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Estrus variation in anxiolytic-like effects of intra-lateral septal infusions of the neuropeptide Y in Wistar rats in two animal models of anxiety-like behavior

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ABSTRACT

Anxiolytic-like effects of intra-lateral septal infusions of the neuropeptide Y (NPY) were assessed during several estrus phases in Wistar rats tested in two animal models of anxiety-like behavior. In a conflict operant test, results showed that during late proestrus, intra-lateral septal nuclei infusions of NPY (1.0 μg/μl, $P < 0.05$; 2.0 μg/μl, $P < 0.05$; 2.5 μg/μl, $P < 0.05$) increased the number of immediately punished responses. During metestrus–diestrus only the highest doses of NPY (2.5 μg/μl, $P < 0.05$) increased the number of immediately punished reinforcers. In the elevated plus-maze test, results showed that during late proestrus, intra-lateral septal nuclei infusions of NPY (1.0 μg/μl, $P < 0.05$; 2.0 μg/μl, $P < 0.05$) produced anxiolytic-like actions. During metestrus–diestrus only the highest doses of NPY (2.0 μg/μl, $P < 0.05$) produced anxiolytic-like actions. Neither NPY nor estrus phases significantly modified the number of closed arms entries in the elevated plus-maze test. It is concluded that anxiolytic-like effects of NPY vary within the estrus cycle in Wistar rats.

1. Introduction

The septal area has been implicated in the modulation of emotions, especially fear and anxiety [74]. Stimulation of the lateral septal nuclei reduces fear-like behavior during aversive states [83]. These effects are similar to the one seen with lateral septal infusions of benzodiazepine drugs [47,63], antagonists of galanin [13], the neurosteroid allopregnanolone [50] and the neuropeptide Y [34].

The neuropeptide Y (NPY) is a 36-amino-acid peptide and produces anxiolytic-like actions when administered centrally [19], i.e., in the amygdala [36], in the locus coeruleus [32], in the dorsal periaqueductal gray matter [33] and in the lateral septal nuclei [34]. In this regard, it is important to note that NPY is abundantly expressed in the lateral septal area [25] but is null in the medial septum [41]. However, until now, the role of lateral septal NPY neurotransmission in the regulation of the stress response is not fully understood.

In a variation of standard Geller and Vogel's conflict tests, the rats may choose between receiving an immediately punished reinforcer or a delayed unpunished reinforcer. This experimental task is sensitive to the anxiolytic-like actions of several drugs which increase the number of immediately punished reinforcers [21]. The behavior in this task depends on the endocrine state, since rats are more sensitive to anxiolytic drugs during the late proestrus when compared to

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the metestrus–diestrus [48]. However, there are no reports related to the effects of the NPY infused into the lateral septal region during the estrus cycle. Therefore, NPY was infused into the lateral septal nuclei during different stages of the estrus cycle and its possible anticonflict-like actions were tested in the operant conflict task. We tested the hypothesis that the anxiolytic-like actions of intra-lateral septal infusions of NPY will vary according to the estrus cycle phases. Considering that NPY may produce nociceptive and sedative actions [46], other groups of female rats were tested in the elevated plus-maze test after receiving intra-lateral septal infusions of the NPY. The rationale in the use of the elevated plus-maze is that the elevated plus-maze task is a well-designed test to detect both the anxiolytic and the locomotor effects of drugs [27].

2. Methods

2.1. Animals

Adult female Wistar rats (250–300 g; at the beginning of the experiments the rats were about 3 months old; $n = 104$) were lodged in housing facilities (12:12-h controlled light–dark cycle; lights on at 07:00 h). Tap water and food (Harlan Teklad, Global Diets; Madison, Wisconsin, USA) were provided ad lib, except for the conflict test groups which were lodged individually and whose food was restricted: approximately 12 h before a training or a testing session, food was removed, and training in the experimental chamber began. After finishing each experimental session, the rats had free access to food again [29]. Water was continuously available. Estrus phases were determined by daily microscopic examination of vaginal smears [47]. Proestrus was identified by the predominance of nucleated epithelial cells, estrus was identified by the presence of dense sheets of cornified epithelial cells, metestrus was identified by the presence of scattered, nucleated, or cornified epithelial cells and leukocytes, and diestrus was identified by the presence of leukocytes [16]. Only those rats showing two consecutive regular estrus cycles (4 or 5 days) were included in the study. All experimental sessions were performed from 8 a.m. to 12 a.m. under standard laboratory conditions. All experiments were performed under strict principles of animal care [53].

2.2. Drugs

Neuropeptide Y (Porcine; American Peptide Company, Sunnyvale, CA; dissolved in an artificial CSF) was infused into lateral septal nuclei.

2.3. Experimental design

The inclusion of rats in any experimental group ($n = 5$ rats each group) was counterbalanced. On the basis of a previous report showing large behavioral differences between late proestrus and metestrus–diestrus in the conflict-operant task [48], only these estrus phases were considered. In the conflict test, an initial series of experiments obtained dose–response curves for intra-lateral septal infusions of NPY (0.5; 1.0; 2.0; 2.5 μg/μl; dissolved in an artificial CSF) for rats tested during late proestrus or during metestrus–diestrus.

In a second series of experiments, using the elevated plus-maze test, dose–response curves for intra-lateral septal infusions of NPY (0.5; 1.0; 2.0 μg/μl; dissolved in an artificial CSF) during late proestrus or during metestrus–diestrus were obtained.

2.4. Behavioral tests

2.4.1. Conflict test

2.4.1.1. Apparatus. The rats ($n = 50$) were trained in an experimental chamber (height: 33.0 cm; length: 30.0 cm; width: 25.0 cm; Coulbourn Apparatus, USA) placed in a ventilated, sound-attenuated cubicle. One wall of the experimental chamber contained a recess in which a dispenser delivered a reinforcer (0.10 ml of condensed milk). Two apertures located 5.0 cm above and 2.5 cm on either side of the recess allowed the placement of a motor-driven retractable lever on each side. The experimental chamber was supplied with four lights (3 W, 24 V each): one situated above each lever, one inside the dispenser, and one in the middle of the ceiling (house light). During punishment periods, a shock generator (Grass S48) delivered electric foot-shocks (0.4 mA, 45 ms). The experimental chamber was wiped clean after each session. Software (Coulbourn Instruments) and a computer accomplished the control of light stimuli and the delivery of reinforcers, and counted the number of responses.

2.4.1.2. Training procedure. The rats were trained as previously described by Hascoët et al. [21]. Briefly, all rats were trained to press either of two levers continuously present in the chamber. At the beginning, a fixed ratio of 1 was used, i.e., the rats received a reinforcer after one lever pressing. After that, the operant conditioning task was increased progressively over a 15-day period from the fixed ratio of 1 to a fixed ratio of 8, i.e., the rats received one reinforcer after eight lever presses. Thereafter, the rats underwent the final conflict training procedure. Final conflict-training sessions were organized in five successive periods totaling 17 min; these periods alternated between unpunished and punished periods. The unpunished periods (duration: 3 min) were periods 1, 3, and 5; the punished periods (duration: 4 min) were periods 2 and 4. Each session began with an unpunished period. During unpunished periods, only the right lever was inserted, and a reinforcer was presented at a fixed ratio of 8. When the unpunished periods stopped, punished periods ensued. These were signaled by illumination of the house light, and the insertion of the left lever. Each press of the left lever was unpunished, and the reinforcer was delivered at a fixed ratio of 8. Each pressing of the right lever was now reinforced according to a fixed ratio of 1, and associated with an electric foot shock. Thus, during punished periods, the rats were presented with a choice of responding, i.e., if the response is followed by punishment, then the rats have the opportunity to avoid shocks by active behavior, such as the unpunished pressing of the associated lever, a clear picture of choice-and-conflict results. Each daily session consisted of five successive periods alternating between unpunished and punished periods. When the rats displayed stable baselines (two to