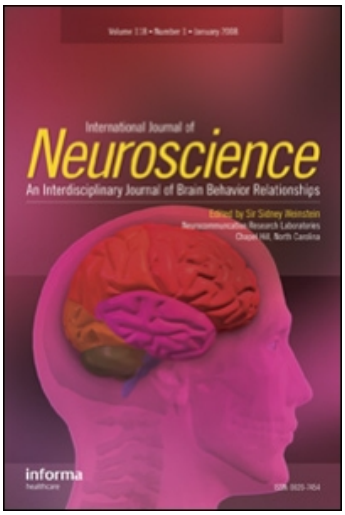

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Amitriptyline Treatment Under Chronic Stress Conditions: Effect on Circulating Catecholamines and Anxiety in Early Maternally Separated Rats

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AMITRIPTYLINE TREATMENT UNDER CHRONIC STRESS CONDITIONS: EFFECT ON CIRCULATING CATECHOLAMINES AND ANXIETY IN EARLY MATERNALLY SEPARATED RATS

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The aim of this work was to determine the effect of amitriptyline (AMI) on peripheral outcomes such as plasma epinephrine (E) and norepinephrine (NE) concentration

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and anxiety-like behavior displayed in the plus maze test in adult maleWistar rats under variable chronic stress and daily oral administration of AMI (5 mg/kg). Animals were previously isolated from the mother for 4.5 hr every day for the first 3 weeks of life. Administration of the antidepressant AMI reduced anxiety-like behavior in animals submitted only to chronic stress but not in early maternally separated (MS) subjects or in animals subjected to the two types of stresses.

Keywords amitriptyline, anxiety, catecholamines, early maternal separation, plus maze test, variable chronic stress

INTRODUCTION

Disturbances in the body, either real or imagined, evoke a stress response, which serves to restore homeostasis and facilitate adaptation. Stressful stimuli are processed in the central nervous system (CNS) that simultaneously activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal medullary (SAM) system (De Boer, Slangen, & Van der Gugten, 1988; Kvetnansky et al., 1995; Tafet & Bernardini, 2003). These two modes of action constitute the basic hormonal response to stress in mammals, which concludes with the secretion of glucocorticoids (GC) and catecholamines (CAs) into the blood flow (Strtakis & Chrousos, 1995; Tafet and Bernardini, 2003). GC and CA are the mechanism of defense for mammals in threatening situations that promote behavioral, metabolic, and physiological changes (Clow, Hucklebridge, & Evans, 1998; De Kloet, Meijer, & Van Haarst, 1998; Strtakis & Chrousos, 1995; Tafet & Bernardini, 2003).

Thus, the stress system is extremely complex but also highly efficient and flexible. Alterations in the ability of the organism to respond to stressors, with responses that are either excessive or inadequate in magnitude or duration, may lead to disease (Strtakis & Chrousos, 1995), especially to emotional disturbances such as depression (Anisman & Matheson, 2005; Ferretti, Blengio, Ricci Gamalero, & Ghi, 1995; Nestler et al., 2002; Willner, 1991), panic, and obsessive-compulsive disorder (Strtakis & Chrousos, 1995; Tafet & Bernardini, 2003). It has been suggested that stress acts as a predisposing and precipitating factor in the onset of depression (Anisman & Matheson, 2005; Ferretti et al., 1995). Apparently, both the HPA axis and the SAM system are constantly activated in patients with depression and other emotional disorders (Fava & Kendler, 2000; Frankenhaeuser, 1971; Nestler et al., 2002; Strtakis & Chrousos, 1995).

The chronic variate stress model designed by Katz in 1981, in which a variety of stressors are applied to the animals for several days, produces a

situation in which unpleasant external stimuli cause a depressed seeming state in the animal. This model appears to be the best suited for predictive purposes in the experimental investigation of stress and depression (Ferretti et al., 1995; Willner, 1991). It has been used in our laboratory also to evaluate the physiologic, endocrine, and behavioral responses to stress and the role of limbic structures as regulating regions of the endocrine system under chronic stress conditions (Molina, Suárez, & Rivarola, 2006; Renard, Suárez, Levin, & Rivarola, 2005; Suárez & Perassi, 1995; 1997; Suárez, Maglianesi, & Perassi, 1998; Suárez, Rivarola, Molina, Levin, et al., 2004; Suárez, Rivarola, Molina, Perassi, et al., 2001). An advantage of this model lies in the fact that it permits the action of chronically administered antidepressants in the animal to be evaluated, since the effect of these drugs can only be assessed in depressed/stressed persons after several days; i.e., the effect of these drugs is specific to the stressed/depressed state (Ferretti et al., 1995; Willner, 1991).

Early childhood stress, as well as stress during adulthood, has been linked to psychopathology later in life. Exposure to early stress programs the individual to display enhanced stress responsiveness. In humans, childhood maltreatment, through promoting an alternative neurodevelopmental pathway, may enhance the emergence of psychiatric illness and behavioral dysfunction (Teicher et al., 2003). Adverse experiences in early life may sensitize specific neurocircuits to subsequent acute stressors thereby increasing individual vulnerability to the onset of physiopathology and psychopathology (Ladd, Thrivikraman, Huot, & Plotsky, 2005).

In rats, acute or repeated long-term separation from the dam is considered to be one of the most potent natural stressors during development, inducing long-term alterations in HPA axis sensitivity and medullo-adrenal secretion (Molina et al., 2006; Suárez et al., 2001, 2004; Wigger & Neumann, 1999).A long-term maternal separation increases HPA activity in pups and may also increase stressor reactivity during adulthood (Anisman, Zaharia, Meaney, & Merali, 1998). Male rats submitted to repeated neonatal separation show greater HPA activity, basally as well as in response to an acute stressor during adulthood (Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; McIntosh, Anisman, & Merali, 1999).

Previous works in our laboratory and from other authors have shown that anxiety-like behavior and plasma CA concentration in male Wistar rats are modified by early maternal separation and by chronic stress (McCarty, 1994; McIntosh et al., 1999; Renard et al., 2005; Suárez et al., 2001, 2004), so we propose that if emotional stress such as maternal separation and chronic stress both affect the plasma level of CAs and anxiety-like behavior, the tricyclic

antidepressant amitriptyline (AMI) could restore the effect of these two types of stresses, by eliciting a decrease of anxiety and norepinephrine (NE) and epinephrine (E) plasma levels.

AMI is a classic tricyclic antidepressant with sedative, analgesic (Bryson & Wilde, 1996), and antihistaminergic (Bendtsen, Jensen, & Olesen, 1996) properties. Its antidepressant effect is a result of the nonspecific inhibition of serotonin and noradrenaline reuptake in the brain, increasing the synaptic availability of these neurotransmitters (Bendtsen et al., 1996; Gould, Altamirano, Javors, & Frazer, 2006; Wasieswski, 2001). It has been demonstrated that, under chronic stress conditions, AMI maintains the ability to downregulate receptor populations (e.g., β-adrenergic and serotonin $5HT_2$) that are known to be affected with chronic stress and depression, reinstating the ability to respond to acute stress (Ferretti et al., 1995).

In this work we propose to investigate the effect of AMI under stressful situations, not on the central system but on peripheral outcomes like plasma CAs (i.e., E and NE) levels and anxiety behavior displayed in a plus maze test, which are known to be affected by early maternal separation and chronic stress (Renard et al., 2005).

MATERIALS AND METHODS

Animals

Male Wistar rats, aged 50 days at the beginning of the chronic stress and antidepressant administration, were used. All animals were subjected to the same conditions. They were housed in a temperature-controlled room (22 $\pm$ 2 °C) under artificial illumination (12:12 hr light/dark; lights on at 07:00 a.m.), with water and food available ad libitum. All rats were handled daily by the same investigator from weaning to minimize stress reactions to manipulation. Handling consisted in picking up each animal from its home cage by placing the hand over its back, with the thumb and forefinger pressing its forelegs toward its head, and then, all subjects were orally administered fresh water with a 1-ml syringe, in order to familiarize the animal to the later administration of the antidepressant. Then each rat was placed briefly in another cage and finally returned to its home cage. On the day of sacrifice, the rat was picked up in the same way, but instead of being placed into a cage, it was immediately decapitated. At the time of decapitation, all the animals were 2.5 months old and weighed 350–400 g. The rats were decapitated between 09:00 and

12:00 a.m.,in order to avoid unwanted variability linked to diurnal fluctuations in circulating hormone levels.

Experiments were performed in full accordance with protocols approved by the animal care committee of the University of Córdoba, Argentina.

Maternal Separation Procedure

On postnatal day 1, litters were culled to eight pups (four females and four males when possible). Pups were deprived daily of their mother for 4.5 hr during the first 3 weeks of life (Ogawa et al., 1994). Each separation consisted of removing the mother from the home cage. The mother was placed alone in a cage in the same room. The litters were kept at room temperature during the separation, with water and food ad libitum. At 4.5 hr, the mother was returned to the home cage. Separations were carried out between 08:00 a.m. and 12:30 a.m. until postnatal day 21. Nonseparated rats remained undisturbed in the maternal cage until the weaning age at postnatal day 25.

After weaning, male rats were selected and housed in standard cages until 50 days of age, at which time they were subjected to the chronic stress model and AMI treatment. Both non–maternally separated (NMS) and maternally separated (MS) rats were randomly divided into two groups: the first was submitted to unpredictable stress, while the second remained unstressed. Unrelated subjects were used to avoid confounding litter effects.

Antidepressant Drug Treatment

The tricyclic antidepressant used in our study, amitriptyline hydrochloride, is presented in 25-mg tablets and is known under the commercial name of Tryptanol. Each tablet was dissolved in 4 ml of water, and 0.2 ml of this solution was administered daily to the respective animals, reaching a final dose of 5 mg/kg of body weight (Ferretti et al., 1995). Vehicle was prepared with the same excipients contained in the tablet. The proportions in one pill are 50% starch, 45% lactose, 1.5% talc, and 1.5% magnesium stearate. These percentages are of the pill weight after subtracting the drug weight. (The final weight of excipients per tablet is 100 mg.) The vehicle was administered in the same way and volume as the antidepressant.

For oral administration of the drug, we used a 1-ml syringe (without the needle), directly introducing 0.2 ml of the solution into the animal's mouth. This mode of drug administration was chosen with the aim of resembling the most frequent treatment prescribed for patients with this disorder. The

Table 1. Variable chronic stress (Katz's modified model) animals were subjected to various stressors for 24 days

Day	Stressor	Time
1	Noise	09:00 to 13:00 hr
2	Immobilization	10:00 to 11:00 hr
3	Ether anesthesia	16:00 hr
4	Two saline injections	11:00 and 15:00 hr
5	Ether anesthesia	10:00 hr
6	Fasting	
7	Rest day	
8	Ether anesthesia	09:30 hr
9	Noise	13:30 to 17:30 hr
10	Two saline injections	11:30 and 15:30 hr
11	Immobilization	15:00 to 16:00 hr
12	Noise	10:00 to 14:00 hr
13	Fasting	
14	Rest day	
15	Ether anesthesia	13:00 hr
16	Immobilization	15:00 to 16:00 hr
17	Noise	09:30 to 13:30 hr
18	Two saline injections	12:00 and 16:00 hr
19	Noise	09:30 to 13:30 hr
20	Fasting	
21	Rest day	
22	Ether anesthesia	11:45 hr
23	Immobilization	10:00 to 11:00 hr
24	Noise	09:00 to 13:00 hr

use of injectable therapy is not common in the treatment of major depressive disorder. In addition, oral administration of antidepressant drugs is also the most commonly applied route in humans. The drug and the vehicle were administered for 21 days from postnatal day 60.

Variable Chronic Stress (VCS)

At 50 days of age, the rats were exposed to a 24-day variable-stressor paradigm (modified Katz's stress model) (Katz, Roth, & Carrol, 1981). Individual stressors are listed in Table 1.

The type of stressor and the day on which it was applied were chosen by using a random number table, except for that on day 24. In this case, noise

was used as stressor on the day preceding the plus maze test to avoid the unpredictability associated with this chronic stress model (García Marquez & Armario, 1987). The stressors used were: (a) noise produced for 4 hr by an alarm bell (85 dB); (b) ether anesthesia until loss of consciousness; (c) two intraperitoneal (IP) injections of 0.5-ml isotonic saline; (d) food deprivation for 24 hr; (e) restraint for 1 hr by placement inside a 6-cm diameter metal cylinder. The stressors used in this paradigm did not affect the rat's body weight.

Elevated Plus Maze

Twenty-four hours after the last stressor, rats were tested in the elevated plus maze apparatus. At the same age, the unstressed group was also tested. The elevated plus maze test is based on creating a conflict between the rat's exploratory drive and its innate fear of open and exposed areas. Thus, decreased open arms exploration was taken to indicate enhanced anxiety-related behavior. As described in detail by Liebsch et al. (1995), the apparatus consisted of a plus-shaped platform elevated 50 cm from the floor. Two of the opposing arms (50×10 cm) were enclosed by 40-cm high side and end walls (closed arms), whereas the other two had no walls (open arms). At the beginning of the test, each rat was placed onto the central area (10×10 cm) of the maze facing a closed arm and was allowed to explore the plus maze freely. During the 5-min exposure, certain parameters were recorded: number of entries into open arms, number of entries into closed arms, and time spent on the open arms. Two indices of anxiety were obtained: the number of entries into open arms, expressed as a percentage of the total number of entries, and the amount of time spent in the open arms, expressed as a percentage of total time. Between each session, the maze was wiped clean. Behavioral testing was conducted in a quiet room. Animals were transported to the experimental room 2 hr before the behavioral test to eliminate the stressor effects of the new environment.

Assays of Hormones

Twenty-four hours after the test, the rats were decapitated with a small guillotine within 5–7 s after being taken from their home cage. Immediately after decapitation, trunk blood was collected and centrifuged. Individual plasma samples were frozen and stored for subsequent determination of epinephrine (E) and norepinephrine (NE) concentration.

Blood for plasma CA assay was poured into plastic tubes containing heparin and kept on ice. The catechols in 500-μl aliquots of plasma were

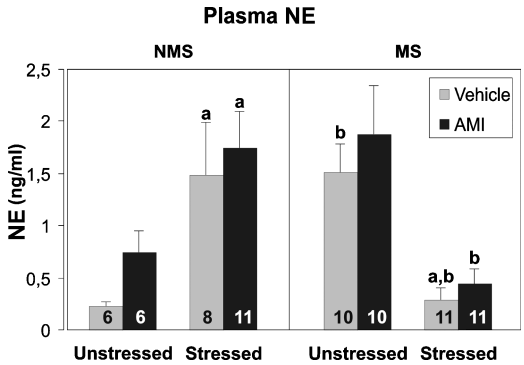

Figure 1. Plasma NE concentration in NMS and MS rats subjected to unpredictable stress under AMI (5 mg/kg) administration. Mean ± *SE* are presented. The number of animals per group is included inside each bar. (a) Significant differences ($p < .05$) vs. respective unstressed. (b) Significant differences ($p < .05$) vs. respective NMS.

partially purified by batch alumina extraction, separated by reverse-phase high-pressure liquid chromatography (RF-HPLC), using a 4.6 × 250 mm Zorbax R × C18 column (New England Nuclear, Du Pont). The quantification was made by current produced upon exposure of the column effluent to oxidizing and then by reducing potentials in series using a triple-electrode system (Coulochem II, ESA) (Eisenhofer et al., 1986). Recovery through the alumina extraction step averaged 70–80% for CAs. Catechol concentrations in each sample were corrected for the recovery of an internal standard dihydroxybenzylamine.

Statistical Analysis

Statistical significance of the data was determined by three-way analysis of variance (ANOVA)—with the factors maternal separation × stress × drug treatment), and individual group means were compared by Tukey's post hoc test. Significance was set at $p < .05$.

RESULTS

The effect of maternal separation and chronic stress on plasma NE under AMI administration is displayed in Figure 1.

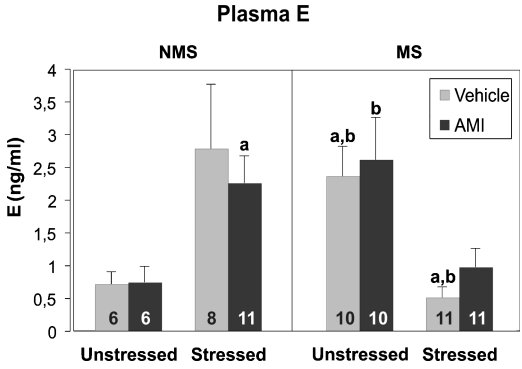

Figure 2. Plasma E concentration in NMS and MS rats subjected to unpredictable stress under AMI (5 mg/kg) administration. Mean ± *SE* are presented. The number of animals per group is included inside each bar. (a) Significant differences ($p < .05$) vs. respective unstressed. (b) Significant differences ($p < .05$) vs. respective NMS.

Plasma concentration of NE was higher in NMS rats under stress situations compared to unstressed controls both in animals administered with vehicle solution and with AMI ($p < .05$ for both).

On the other hand, unstressed rats administered with vehicle and MS showed a significant rise in NE plasma level ($p < .05$) against the NMS group. MS stressed animals also had less concentration of NE than unstressed controls, but this was significant only for vehicle-administered animals ($p < .05$). Then, in MS animals submitted to stress, with vehicle and with AMI administration, NE plasma levels were lower than in NMS stressed rats ($p > .05$ for both).

Figure 2 shows plasma E concentration in early MS and stressed rats under AMI administration.

As well as NE levels, E concentration in NMS stressed was higher than in NMS unstressed rats, although this difference was significant only in AMI-treated individuals ($p < .05$).

Regarding MS rats, both vehicle and AMI unstressed groups showed significantly higher ($p < .05$) levels of plasma E than NMS unstressed animals. Then, when comparing MS stressed groups, vehicle-administered rats showed lower E plasma concentration than both MS unstressed and NMS stressed animals ($p < .05$ for both), while MS stressed animals with AMI treatment showed differences neither with unstressed nor NMS stressed groups.

Figures 3 and 4 display the effects of maternal separation and stressful conditions under AMI treatment on behavioral parameters during a 5-min exposure to the elevated plus maze and their respective controls.

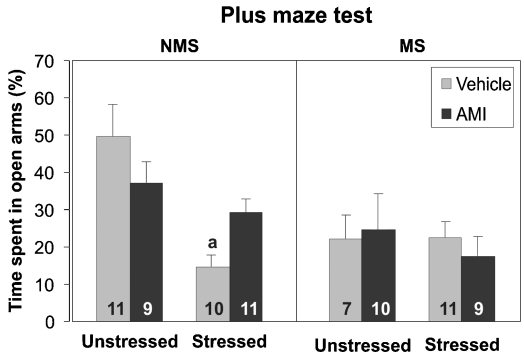

Figure 3. Time spent in the open arms (expressed as percentage) during a 5-min exposure to the elevated plus maze test of NMS and MS rats subjected to unpredictable stress under AMI (5 mg/kg) administration. Mean ± *SE* are presented. The number of animals per group is included inside each bar. (a) Significant differences ($p < .05$) vs. respective unstressed.

Statisticalthree-way ANOVA revealed that stressed animals spent less time in open arms than unstressed individuals [$F(1,70) = 4{,}34$ ($p < .05$)], and MS rats also spent less time in open arms than NMS ones [$F(1,70) = 7{,}33$ ($p < .05$)]. The post hoc test showed that NMS stressed animals with saline administration spent less time in open arms than NMS unstressed controls ($p < .05$). The AMI-treated ones did not differ from the unstressed group but

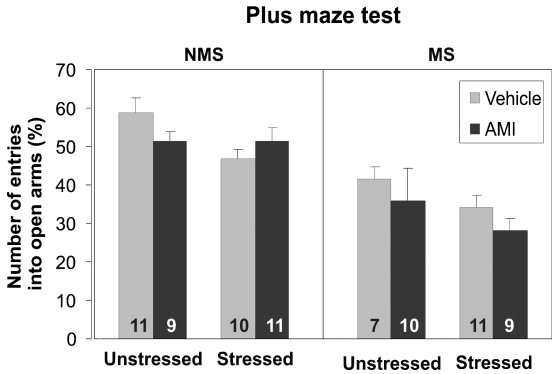

Figure 4. Number of entries into the open arms (expressed as percentage) during a 5-min exposure to the elevated plus maze test of NMS and MS rats subjected to unpredictable stress under AMI (5 mg/kg) administration. Mean ± *SE* are presented. The number of animals per group is included inside each bar.

spent more time in the open arms than the vehicle stressed group, although this was not significant (Figure 3).

Number of entries into open arms was lower in stressed animals [$F(1,70) = 4,76$] and in MS ones [$F(1,70) = 31, 12$] than their respective controls. AMI treatment had no differential effect (Figure 4).

DISCUSSION

The present study evaluates the effect of the tricyclic antidepressant AMI on endocrine and behavior parameters in rats that are known to be affected by stressful situations such as early maternal separation and chronic stress. These parameters (i.e., plasma CAs and anxiety behavior) are also altered in persons with stress-related disorders such as depression (Corona et al., 1987, 1989; Lister, 1990; Olivier et al., 1994; Stoney & Hughes, 2001; Tafet & Bernardini, 2003).

In NMS rats, chronic stress caused a rise in E and NE levels and AMI had no significant effect on this, although E concentration was lower in animals with chronic stress administered with the drug. Then, in early MS rats, animals under chronic stress showed lower levels of these two hormones than separated unstressed rats, but that is not attributable to AMI, since vehicle-treated animals under the same conditions also had lower levels of these CAs than their respective controls.

The rise in NE (86% vehicle-treated animals, 59% AMI-treated) and E (75% vehicle, 68% AMI) concentrations in NMS stressed rats is attributable to the SAM activation after stress and is in agreement with other studies (McCarty, 1994; Suárez et al., 2001, 2004; Tafet & Bernardini, 2003). Although this rise in E was not significant for vehicle-treated animals, due to the high experimental error in these groups, AMI-treated animals under stress conditions showed lower levels of E with respect to the vehicle group, but this too was not significant.

Maternal separation also modified medulloadrenal secretion in adulthood, as could be seen in MS animals, whose NE levels were 86% and 60% higher in vehicle- and AMI-administered rats respectively. Concentration of E was 71% and 72% higher (vehicle and AMI respectively) when animals were early MS compared with those not separated. Similar effects were reported by other researchers in our laboratory for males as well as for females (Renard et al., 2005; Suárez et al., 2001, 2004). Also in this situation, the antidepressant had no effect on these hormones' concentrations.

Regarding these CA levels in MS stressed animals, as in previous works with females (Suárez et al., 2004), the concentration of these hormones after maternal separation and chronic stress was lower than in MS unstressed animals, reaching the basal concentration, both in vehicle- and AMI-administered rats, so in this case too, AMI had no differential effect.

The rise of NE and E levels in those groups submitted only to variable chronic stress is due to the degree of uncertainty of the stressor type and duration, which prevent attenuation of plasma CA responses to stress (McCarty, 1994). However, this response was not reversed by AMI. Other authors have demonstrated that this drug, like other tricyclic antidepressants, can restore plasma levels of the principal stress hormone, corticosterone, in mice and rats (Katz & Hersh, 1981; Roth & Katz, 1981; Soblosky & Thurmond, 1986). It has also been proved that AMI can restore β-adrenergic receptor population in the rat brain cortex, which is related to the response to chronic stress, restoring the ability to respond to subsequent stress (Ferretti et al., 1995). But on the other hand, it has been suggested that NE, which is diminished in the brain during variable chronic stress, is not restored with this antidepressant treatment (Soblosky & Thurmond, 1986). In view of this, perhaps if AMI had an effect on the sympathetic system during stress, this could be elicited in receptor systems that are altered under chronic stress conditions instead of hormone secretion.

There is no evidence that treatment with this or any other antidepressant during adulthood may reverse the endocrine changes evoked by repeated maternal separation as neonates. In this work, administration with AMI exerted no effect on CA concentration in early maternally separated animals, perhaps due to the same explanation given for chronic variable stress.

Concerning the lack of NE and E responses to the Katz's paradigm in MS rats, as was shown in previous works in our laboratory, this might reflect exhaustion of the SAM system or deficient response throughout the chronic stress (Suárez et al., 2004). On the other hand, it is known that exposure to inescapable or uncontrollable stress may lead to dysfunction of the NE regulation center in the brain (nucleus locus coeruleus), leading to depressed NE release in the brain, which is associated with a learned helplessness state (Tafet & Bernardini, 2003). Perhaps a similar dysfunction underlies this lack of plasmatic CA response when these two stresses overlap; i.e., the endocrine pattern developed as a consequence of maternal separation could lead, through the lack of plasma CA response, to the inability to face chronic stress situations during adulthood, since one of the principal effects of these hormones is on coping behavior in stressful situations (Frankenhaeuser, 1971). Nevertheless, this decrease in NE and E concentrations was not reversed by AMI, although

it is known that learned helplessness can be ameliorated by this and other tricyclic antidepressants (Soblosky & Thurmond, 1986; Telner & Singhal, 1981 Vollmayr & Henn, 2003). Despite these results, it has been suggested that antidepressants may play a role in normalizing biological rhythms (Rota et al., 2005), so we cannot discard an effect of AMI on circadian rhythms of these hormones. If that occurs, our data are only preliminary, and we cannot say that AMI has no effect on these CAs.

In addition to the effect on medulloadrenal secretion, animals under chronic stress and repeated maternal separation also differed in parameters believed to reflect levels of anxiety and emotionality on the plus maze test, such as the number of open arms entries and time spent in the open arms (Handley & McBlane, 1993). In this study, stressed NMS animals treated with vehicle solution spent less time in open arms than their respective unstressed group, thus showing enhanced anxiety behavior. On the other hand, the AMI-stressed group had no difference as compared with the AMI-unstressed group, and they were less anxious than the vehicle-stressed group, but this was not significant, so, supported by the trend displayed in the statistical analysis, we can say that AMI in this study elicited a slight anxiolytic effect.

In early MS animals, both unstressed and stressed submitted animals showed increased anxiety-related behavior after maternal separation, which is in agreement with other results (Renard et al., 2005; Suárez et al., 2004).

What is remarkable in this work is that the anxiolytic effect is only exerted in NMS rats, whereas in the more anxious MS ones, the antidepressant could not reverse this behavioral change. Perhaps the alternative neurocircuits developed as a consequence of the neonatal maternal separation (Ladd et al., 2005; Teicher et al., 2003) promote this anxiety-like behavior in a way that could not be affected later in life by an anxiogenic situation, such as chronic stress, or by an anxiolytic treatment, such as AMI administered during adulthood. Maybe the neurocircuits underlying this maternal separation anxiety are not the same as those involved in the anxiety provoked by chronic variable stress, and once they are consolidated, they would be resistant to changes. So this maternal separation anxiety is neither enhanced by chronic stress nor reversed by AMI.

In summary, maternal separation and chronic variable stress altered SAM activity and anxiety-related behavior, and the antidepressant could only exert a slight anxiolytic effect on NMS rats submitted to the variable chronic stress paradigm, which was evidenced by the reduction in E levels and the increased time spent in open arms of the plus maze in chronically stressed animals.

Although some of the results of this study were not as expected, they raise issues that need additional research. Further investigation regarding AMI in

early maternal separation and chronic variable stress is needed to understand the real effects of this drug on such situations. These studies should try to test different doses and influence on peripheral receptor systems or on biological rhythms.

Perhaps the reason why this study did not reach the results we expected in all the measured parameters may be due to the low dose of AMI used. Nevertheless, we found that AMI exerts an anxiolytic effect only in certain circumstances. MS rats were resistant to this effect, so it may be interesting to perform further investigation with higher doses so as to reinforce this observation and so reach more solid conclusions about the effect of AMI in these stressful situations.

REFERENCES

Anisman, H., & Matheson, K. (2005). Stress, depression and anhedonia: Caveats concerning animal models. *Neuroscience and Biobehavioral Reviews*, *29*, 525–246.

Anisman, H., Zaharia, M. D., Meaney, M. J., & Merali, Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, *16*, 149–164.

Bendtsen, L., Jensen, R., & Olesen, J. (1996). A non-selective (amitriptyline), but not a selective (citalopram), serotonin reuptake inhibitor is effective in the prophylactic treatment of chronic tension–type headache. *Journal of Neurology, Neurosurgery, and Psychiatry*, *61*, 285–290.

Bryson, H. M., & Wilde, M. I. (1996). Amitriptyline: A review of its pharmacological properties and therapeutic use in chronic pain states. *Drugs and Aging*, *8*, 459–476.

Clow, A., Hucklebridge, F., & Evans, P. (1998). The role and regulation of monoamines in stress. In A. Levy, E. Grauer, D. Ben-Nathan, & R. de Kloet (Eds.), *New frontiers in stress research: Modulation of brain function* (pp. 21–31). Amsterdam, The Netherlands: Harwood Academic Publishers.

Corona, G. L., Cucchi, M. L., Frattini, P., Santagostino, G., Schinelli, S., & Comincioli, V. (1989). Noradrenergic output and clinical response in depressed women during amitriptyline therapy. *Pharmacopsychiatry*, *22*, 144–151.

Corona, G. L., Cucchi, M. L., Frattini, P., Santagostino, G., Schinelli, S., Tosca, P., et al. (1987). Amitriptyline action on sympathetic neuronal function in depressed women. *Clinical and Experimental Pharmacology and Physiology*, *14*, 877–885.

De Boer, S. F., Slangen, J. L., & Van der Gugten, J. (1988). Adaptation of plasma catecholamine and corticosterone responses to short term repeated noise stress in rats. *Physiology and Behavior*, *44*, 273–280.

De Kloet, E. R., Meijer, O. C., & Van Haarst, A. D. (1998). Corticosteroid hormones and the organization of stress response system. In A. Levy, E. Grauer, D. Ben-Nathan,

& R. de Kloet (Eds.), *New frontiers in stress research: Modulation of brain function* (pp. 1–19). Amsterdam, The Netherlands: Harwood Academic Publisher.

Eisenhofer, G., Goldstein, D. S., Stull, R., Keiser, H. R., Sunderland, T., Murphy, D. L., et al. (1986). Simultaneous liquid chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines and 3,4-dihydroxyphenylalaline in plasma, and their responses to inhibition of monoamine oxidase. *Clinical Chemistry*, *32*, 2030–2032.

Fava, M., & Kendler, K. S. (2000). Major depressive disorder. *Neuron*, *28*, 335–341.

Ferretti, C., Blengio, M., Ricci Gamalero, S., & Ghi, P. (1995). Biochemical and behavior changes induced by acute stress in a chronic variate stress model of depression: The effect of amitriptyline. *European Journal of Pharmacology*, *280*, 19–26.

Frankenhaeuser, M. (1971). Behavior and circulating catecholamines. *Brain Research*, *31*, 241–262.

García Marquez, B., & Armario, A. (1987). Chronic stress depresses exploratory activity and behavioral performance in the forced swimming test without altering ACTH response to a novel acute stressor. *Physiology and Behavior*, *40*, 33–38.

Gould, G. G., Altamirano, A. V., Javors, M. A., & Frazer, A. (2006). A comparison of the chronic treatment effects of venfalaxine and other antidepressants on serotonin and norepinephrine transporters. *Biological Psychiatry*, *59*, 408–414.

Handley, S. L., & McBlane, J. W. (1993). An assessment of the elevated X-maze test for studying anxiety and anxiety modulating drugs. *Journal of Pharmacological and Toxicological Methods*, *29*, 129–139.

Katz, R. J., & Hersh, S. (1981). Amitriptyline and scopolamine in an animal model of depression. *Neuroscience and Biobehavioral Reviews*, *5*, 265–271.

Katz, R. J., Roth, K. A., & Carrol, R. J. (1981). Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neuroscience and Biobehavioral Reviews*, *5*, 247–251.

Kvetnansky, R., Pácak, K., Fukuhara, K., Viskupic, E., Hiremagalur, B., Nankova, B., et al. (1995). Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenocortical system. In G. Chrousos, R. McCarty, K. Pacak, G. Cizza, E. Sternberg, P. Gold, et al. (Eds.), *Stress: Basic mechanisms and clinical implications* (pp. 131–158). New York: New York Academy of Sciences.

Ladd, C. O., Thrivikraman, K. V., Huot R. L., & Plotsky, P. M. (2005). Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates. *Psychoneuroendocrinology*, *30*, 520–533.

Liebsch, G, Landgraf, R, Gerstberger, R, Probst, J. C., Wotjak, C. T., Engelmann, M., et al. (1995). Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regulatory Peptides*, *59*, 229–239.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology & Therapeutics*, *46*, 321–340.

Liu, D., Caldji, C., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology*, *12*, 5–12.

McCarty, R. (1994). Regulation of plasma catecholamine responses to stress. *Seminars in Neuroscience*, *6*, 197–204.

McIntosh, J., Anisman, H., & Merali, Z. (1999). Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: Gender-dependent effects. *Developmental Brain Research*, *113*, 97–106.

Molina, S. M., Suárez, M. M., & Rivarola, M. A. (2006). Behavioral and hypothalamic-pituitary-adrenal responses to anterodorsal thalami nuclei lesions and variable chronic stress in maternally separated rats. *International Journal of Developmental Neuroscience*, *24*, 319–326.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of Depression. *Neuron*, *34*, 13–25.

Ogawa, T., Mikuni, M., Kuroda, Y., Muneoka K., Mori, K. J., & Takahash, K. (1994). Periodic maternal deprivation alters stress response in adult offspring: Potentiates the negative feedback regulation of restraint stress–induced adrenocortical response and reduces the frequencies of open field–induced behaviors. *Pharmacology Biochemistry and Behavior*, *49*, 961–967.

Olivier, B., Molewijk, E., van Oorschot, R., Van Der Poel, G., Zethof, T., Van Der Heyden, J., et al. (1994). New animal models of anxiety. *European Neuropsychopharmacology*, *4*, 93–102.

Renard, G. M., Suárez, M. M., Levin, G. M., & Rivarola, M. A. (2005). Sex differences in rats: Effects of chronic stress on sympathetic system and anxiety. *Physiology and Behavior*, *85*, 363–369.

Rota, E., Broda, R., Cangemi, L., Migliaretti, G., Paccotti, P., Rosso, C., et al. (2005). Neuroendocrine (HPA axis) and clinical Correlates during fluvoxamine and amitriptyline treatment. *Psychiatry Research*, *133*, 281–284.

Roth, K. A., & Katz, R. J. (1981). Further studies on a novel animal model of depression: Therapeutic effects of a tricyclic antidepressant. *Neuroscience and Biobehavioral Reviews*, *5*, 253–258.

Soblosky, J. S., & Thurmond, J. B. (1986). Biochemical and behavioral correlates of chronic stress: Effects of tricyclic antidepressants. *Pharmacology Biochemistry and Behavior*, *24*, 1361–1368.

Stoney, C. M., & Hughes, J. W. (2001). Catecholamine stress responses in arterialized blood. *Psychophysiology*, *38*, 590–593.

Strtakis, C. A., & Chrousos, G. P. (1995). Neuroendocrinology and pathophysiology of the stress system. In G. Chrousos, R. McCarty, K. Pacak, G. Cizza, E. Sternberg, P. Gold, et al. (Eds.), *Stress: Basic mechanisms and clinical implications* (pp. 1–18). New York: New York Academy of Sciences.

Suárez, M. M., Maglianesi, M. A., & Perassi, I. (1998). Involvement of the anterodorsal thalami nuclei on the hypophysoadrenal response to chronic stress in rats. *Physiology and Behavior*, *64*, 111–116.

Suárez, M. M., & Perassi, N. I. (1995). Adrenal function response to chronic stress in rats with anterodorsal thalami nuclei lesion. *Life Sciences*, *56*, 163–165.

Suárez, M. M., & Perassi, N. I. (1997). Influence of anterodorsal thalami nuclei on ACTH release under basal and stressful conditions. *Physiology and Behavior*, *62*, 373–377.

Suárez, M. M., Rivarola, M. A., Molina, S. M., Levin, G. M., Enders, J., & Paglini, P. (2004). The role of the anterodorsal thalami nuclei in the regulation of adrenal medullary function, beta-adrenergic cardiac receptors and anxiety responses in maternally deprived rats under stressful conditions. *Stress*, *7*, 195–203.

Suárez, M. M., Rivarola, M. A., Molina, S. M., Perassi, N. I., Levin, G. M., & Cabrera, R. (2001). Periodic maternal deprivation and lesion of anterodorsal thalami nuclei induce alteration on hypophyso adrenal system in adult rats. *Life Sciences*, *69*, 803–813.

Tafet, G. E., & Bernardini, R. (2003). Psychoneuroendocrinological links between chronic stress and depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *27*, 893–903.

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience and Biobehavioral Reviews*, *27*, 33–44.

Telner, J. I., & Singhal, R. L. (1981). Effects of nortriptyline treatment on learned helplessness in the rat. *Pharmacology Biochemistry and Behavior*, *14*, 823–826.

Vollmayr, B., & Henn, F. A. (2003). Stress models of depression. *Clinical Neuroscience Research*, *3*, 245–251.

Wasieswski, W. W. (2001). Preventive therapy in pediatric migraine. *J. Child Neurology*, *16*, 71–78.

Wigger, A., & Neumann, I. D. (1999). Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. *Physiology and Behavior*, *66*, 293–302.

Willner. (1991). Animal models as simulations of depression. *Trends in Pharmacological Sciences*, *12*, 131–136.