido de um evento estressante (Willner 2005). A validade de face desse modelo portanto é considerada muito boa, além disso a reversão da anedonia no modelo de CMS é obtida somente com tratamento antidepressivo prolongado (2-4 semanas), da mesma forma que ocorre na clínica (Paykel 2003). Entretanto o modelo guarda algumas limitações de reprodutibilidade, especialmente no que toca à adaptação do animal aos eventos estressantes (variabilidade individual) e definição dos eventos estressantes.

Baseado nessas evidências e trabalhos demonstrando que a redução do BDNF hipocampal por corticosterona (Dwivedi et al 2006) e os efeitos da dexametasona sobre neurônios hipocampais e estriatais (Haynes et al 2001) e ainda considerando que, em último passo, a apresentação de eventos estressantes causa a liberação de GC endógenos e estes podem ser responsáveis pela atrofia hipocampal e precipitação do episódio depressivo, postulamos a hipótese de que a administração de um agonista de receptores glucocorticóides (GR), como a dexametasona, poderia desencadear o mesmo efeito, porém sem as dificuldades de reprodutibilidade do modelo original, tanto na modificação comportamental dos animais (anedonia) quanto nos níveis de BDNF no hipocampo.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Avaliar o papel de GC em modelos animais de depressão

2.2 OBJETIVOS ESPECÍFICOS

Avaliar o efeito da dexametasona:

- No teste de preferência por sacarose e sua evolução ao tratamento com drogas antidepressivas;

- No nível de BDNF hipocampal após administração de dexametasona e no decorrer do tratamento com drogas antidepressivas.

3. ARTIGO 1: Repeated paroxetine treatment reverses anhedonia induced in rats by chronic mild stress or dexamethasone

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Abstract

The present study was designed to assess the effect of dexamethasone, a synthetic glucocorticoid receptor agonist, in the sucrose preference test in rats. Rats treated acutely with dexamethasone (5-10 mg/kg) showed a significant decrease in sucrose preference (anhedonia) in comparison to vehicle treated rats, although 1 mg/kg dexamethasone did not alter the sucrose preference. Daily paroxetine treatment (10 mg/kg, ip, 14 days) reversed the anhedonic effect of acute dexamethasone (5 mg/kg), while causing no increased sucrose preference in rats that received dexamethasone vehicle. The paroxetine vehicle treated rats showed anhedonia even 14 days after acute dexamethasone administration. Paroxetine (10 mg/kg, ip for 28 days) also reversed anhedonia induced by chronic mild stress (8 weeks). In conclusion, acute dexamethasone induced an enduring anhedonic state that was reversed by repeated paroxetine treatment. Thus, the present study adds new data to the evidence supporting an important role for glucocorticoid in depression.

Key words: anhedonia, antidepressant, depression, glucocorticoid, paroxetine, rat

1. INTRODUCTION

The precipitation of a depressive episode has been linked to stressful life events (Paykel, 2003), which can activate the hypothalamic-pituitary-adrenal (HPA) axis leading to an increase in plasma cortisol. Furthermore, dysregulation of HPA; such as lack of cortisol suppression by dexamethasone administration, increased cortisol secretion and blunted adrenocorticotropic hormone response to exogenous corticotropin-releasing hormone; are frequently associated with depression, suggesting that depression is related to a failure in HPA negative feedback, which would result in higher cortisol levels (Checkley, 1992; Holsboer, 2001; Barden, 2004; Juruena et al., 2004). This stress-induced increase in cortisol secretion is one underlying mechanism proposed for the stress-depression association (Holsboer, 2001; Mello et al., 2003; Paykel, 2003). The hippocampus, which shows signs of atrophy in patients with prolonged depression (Campbell and MacQueen, 2004), is vulnerable to stress and increased glucocorticoid levels (Campbell and MacQueen, 2004; McEwen, 2005). Since the hippocampus exerts a negative control over the hypothalamic-pituitary-adrenal (HPA) axis, its atrophy may induce impairment in HPA control, leading to cortisol hypersecretion (Holsboer, 2001; Barden, 2004). Thus, glucocorticoid is thought to play a major role in hippocampal atrophy and depressive symptoms. In this context, chronic antidepressant administration was shown to increase corticosteroid receptors, which can restore HPA negative feedback and normalize cortisol levels and HPA function (Barden, 2004). Furthermore, an abnormal dexamethasone suppression test after treatment-induced clinical improvement is associated with a higher risk of relapse and may present prognostic value for treatment (Dratcu and Calil, 1989; Ribeiro et al., 1993). Thus, it appears that there is an interrelationship between stress, high glucocorticoid levels and depression.

Several animal models of depression, such as the forced swim test, learned helplessness and anhedonia induced by unpredictable chronic mild stress, involve behavioral responses to stressful procedures. The anhedonia induced by unpredictable chronic mild stress consists in the repeated exposure of animals to unpredictable mild stress: tilted cage, food deprivation, paired caging, reduction of cage area, etc.; leading to a reduction in self-stimulation of

rewarding areas or in the consumption of palatable food or liquids, i.e. anhedonia. This model yields good similarity with clinical depression, since it was found that stressful life events frequently precede major depression episodes (Paykel, 2003; Willner, 2005). This model has also good face validity, since anhedonia, described as a marked diminished interest or pleasure in events that would normally be enjoyable, is a core symptom of major depression episodes according to DSM-IV criteria (APA, 1994; Willner, 2005). Furthermore, the reversal of anhedonia induced by unpredictable chronic mild stress requires 2-4 weeks of daily antidepressant treatment (Willner, 1997; Stout et al., 2000; Willner 2005), again showing good parallels with clinical data. Therefore, the chronic mild stress paradigm is adequate for providing insight into the neurobiology of depression (Willner, 1997; 2005). However, the anhedonia induced by unpredictable chronic mild stress was not reliably observed in some experiments (Harris et al., 1997; Nielsen et al., 2000; Bielajew et al., 2002). Another problem is that although some studies found an increase in plasma corticosterone in this model (Ayensu et al., 1995; Harris et al., 1997; Bielajew et al., 2002; Froger et al., 2004; Grippo et al. 2005a; Song et al., 2006), this effect was not consistently observed (Harris et al., 1997; Stout et al., 2000; Grippo et al. 2005b). Although these inconsistencies could be related to the strain of the experimental animals or procedural differences (e.g. nature, duration or frequency of stress) among the studies, they may be also related to individual variability and styles of coping with stress (Nielsen et al., 2000; Bielajew et al., 2002; Veenema et al., 2003; Anisman and Matheson, 2005). The administration of exogenous glucocorticoid would avoid some of these variables in the study of the role of glucocorticoid in stress-induced depression.

Thus, the main objective of the present study was to evaluate the effect of acute administration of dexamethasone, a synthetic glucocorticoid that binds to glucocorticoid receptors, on the sucrose preference of rats; a measure of anhedonia. If the dexamethasone-effect on anhedonia plays a significant role in depression neurobiology, it should be reversed by repeated antidepressant administration. Therefore, the influence of chronic treatment with paroxetine, a clinically effective antidepressant drug, regarding the effect of dexamethasone on anhedonia was also studied.

2. METHODS

2.1 Subjects

Adult male Wistar rats weighing between 200-300 g were used. The rats were housed in polypropylene cages with wood shavings as bedding, under controlled room conditions of light (12-h light-dark cycle, lights on at 7:00 a.m.) and temperature (22± 2^{o}C), with free access to food and water, except prior to the sucrose preference test or when they were submitted to chronic mild stress (see below). Two rats were housed in each cage (cage size: 41 x 32 x 16.5 cm) but they were isolated by a central aluminum wall, which divided the cage in two equal compartments and permitted minimal contact between them, but neither one consumed the food or water/ sucrose solution of the other. Thus, the rats were not absolutely isolated. All procedures were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals (Committee to Revise the Guide for the Care and Use of Laboratory Animals, 1996).

2.2 Drugs

Paroxetine (Eurofarma, São Paulo, Brazil) was dissolved in distilled water. Dexamethasone-acetate (DEG, Curitiba, Brazil) was suspended in saline containing 0.2% Tween 80. The vehicle of each drug was administered in the respective control rats. All drugs were administered intraperitoneally (i.p.) at a constant volume of 1.0 ml/kg. Dexamethasone or its vehicle were administered once, while paroxetine or its vehicle were administered for 14 (dexamethasone-induced anhedonia) or 28 days (chronic mild stress experiment). The paroxetine dose was chosen on the basis of previous studies in our laboratory (Consoni et al., 2006; Beijamini and Andreatini, 2003).

2.3 Sucrose preference test.

In all experiments, prior to the first sucrose preference test, all the rats were submitted to 48 h of forced exposure to 1% sucrose solution in order to habituate to them, during which sucrose solution was the only fluid available for consumption, followed by two days of free access to food and water. After this, the rats were submitted to water deprivation for 16h prior to performing the sucrose preference test; baseline test at day zero. The sucrose preference test

was performed in the rat's home cage: two pre-weighted bottles, one containing tap water and another containing 1% sucrose solution, were presented to each rat. The bottles were weighed again after 1h and the weight difference was considered to be the rat intake from each bottle. The sum of water and sucrose intake was defined as total intake and the sucrose preference was expressed as the percentage of sucrose intake from the total intake following the formula:

% sucrose preference = sucrose intake x100/ total intake

All tests were carried out weekly (each Tuesday) between 8:00 and 10:00 am, with a variable sequence of bottle positioning (for each rat, the side of sucrose or water bottles were changed from one test to another), in order to avoid habituation. After the sucrose preference test, all the rats received free access to food and water. After the baseline sucrose preference test, and prior to drug treatment or stress administration, the rats were paired according their preference and then distributed in experimental groups to form paired (matched) groups.

2.4 Chronic Mild Stress (experiment 1)

The rats were initially divided into two groups: stressed and nonstressed. The stressed group received a stress regimen over an eight-week period, consisting of weekly "unpredictable" (in fact, a pseudorandom sequence) mild stress, such as food and/or water deprivation, an overnight cage tilt, an overnight soiled cage, space reduction and continuous overnight illumination (Table 1). The pseudorandom sequence of stressors was used to avoid the rats developing any habituation to repeated mild stress. The nonstressed (control) group remained undisturbed, except for the previously described deprivation before the weekly sucrose consumption (each Tuesday, between 8:00 and 10:00 am) test and the handling necessary for animal care (cleaning cages) and drug administration (weighing, tail marking and drug administration).

After four weeks the stressed and nonstressed groups were subdivided into two paired subgroups (see above, n=6-8 rats/group): (1) stressed administered 10 mg/kg paroxetine; (2) stressed administered distilled water as vehicle; (3) nonstressed administered paroxetine and; (4) nonstressed

administered vehicle. Both treatments were administered for 28 days, intraperitoneally (i.p.), at a constant volume of 1.0 ml/kg.

2.5 Dexamethasone-induced anhedonia (experiment 2)

This experiment was performed to assess the possible role of glucocorticoid in anhedonia in rats. The rats (n=20) were submitted to a baseline sucrose preference test and then allocated to one of four paired groups: vehicle (control), 1, 5, and 10 mg/kg dexamethasone. Twenty-four and forty-eight hours later, respectively, the rats were submitted to a second and third sucrose preference test.

2.6 Effect of chronic paroxetine treatment on dexamethasone-induced anhedonia (experiment 3)

In this experiment, after a baseline sucrose preference test, 20 rats were divided into two paired groups (n=10/group), receiving a single dose of dexamethasone or vehicle. Then, following a second sucrose preference test (48 h), these groups were further subdivided into two paired groups (n=5/group), one treated with distilled water and the other with 10 mg/kg paroxetine. Thus, 4 groups were formed: (1) vehicle plus distilled water; (2) vehicle plus 10 mg/kg paroxetine; (3) dexamethasone plus distilled water; and (4) DEX plus 10 mg/kg paroxetine. On the experimental day, the paroxetine administration occurred 2 h after the sucrose preference test, which occurred weekly, each Tuesday, between 8:00 and 10:00 am.

2.7 Body Weight Gain:

The body weight gain was calculated as the difference between the final and baseline (day 0) body weight.

2.8 Statistical analysis

Each experiment of the sucrose preference test was submitted to two-way ANOVA with repeated measures, with drug treatment as an independent factor and treatment weeks as a dependent factor. Whenever a significant treatment x trial interaction was found, intergroup comparisons were realized for each week using one-way ANOVA, followed by the Newmann-Keuls *post-hoc*

test. Intragroup comparison was carried out by repeated measures ANOVA followed by the Newmann-Keuls *post-hoc* test. Body weight gain was analyzed by one-way ANOVA followed by the Newman-Keuls *post-hoc* test. Statistical significance was considered when $p < 0.05$.

Table 1. Stressors schedule applied to stressed group

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
sucrose preference test			8:00 – 10:00				
cage tilt		18:00------10:00				18:00-------10:00	
continuous overnight illumination				18:00------10:00			18:00------10:00
space reduction					18:00------10:00		
soiled cage			18:00----8:00				18:00------10:00
water deprivation				18:00----------18:00			
food deprivation					18:00----------18:00		
food + water deprivation	10:00----------10:00						

3. RESULTS

3.1 The effect of paroxetine on chronic mild stress-induced anhedonia:

Figure 1 shows the effect of chronic mild stress on the sucrose preference test. Two-way ANOVA indicated a significant effect for treatment: [$F(3, 25)= 31.72$, $p<0.001$]; weeks [$F(8, 200)= 9.65$, $p<0.001$] and interaction [$F(24, 200)= 4.48$, $p<0.001$]. One-way ANOVA revealed a significant difference in sucrose preference between groups on day 14 [$F(3, 25)= 3.84$, $p< 0.03$], day 21 [$F(3, 25)=, 10.39$, $p< 0.001$], day 28 [$F(3, 25)= 10.30$, $p< 0.001$], day 35 [$F(3, 25)= 14.80$, $p<0.001$], day 42 [$F(3, 25)= 10.68$, $p< 0.001$], day 49 [$F(3, 25)= 11.60$, $p< 0.001$] and day 56 11.19, $p< 0.001$]. No significant effect was seen between baseline [day 0: $F(3, 25)= 0.07$, NS] and day 7 [$F(3, 25)= 0.78$, NS]. Prior to paroxetine treatment onset (day 28), a significant reduction in sucrose preference (anhedonia) occurred in both stressed groups in comparison to nonstressed groups on the same day (both $p <0.01$), an effect that was sustained until day 42 for paroxetine (all $p < 0.01$) treated rats and until day 56 for water treated rats (all $p < 0.05$). On day 56 the stressed group treated with paroxetine showed no difference in comparison with the nonstressed group, but differed significantly from stressed rats treated with vehicle ($p<0.001$). Surprisingly, a significant effect occurred between the stressed plus paroxetine and stressed plus water groups on day 14 and 21 (both $p < 0.02$). Furthermore, a significant difference occurred between nonstressed rats and stressed plus water rats on day 21 (both $p <0.001$).

A difference in sucrose preference occurred throughout the experiment in all groups (nonstressed + water: $F(8, 40)= 2.80$, $p<0.02$; nonstressed + paroxetine: $F(8, 56)= 7.26$, $p< 0.001$; stressed + water: $F(8, 48)= 3.81$, $p< 0.01$; stressed + paroxetine: $F(8, 56)= 11.47$, $p< 0.001$). *Post-hoc* analysis showed that no significant difference occurred between baseline and any other day. In contrast, a significant reduction occurred in sucrose preference on some day when compared to baseline results in both stressed groups. In stressed rats treated with water, the sucrose preference was significantly lower that baseline on days 14, 21, 24, 28, 49 and 56 (all $p < 0.03$). On day 35 and 42 the differences were almost significant (both $p= 0.06$). In stressed rats treated with

paroxetine, the sucrose preference was significantly lower than baseline on day 14, 21 and 28 (all $p< 0.05$).

The CMS procedure led to a significant change in the total fluid intake throughout the experiment [$F (8, 200)= 6.588$, $p< 0.001$], but not between drug treatments [$F (3, 25)= 2.615$, $p> 0.05$] or treatment x weeks interaction [$F (24, 200)= 0.951$, $p> 0.05$].

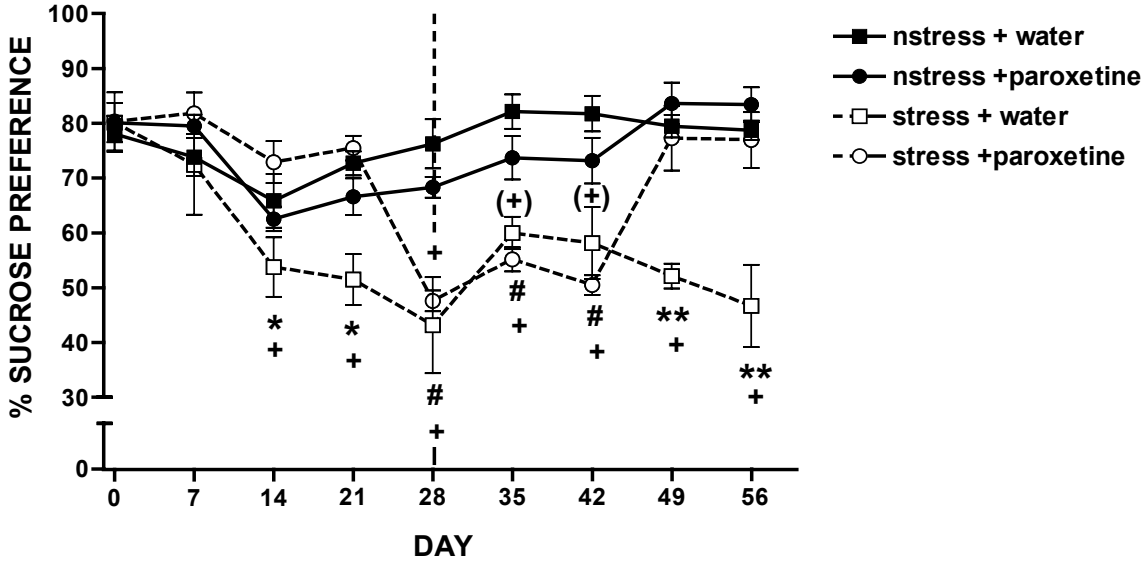

Fig 1. Effect of repeated paroxetine (10 mg/kg, i.p., 28 days) on chronic mild stress-induced anhedonia. stress: stressed rats; nstress: nonstressed rats. Data expressed as mean ± SEM (n=6-8 / group). Vertical dotted line indicates the onset of paroxetine treatment. Water: distilled water

* $p<0.05$, stressed plus water from stressed groups plus paroxetine on same day.

# $p<0.05$, stressed (plus water or paroxetine) from nonstressed (plus water or paroxetine)

** $p<0.05$ stressed plus water from all other groups

+ $p<0.05$ from baseline (day 0) for the same group.

(+) $0.05< p<0.07$ from baseline (day 0) for the same group

***3.2 Dexamethasone-induced anhedonia*:**

Two-way ANOVA indicated a significant effect for treatment: [$F (3, 16)= 5.34$, $p<0.0019$]; weeks [$F (2, 32)= 11.21$, $p<0.0001$] and interaction [$F (6, 32)= 2.44$, $p<0.0020$]. As shown in Figure 2, significant group differences occurred

after 24h [F (3, 16)= 5.98, p<0.01] and 48 h [F (3, 16)= 7.09, p<0.01] of dexamethasone administration. On both occasions a dose-dependent anhedonia induced by dexamethasone occurred, with a significant difference between the 5 and 10 mg/kg dexamethasone groups from the control group (all p<0.01). No significant difference was seen in baseline [F (3, 160)= 1.24, NS]

Significant reductions also occurred in sucrose preference from baseline (day 0 of the same group) in rats treated with dexamethasone 5 mg/kg [F (2, 8)= 11.37, p<0.01] and 10 mg/kg [F (2, 8)= 28.26, p<0.001]. The group treated with 1 mg/kg dexamethasone exhibited no difference from the control group at 24 and 48 h or from baseline [F (2, 8)= 1.25, NS].

The anhedonia exhibited by the 5 and 10 mg/kg dexamethasone groups was stable for one week (data not shown), when the experiment was finished. However, 60% of the rats that were administered 10 mg/kg dexamethasone died. Since a 5 mg/kg dexamethasone dose was able to induce anhedonia without mortality or other external signs of toxicity, this dose was chosen to perform subsequent experiments.

Dexamethasone administration presented a significant effect on the total fluid intake throughout the experiment [F (92, 32)= 30.22, p<0.001] and week x treatment interaction [F (6, 32)= 2.89, p<0.03], but not for treatment [F (3, 16)= 2.56, p> 0.05]. However, no significant difference was seen among treatments in any sucrose preference test [baseline: F (3, 16)= 2.83; 24 h: F (3, 16)= 2.64; 48 h: F (3, 16)= 2.47; all p>0.05].

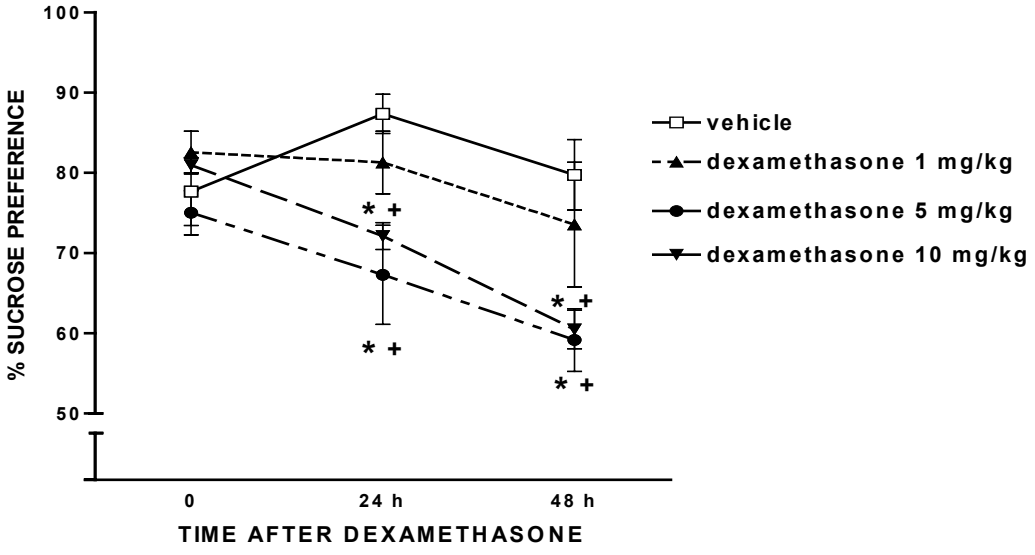

Fig.2 Dose-dependent Dexamethasone-induced anhedonia. Results expressed as means ± SEM (n=5/group).

* $p<0.05$ from vehicle group at same time.

$^{+}$ $p<0.05$ from baseline (time 0) results for the same group.

3.3 The effect of paroxetine on dexamethasone-induced anhedonia

Two-way ANOVA indicated a significant effect for treatment: [$F(3, 16)= 68.07$, $p<0.001$] weeks [$F(3, 48)= 39.59$, $p<0.001$] and interaction [$F(9, 48)= 13.11$, $p<0.001$]. Figure 3 shows the effect of paroxetine (10 mg/kg) on dexamethasone-induced anhedonia. No significant difference occurred between the groups in baseline [$F(3, 16)= 0.9523$, $p>0.10$]. However, significant differences were found at 2 [$F(3, 16)= 193.35$, $p<0.0001$], 9 [$F(3, 16)= 14.81$, $p< 0.0001$] and 16 days [$F(3, 16)=21.86$, $p < 0.0001$] after dexamethasone treatment. On day 2, a significant difference occurred between both groups treated with dexamethasone and the groups treated with dexamethasone-vehicle (all $p< 0.001$). On day 9, 7 days after the onset of paroxetine treatment, this difference was sustained (all $p < 0.001$). On day 16, after 14 days of paroxetine treatment, only the dexamethasone plus distilled water group differed from the other groups (all $p< 0.001$).

Repeated measures ANOVA indicated that a significant change occurred in sucrose preference in both dexamethasone treated groups [dexamethasone plus saline: $F(3, 12)= 57.11$, $p<0.001$; dexamethasone plus paroxetine: $F(3, 12)= 22.63$, $p<0.001$], but not in dexamethasone-vehicle [dexamethasone-vehicle plus paroxetine: $F(3, 12)= 2.54$, NS; dexamethasone-vehicle plus distilled water: $F(3, 12)= 1.53$, NS]. Both dexamethasone groups showed a reduction in sucrose preference 48 h after dexamethasone administration when compared to their baselines ($p<0.001$). Seven days of paroxetine treatment (day 9) was unable to modify this result ($p<0.001$), but 14 days of paroxetine treatment (day 16) reversed the anhedonic-like state induced by dexamethasone. However, the dexamethasone plus distilled water treated

group exhibited a persistent anhedonia even 16 days after dexamethasone treatment (day 14 of distilled water treatment; $p<0.001$).

No significant effect was seen in total fluid intake for treatment [$F(3, 16)= 1.71$, $p > 0.10$], week [$F(3, 48)= 2.79$, $p>0.05$] or treatment x week interaction [$F(9, 48)=1.42$, $p>0.10$].

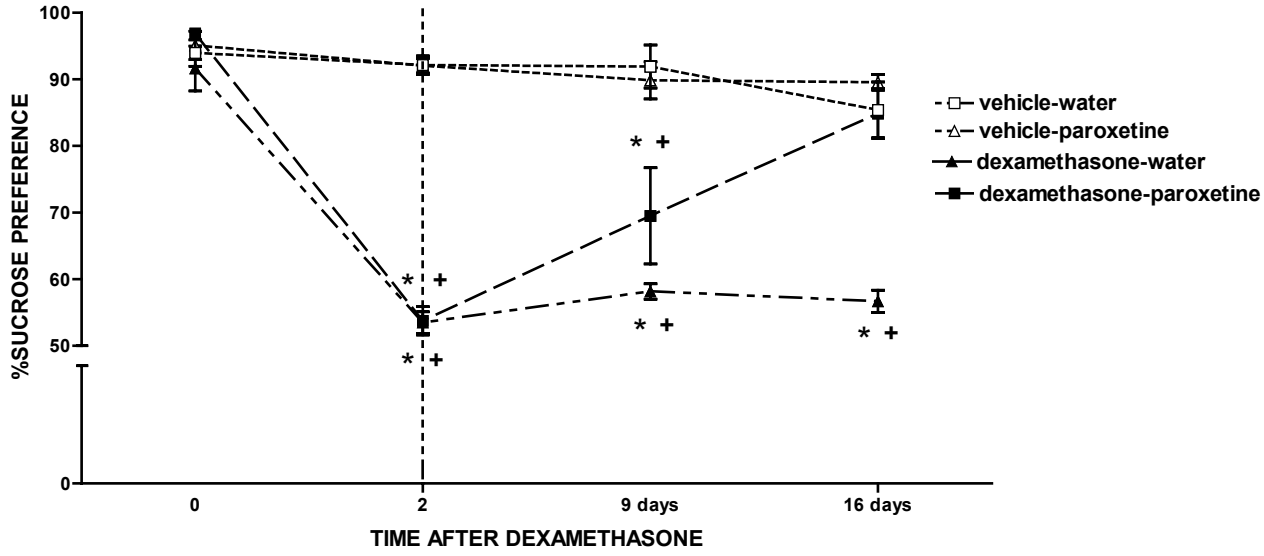

Fig. 3 Repeated treatment with Paroxetine (10 mg/kg, ip, for 14 days) reverses the Dexamethasone-induced anhedonia (5 mg/kg, one injection ip at time 0). Vertical dotted line indicates the onset of paroxetine treatment. Data expressed as mean ± SEM (n=5 / group). Water: distilled water

***** $p<0.05$ from control groups (vehicle plus water or paroxetine) at same time.

+ $p<0.05$ from baseline (time 0) measure for the same group.

3.4 Body Weight Gain:

In the CMS procedure (experiment 1), a significant effect occurred in body weight gain [nonstressed + water: 71.4 ± 9.3 g; nonstressed + paroxetine: 51.3 ± 7.4 g; stressed + water: -21.4 ± 11.6 g; stressed + paroxetine: 5.0 ± 9.4 g; mean ± SEM; $F(3, 25)= 19.13$, $p<0.0001$], which resulted from a higher weight gain in nonstressed rats (saline or paroxetine treated) than stressed saline treated rats (both $p<0.001$). Moreover, observation also revealed a reduced weight gain in the stressed group treated with paroxetine when compared to nonstressed saline treated rats (both $p<0.01$).

In dexamethasone-induced anhedonia (experiment 2), all the dexamethasone treated groups exhibited a significant reduction in body weight gain [vehicle: 24.6 ± 2.1 g; DEX 1 mg/kg: 3.8 ± 7.2 g; DEX 5 mg/kg: - 6.0 ± 6.9

g; DEX 10 mg/kg: - 13.4 ± 3.2 g; mean ± SEM; F (3,16)= 9.44, p<0.001] compared to vehicle treated rats (all p<0.02).

In experiment 3 (paroxetine effect on dexamethasone-induced anhedonia), a significant effect on body weight gain also occurred [vehicle + water: 24.0 ± 2.5 g; vehicle + paroxetine: 19.0 ± 5.8 g; DEX + water: - 7.0 ± 14.8 g; DEX + paroxetine: 1.0 ± 7.9; mean ± SEM; F (3,16)= 5.75, p< 0.01]. *Post-hoc* comparison showed that the vehicle plus vehicle group presented a higher body weight gain than both dexamethasone treated groups (plus saline or paroxetine; both p < 0.05). Furthermore, the vehicle plus paroxetine group presented a higher body weight gain than dexamethasone plus distilled water (p< 0.05).

4. DISCUSSION

The main finding of the present study is that dexamethasone induced a decrease in sucrose preference, which is suggestive of an anhedonic state. This anhedonia was similar to that found in the chronic mild stress model. Additionally, repeated, but not acute, administration of paroxetine (a clinically effective antidepressant drug) gradually restored the sucrose preference, indicating that paroxetine was able to reverse the dexamethasone-induced anhedonia, showing a similar profile to repeated paroxetine administration on anhedonia induced by chronic mild stress. Since no significant difference in total fluid consumption between the groups was observed, these effects were specific to sucrose preference.

The fact that anhedonia is one of the core criteria for major depression diagnosis and that it can be induced in rats by dexamethasone administration, supports the view that corticosteroid plays an important role in the neurobiology of depression. However, in contrast to the dexamethasone-induced anhedonia found in the present study, corticosteroid is also associated with rewarding effects. For example, chronic corticosterone administration reduced the current threshold for hypothalamic self-stimulation (Barr et al., 2000). This discrepancy may be related to methodological differences, like the difference in sensitivity of the hedonic indices (Nielsen et al., 2000) and corticosteroid administration; acute dexamethasone or chronic corticosterone administration. On the other hand, the anhedonic effect of dexamethasone observed in the present study is in agreement with other data, which found that high corticosteroid administration

reduces sexual performance (Gorzalka and Hanson, 1998), a behavioral change that also is included in the anhedonia criteria for a major depressive episode (APA, 2000). It is interesting that in depressive patients, failure in the dexamethasone suppression test was associated with anhedonia and suicide ideation (Oei et al., 1990). Furthermore, antidepressant treatment can normalize hypothalamic-pituitary-adrenal dysfunctions in patients with major depression and increase brain corticosteroid receptors in both animals and humans (Holsboer, 2001; Calfa et al., 2003; Barden, 2004; Juruena et al., 2004). Reinforcing the glucocorticoid role in depression, it has been shown that strategies that reduced glucocorticoid effects exerted an antidepressant-like effect in animal models (Veldhuis et al., 1985; De Kloet et al., 1988; Mitchel and Meaney, 1991; Peeters et al., 1992; Papolos et al., 1993; Baez and Volosin, 1994; Peeters and Broekkamp, 1994; Korte et al., 1996; Bachmann et al., 2005; Gregus et al., 2005; Rogoz et al., 2005; Johnson et al., 2006) and an antidepressant effect in patients (Young, 2006; Berton and Nestler, 2006).

The acute administration of dexamethasone induced an anhedonic state 24 h after treatment and this effect persisted until the end of the study; 16 days after dexamethasone administration. Thus this effect was not due to the presence of the drug, but the consequence of a more stable alteration induced by glucocorticoid. One structure that may mediate this deleterious effect of high glucocorticoid levels is the hippocampus, since a reduction in its volume has been frequently associated with depression and it is sensitive to high glucocorticoid levels (Graeff et al., 1996; Haynes et al., 2001, 2004; Campbell and MacQueen, 2004; McEwen, 2005). Considering the glucocorticoid receptor activity, the dexamethasone doses used in the present study were high when compared directly to a corticosterone dose used to mimic its rise in stressful procedures (Andreatini and Leite, 1994; Retana-Marquez et al., 2003), to block neurogenesis (Hellsten et al., 2002; Mayer et al., 2006), to increase immobility time (Johnson et al., 2006) or to reinstate the immobility time of adrenalectomized rats in the forced swimming test (Jefferys et al., 1983; Veldhuis et al., 1985; Mitchell and Meaney, 1991; Peeters et al., 1992; Peeters and Broekkamp, 1994). However, it is within the dose range of DEX used to induce hippocampal damage; 0.7 to 20 mg/kg (Haynes et al., 2001; Haynes et al., 2004). Interestingly, this dexamethasone-induced hippocampal damage was

attenuate by chronic pretreatment with antidepressants of different classes (Haynes et al., 2004). Moreover, factors other than receptor potency (e.g. multidrug-resistant P-glycoprotein on the blood brain barrier) may contribute to the magnitude of the glucocorticoid effect on the brain (Buckingham, 2006), which could partially explain this discrepancy between the dexamethasone and corticosterone dose range. In future experiments, it will be interesting to evaluate the effect of repeated low doses of corticosterone or dexamethasone on the sucrose preference test. The cellular mechanisms through which dexamethasone induces such effects were not addressed in the present study, but these effects may be mediated by brain derived neurotrophic factor, since glucocorticoid and stress decrease this factor and such effects are reversed by chronic antidepressant treatment (Dwivedi et al., 2006). Similarly, electroconvulsive seizures found in an animal model of electroconvulsotherapy in humans, reverse the reduction of neurogenesis induced by corticosterone administration (Hellsten et al., 2002). The time for paroxetine reversal of dexamethasone-induced anhedonia (14 days) is shorter than for CMS-induced anhedonia (21 days). This may be due to the fact that in dexamethasone-induced anhedonia, no other DEX administration occurred during the treatment with paroxetine, thus the rats were exposed only to one episode of high glucocorticoid plasma level; while in CMS-induced anhedonia, the stressful events remain during the drug treatment, thus these rats were submitted to repeated episodes of increased glucocorticoid plasma levels. Another possibility that could explain this difference is inter-experiment variability. On the other hand, the prolonged anhedonic-state of at least 2 weeks induced by one high dexamethasone dose administration showed that concurrent high glucocorticoid levels and depressive symptoms are not a requirement, which may partially explain the fact that high cortisol levels are not found in all depressed patients.

In view of the fact that certain reproducibility problems with anhedonia induced by pseudorandomized chronic mild stress present some difficulties with the use of this model (Vollmayr and Henn, 2003; Willner, 1997), the results of experiment 1 showed that the procedure was able to induce anhedonia, under the experimental conditions studied. Moreover, repeated, but not acute, paroxetine treatment reversed this anhedonia, with no alteration in total fluid intake. Although the reversal of CMS-induced anhedonia has already been

shown with other SSRI, like fluoxetine, sertraline, citalopram and escitalopram (Muscat et al, 1992; Marona-Lewicka and Nichols, 1997; Przegalinski et al, 1995; Montgomery et al, 2001) to our knowledge, this is the first report showing the ability of chronic paroxetine treatment to reverse anhedonia induced by chronic mild stress, which increases the pharmacological validity of the model.

Since both CMS (Matthews et al., 1995) and high glucocorticoid administration (Andreatini and Leite, 1994) can lead to body weight change and that controversy exists regarding the influence of body weight change on sucrose solution consumption (Matthews et al., 1995; Willner et al., 1996), it is important to measure this variable. In the present study, the CMS procedure led to lower body weight gain compared to saline treated nonstressed animals. All dexamethasone doses also led to reduced body weight gain compared to vehicle treated rats. In the last experiment regarding paroxetine reversal of DEX-induced anhedonia, both DEX treated groups also showed lower body weight gain compared to vehicle treated rats. These data are in agreement with previous data showing that chronic mild stress and high glucocorticoid administration lead to reduced body weight gain, which could be viewed as a confounding variable in the anhedonia results and, thus, could lead to false results. However, some points may indicated that this is not the case in the present study. First, it was suggested that although absolute sucrose consumption may be influenced by body weight gain, sucrose preference may not influenced, which makes this latter parameter a better index of anhedonia (Matthews et al., 1995). Second, the CMS and DEX administration induced a reduced weight gain both in water and paroxetine treated rats, although only the former showed a decrease in sucrose preference at the end of experiments. These results suggest some dissociation between body weight change and anhedonia. Finally, it is important to note that body weight reduction is also a diagnostic criterion for a major depressive episode in DSM-IV (APA, 1994) and their occurrence in the CMS procedure may be considered another indication of the face validity of the CMS procedure, instead of only a confounding variable.

In conclusion, the present results suggest that high glucocorticoid levels induce an anhedonic state, which can be reversed by repeated antidepressant treatment. These results reinforce the hypothesis that glucocorticoids play an important role in stress-induced depression and indicate that high glucocorticoid

levels can lead to depression and not the opposite. However, depression is a multifactorial disorder and hypothalamic-pituitary-adrenal dysfunction must be viewed as only one of many contributing factors. Additionally, the procedure described here is a useful approach for studying the role of glucocorticoid in depression.

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4. ARTIGO 2: The dexamethasone-induced anhedonia: decrease of brain derived neurotrophic factor hippocampal level is reversed by repeated paroxetine treatment.

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Abstract

The present study was designed to assess the effect of dexamethasone, a synthetic glucocorticoid receptor agonist, in the hippocampal brain derived neurotrophic factor (BDNF) and sucrose preference in rats. Rats treated acutely with dexamethasone (5 mg/kg) showed a significant decrease in BDNF levels in comparison to vehicle treated rats. Daily paroxetine treatment (10 mg/kg, ip, 14 days) enhanced the hippocampal BDNF levels in rats treated with dexamethasone and reversed the anhedonic effect of acute dexamethasone (5 mg/kg). Paroxetine treatment did not increase sucrose preference nor alter hippocampal BDNF level in rats that received dexamethasone vehicle. The dexamethasone plus vehicle treated rats showed anhedonia even 14 days after acute dexamethasone administration, although the BDNF level was not different from control groups. In conclusion, acute dexamethasone induced a decrease in BDNF levels in hippocampus and an enduring anhedonic state that was reversed by repeated paroxetine treatment. Thus, the present study adds new data to the evidence supporting an important role for glucocorticoid in depression.

Key words: anhedonia, antidepressant, depression, glucocorticoid, paroxetine, rat, BDNF

1. INTRODUCTION

Several studies suggest a critical role of glucorticoids (GC) in the pathogenesis of depression, indicating a relationship between this disorder, HPA axis dysfunction and antidepressant action. Although the exact role of corticoids in depression remains unclear, some studies showed a link between corticoid hypersecretion, depression and brain derived neurotrophic factor (BDNF) levels in hippocampus (Nestler et al 2002).

Glucocorticoid release is controlled by the hypothalamic–pituitary–adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) released by the paraventricular nucleus of the hypothalamus stimulates the release of corticotrophin (ACTH) from the anterior pituitary, which, in turn, stimulates glucocorticoid secretion from the adrenal cortex. The HPA axis is an essential component of an individual's capacity to cope with stress. It was observed that stressful life events frequently preceded unipolar depressive episodes (Holsboer 2001; Paykel 2003) and corticosteroids may mediate the remembering of negative events in depressive patients (Peeters and Broekkamp 1994). Hyperactivity of the HPA axis is commonly observed in patients with depression, as manifested by increased expression of CRH in the hypothalamus, increased levels of CRH in the cerebrospinal fluid (CSF) and plasma cortisol, and reduced feedback inhibition of the axis by CRH and glucocorticoids. Thus, the stress-induced HPA hyperactivity may play an important role in pathophysiology of depression (Barden 2004; Holsboer 2000; Holsboer 2001)

In animal models, glucocorticoids (corticosterone or dexamethasone) exerted a depressive role, such as increase immobility in the forced swimming test (Baez and Volosin 1994) and treatments that reduce glucocorticoid function (eg. mifepistrone, a glucocorticoid receptor antagonist, or metyrapone, a corticosterone synthesis inhibitor) induced an antidepressant-like effect, such as decrease immobility time in the forced swimming test (Bachmann et al 2005). Previous study in our lab observed a decrease in sucrose preference by dexamethasone administration, which suggests that glucocorticoids can play a role in anhedonia, a core symptom of major depressive episode. This anhedonic state can be reversed by repeated, but not acute, paroxetine administration (Casarotto and Andreatini, *in press*). Exogenous glucocorticoids

(dexametashone or corticosterone) can lead to hippocampal damage as well as inhibit the birth of new granule cell neurons in the hippocampal dentate gyrus and hippocampal gliogenesis. Many of these changes can be prevented by antidepressant treatment (Haynes et al 2004; Haynes et al 2001; Hellsten et al 2002; Mayer et al 2006; Wennström et al 2006; Wong and Herbert 2005). Thus, Excessive glucocorticoids could, therefore, be a causative factor for the small reductions in hippocampal volume that have been reported in patients with depression (Dubovsky 2003). In this line, patients receiving chronic corticosteroid treatment showed smaller hippocampal volume compared to controls (Brown et al 2004).

The neurotrophin family of signaling proteins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5, is crucially involved in regulating the survival and differentiation of neuronal populations during development. Brain-derived neurotrophic factor (BDNF) is a 27-kDa polypeptide that is recognized as playing an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and in adulthood. It is well known that BDNF participates in use-dependent plasticity mechanisms such as long-term potentiation, learning, and memory (Aleisa et al 2006; Hashimoto et al 2004). Neurotrophins activate one or more receptor tyrosine kinases of the tropomyosin-related kinase (Trk) family. NGF binds preferentially to TrkA, BDNF and NT-4 to TrkB, and NT-3 to Trk C. In addition to Trk receptors, all neurotrophins bind to the p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor superfamily. The role of p75NTR is slowly beginning to emerge. One important function may be facilitation of Trk activation, either by presenting the neurotrophin to Trks or by inducing a favorable conformational change in the receptor (Bramham and Messaoudi 2005). The neurotrophic hypothesis of depression and antidepressant action was originally based on findings in rodents that acute or chronic stress decreases expression of BDNF in the hippocampus and that diverse classes of antidepressant treatment produce the opposite effect and prevent the actions of stress (Duman et al 2001). These observations led to the suggestion that perhaps such changes in BDNF could in part mediate the structural damage and reduced neurogenesis seen in the hippocampus after stress. On autopsy, reduced BDNF levels in the

hippocampus have been reported in some patients with depression — an abnormality not seen in patients treated with antidepressants (Berton and Nestler 2006). It is interesting that exogenous administration of corticosterone decreased hippocampal BDNF level, an effect that was reversed by repeated antidepressant administration (Dwivedi et al 2006).

Based on the above findings, we hypothesized that a synthetic GC, such dexamethasone, could induce (a) anhedonia that can be reversed by repeated antidepressant administration; (b) a BDNF decrease, which could be reversed by a clinical-used antidepressant.

2. MATERIAL AND METHODS

2.1 Subjects.

Adult male Wistar rats (weighting between 200-300 g) were used. The animals were housing individually in polypropylene cages with wood shavings as bedding, under controlled room conditions of light (12-h light-dark cycle, lights on at 7:00 a.m.) and temperature (22 ± 2^{o}C), with free access to food and water, except prior sucrose preference test (see below). Two animals were housed in each cage (cage size: 41 x 32 x 16.5 cm) and an aluminum wall separated them. All procedures were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals (Committee to Revise the Guide for the Care and Use of Laboratory Animals, 1996).

2.2 Drugs.

Paroxetine (Eurofarma, São Paulo, Brazil) was dissolved in distilled water. Dexamethasone-acetate (DEG, Curitiba, Brazil) was suspended in saline containing Tween 80 at 0.2%. The vehicle of each drug was administered in the respective control rats. All drugs were administered intraperitoneally (i.p.) at constant volume (1.0 ml/kg), between 11:00 – 12:00 am.

2.3 Sucrose preference test.

In all experiments, before the first sucrose preference test all rats were submitted to 48 h period of forced exposition to 1% sucrose solution in order to habituate to it. During the forced exposition period, sucrose solution was the only fluid available for consumption; this period was followed by two days of free

access to food and water. After this habituation, the rats were submitted to water deprivation for 16h prior to performing the sucrose preference test (baseline test at day zero). The sucrose preference test was performed in the rat's home cage: two pre-weighted bottles, one containing tap water and another containing 1% sucrose solution, were presented to each rat. The bottles were weighed again after 1h and the weight difference was considered to be the rat intake from each bottle. The sum of water and sucrose intake was defined as total intake and the sucrose preference was expressed as the percentage of sucrose intake from the total intake following the formula:

% sucrose preference = sucrose intake x100/ total intake

All tests were carried out between 8:00 and 10:00 am. After the sucrose preference test, all the rats received free access to food and water. After the baseline sucrose preference test, prior to any drug treatment, the rats were paired according their preference and then distributed in experimental groups to form paired (matched) groups.

2.4 Tissue collection, sample preparation and BDNF assay.

The same procedure was used in both Experiment One and Two. Following decapitation, meninges were removed and hippocampi were isolated on ice. Punches from each animal were processed for the quantification of BDNF protein by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Promega, BDNF Emax® Immunoassay System, Cat # G7610). Briefly, tissue was sonicated to achieve homogenate in microcentrifuge tubes in lysis buffer containing a cocktail of protease inhibitors (137 mM NaCl, 20 mM Tris, 1% Nonidet P-40, 10% glycerol, 1 mM PMSF, 10 mg/ml aprotinin, 1 mg/ml leupeptin, 0.5 mM Na-orthovanadate; all from Roche – Sao Paulo – SP). Following homogenization, samples were centrifuged at 6000 g cycles for 30 min at 4^{o}C to pellet cellular debris. Supernatant was collected, diluted 5-fold with DPBS (2.7 mM KCl, 0.137 M NaCl, 1.47 mM KH_2PO_4, 8.1mM Na_2HPO_4, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, pH 7.35), an aliquot was removed from each sample to determine protein concentration using the colorimetric method of Bradford (Bradford 1976). Briefly, protein concentration was quantified by comparing the colorimetric intensity of the reaction product from

each sample with a serie of protein standard dilution (bovine serum albumin – BSA). This allowed for expression of BDNF levels to be normalized to total protein content and, thus, results will be expressed as pg of BDNF per µg of protein. ELISAs were performed in 96-well plates as per kit instruction. The antibodies used in this kit have very little cross-reactivity (<3%) with related growth factors (i.e. NGF, NT-3, NT-4/5). All samples were assayed in triplicate along with a known dilution series ranging from 0 to 500 pg/ml of BDNF standard (supplied by the kit). Colorimetric detection of peroxidase activity was achieved by adding tetramethylbenzidine (TMB One) solution and incubating for 10 min at room temperature. The enzymatic reaction was stopped with chloridric acid (HCl) 1 M and the optical density of each well was measured at 450 nm using a plate reader. A standard curve was generated using values from the dilution series and was used to determine the concentration of BDNF in each of the tissue samples.

2.5 Experiment One.

In this experiment 10 animals were divided in 2 groups receiving a single dose of dexamethasone (5 mg/kg ip) or vehicle and monitored with the sucrose preference test before (day 0), 24 and 48 hours after the drug treatment and than decapitated to perform tissue collection and sample preparation as described above.

2.6 Experiment Two.

In this experiment 20 animals were divided in 2 groups receiving a single dose of dexamethasone (5 mg/kg ip) or vehicle and monitored with the sucrose preference test as done in Experiment One. After 48h the animals were subdivided in another 2 groups: vehicle receiving distilled water (*vehicle-water*), vehicle receiving paroxetine (vehicle-paroxetine), dexamethasone receiving distilled water (dexamethasone-water) and dexamethasone receiving paroxetine (dexamethasone-paroxetine); with 5 animals in each group. The animals were treated for 14 days and monitored by the sucrose preference test weekly. At the 14th day after the beginning of paroxetine or water treatment the animals were decapitated 2h after the last injection to perform sample preparation as described above.

2.7 Body Weight Gain:

The body weight gain was calculated as the difference between the final and baseline (day 0) body weight.

2.8 Statistical analysis

The sucrose preference tests were submitted to a two-way ANOVA with repeated measures with drug treatment as independent factor and treatment weeks as dependent factor. Whenever a significant treatment x trial interaction was found, inter-group comparisons were made at each week using one-way ANOVA, followed by Newmann-Keuls test. The analysis in sucrose preference change across the experiment within treatment group was performed by one-way ANOVA for repeated measures followed by Newmann-Keuls test. Acute dexamethasone treatment effects on hippocampal BDNF levels and sucrose preference were evaluated by Student's t-test for independent samples. Levels of statistical significance were considered when $p < 0,05$.

3 RESULTS

3.1 Experiment One:

3.1.1 Dexamethasone-induced anhedonia

As showed in Figure 1 there is an anhedonic-like state induced by a single dose of dexamethasone over the animal sucrose preference, with a significant difference between dexamethasone and vehicle groups 48h after the treatment [$t(6) = 2,74370$, $p < 0,026$]. On the other hand, no significant effect was seen on sucrose preference after 24h [$t(6) = 0.898$, NS]

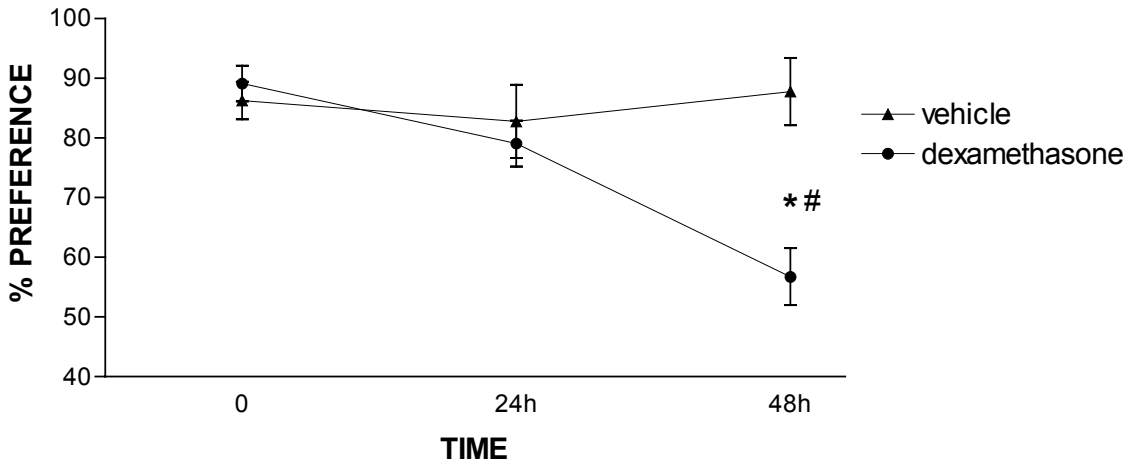

Fig. 1. Effect of acute dexamethasone (5mg/kg, ip, single injection) on preference sucrose test. Data expressed as Means ± SEM (n=5/group).

*** p< 0.05 from *vehicle* group.**

 # p< 0.05 from basal (0) in the same group.

3.1.2 Hippocampal BDNF levels after dexamethasone acute treatment.

As seen in Figure 2 there is a BDNF protein levels decrease 48h after dexamethasone treatment [t(6)= 3,223, p< 0,017].

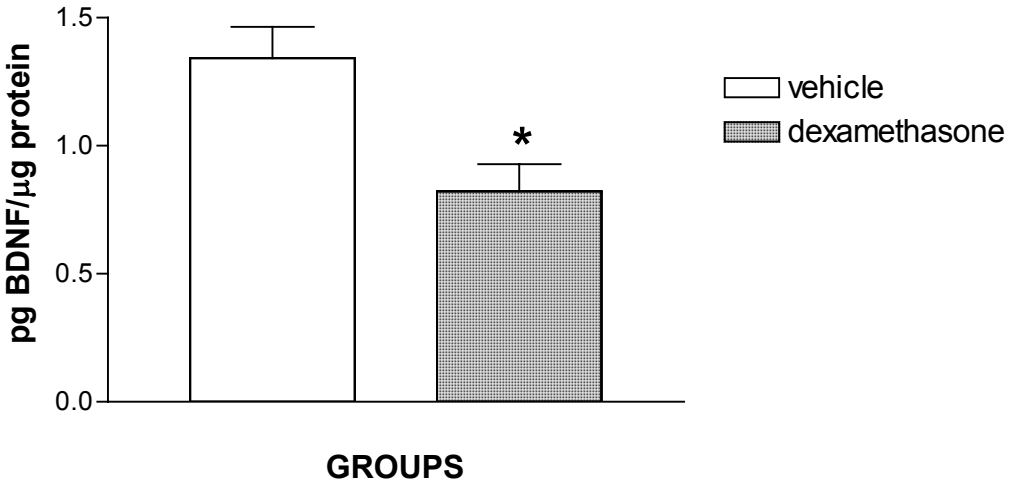

Fig. 2 Effect after 48h of dexamethasone (5mg/kg, ip, single injection) on hippocampal BDNF levels (pg/□g of total protein). Data expressed as Means ± SEM (n=5/group).

*** p< 0.05 from *vehicle* group.**

3.2 Experiment Two

3.2.1 Repeated Paroxetine treatment on dexamethasone-induced anhedonia.

The two-way ANOVA indicated a significant effect for treatment [F(3,16)= 13,213; p< 0,0001] weeks [F(3,48)= 4,97, p< 0,0044] and interaction [F(9,48)= 3,16; p< 0,0045]. The Figure. 3 shows the Paroxetine (10 mg/kg) reversion of dexamethasone-induced anhedonia. The time points analysis showed differences at day 2 [F(3,16)= 8,82; p< 0,0011], day 9 [F(3,16)= 6,57; p< 0,0042], and day 16 [F(3,16)= 15,17; p< 0,0001]. At day 2 and 9 the dexamethasone treated groups (plus water or paroxetine) exhibited a reduced sucrose preference when compared to control groups [at day 2: F(3,16)=8.82; p< 0.0011; at day 9: F(3,16)=6.57; p<0.0042]. Furthermore, at day 9 there is also a significant difference between dexamethasone groups ($p < 0.05$). At day 16 only the dexamethasone-water group exhibited difference from others.

In dexamethasone-paroxetine group, the within analysis showed that dexamethasone induce an anhedonic state at days 2 and 9, that was reversed at day 16 (14 days of paroxetine treatment). The dexamethasone-water group exhibited a persistent anhedonia from day 2 to day 16. The control groups (vehicle-water and vehicle-paroxetine) didn't show any alteration in the sucrose preference through the experiment time [vehicle-water F(3,12)= 0.08; vehicle-paroxetine F(3,12)= 2.14].

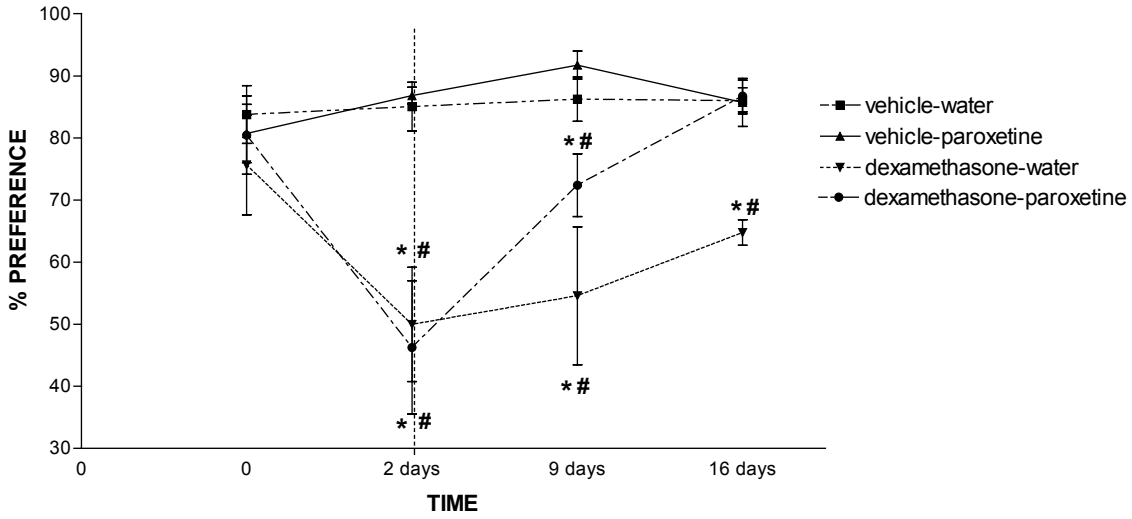

Fig. 3 Effect of repeated Paroxetine (10 mg/kg, ip, for 14 days) treatment in the Dexamethasone-induced anhedonia (5 mg/kg, single injection, ip, at time 0). Data expressed as mean ± SEM (n=5 / group). Vertical dotted line indicates the onset of paroxetine treatment. Water: distilled water.

*** p< 0.05 from control groups (dexamethasone-vehicle treated groups) at same time.**

 # p< 0.05 from baseline (time 0) measure in the same group.

3.2.2 Repeated Paroxetine treatment on dexamethasone-induced BDNF decrease.

As seen in Fig 4 repeated paroxetine treatment was able to elevate the BDNF levels in dexamethasone-paroxetine group but not in the vehicle-paroxetine group [$F(3,8)= 7.26$; $p< 0.0113$].

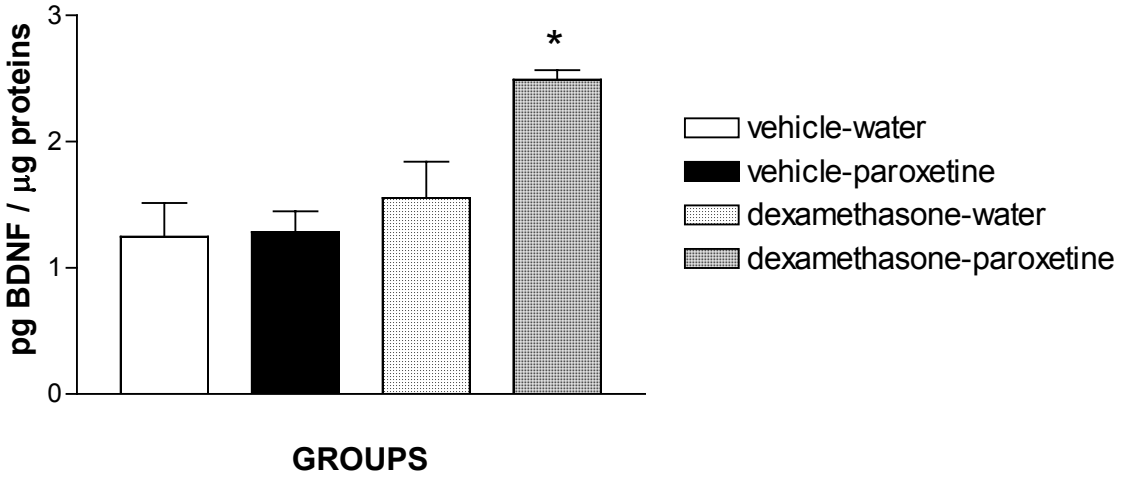

Fig. 4 Effect of Paroxetine (10mg/kg, ip, 14 days) on hippocampal BDNF levels (pg/□g of total protein). Data expressed as Means ± SEM (n=3/group). Water: distilled water

*** p< 0.05 from other groups.**

3.3 Body weight gain and total fluid intake.

In Experiment One there was a significant difference in body weigh gain [vehicle group: 6.0 ± 2.45g; dexamethasone group: -15.0 ± 2.24g; mean ± SEM; t= 6.33; p< 0.001]. No significant difference in total fluid intake [t= 0.97; p< 0.35 at time 0; t= 1.46; p< 0.18 at 24 hours; t= 1.52; p< 0.16 at time 48 hours].

In Experiment Two there was a significant difference in body weight gain also occurred [vehicle-water: 21.4 ± 1.6 g; vehicle-paroxetine: 24.2 ± 3.4 g; dexamethasone-water: -26.0± 5.5 g; dexamethasone-paroxetine: -3.4 ± 2.7 g; mean ± SEM; F (3,16)= 45.15; p< 0.01]. Post-hoc comparison showed that the vehicle-water and vehicle-paroxetine groups presented a higher body weight gain than both dexamethasone treated groups (plus water or paroxetine; both p < 0.05). Furthermore, the dexamethasone-paroxetine group presented a higher body weight gain than dexamethasone-water (p< 0.05). The total fluid intake was not affected during the experiment [F (9,48)= 0.56; p< 0.82].

4. DISCUSSION

4.1 Experiment One.

The main finding of this experiment is that the BDNF protein level was lower in hippocampus of rats 48h after a single injection of dexamethasone (5mg/kg), which is in parallel with reduction in sucrose preference. The BDNF data is in agreement with earlier reports about the effects of corticoids on BDNF mRNA, specially in hippocampal structures (Schaaf et al 1998). Some studies suggest that stress, activating the HPA-axis and thus elevating glucocorticoids levels, decreases hippocampal BDNF mRNA in rats (Smtih et al 1995) and induces changes in GR expression and function (Mizoguchi et al 2001). Moreover it was verified a stress-induced decrease in cell proliferation in hippocampus (Malberg and Duman 2003).

In this study a high dose of dexamethasone was used, it means that there was, preferentially, activation of GR instead mineralocorticoid receptor (MR), but how dexamethasone could induce neuronal sublethal damage on hippocampus remains unclear. The BDNF decrease could be expected following the dexamethasone-induced neuronal loss (Haynes et al 2001) by two ways: (a) direct effect or (b) by a “chemical adrenalectomy”, these two hypothesis does not abolish each other. Following the first one dexamethasone could induce neuronal death by inhibiting the glucose transport, decreasing the cell energy supply and compromising the glutamate release and uptake (Brooke et al 1998), triggering the calcium-dependent proteases cascades. Glucocorticoids could also reduce the antioxidant enzymes capacity (McIntosh and Sapolsky 1996) contributing for neuronal degeneration and consequently BDNF decrease.

The second hypothesis explain the neurotoxocity by an “indirect way”: since dexamethasone is able to suppress the HPA-axis function would be acceptable that this GR agonist suppress the corticosterone releasing and its effects on hippocampal cells’ MR (Maclennan et al 1998). The MR signaling pathway promotes cell surviving in hippocampus (De Kloet et al 1994), once this receptor activation induces the expression of bcl-2 mRNA (McCullers and Hermann 1998).

Several animal models of depression employed stress-induced behavioral changes that were sensitive to antidepressant administration. In the chronic mild stress procedure (CMS), anhedonic state was induced by repeated (3-4 weeks) unpredictable mild stress (Willner 2005). In the present study, it was found that DEX induced anhedonia faster than CMS procedure. Thus, our study suggests that the BDNF protein decrease follows the dexamethasone-induced neuronal damage described in literature and is parallel to the anhedonic-like state exhibited by the animals in the sucrose preference test, which suggests that glucocorticoids may play an important role in the pathophysiology of depression.

4.2 Experiment Two.

Two new hypotheses, which are complementary rather than mutually exclusive, have been forwarded to explain how antidepressants work at the neurobiological level. One hypothesis focused on the effects of activation of the cAMP (cyclic adenosine monophosphate) cascade through cell membrane receptors, followed by enhanced induction of CREB (cAMP Response Element Binding Protein) and hippocampal BDNF. Based on this hypothesis, the stress (or DEX)-induced decrease in BDNF is reversed by antidepressants, such as paroxetine, because the BDNF gene contains a cAMP response element (CRE), that, following CREB activation enhances BDNF transcription (Carlezon et al 2005). This neurotrophic factor, when injected centrally produces “antidepressant-like” behavioral changes in rats in the forced swimming test (Hoshaw et al 2005). But this theory does not explain the ineffectiveness of paroxetine to enhance the vehicle-paroxetine group BDNF level or the absence of difference between dexamethasone-water group and vehicle treated groups after 14 days found in the present study. However, although several studies showed an antidepressant-induced up-regulation of BDNF level that was cited as a strong evidence for the molecular hypothesis of depression, some studies did not found this effect (Duman and Monteggia 2006). This discrepancy could be due to difference in drug treatment such as dose or time schedule (Duman and Monteggia 2006). The difference in hippocampal BDNF levels between paroxetine treated groups found in the present study can be explained by a need of a lesion signal plus antidepressant treatment to increase BDNF level.

The recovery of BDNF level 16 days after DEX treatment, in contrast to decrease found 48 hours after DEX administration, may suggest that acute DEX acts a trigger for hippocampal function change, accomplished by hippocampal BDNF reduction and anhedonia, and that BDNF normalization was not sufficient to restore hedonic response.

The second theory establishes that antidepressants act improving corticosteroid receptors (CR) function (Holsboer 2000). This theory supports better the observed results. Supposing that the dexamethasone treatment induces changes in HPA-axis set-point by desensitization of CR in hippocampal cells it is expected that, based in this hypothesis, paroxetine exerts its effects in dexamethasone-paroxetine group but not in the vehicle-paroxetine group. The anhedonic-like state exhibited by dexamethasone-water group could be also explained by this theory once the CR function remains unaltered (desensitized) after dexamethasone administration, and BDNF expression can be regulated by other mechanisms.

As said previously, these two theories are not mutually exclusive. Thus, there is substantial cross-talking between CR signaling and CREB phosphorylation pathways. Further studies are necessary to explain the relationship between CR, CREB, hippocampal function and pathogenesis of depression.

5. CONCLUSION

It was found that dexamethasone was able to induce BDNF decrease and anhedonia, effects that were reversed by repeated paroxetine administration, a clinically used antidepressant. These results indicate an important role for glucocorticoids in depression and a possible link between glucocorticoid and BNDF in the neurobiology of depression and in the cellular effects of antidepressant. Moreover, the acute dexamethasone administration appears to be a valid animal model for studies concerning depression and the role of glucocorticoids in this pathology.

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5. CONCLUSÃO

O presente estudo demonstrou a regulação dos níveis de BDNF por um agonista glucocorticóide exógeno. Nós observamos que a dexametasona foi capaz de induzir alterações tanto comportamentais quanto bioquímicas nos animais submetidos a uma única administração da mesma e que o tratamento com paroxetina foi capaz de alterar os mesmos parâmetros de modo similar ao observado na anedonia induzida por estresse crônico, o que contribui para a validação do modelo. Esperamos que num futuro próximo a administração de dexametasona possa ser empregada como modelo animal para estudos envolvendo tais patologias e o papel dos glucocorticóides nas mesmas.

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7. APOIO FINANCEIRO

CAPES e CNPQ

8. ANEXOS

8.1 EXPERIMENTOS COMPLEMENTARES

ANÁLISE ESTATÍSTICA

Os experimentos 8.1.1 e 8.1.2.3 foram submetidos a ANOVA de 2 vias com 2 fatores (tempo e tratamento), seguido de *post-hoc* de Newmann-Keuls. Quando um nível de significância estatística foi identificado ($p<0,05$) o experimento foi submetido a ANOVA de 1 via seguido de *post-hoc* de Newmann-Keuls. Os experimentos 8.1.2.1 e 8.1.2.2 foram submetidos ao teste T de Student.

8.1.1 Tratamento repetido com desipramina para reversão da anedonia induzida por dexametasona.

O presente estudo foi realizado para avaliar a extensividade do modelo de anedonia induzida por dexametasona a drogas antidepressivas noradrenérgicas. Os animais utlizados são da mesma procedência dos utilizados nos outros experimentos e submetidos ao mesmo protocolo experimental dos experimentos com paroxetina.

Os animais (n=28) foram igualmente divididos em 4 grupos: *veículo-água, veículo-desipramina, dexametasona-água, dexametasona-desipramina* 48h após a administração de dexametasona (5mg/kg) os animais foram tratados por 14 dias com desipramina (10mg/kg) e monitorados pelo teste de preferência por sacarose semanalmente.

DROGAS:

- Dexametasona (DEG, Curitiba) suspensa em Tween 80 0,2% em salina;
- Desipramina (Sigma, São Paulo) dissolvida em água destilada;

Todas as drogas foram injetadas por via intraperitoneal (ip) em volume constante de 1,0 ml/kg/animal entre as 11:00 e 13:00 horas.

A análise ANOVA de 2 vias mostrou diferença significante no fator tratamento [F(3, 23)= 24.77, P< 0.0001], tempo [F(3, 69)= 6.71, P< 0.0005] e interação [F(9, 69)= 7.95, P< 0.0001], como mostrado na Fig. 1. Não houve diferença quanto aos valores basais dos grupos [F(3, 24)= 2.33, P> 0.05]. Foram observadas diferenças nos dias 2 (48h), 9 e 16 após o tratamento com dexametasona. No dia 2 houve diferença entre os grupos tratados com dexametasona e os tratados com veículo (p<0.001). No dia 9 (7 dias após o início do tratamento com desipramina) tal diferença ainda era verificada. No 16º dia, ou seja, 14 dias após o início do tratamento com desipramina, somente o grupo *dexametasona-água* apresentou diferença em relação aos grupos controle (p<0.001).

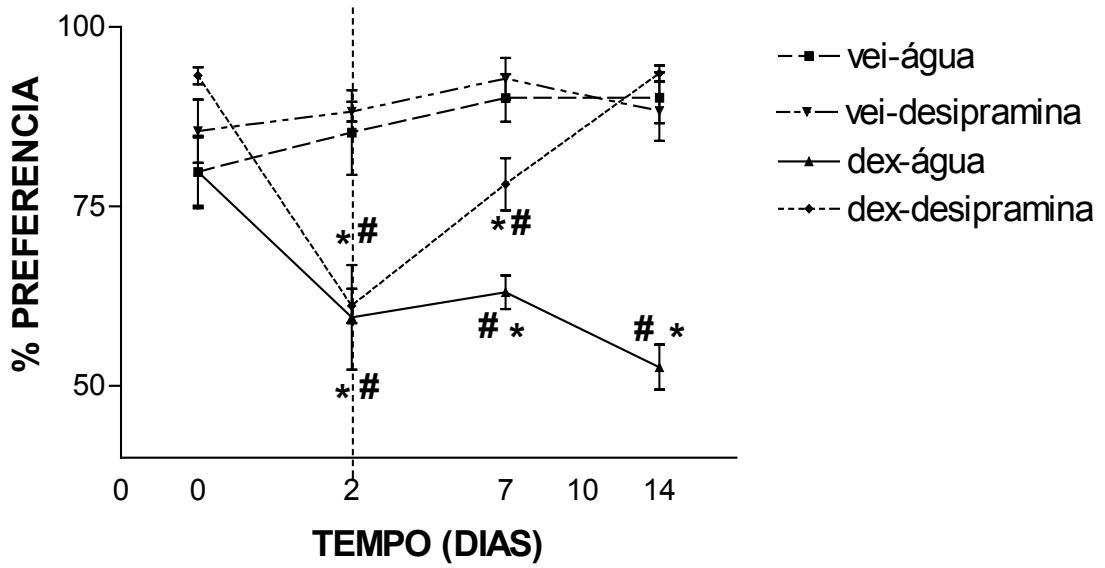

Fig. 1. Efeito do tratamento repetido com desipramina (10 mg/kg, ip, por 14 dias) sobre a anedonia induzida por dexametasona (5 mg/kg, ip no tempo= 0). Linha vertical indica o início do tratamento com desipramina.

Dados expressos como Média ± SEM (n=7 / grupo).

Água: água destilada.

* p<0,05 em relação aos grupos *vei-água ou vei-desipramina* no mesmo período.

# p<0,05 em relação ao valor basal (0) no mesmo grupo.

8.1.2 Experimentos preliminares para elucidação do mecanismo de indução de anedonia pela dexametasona.

Os experimentos subseqüentes foram realizados para elucidação dos mecanismos envolvidos na indução de anedonia por dexametasona. As duas principais hipóteses são:

1. A dexametasona atuaria promovendo uma "adrenalectomia química", comprometendo o hipocampo por supressão do sinal de sobrevivência via receptores mineralocorticóides;
2. A dexametasona atuaria diretamente sobre as células hipocampais, via receptores glucocorticóides.

As duas hipóteses não são mutuamente excludentes, entretanto foram testadas em experimentos separados. Para a primeira foram realizados experimentos de administração aguda de metirapone, na tentativa de suprimir o eixo HPA, simulando um quadro de adrenalectomia. No teste da segunda hipótese os animais foram tratados com mifepristone antes de receber dexametasona, no intuito de bloquear os receptores glucocorticóides, ou receberam uma dose aguda de corticosterona, para ativação de receptores gluco e mineralocoticóides.

Para todos foram utilizados animais de mesma procedência dos experimentos anteriores, nas mesmas condições também citadas.

DROGAS:

- Corticosterona (Sigma, São Paulo) suspensa em Tween 80 0,2% em salina;
- Metirapone (Sigma, São Paulo) dissolvida em PEG 400;
- Dexametasona (DEG, Curitiba) suspensa em Tween 80 0,2% em salina ou dissolvida em PEG 400;
- Mifepristone (Sigma, São Paulo) dissolvida em PEG 400.

Todas as drogas foram injetadas por via intraperitoneal (ip) em volume constante de 1,0 ml/kg/animal entre as 11:00 e 13:00 horas.

8.1.2.1: administração aguda de metirapone.

A administração de uma dose única (50 mg/kg, ip) de metirapone, um inibidor da síntese de corticóides das glândulas adrenais; não foi capaz de induzir perda de preferência 48 horas após a administração, em relação ao grupo *veículo* [t= 0.233; p> 0.82]. Como mostrado na Fig. 2.

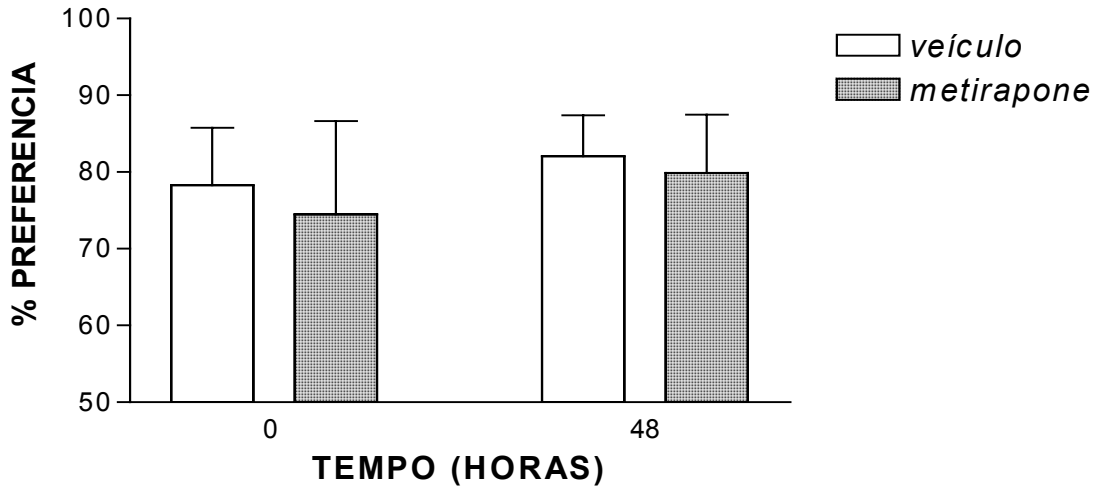

Fig. 2. Efeito da administração aguda de metirapone (50 mg/kg, ip, dose única) no teste de preferência por sacarose. Dados expressos como Média ± SEM (n=5/grupo).

8.1.2.2: administração aguda de corticosterona.

A administração de uma dose única (50 mg/kg, ip) de corticosterona não foi capaz de induzir perda de preferência em relação ao grupo *veículo* [t= 0.344; p> 0.74] como mostrado na Fig. 3.

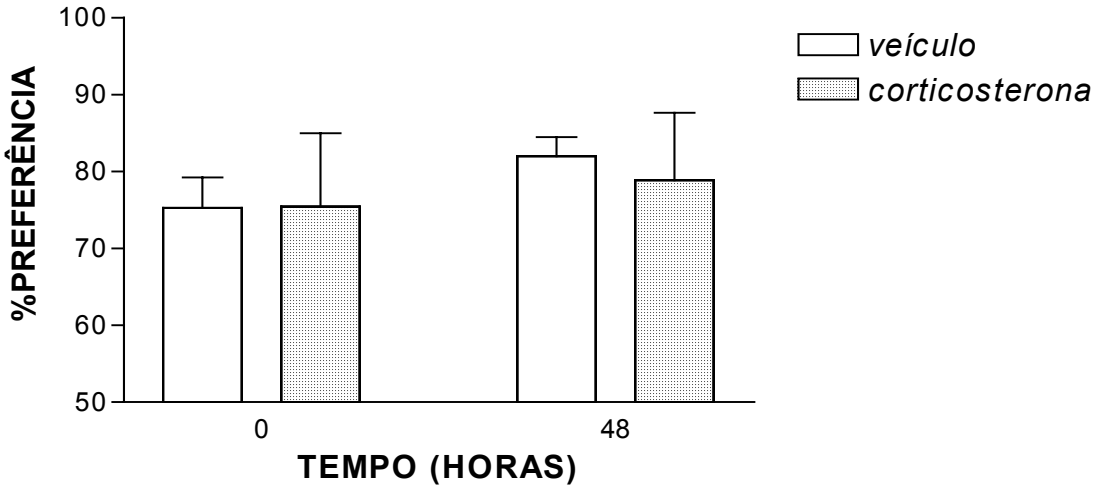

Fig. 3. Efeito da administração aguda de corticosterona (50 mg/kg, ip, dose única) no teste de preferência por sacarose. Dados expressos como Média ± SEM (n=4/grupo)

8.1.2.3 pré-tratamento com mifepristone.

A administração de mifepristone, um antagonista de receptores glucocorticóides, na dose de 8 mg/kg, foi capaz de retardar a indução e impedir a manutenção da anedonia induzida por dexametasona (5 mg/kg) administrada 1h depois da mifepristone, ambas foram dissolvidas em PEG 400.

A análise ANOVA de 2 vias mostrou diferença significante no fator tratamento [$F(3, 23)= 14.82$, $P< 0.0001$], tempo [$F(4,92)= 3.69$, $P< 0.0078$] e interação [$F(12,92)= 2.20$, $P< 0.0180$], como mostrado na Fig. 4. Não houve diferença quanto aos valores basais dos grupos [$F(3, 23)= 0.52$, $P> 0.05$]. Foram observadas diferenças nos dias 2, 7, 14 e 21 após o tratamento com dexametasona. No dia 2 houve diferença entre o grupo tratado com dexametasona e os tratados com veículo, inclusive em relação o grupo pré-tratado com mifepristone ($p<0.001$). No dia 7 tal diferença ainda era verificada, entretanto houve diferença do grupo *mifepristone-dexa* em relação aos grupos *veículo-veículo* e *mifepristone-veículo*, porém sem diferença em relação ao grupo *veículo-dexa*. No 14º e 21º dia, somente o grupo *veículo-dexa* apresentou diferença em relação aos outros grupos ($p<0.001$).

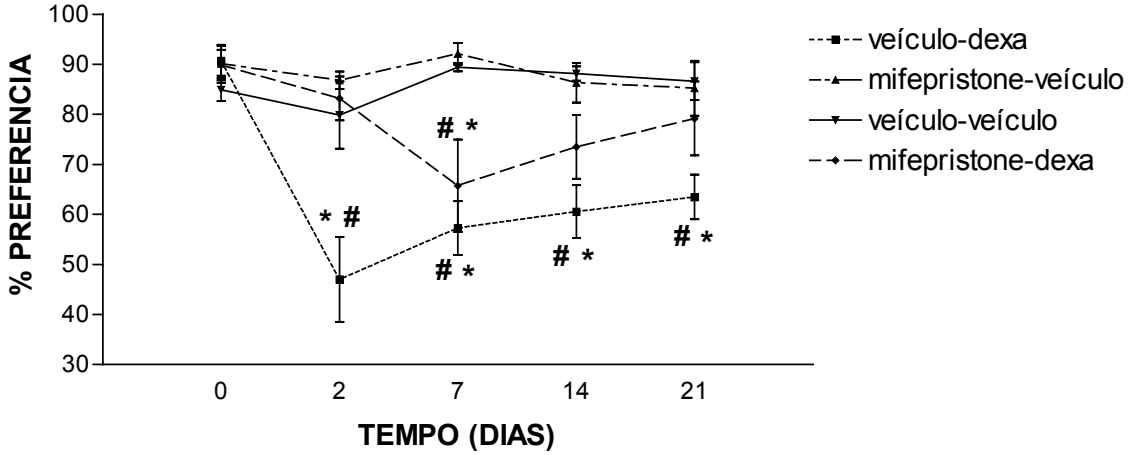

Fig. 4. Efeito do pré-tratamento com mifepristone (8 mg/kg, ip, dose única) sobre a anedonia induzida por dexametasona (5 mg/kg, ip,dose única). Dados expressos como Média ± SEM (n=7-9/grupo).

* p< 0.05 em relação aos grupos controle (*mifepristone-veículo* e *veículo-veículo*) no mesmo tempo.

# p< 0.05 em relação aos valores basais (0) do mesmo grupo.

8.2 DISCUSSÃO

Uma vez que não foi verificado efeito indutor de anedonia com a utilização de inibidores de síntese de corticóides adrenais, como a metirapone, a hipótese de que a anedonia seria decorrente de uma adrenalectomia química e conseqüente abolição do sinal de sobrevivência neuronal por ativação de receptores mineralocorticóides perde força. Entretanto tal hipótese não pode ser descartada, pois alterações metodológicas, como doses mais altas ou tratamentos repetidos, poderiam resultar em efeitos diferentes. A utilização de outros inibidores de síntese de corticóides, como cetoconazol, seria outra alternativa para validar a hipótese.

As observações do efeito do pré-tratamento com mifepristone sugerem que a indução de anedonia por dexametasona decorre da ativação de receptores glucocorticóides, pois o bloqueio dos mesmos foi capaz de retardar as alterações comportamentais induzidas por dexametasona. Além disso, a administração aguda de corticosterona não foi capaz de alterar a preferência dos animais, o que sugere que a ativação de receptores mineralocorticóides, preferencialmente ativados pela corticosterona, poderia proteger o hipocampo

dos efeitos lesivos da ativação de receptores glucocorticóides. Entretanto os resultados não permitem afirmar que o efeito é somente decorrente do bloqueio dos receptores glucocorticóides no hipocampo, uma vez que as drogas foram injetadas sistemicamente.

O retardo promovido pela mifepristone sobre o efeito da dexametasona pode ser explicado com base na farmacocinética das drogas. Uma vez que a meia-vida da dexametasona é maior que da mifepristone, após a depuração da mifepristone ainda haveria dexametasona no organismo suficiente para exercer seus efeitos, entretanto não suficiente para alteração prolongada do comportamento. Por outro lado, as características farmacocinéticas não são as únicas possibilidades. Devido aos efeitos genômicos de ambas as drogas, seria possível que mesmo não havendo qualquer delas presente no organismo seus efeitos seriam prolongados.

Quanto ao efeito do tratamento prolongado com desipramina, pode-se inferir que o modelo também é sensível a drogas noradrenérgicas, não sendo restrito a drogas serotoninérgicas, aumentando assim a validade do modelo.

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