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Effects of paradoxical sleep deprivation and cocaine on genital reflexes in hyperlipidic-fed rats

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Abstract

The present study was designed to investigate the effects of a hyperlipidic diet (HD) on penile erection (PE) and ejaculation (EJ) induced by cocaine in paradoxical sleep deprived (PSD) rats. Secondly, we aimed to verify the influence of HD cafeteria diet on steroid hormone levels. Twenty-one day-old male Wistar rats were randomly assigned into two groups: rats fed with commercial chow diet and rats fed with a palatable HD containing chow mixed with peanuts, milk chocolate and sweet cookies in the proportion of 3:2:2:1. After nine weeks of treatment, the animals were submitted to PSD or maintained as home cage control group for 96 h and challenged with cocaine (7 mg/kg, ip). Results showed that the HD led to a reduction in the frequency of erection in the PSD+cocaine group when compared to the PSD+cocaine fed with standard diet. Regardless of the diet, testosterone concentrations were significantly lower and progesterone was higher in the PSD rats than in the respective home-cage control rats. Although there were no hormonal alterations, the findings showed that a long-term HD might modify the stimulating effects of cocaine in potentiating genital reflexes in PSD rats.

Keywords: Paradoxical sleep deprivation; Hyperlipidic diet; Genital reflexes; Cocaine; Testosterone; Progesterone

1. Introduction

Obesity has become ubiquitous in the past two decades and has become epidemic in several countries. In the years 1999 and 2000, it was estimated that 31% of the adult American population 20 years of age and over was obese (body mass index-BMI of 30 and over).

Eating habits have increasingly drawn the attention of investigations since these have changed dramatically in the last decades in view of the alterations in people's life styles, who have had less time to eat adequate and balanced meals resorting instead to fast foods, normally containing an unnecessary high fat content and calories. Moreover, these foods are highly palatable that stimulate their intake aggravating the situation even further, especially in children and adolescents who may acquire poor eating habits that are kept throughout their entire lives leading them to obesity (Dâmaso et al., 2003).

Feeding rats a cafeteria diet (Scalfani and Springer, 1976; Mandenoff et al., 1982) has been used extensively as an experimental model to study obesity and energy balance expenditure (Coatmellec-Taglioni et al., 2000). In this protocol, a choice of several energy dense human foods is offered to the animals to induce voluntary hyperphagia, which can thus result in increases in body weight and adiposity (Scalfani and Springer, 1976) but occasionally this

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consequence is controversial (Hill et al., 1983). Several hormonal alterations may occur in response to the ingestion of high lipidic diets.

Since erections are dependent on testicular androgen, and testosterone exerts motivational effects necessary for the display of male sexual behavior (Roselli and Chambers, 1999), we have been investigating the effects of paradoxical sleep deprivation (PSD) that affects several behavioral responses (Frussa-Filho et al., 2004; Silva et al., 2004a,b,c; Andersen et al., 2005), including sexual behavior (Andersen et al., 2003, 2004a) in combination with cocaine, a drug of abuse that increases sexual drive and pleasure (Buffum et al., 1988). Indeed, PSD modified the effects of steroid hormones on the sexual behavior of rats (Velazquez-Moctezuma et al., 1989) and also led to reduced testosterone concentrations and increased progesterone in male rats (Andersen et al., 2003, 2004a, 2005) besides having induced marked cocaine-enhanced genital reflexes in male PSD rats. Sleep deprivation is a major social concern with important implications in health and performance and leads to several behavioral changes in humans and animals. Much literature documents the negative effects of sleep deprivation on a wide range of psychomotor and cognitive performance, mood variables, and brain alterations. However, as described in our experiments, PSD showed a facilitatory effect on the occurrence of genital reflexes in male rats, in addition to the well-documented anti-depressant effects.

Considering that dopaminergic mechanisms are involved with feeding behavior (Rothwell et al., 1982), and dopamine exerts great influence in male sexual behavior (Hull et al., 1999), we proposed to verify if altered food consumption induced by a highly palatable diet could modify genital responses of rats after being sleep deprived and challenged with cocaine. Secondly, we aimed to investigate if sex hormones over behavior could be involved in these reflexes. Thus, the objective was firstly to investigate whether PSD and cocaine administration would also induce spontaneous genital events in male rats after long term HD, focusing on penile erection (PE) and ejaculation (EJ). Secondly, to verify the influence of HD on steroid hormones.

2. Methods

2.1. Animals

Eighty Wistar male rats from our breeding facilities were used in this experiment. The vivarium was maintained on a 12 h light–dark photoperiod (lights on 0700–1900 h) at an ambient temperature of 23 °C. All procedures used in the present study complied with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals (Andersen et al., 2004b).

2.2. Hyperlipidic diet (HD)

At 21 days after weaning, the rats were randomly distributed into two groups. The first group ($n=40$), on a normal diet, received standard chow (17.03 kJ/g). The second group, HD-fed group ($n=40$), was given a palatable hyperlipidic diet that consisted of powdered commercial standard chow mixed with sweet cookies, peanuts and milk chocolate that were made into pellets. The approximate composition of the HD was 20% protein, 20% fat and 48% carbohydrate. The caloric densities were 21.40 kJ/g (35% of calories as fat) and, according to Estadella et al. (2004), this diet induces an excessive accumulation of body fat. Pilot studies demonstrated that 210 g of food daily allowed the three animals per cage to have chow ad libitum, so this was the amount both groups were fed. Animals were fed for 9 weeks before being studied. The animals were weighed three times per week during the nine-week treatment yet statistical differences were not observed between the groups. Still, a significant accumulation of adipose tissue, mainly in the abdominal region and peritoneal cavity was observed.

2.3. Paradoxical sleep deprivation (PSD)

Naive rats were submitted to the PSD procedure, which involved placing ten rats on 14 narrow circular platforms placed inside a tiled tank ($123 \times 44 \times 44$ cm) filled with water to within 1 cm of their upper surface. At the onset of each paradoxical sleep (PS) episode, the animal experiences a loss of muscle tone and falls into the water, thus being awakened. The cage control group was maintained in the same room as the experimental rats for the duration of the study. Previous work from this laboratory has demonstrated that the rats are deprived of all phases of sleep on the first day, followed by a more selective and complete loss of PS on subsequent days. Therefore, our aim in choosing this technique was to evaluate genital reflexes that would reflect a predominant suppression of PS over four days. Throughout the study, the experimental room was maintained under controlled temperature (23 ± 1 °C) and a light–dark cycle (lights on at 0700 h and off at 1900 h). Food and water were provided ad libitum by placing chow pellets and water bottles on a grid located over the tank. The water in the tank was changed daily throughout the PSD period.

2.4. Experiment 1: genital reflexes evaluation challenged with cocaine

Behavioral observations were carried out at 0900 h in a temperature-controlled room, where the animals were monitored by trained observers unaware of which group the rats belonged to (PSD or home-cage or diet). Observations of genital reflexes were made immediately after the cocaine injection, which was applied immedi-

ately upon removing each rat from the deprivation tank or control home-cage. Forty rats were tested for spontaneous genital reflexes after receiving an acute i.p. injection of cocaine (7 mg/kg, Sigma Chemical Co., USA) freshly dissolved in saline solution. The number of spontaneous PE and EJ was assessed for 60 min. A PE was counted only when the rat displayed and bent down to lick its penis in full erection and EJ was scored by the number of ejaculatory plugs. The following parameters of genital reflexes were recorded: 1) the number of PE and EJ displayed during the test; 2) latency to the first PE and EJ.

2.5. Experiment 2: collection of blood samples and hormone analysis

Naïve PSD or home-cage animals (without injection) were sacrificed individually by decapitation with a minimum of disturbance in an adjacent room. Blood samples were immediately collected and stored individually. For testosterone and progesterone analysis, blood was collected in glass tubes and centrifuged at 3500 rpm for 10 min at room temperature, then frozen at −20 °C until assayed. Intra-assay coefficient variations are given in parentheses. Testosterone concentration (7.7%) and progesterone (9.8%) were measured by chemiluminescent enzyme immmunoassay (Advia Centaur, Bayer Corporation, Tarrytown, NY, USA). Duplicate serum aliquots were used. These assays were performed with automated procedures routinely performed in our clinical laboratory.

2.5.1. Statistical analysis

Statistical evaluation of the frequency of genital reflexes and hormone assay results were performed by analysis of variance (ANOVA), followed by Duncan's test for the comparison between the groups. The level of significance was set at $p<0.05$.

3. Results

3.1. Experiment 1: Genital reflexes evaluation

In experiment 1 (Fig. 1), two-way ANOVA for penile erections revealed a significant group (CTRL or PSD) and diet (normal or HD) effect [$F_{(1,36)}=31.45$, $p<0.0001$] and [$F_{(1,36)}=4.15$, $p<0.04$]. Although analysis of variance did not reveal significant interaction between these factors, the two-way ANOVA showed a tendency ($p<0.08$) and suggests that the association of the stimulating effect of PSD and HD induced alterations in frequency of genital reflexes in cocaine challenged rats.

Post-hoc Duncan analysis demonstrated that PSD potentiated cocaine-induced genital reflexes in both normal diet (ND) and HD rats in relation to respective home cage control groups ($p<0.0001$ and $p<0.01$, respectively). Moreover, the PE frequencies displayed by the HD-fed PSD group were significantly lower than in the standard diet group ($p<0.01$), with the frequency of events falling from 3.0 ± 0.5 to 1.5 ± 0.6.

ANOVA followed by Duncan test for ejaculatory behavior [$F_{(1,36)}=17.72$; $p<0.0001$] revealed that the EJ frequencies observed in the PSD group, independently of the diet, were significantly higher than in home-cage rats (ps<0.01). The EJ frequencies observed in the PSD groups were 0.9 ± 0.3 (ND) and 0.6 ± 0.2 (HD). No statistical differences were seen among the PSD groups. Neither of the two home-cage groups displayed EJs during the 60-min observation period.

No significant alterations were seen in the latency of PE or EJ reflexes (time elapsed between the injection to the first erection/EJ) in the four groups evaluated.

3.2. Experiment 2: hormone levels

Since a high fat diet resulted in an increased mean concentration of serum testosterone in rats (Clinton et al.,

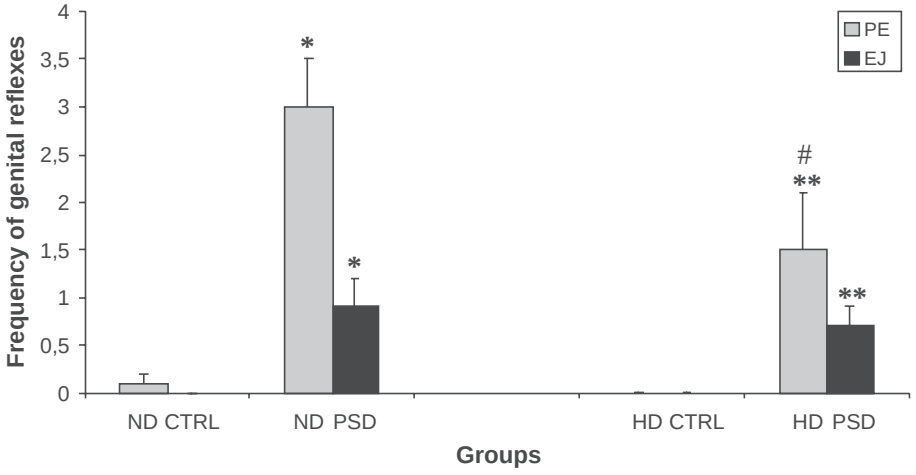

Fig. 1. Effect of i.p. cocaine (7 mg/kg) on penile erection (PE) and ejaculation (EJ) in home-cage control (CTRL) and paradoxical sleep deprived (PSD) rats. Frequency of PE events expressed as mean±SEM of 10 rats per group. *Different from respective normal diet (ND) control rats; **Different from respective hyperlipidic diet (HD) control rats; #Different from normal diet (ND) PSD rats; (two-way ANOVA followed by Duncan's test). See text for p values.

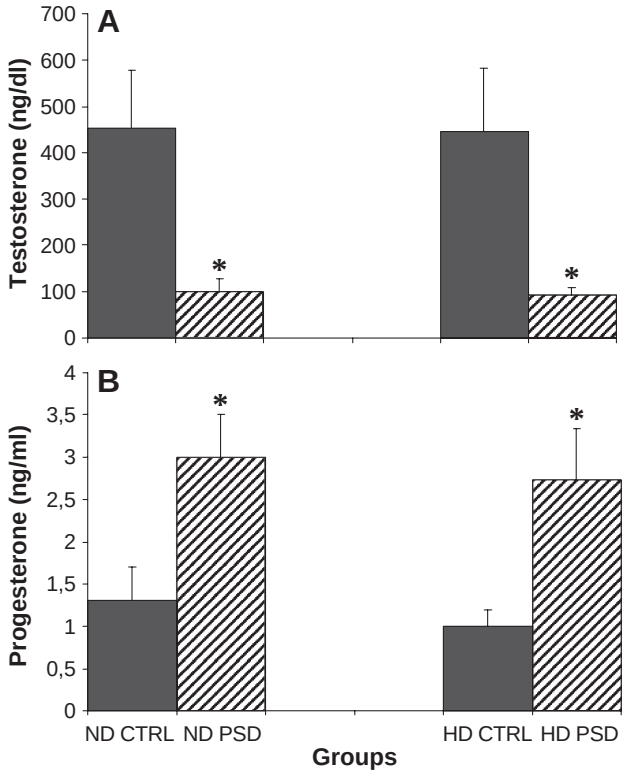

Fig. 2. Serum testosterone (ng/dL) and progesterone (ng/mL) in control, and paradoxically sleep deprived (PSD) rats (without injection). Values are expressed in mean±SEM for 10 rats. *Different from respective control rats (two-way ANOVA followed by Duncan's test).

1997), we elected to evaluate this hormone in our study. PSD resulted in a significant decrease in testosterone mean levels as depicted in Fig. 2A. ANOVA revealed a significant group effect [$F_{(1,36)}=13.82$; $p<0.001$] although no other significant effects were detected. The Duncan test showed that regardless of the diet, testosterone concentrations were significantly lower in the PSD rats than in the control rats ($p<0.01$). Mean (±SEM) levels for the ND groups were: control, 452.1±126.8 ng/dl and PSD, 99.3±28.7 ng/dl; and for HD, control, 446.0±137.3 ng/ml and PSD, 93.8±13.9 mg/dl.

We added progesterone analysis to the present study in view of our previous data that demonstrated a relevant role of this hormone in genital reflexes. Analysis of progesterone serum concentrations revealed significant differences between the groups [$F_{(1,34)}=12.92$, $p<0.001$]. No statistical differences were seen for diet or interaction effects. Sleep deprived rats submitted to ND or HD showed higher progesterone concentrations than the respective home-cage control rats ($p<0.02$ and $p<0.01$, respectively) as shown in Fig. 2B.

4. Discussion

High caloric and palatable foods (ad libitum) were offered to the animals in an attempt to induce voluntary hyperphagia (Scalfani and Springer, 1976; Estadella et al., 2003, 2004). Indeed, such diet after weaning led to increased adiposity and lipid content (Estadella et al., 2004) as well as biochemical alterations related to the cardiovascular system (D'Almeida et al., submitted for publication). After nine weeks on HD, the rats were submitted to sleep deprivation and challenged with cocaine to evaluate the frequency of genital reflexes. Corroborating our previous studies (Andersen and Tufik, 2002; Andersen et al., 2003) a single cocaine injection did not produce a marked increase in the frequency of genital events in control rats, and the present study complements the data in that regardless of the diet genital reflexes did not occur in home-cage rats. Moreover, when exposed to PSD, HD-fed rats showed a significant reduction in the frequency of erections when compared to sleep-deprived rats that were fed a standard diet during the same period.

This finding corroborates the existence of a relationship between alterations in feeding behavior and sexual dysfunction. It also validates the cafeteria/hyperlipidic diet model for studies of adiposity associated to increased food intake in rats (Estadella et al., 2004). Of note, 98% of obesity cases are caused by excessive ingestion of calories associated to a sedentary lifestyle (Sande and Mahan, 1991).

The search for the consequences of dietary habits has involved the study of various animal models. Cafeteria-diet data are commonly used in many laboratories because of the similarities of human obesity in developed countries (Lladó et al., 1997). However, little behavioral work has been done with dietary factors related to sexual behavior. Although in humans obesity is characterized by a variety of severe co-morbidities and it imposes a tremendous burden to the health care system of western societies, sexual dysfunction as a consequence of obesity is less recognized (Trischitta, 2003).

A model for genetic obesity can be found in the Zucker rat. This strain presented reduced reproductive capacity (Piser et al., 1981). One contributing factor to this infertility lies in the reduced sexual behavior of the male, described in young as well as in old obese rats (Edmonds and Withyachumnarnkul, 1980). In view of the markedly reduced sexual activity verified in this strain along with the difficulty of keeping them on narrow platforms (6,5 cm in diameter), we elected to investigate the interaction of HD-diet associated to sleep deprivation on genital reflexes in rats which were exclusively fed a HD that simulates the worrisome condition of a high caloric intake. Since it is known that a positive energetic imbalance has an extreme influence in the etiology of exogenous obesity, food in excess becomes a highly relevant factor.

Male obese rats are infertile in spite of normal sperm motility and testicular anatomy (Piser et al., 1981). Decreased sexual behavior is not a consequence of obesity itself, since it persists in obese animals given a restricted diet (Edmonds and Withyachumnarnkul, 1980). Moreover, the data show that sperm from obese males is normal in fertilizing capability leading to inadequate sexual behavior as the

primary factor that limits their reproductive potential. These findings suggest that reproductive dysfunction of obese males is behavioral rather than functional (Edmonds et al., 1982).

The current study demonstrates that animals submitted to HD and later exposed to PSD and to a single cocaine injection displayed a significant lower frequency of erection compared to PSD+cocaine rats fed with standard diet. The method of PSD associated to cocaine seems to exert a favorable effect over male sexual reflexes as in agreement with our previous data (Andersen and Tufik, 2002; Andersen et al., 2003, 2004a). Although this facilitatory effect was observed in HD fed PSD rats in relation to their respective non sleep-deprived groups, the incidence of the occurrence of the erectile events was lower, falling from 3.0±0.5 (ND) to 1.5±0.6 (HD, Fig. 1) observed in PSD rats. Thus, it seems that the stimulating action of cocaine in potentiating the sexual effects in PSD rats does not override the inhibitory effects of high fat diet on sexual behavior.

For a long time dopamine has been documented to play a central role in male sexual motivation and behavior (Melis and Argiolas, 1995) and further, supersensitivity of dopaminergic receptors, with consequent behavioral changes in response to dopaminergic drugs following PSD have been reported (Tufik et al., 1978). In regards to our model, we do not exclude the possibility that other neurotransmitters may play an important role in modulating this behavior since cholinergic, GABAergic, noradrenergic and opioid systems and dopaminergic as well have also been involved with genital reflexes.

Although testosterone male sexual motivational effects have been proposed (Roselli and Chambers, 1999), the cause and effect relationship between this hormone and sexual responses in males cannot be established from the our findings since sleep-deprived rats showed significantly lower testosterone levels besides a high frequency of erections (Andersen et al., 2003, 2004a). Furthermore, testosterone and progesterone were not modified by HD. Thus, it could be speculated that further mechanisms may be involved in such complex behavior. Of note, the current finding indicates that although HD reduces genital reflexes they are still present reflecting the reliability of the model proposed by Andersen and coworkers.

Recently much attention has been focused on the interactions between diet and sex hormones. Nutrition and diet have the potential to effect sex hormone production and metabolism in men (Hamalainen et al., 1984). Obesity is associated with low plasma testosterone (Glass et al., 1977; Kley et al., 1980). In contrast, intentional weight loss in a program including very-low-energy-diet increases testosterone but it does not affect the scores of a questionnaire measuring sexual function (Kaukua et al., 2003).

In rats, the diminished male sexual behavior was reported in both young obese rats, which have normal levels of circulating testosterone, and in older rats that show a moderate decline in testosterone (Edmonds et al., 1982). According to these authors, the reduced plasma testosterone concentrations cannot account for the reproductive inadequacy of obese male rats since sexual behavior was inadequate and natural insemination rate was reduced despite normal testosterone levels.

The increase of fat in one's diet may induce alterations in the concentrations of testosterone in rats as well as in humans (Hamalainen et al., 1984; Clinton et al., 1997, and references therein). It seems unlikely that the frequency of genital reflexes is driven by a variation in circulating testosterone concentrations. In fact, sleep-deprived young (Andersen et al., 2003), adult (Andersen et al., 2004a) and aged rats (Andersen et al., 2004c) showed significantly lower testosterone concentrations at the time-points analyzed, indicating that testosterone per se could not be accounted for as the main factor governing genital behaviors observed in the PSD groups. Although testosterone male sexual motivational effects have been proposed (Roselli and Chambers, 1999) the cause and effect relationship between this hormone and sexual responses in males cannot be determined by analysing present data.

In accordance with our previous data, high progesterone levels were found in sleep-deprived groups regardless of the diet treatment. The present data extend the information that progesterone concentrations were significantly higher not only in PSD given a ND but also in the PSD group fed with HD in comparison with the respective home-cage group.

The physiological role of progesterone in male sexual behavior is still not completely understood. Data from our lab indicate that progesterone treatment facilitates PE in PSD castrated rats (Andersen et al., 2004d) and furthermore, a single injection of mifepristone, an antiprogestin before cocaine administration significantly reduced the frequency of PSD rats displaying PE compared to control rats (Andersen and Tufik, 2005). In light of this evidence, we may speculate that progesterone might influence the modulation of male genital reflexes displayed by sleep-deprived rats; nevertheless, such a complex phenomenon as sexual behavior should not be attributed to one single factor.

Our data, as a whole, suggest that PSD and cocaine stimulates genital reflexes in HD-fed rats, however, not at the same magnitude seen in standard diet fed rats, suggesting that a high fat diet may influence sexual behavior in a negative way. Further research conducted in animal models can provide evidence and may prove helpful in the understanding of the stimulatory role sleep deprivation and hormones have on events related to sexual behavior.

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