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Effects of acute stress on the day of proestrus on sexual behavior and ovulation in female rats: Participation of the angiotensinergic system

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Abstract

Physical or emotional stress can affect the female reproductive physiology and angiotensin II (Ang II) is a hormone that participates in the stress response and also in the control of reproductive hormones. The present study aimed at evaluating the effects of acute stress in the morning and afternoon of proestrus on sexual behavior and ovulation and the participation of Ang II in the stress-induced effects. Female rats with regular estrous cycles were used. Several different stress protocols were tested in the morning and in the afternoon of proestrus: restraint stress 10 min; restraint stress 1 h and ether stress, respectively. The participation of Ang II was evaluated by injecting Ang II receptor antagonists (losartan and PD123319) 15 min before stress. The lordosis quotient was recorded and the number of oocytes was counted. Plasma levels of luteinizing hormone, progesterone, prolactin and corticosterone were measured. All types of stress in the morning of proestrus induced a reduction in the number of oocytes. Restraint stress (1 h) in the afternoon of proestrus induced a significant reduction in the lordosis quotient. Peripheral and central losartan, but not PD123319, injections partly reverted the effects of stress on ovulation in the morning of proestrus. Acute stress in the morning of proestrus also reduced luteinizing hormone, progesterone and prolactin surges later on the same day. In conclusion, acute stress on the day of proestrus can affect female reproductive physiology. Moreover, the angiotensinergic system, through AT_1 receptors, participates in the effects of acute stress in the morning of proestrus.

Keywords: Stress; Ovulation; Sexual behavior; Female rats; Estrous cycle; Angiotensin II; LH; Progesterone; Prolactin

1. Introduction

Reproductive functions such as luteinizing hormone (LH) secretion and sexual behavior can be affected by stressful experiences [1,2]. Stress activates the hypothalamic–pituitary–adrenocortical (HPA) axis and disrupts the hypothalamic–pituitary–gonadal (HPG) axis, leading to suppressive effects on female reproductive physiology and behavior [1,3]. The suppressive effect of stress on the HPG axis, especially in chronic stress models, is believed to be due primarily to the influence of elevated levels of corticotropin-releasing hormone (CRH) and glucocorticoids [4]. Women submitted to intense exercise, which is considered physiological stress, present many reproductive abnormalities including delayed menarche, amenorrhea and infertility [5].

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The preovulatory gonadotropin surge in proestrus, a key event for ovulation, depends on both specific neural signals to initiate the surge and the positive-feedback actions of preovulatory estradiol (E_2) secretion [6]. Besides controlling ovulation, the ovarian steroid hormones E_2 and progesterone (P) have a profound modulatory influence on neural circuits that regulate sexual behavior since, during the estrous cycle, sexual behavior depends on an increase in serum E_2 followed by an increase in P concentration [7,8]. On the other hand, the preovulatory LH surge in the proestrus is also preceded by other important events such as an increased LH-releasing hormone secretion [6], an increased noradrenaline turnover rate in hypothalamic areas [9], as well as an angiotensin II (Ang II) rise prior to the LH surge in the afternoon of proestrus [10].

Although it is well established that chronic stress affects reproductive functions such as LH secretion, the effects of acute stress remain controversial. Acute stress can elicit variable patterns of LH release, for instance increased, unaltered or decreased circulating LH levels, depending on the paradigm and the magnitude of the stress [11]. One of the major stress hormone, Ang II, increases in the plasma as well as in the central nervous system in response to stress stimulation [12]. Stress increases the density of Ang II binding sites in the hypothalamic paraventricular nucleus and the subfornical organ of rats [13], and also increases renin activity [14]. Indeed, the Ang II receptor blockade completely abolishes the HPA response to stress, strongly supporting a role for Ang II as a major stress hormone [15–17]. Moreover Ang II involvement in the control of reproductive functions [18], including the regulation of LH secretion, has been well-established [19]. Ang II seems to play a crucial role in the mechanisms involved in ovulation, since there is a physiological rise of Ang II in the central nervous system prior to the LH surge during the afternoon (between 1200 and 1330 h) of the proestrus [10] and treatment with the Ang II receptor antagonist saralasin has been shown to reduce LH release and inhibit ovulation [20].

Considering the controversial results of acute stress on LH secretion and the evidence that activation of the HPA axis can disrupt the HPG axis, leading to suppressive effects on female reproductive physiology, the present study aimed at testing the effects of different acute stress paradigms on the day of proestrus on ovulation, sexual behavior and reproductive hormone release, such as LH, P and prolactin. Moreover, we studied the participation of the angiotensinergic system on stress-induced effects, since Ang II is a major stress hormone and also influences LH secretion and ovulation.

2. Materials and methods

2.1. Animals

Adult female (180–280 g) Wistar rats were obtained from the colony of the Federal University of Rio Grande do Sul (Porto Alegre, Brazil). Animals were housed individually in a temperature-controlled room (22 ± 1 °C) with a 12:12 h light-dark cycle (lights on at 0600 h) and free access to food (Rodent chow — Nutrilab, Colombo, PR, Brazil) and water. All animal procedures were carried out in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals [21] and the Research Committee of the University approved them.

2.2. Experimental design

In all experiments vaginal smears were taken daily after the 70th day of age and only rats showing at least 3 consecutive regular 4-day estrous cycles were used.

2.2.1. Experiment 1: effects of acute stress in the morning and in the afternoon of proestrus on sexual behavior and number of oocytes

Adult female rats with 3 regular estrous cycles were submitted to acute stress in the morning (1000 h) or in the afternoon (1600 h) of proestrus. The animals were submitted to one of the 3 different stress paradigms tested: restraint stress 10 min, restraint stress 1 h and ether stress 1 min. Different animals were used in each stress paradigm group tested. In the evening of proestrus (from 2000 to 2100 h) sexual behavior was recorded for 15 min and the total number of lordosis and the total number of mounts and intromissions were analyzed in all experimental groups. In the morning of estrus (9000 h) animals were decapitated, the ovaries removed and the oviduct dissected and squashed between two microscope slides. The number of oocytes of both oviduct ampullae was counted under the microscope (Zeiss, Goettingen, Germany) with a 2.5× lens.

2.2.2. Experiment 2: participation of the angiotensinergic system in the effects of acute stress in the morning of proestrus on the number of oocytes

Adult female rats with 3 regular estrous cycles were used in all cases. In order to test the participation of the angiotensinergic system animals were submitted to peripheral or central Ang II antagonist injections. Intraperitoneal injections were performed 15 min before stress according to the following experimental groups: control (no stress and no injection); saline (0.9%) injection, no stress; saline + stress; PD 123319 (3 mg/kg) + stress and Losartan (10 mg/kg) + stress. In order to perform central injections animals had a guide cannula implanted into the right lateral ventricle by stereotaxic surgery. In the third regular estrous cycle after the surgery, animals were submitted to an intracerebroventricular (ICV) injection 15 min before stress according to the following experimental groups: control (no surgery and no stress); surgery, no stress; surgery + stress; saline ICV (0.9%) + stress and Losartan ICV (1 μM; 2 μl) + stress. In all cases the stress paradigm used in this experiment was restraint 1 h in the morning (1000 h) of proestrus. In the morning of estrus (9000 h) animals were decapitated, the ovaries removed and the number of oocytes was counted as described in Experiment 1.

2.2.3. Experiment 3: effect of acute stress in the morning of proestrus on LH, P, PRL and corticosterone plasma concentrations

In the afternoon of diestrus, between 1600 and 1800 h, the animals with three regular estrous cycles were submitted to