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Research article

Progesterone modifies the responsivity of the amygdala-mPFC connection in male but not female Wistar rats

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HIGHLIGHTS

- Basal amygdala stimulation produced a brief excitatory response in PL and IL neurons, followed by a long-lasting inhibitory response.
- The highest inhibitory response of the BA-mPFC occurred in the PL region in males.
- Progesterone reduced the response in the PL region in males, with an opposite response in females.
- Progesterone reduced the responsivity of the PL and IL to BA stimulation only in males.
- Progesterone negated the sex difference in the responsivity of the BA-PL connection.

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ABSTRACT

Amygdala-medial prefrontal cortex (mPFC) connections partially regulate fear, anxiety, and the acquisition of conditioned fear. Progesterone exerts some effects on anxiety and fear. Currently unknown, however, are the actions of progesterone on the responsivity of amygdala-mPFC connections and possible sex differences. We performed single-unit extracellular recordings from the prelimbic (PL) and infralimbic (IL) cortices of the mPFC during stimulation of the basal amygdala (BA) in anesthetized male and diestrus female rats. Basal amygdala stimulation produced an initial excitatory paucisynaptic response that was similar between sexes and unaffected by progesterone. A long-lasting inhibitory response followed the initial brief excitatory response, which was more pronounced in the PL region in males. The unit activity ratio analysis indicated that progesterone negated the sex difference in the PL region response to BA stimulation. The results suggest that progesterone decreases the responsivity to amygdala stimulation, particularly in males compared with diestrus females, which may be related to sex differences in the strategies to cope with threatening situations.

1. Introduction

In rats, the medial prefrontal cortex (mPFC) is composed of a dorsal portion, the anterior cingulate cortex, and two ventral regions (prelimbic [PL] and infralimbic [IL] regions). However, because of its different connections and functions, the actions of these ventral regions are different with regard to fear regulation [18] and other behaviors [31]. Based on reciprocal connections between the PL/ILregions and amygdala nuclei [14], the present study considered the excitatory input from the basal amygdala (BA) [2]. We explored the responsivity of connections from the amygdala to PL and IL, in which the PL participates in the acquisition of conditioned fear, and the IL participates in the extinction of conditioned fear [28,29]. This suggests specific roles of the responsivity of BA-PL and BA-IL connections in coping styles and possible sex differences.

Among behavioral sex differences, male animals are significantly more vulnerable to acute and chronic stress than females [5], which may be related to both the organizing and activating effects of estradiol in females [20]. The steroid progesterone protects emotional and cognitive performance, independent of the participation of estrogens and corticosterone [23]. Progesterone and its metabolite allopregnanolone produce anxiolytic actions [10,26] that are mediated by progestin receptors and/or γ-aminobutyric acid-A ($GABA_A$) receptors [1]. Therefore, we performed single-unit extracellular recordings in the PL and IL during stimulation of the BA in anesthetized rats. We hypothesized that progesterone would modify the responsivity of the BA-PL and BA-IL, and such changes would present sex differences.

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2. Materials and methods

The animal procedures were performed in strict accordance to National Institutes of Health guidelines for the care and use of animals in research [8]. Authorization was obtained from the Biomedical Research Institute (National Autonomous University of Mexico) Ethical Committee to perform the study.

2.1. Animals and housing conditions

Wistar rats (8–12 weeks of age, weighing 200–250 g) were housed in groups of five in Plexiglas boxes (44 cm width × 33 cm length × 20 cm height) in local housing facilities. The colony room was maintained at a mean temperature of 25 °C with a 12 h/12 h light/dark cycle (lights on at 7:00 AM) with free access to food (rat chow, Purina, México City, Mexico) and purified water.

2.2. Experimental groups

To achieve similar values for basal plasma steroids among the experimental groups, only diestrus female rats were included in the study, presenting a predominance of leukocytes in vaginal smears [32]. A pilot study was conducted to determine the effects of a single injection of progesterone (1 mg/kg) on plasma concentrations of three steroids. The pilot study included 11 diestrus female Wistar rats ($n = 4$, vehicle group; $n = 7$, steroid determination group). Two hours after progesterone administration, the rats received a lethal dose of pentobarbital. Blood samples (3 ml) were obtained from the left ventricle. Conventional enzyme-linked immunosorbent assay (ELISA) procedures and commercial kits (Enzo Life Sciences, Farmingdale, NY, USA) allowed the determination of plasma progesterone (catalog no. ADI-900-011), estradiol (catalog no. ADI-900-174), and corticosterone (catalog no. ADI-900-097) 2 h after the injection.

The single-unit extracellular recording study included 12 males (vehicle, $n = 6$; progesterone, $n = 6$) and 12 diestrus females (vehicle, $n = 6$; progesterone, $n = 6$). Progesterone (Sigma, St. Louis, MO, USA) was injected subcutaneously at 1 mg/kg [21,26] dissolved in 1 ml/rat. Corn oil was used as the vehicle (1 ml/rat). Single-unit extracellular recordings began 2 h after the single injection of progesterone or corn oil at approximately 12:00 PM.

2.3. Single-unit extracellular recordings

The nature of two anatomically related neural regions may be assessed using a model [3] in which one structure is stimulated (BA) while another anatomically related neuronal region is recorded (*i.e.*, PL and IL regions of the mPFC), thus allowing exploration of the responsivity of such neural connections. The general procedure has been described previously [7]. Urethane (1 g/kg, intraperitoneally; Sigma, St. Louis, MO, USA) was used as an anesthetic. Single-unit extracellular recordings from the PL and IL (anterior/posterior, 3.2 mm; lateral, −0.6 mm; dorsal/ventral, −1 to −5 mm [24]) were performed using a glass micropipette that was filled with 1 M KCl (4–5 MΩ) that contained Pontamine blue (4%; Chicago Sky Blue, Sigma Chemicals, St. Louis, MO, USA). Basal amygdala (anterior/posterior, 2.8 mm; lateral, 5.0 mm; dorsal/ventral, −6.5 to −7.5 mm) stimulation (1 ms, 0.3 Hz, 5 min) was performed using a stainless-steel bipolar concentric electrode (∼100 kΩ resistance, 1 mm insulation uncovered at the inner tip, 100 μm diameter).

The micropipette signal was connected in series to a 7P511L Grass amplifier (Quincy, MA, USA; bandwidth pass filters: 300 Hz–3 kHz), oscilloscope (model 5111A, Tektronix, Beaverton, OR, USA), and audio amplifier. The signal was then sent to an interphase (CED MICRO 1401; Cambridge Electronic Design, Cambridge, United Kingdom) that transformed the analog signal to a digital signal. Spike2 5.20 software delivered digital data for the statistical analysis. The firing rate was analyzed by peristimulus histograms (base, 250 ms; bin width, 1 ms) that were obtained during 5 min of BA stimulation (60 stimuli).

2.4. Histological analysis

The last recorded point (PL or IL) was marked by Pontamine blue staining by electrophoresis (−20 μA, 20 min), and the stimulated point (BA) was marked by a direct current (0.3 mA, 30 s each polarity). After a lethal dose of pentobarbital, the rats were intracardially perfused with 0.9% saline (50 ml), followed by 37% formaldehyde (50 ml). After removal, the brain was frozen at −20 °C, cut into 40 μm thick sections with a cryocut microtome (Leica-Jung, Nussloch, Germany), and dyed using the Nissl technique to reconstruct the path that was followed by the micropipette and stainless-steel electrodes with the aid of stereotaxic coordinates [24]. After sectioning, only the brains for which we recognized a clear mark that was left by the electrodes in the recording and stimulating points were included in the data analysis.

2.5. Data analysis

A complete database was constructed. The cumulative peristimulus histograms (250 ms pre and 250 ms post-stimulus) that were obtained included all of the recorded neurons for each experimental group. We used the method that was described by Thomas et al. [30], with slight modifications. The original procedure obtains a quotient by comparing the difference in the frequency of firing during the post-stimulation and pre-stimulation periods divided by the sum of the two periods (*Post-Pre)/(Post + Pre*). A value of −1.00 indicates total inhibition, and a value of +1.00 indicates total excitation, with zero indicating no change. We performed the analysis of the unit activity ratio using cumulated peristimulus histograms that contained data from all neurons that were recorded for each experimental group. Using this procedure, we obtained a statistical total profile of all recorded neurons. The unit activity ratio was analyzed in 10 ms blocks of firing and as a total.

2.6. Statistical analysis

Plasma levels of steroids were analyzed by Student's *t*-test. Basal firing rate was analyzed by three-way ANOVA, with sex (male, female), treatment (vehicle, progesterone), and mPFC region (PL,IL) as factors. For the 10 ms blocks of the unit activity ratio, ranked data were analyzed by two-way analysis of variance (ANOVA), with treatment (progesterone and saline) and sex (males and diestrus females) as factors for separate analyses of mPFC regions (PL and IL), followed by the Holm–Sidak *post hoc* test. Values of $p \leq 0.05$ were considered statistically significant. The total unit activity ratios were analyzed using one-way repeated-measures Friedman ANOVA, followed by the Wilcoxon *post hoc* test. The results are expressed as the mean ± standard error of the mean.

3. Results

We recorded 270 neurons in the experimental groups (diestrus females, $n = 140$ neurons; males, $n = 130$ neurons). Of these, 163 single-unit extracellular recordings were obtained from the PL ($n = 38$ vehicle females, $n = 42$ vehicle males, $n = 46$ progesterone females, $n = 37$ progesterone males) and 107 from the IL ($n = 28$ vehicle females, $n = 26$ vehicle males, $n = 28$ progesterone females, $n = 25$ progesterone males). The three-way ANOVA of basal firing rate indicated no significant effects of sex ($F_{1,263} = 0.277$,