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Interaction between estradiol replacement and chronic stress on feeding behavior and on serum leptin

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Abstract

Exposure to stress may cause either an increase or a decrease in food intake. Behavioral and physiological responses to stress, including alterations in feeding behavior, are sexually dimorphic. This study aimed to evaluate the interaction between estradiol levels and chronic variate stress on the intake of sweet food and on serum levels of leptin, a hormone secreted by the adipose cells with a role in the regulation of body weight. Adult female Wistar rats were used. After ovariectomy, the animals received estradiol replacement (or oil) subcutaneously. Rats were then divided in controls and stressed (submitted to 30 days of variate stress). Consumption of sweet food and of serum leptin was measured. Although animals receiving estradiol replacement presented smaller weight gain, they showed an increased consumption of sweet food. Chronic variate stress decreased sweet food intake at 30, but not at 20, days of treatment. Estradiol replacement in the stressed group prevented both the reduction observed in sweet food intake and the increase in leptin levels. These results suggest that there is an interaction between chronic stress and estradiol replacement in feeding behavior concerning sweet food consumption, and this interaction may be related to altered leptin levels.

Keywords: Estradiol; Chronic variate stress; Feeding behavior; Sweet food; Leptin; Female rats

1. Introduction

Food intake depends on several internal and external variables. Emotional changes such as those induced by the exposure to stress situations can influence feeding behavior, causing either an increase or a decrease in food intake, depending upon the nature of the stress (Varma et al., 1999; Ely et al., 1997; Gamaro et al., 2003a; Torres et al., 2002). Several studies have demonstrated that chronic exposure to stressors may alter food intake and body weight of rats (Harris et al., 2002; Kant and Bauman, 1993; D'áquila et al., 1997; Gamaro et al., 2003a,b). Inescapable shock, for example, can affect food intake and reduce weight gain with shocked rats gaining significantly less weight than restrained and untreated rats (Dess et al., 1988). Moreover, models of chronic mild stress have been reported to have different effects on feeding behavior depending on the model. Animals repeatedly stressed by restraint show increased ingestion of sweet food (Ely et al., 1997), while models of chronic variate stress have been observed to induce a decreased appetite for sweet food or palatable solutions (Gamaro et al., 2003a,b; Willner, 1991). It should be pointed out, however, that although in humans women are more sensitive to disturbances in feeding behavior than men (Kornstein, 2002; Oliver and Wardle, 1998), most of these studies in animal models have been carried out in males (Kelly et al., 1999; Harris et al., 2002).

Several sources of data indicate that behavioral and physiological responses to stress are sexually dimorphic, including alterations in feeding behavior (Ely et al., 1997; Faraday, 2002; Kelly et al.,1999; Than et al., 1994). Ovariectomy produces an increase in body weight and in meal size in rats (Butera et al., 1993; Bonavera et al., 1994), which is attenuated by estradiol treatment for 7 days (Shimizu et al., 1996), and the normal cyclic pattern of ovarian estradiol secretion leads to tonic and phasic inhibitions of feeding (Geary and Asarian, 1999).

Leptin is a hormone secreted mainly by the adipose cells with a role as a metabolic adaptor in the regulation of body weight. It is believed to establish a feedback loop between the energy reserves and the hypothalamic centers that

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control food intake (Prolo et al., 1998; Loftus, 1999; Inui, 1999). Recent data suggest also that leptin interacts with other endocrine systems to provide critical information about the size of the fat stores (Lotfus, 1999; Sandoval and Davis, 2003). As well as participating in the expression of CRH in the hypothalamus, leptin also interacts with ACTH in the adrenals and its levels are regulated by glucocorticoids (Spinedi and Gaillard, 1998; Oates et al., 2000). Thus, this hormone is probably influenced by activation of the hypothalamo–pituitary–adrenal (HPA) axis when animals are exposed to stress situations. The fact that leptin levels are always higher in females, even after correcting for body fat content (Chudek et al., 2002; Machinal-Quelin et al., 2002), suggests that the interaction between the adipose tissue and the reproductive system is modulated in a different way in males and females by androgenic and estrogenic hormones. While some androgens are inhibitors of leptin secretion, estradiol induces a strong stimulation in adipose tissue (Kristensen et al., 1999; Machinal-Quelin et al., 2002).

Although previous studies indicate that exposure to stress presents different effects on feeding behavior, depending on the gender, little is known about the ability of ovarian hormones to modulate the responses induced by chronic stress on feeding behavior or how they influence leptin levels in these animals. The study presented herein was conducted to investigate the interaction between estradiol levels and chronic variate stress on feeding behavior, on the intake of sweet food, and on serum leptin levels.

2. Experimental procedures

2.1. Animals

Adult female Wistar rats (60 days old; 180–230 g of weight) were used. Thirty-eight rats were used in the behavioral measurements and 28 of these animals were used to measure leptin levels. The animals used to evaluate leptin levels in each group were randomly chosen. The experimentally naive animals were housed in groups of four or five in home cages made of Plexiglas material ($65 \times 25 \times 15$ cm) with the floor covered with sawdust. They were maintained under a standard dark–light cycle (lights on between 7:00 and 19:00 h) at a room temperature of 22 ± 1 °C. The rats had free access to food (standard rat chow) and water, except for the stressed group during the period when the stressor applied required no food or water. During the first days of treatment, the ingestion of rat chow was monitored. Body weight was measured at the beginning, at the middle, and at the end of the treatment. All animal treatments were in accordance with the institutional guidelines and according to the recommendations of the International Council for Laboratory Animal Science (ICLAS), and all efforts were made to minimize animal suffering as well as to reduce the number of animals.

2.2. Surgery

Overiectomy (OVX) was performed in the morning. Rats were anesthetized with 120 mg/kg ketamine HCl (Dopalen: Agribrands, Campinas, SP, Brazil) and 16 mg/kg xylazine (Anasedan: Agribrands), and bilateral ovariectomy was performed through a single abdominal incision. After a recovery period of at least 1 week, the animals were submitted to estradiol replacement or to a sham surgery.

2.3. Estradiol replacement

Briefly, 15 mm medical grade tubing (1.02 mm i.d. × 2.16 mm o.d.; Medicone, Multiplast, Porto Alegre, RS, Brazil) was filled with 10 μl of 5% (w/v) β-estradiol 3-benzoate (Sigma, St. Louis, MO) in corn oil and sealed with silicone. Capsules were soaked in sterile saline overnight and implanted subcutaneously between the scapulae under anesthesia. Sham animals were implanted with capsules containing just oil.

2.4. Stress model

Chronic variate stress was modified from other models of variate stress (Willner et al., 1987; Konarska et al., 1990; Papp et al., 1991), as described in Gamaro et al. (2003a). Animals with estradiol replacement and sham animals were subdivided in two groups: control and stressed. Controls were kept undisturbed in their home cages during the treatment, except for the cleaning of the cages and the exposure to the behavioral proceedings to measure consumption of sweet food. A 30-day variate-stressor paradigm was used for the animals in the stressed group. The following stressors were used: (a) 24 h of food deprivation, (b) 24 h of water deprivation, (c) 1–3 h of restraint, as described below, (d) 1.5–2 h of restraint at 4 °C, (e) inclination of home cage during 4 or 5 h, as described below, (f) flashing light during 120–210 min, and (g) isolation (2–3 days). Stress application started at different times everyday in order to minimize its predictability.

Restraint was carried out by placing the animal in a 25×7-cm plastic tube and adjusting it with plaster tape on the outside so that the animal was unable to move. There was a 1-cm hole at the far end for breathing. Exposure to flashing light was performed by placing the animal in a 50-cm high, 40×60-cm open field made of brown plywood with a frontal glass wall. A 40-W lamp, flashing at a frequency of 60 flashes per minute, was used.

2.5. Consumption of sweet food

After 20 and 30 days of treatment, consumption of sweet food was measured. The animals were placed in a lightened rectangular box ($40 \times 15 \times 20$ cm) with a glass ceiling, floor, and sidewalls made of wood. Ten Froot loops (Kellogg's: pellets of wheat and cornstarch and sucrose) were

placed in one extremity of the box. Animals were submitted to 3-min trials, one per day during 5 days, in order to become familiarized with this food (Ely et al., 1997). After being habituated, the animals were exposed to two test sessions, 3 min each, when the number of ingested pellets was measured. A protocol was established so that when the animal ate part of the Froot loops (e.g., 1/3 or 1/4), this fraction was considered.

2.6. Leptin measurement

The animals were sacrificed by decapitation 24 h after the last stress session. Trunk blood was collected and serum separated and frozen until the day of the analysis. Measurement of serum leptin was performed with a commercial mouse leptin ELISA kit (Crystal Chem., Chicago, IL).

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. Data were analyzed using two-way ANOVA.

3. Results

Body weight was evaluated in the four groups before stress and estradiol treatments started, after 15 and after 30 days of treatment. Weight gain during the treatment is shown in Fig. 1. Repeated measure ANOVA showed an effect of estradiol treatment [$F(1,16) = 15.31$; $P = .01$], showing that rats treated with estradiol gained less weight than animals receiving just the vehicle. There was a significant Estradiol × Time interaction [$F(1,16) = 5.94$; $P < .05$] and also a significant Stress × Time interaction [$F(1,16) = 9.98$; $P < .01$], signifying that the difference in weight gain, compared to controls, was higher at the beginning of the treatments both for stressed and estradiol-treated animals.

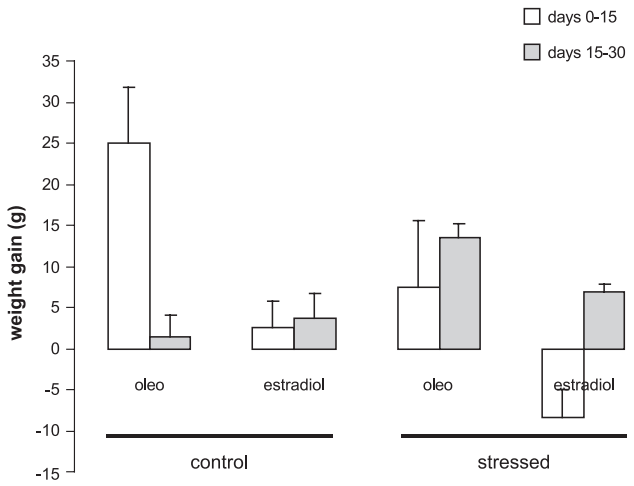

Fig. 1. Mean weight gain during the first 15 days of treatment and during the last 15 days of treatment. Data expressed as mean ± S.E.M. of weight gain in grams. A repeated measures ANOVA revealed a significant effect of estradiol replacement [$F(1,16) = 15.31$; $P = .01$], a significant Estradiol × Time interaction [$F(1,16) = 5.94$; $P < .05$], and a significant Stress × Time interaction [$F(1,16) = 9.98$; $P < .01$].

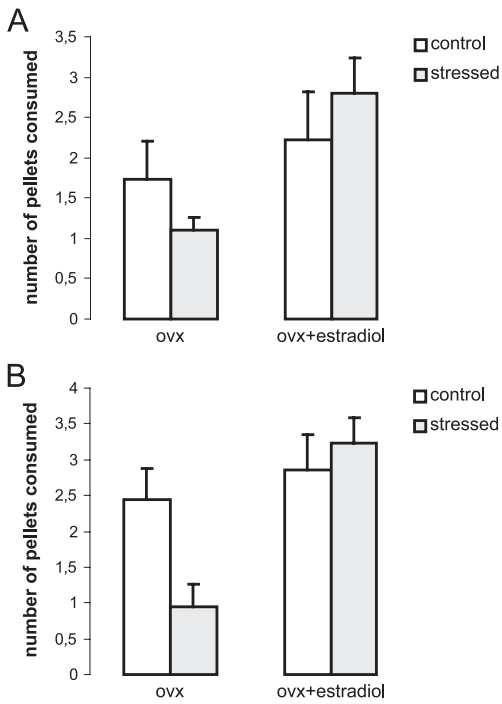

Fig. 2. Mean intake of sweet food (Froot loops) in ovariectomized female rats with or without estradiol replacement after chronic variate stress. (A) After 20 days of exposure to stress; (B) after 30 days of exposure to stress. Data expressed as mean ± S.E.M. $N = 9–10$ animals/group. A two-way ANOVA revealed a significant effect of estradiol replacement at both times of treatment [20 days, $F(1,34) = 6.270$, $P < .02$; 30 days, $F(1,34) = 11.872$, $P < .002$]. At 30 days of treatment (B), a significant interaction between estradiol replacement and exposure to chronic stress was also observed [$F(1,34) = 5.682$, $P < .02$].

The effect of chronic variate stress upon the intake of Froot Loops in ovariectomized female rats with or without estradiol replacement is shown in Fig. 2. Consumption of this type of sweet food was measured both at 20 and 30 days of stress treatment. A two-way ANOVA revealed a significant effect of estradiol replacement at both times of treatment [$F(1,34) = 6.270$, $P < .02$ for 20 days of treatment, and $F(1,34) = 11.872$, $P < .002$ for 30 days of treatment], with the hormone determining an increased intake. At 30 days of treatment, a significant interaction between estradiol replacement and exposure to chronic stress was also observed [$F(1,34) = 5.682$, $P < .02$], i.e., while chronic variate stress induced decreased consumption in OVX animals, this effect was not observed in rats with estradiol replacement.

The effect of chronic varied stress in decreasing sweet intake could also be secondary to a decrease in body weight. Therefore, we compared the ratio consumption of sweet food/body weight. The results presented a similar effect to the consumption only (OVX group: 0.0116 ± 0.0034; OVX + estradiol: 0.0124 ± 0.0037; OVX + stress: 0.0038 ± 0.0016; OVX + stress + estradiol: 0.0149 ± 0.002). A two-way

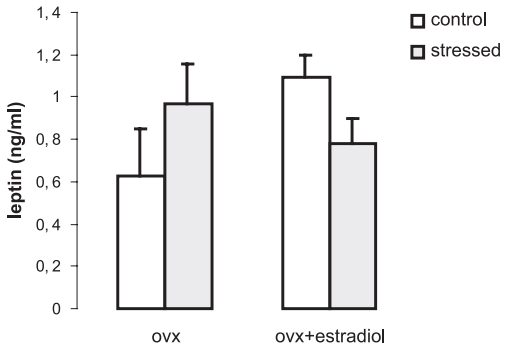

Fig. 3. Serum leptin levels in ovariectomized female rats with or without estradiol replacement after 30 days of chronic variate stress. Data expressed as mean ± S.E.M. $N = 5–9$ animals/group. A two-way ANOVA revealed a significant interaction between stress exposure and estradiol replacement [$F(1,24) = 4.396$, $P < .05$], with no effect of the treatments (stress and estradiol replacement) when separately analyzed.

ANOVA revealed a significant effect of estradiol replacement [$F(1,34) = 6.270$, $P < .02$ for 20 days of treatment, and $F(1,19) = 4.956$, $P < .05$], with the hormone determining an increased ratio. A marginal interaction between estradiol replacement and exposure to chronic stress was also observed [$F(1,19) = 3.753$, $P = .068$].

The effect of chronic variate stress on serum leptin levels in ovariectomized female rats with or without estradiol replacement is shown in Fig. 3. In OVX rats not submitted to chronic stress, estradiol replacement increased leptin levels (marginal significance, Student's t test, $P = .06$). A two-way ANOVA revealed a significant interaction between stress exposure and estradiol replacement [$F(1,24) = 4.396$, $P < .05$], with no effect of the treatments (stress and estradiol replacement) when separately analyzed.

4. Discussion

All animals used in this study gained weight with time; however, the weight gain differed among the groups. The interaction between time and stress indicates that stressed females gain weight differently than nonstressed females, and this is consistent with other studies (Konarska et al., 1990; Harro et al., 2001; Bowman et al., 2002). In addition, estradiol-treated rats weighed less than their controls, a characteristic of the hypophagic effect of estrogen (Bowman et al., 2002).

The results of the present study show that the effect of chronic variate stress on sweet food consumption in ovariectomized rats can be modulated by hormonal replacement. This observation suggests a role of estradiol in the anorectic properties of chronic variate stress with respect to ingestion of sweet food.

Consistent with previous findings, when males were used (Gamaro et al., 2003a), chronic variate stress decreased ingestion of sweet food after 30 days of treatment. Non-ovariectomized females submitted to the same procedure also present a reduction in sweet food intake (Gamaro et al., 2003b). Feeding behavior after exposure to stress may depend on the nature and the predictability of the stressor (Hargreaves, 1990; Paré and Redei, 1993; Pucilowski et al., 1993; Martí et al., 1994; Morley et al., 1986). Models of chronic mild stress have been reported to lead to behavioral disturbances (Katz et al., 1981; Willner, 1991; Basso et al., 1993), including decreased responses to rewarding stimuli, as demonstrated by decreased sucrose consumption (Willner et al., 1987; Ferretti et al., 1995) and place preference conditioning (Papp et al., 1991, 1992). The absence of predictability of the stressor applied is an important characteristic of this model and may be related to the different effects observed in these animals when compared to other models in which repeated stress is used and higher consumption of sweet food is observed (Ely et al., 1997; Silveira et al., 2000). The present results also show no significant effect of stress after 20 days of treatment, although a nonsignificant decrease in sweet food ingestion may be observed. Therefore, it appears that behavioral changes such as the one observed here in animals submitted to chronic variable stress may require some time to develop.

Other studies have observed higher intakes of very sweet mash and lower rates of eating in animals submitted to chronic exposure to mild unpredictable stress relative to controls (Sampson et al., 1992). These results are somewhat different from the results obtained herein, possibly because the session length was very different. Furthermore, in the present data, it is important to point out that the measurement of sweet food consumption was made in a 3-min section. Therefore, the consumption observed represent the initial drive of the animals in relation to sweet food, and agreeing with other studies, this effect may represent decreased reactivity to sweetness following chronic exposure to stress.

In addition to its role in the stress response, the HPA axis and particularly the glucocorticoid hormone corticosterone have been shown to play an important role in appetitively motivated behavior (Barr et al., 2000; Piazza and Le Moal, 1997). Corticosterone is known to modulate feeding behavior, having a stimulatory effect on food intake, particularly on carbohydrates intake (Tempel and Leibowitz, 1989). This effect of corticosterone enhancing carbohydrate intake is opposite to the effect observed in the present study. It is possible that in rats exposed to chronic stress during a long treatment, opponent processes prevail, causing a hedonic shift in the other direction (Solomon and Corbit, 1974). On the other hand, activation of HPA axis also releases CRH, which is known to reduce feeding behavior (Koob and Heinrichs, 1999). Presently, it remains unclear if some of these different effects of HPA axis stimulation on feeding behavior is, at least in part, responsible for the present results.

It should be observed, however, that the pattern of consumption responses exhibited following stress treatment was dependent on whether or not subjects received hor-

monal replacement. After 30 days of variate stress, the suppressive effects of this treatment on sweet food intake was evident in the OVX group; however, it was not exhibited by the group receiving estradiol replacement. An overall effect of estradiol was also observed. Surprisingly, estradiol replacement induced a small, but significant, increase in the consumption of sweet food, although no effect was observed in the standard chow consumption (data not shown). In addition, weight gain was smaller in animals treated with estradiol in accordance with the known anorectic effect of this hormone. Thus, under the conditions of this task, estradiol replacement led to an increased appetite for sweet palatable food, although it reduced weight gain.

This differential pattern of ingestive behavior by the OVX + estradiol stressed group suggests that this model of chronic variate stress is able to induce a pronounced suppressive effect on ingestion in ovariectomized rats not receiving hormonal replacement; while in those rats receiving hormonal replacement, this effect of chronic variate stress was prevented. It has been hypothesized that chronic variate stress reduces intake of sweet food by promoting decreased responses to rewarding stimuli, a characteristic of a state of depression (anhedonia) (Willner, 1991; Murua and Molina, 1992). In the present study, estradiol replacement modified the effect of stress treatment on sweet food ingestion by increasing ingestive responses, an effect observed particularly in the stressed group. This effect may be related to an altered response to the sweet stimulus. Although estradiol is known to reduce intake (Ganesan, 1994; Wade and Schneider, 1992), gustatory and food habit changes have been observed during the menstrual cycle in women (Alberti-Fidanza et al., 1998) and the estral cycle in rats (Clarke and Ossenkopp, 1998b). Sensitivity to sweet taste increases with an increase of estradiol. At the same time, in these studies, with the highest estradiol values, there was a tendency towards lower energy intake, predominantly provided by carbohydrates (such as bread) (Alberti-Fidanza et al., 1998).

Estradiol replacement has been shown to decrease meal size in rats (Geary et al., 1994; Hrupka et al., 1997). Studies of meal microstructure after estradiol replacement have shown that the rate of licking was not changed during the beginning of the meal but was significantly slower during the remainder of the meal. Burst size, cluster size, and interburst interval were lower after estradiol replacement during 5–7 min of the test meal (Hrupka et al., 1997). In addition, estradiol is known to reduce meal size and food intake in female rats, at least in part, by increasing the satiating potency of CCK (Clarke and Ossenkopp, 1998a; Eckel et al., 2002), possibly by increasing the central processing of the vagal CCK satiation signal (Eckel et al., 2002), and this mechanism of action would explain why estradiol effects do not occur during the first minutes of a meal. Since the task used in this study to evaluate feeding behavior lasted for just 3 min, it is possible that longer periods would present different effects, with the manifestation of the anorectic effects of estradiol. In these first minutes, however, the main effect was an increase in consumption, maybe as a result of altered sensitivity to sweet taste, particularly in the chronically stressed group. In this case, estradiol prevented the reducing effect of chronic variate stress on sweet food consumption.

The effect of chronic variate stress to decrease sweet intake has also been hypothesized to be secondary to a decrease in body weight. The present data are relevant to this debate (e.g., Willner et al., 1996). Therefore, we evaluate the ratios sweet food consumption/body weight. The results show the same pattern of effect of the results observed in Fig. 2, suggesting that the reduction in body weight is not enough to explain the decreased consumption of sweet food.

In OVX rats not submitted to chronic stress, estradiol replacement increased leptin levels, consistent with the known anorectic effects of estradiol (Ganesan, 1994; Wade and Schneider, 1992). Females are characterized by significantly higher plasma leptin concentration than males (Chudek et al., 2002). Recent studies have shown that 17β-estradiol increases ob mRNA expression and leptin release in more than 80% (Tanaka et al., 2001; Machinal-Quelin et al., 2002), although others have reported that plasma concentration of estradiol contributes to leptinemia only to a minor degree (Chudek et al., 2002).

In the present study, chronically stressed rats presented a nonsignificant increase in leptin levels; while at the same time, chronic stress induces depressive effects on ingestive behavior. Hormonal factors, including glucocorticoids, the hormones secreted during stress, are known to modulate leptin secretion (Considine et al., 1997). Dexamethasone, for example, is a powerful stimulator of leptin secretion and leptin mRNA expression in rat adipose tissue in vitro. The time lag and the similarity of these two parameters indicate that dexamethasone presumably regulates leptin at the transcriptional level (Kristensen et al., 1999) in such a way that it probably has a long-lasting effect. In addition, in our chronically stressed group, no effect of estradiol replacement was observed as opposed to the effect observed in the control group (as expressed by the significant interaction between the treatments). These results suggest an interaction between the mechanisms of action of these two treatments with regard to their effects on serum leptin levels. Interestingly, estradiol replacement, in the stressed group, prevented both the reduction observed in sweet food intake and the increase in leptin.

Naturally, although it is possible that leptin could play a role in the behavioral effects described in the present study, it becomes clear, by the analysis of the two sets of data, behavioral and biochemical, that leptin levels are not completely consistent with the behavioral effects, particularly the estradiol groups. This interpretation is further complicated by the fact that the neural mechanisms by which estradiol interferes with food intake remain to be determined. The effects observed herein may be related to the known effects of estradiol on leptin biosynthesis and secre-

tion (Mystkowski and Schwartz, 2000) and indicate that, at least in estradiol-treated groups, other factors are certainly involved in the observed effects.

Estradiol treatment has been observed to decrease anxious behavior (Bowman et al., 2002), an effect which may be mediated, at least in part, by modifications in the 5-HT system in response to estradiol treatment (Williams and Uphouse, 1989; Bowman et al., 2002). As observed above, estradiol increases the secretion of leptin. In addition, a greater effect of estrogen on leptin secretion has been reported in dexamethasone-stimulated cells (Kristensen et al., 1999). Conversely, the effects of leptin on food intake have been suggested to be mediated in part by the midbrain serotonergic systems. Leptin can be selectively accumulated by serotonergic neurones in the raphe nuclei (Fernandez-Galaz et al., 2002), and its perfusion induces a significant increase in 5-HIAA overflow from the hypothalamus (Hastings et al., 2002). In addition, leptin treatment regionally down-regulates serotonin transporter binding sites in the brain (Charnay et al., 2000). Thus, a possible interaction between estradiol-induced leptin secretion and the serotonergic system would be a potential mechanism by which estradiol may interfere with the effects of chronic stress observed herein since some models of chronic-mild stress (such as the one used in the present study) have been proposed as models of depression in animals studies (Pucilowski et al., 1993; Katz et al., 1981; Willner, 1990, 1991) and serotonin has been implicated in the pathophysiology of depression (Van Praag et al., 1990). This possibility requires further study.

In conclusion, an alteration in leptin levels may contribute, at least in part, to the interaction observed between chronic stress and estradiol replacement in feeding behavior. The exact neurobiological mechanism involved in this effect, after chronic stress, remains to be clarified. In addition, the existence of a relationship between estradiol and the anorexic effect of chronic stress also requires further study.

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