Autonomic Neuroscience: Basic and Clinical 180 (2014) 48–52

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Effects of electrical stimulation of autonomic nerves to the ovary on the ovarian testosterone secretion rate in rats

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ARTICLE INFO

Article history:
Received 15 August 2013
Received in revised form 26 September 2013
Accepted 21 October 2013

Keywords:
Ovarian testosterone secretion
Superior ovarian nerve
Ovarian nerve plexus
Alpha-adrenoceptors
Rat

ABSTRACT

Previously, we demonstrated that electrical stimulation of the superior ovarian nerve (SON), but not the ovarian nerve plexus (ONP), reduces the secretion rate of estradiol from the ovary via activation of alpha 2-adrenoceptors in rats. The inhibitory effect of SON on estradiol secretion may be due to reduced production of testosterone, a direct precursor of estradiol. Here, we examined the effects of electrical stimulation of the SON and the ONP on ovarian testosterone secretion in rats. On the day of estrous, ovarian venous blood samples were collected intermittently from the ovarian vein. The secretion rate of testosterone from the ovary was calculated from the difference in the testosterone concentration between ovarian venous plasma and systemic arterial blood plasma, and the rate of ovarian venous plasma flow. Stimulation of either the SON or ONP reduced the secretion rate of testosterone from the ovary. The reduction of the testosterone secretion rate by SON stimulation was not influenced by an alpha 2-adrenoceptor antagonist (yohimbine), but it was abolished by an alpha 1-adrenoceptor antagonist (prazosin). Our results show that ovarian nerves have an inhibitory role in ovarian testosterone secretion, via activation of alpha 1-adrenoceptors, but not alpha 2-adrenoceptors. This, therefore, indicates that the reduction of estradiol secretion by SON stimulation is independent of the reduction of testosterone secretion.

1. Introduction

The ovary receives extrinsic adrenergic innervation (Burden, 1972, 1985; Owman and Stjernquist, 1988; Traurig and Papka, 1993). Previously, we demonstrated that electrical stimulation of adrenergic nerve to the ovary reduces the ovarian estradiol secretion rate in anesthetized rats (Kagitani et al., 2008, 2011). Ovarian estradiol production is considered synonymous with release because steroid hormones, once produced, can freely cross the cell membrane without having to be packed in granules and actively exocytozed (Ojeda and Kovacs, 2012). Estradiol is synthesized from testosterone by aromatization in the ovary (Hillier et al., 1994; Ojeda, 2012). The inhibitory effect of SON on estradiol secretion may be due to reduced production of testosterone, a direct precursor of estradiol. There have been some studies on pituitary hormonal control of ovarian testosterone secretion rate (Hilliard et al., 1974), but there have been no studies on its autonomic control. Therefore, our present study was designed to clarify the effect of electrical stimulation of adrenergic nerves to the ovary on the secretion rate of testosterone from the ovary in rats.

Adrenergic nerves to the rat ovary course through the superior ovarian nerve (SON) in the suspensory ligament and the ovarian nerve plexus (ONP) along the ovarian arterioles (Baljet and Drukker, 1979; Lawrence and Burden, 1980). Recently, we demonstrated that the secretion rate of estradiol from the ovary is reduced by SON stimulation, but is not influenced by ONP stimulation in rats (Kagitani et al., 2008). On the other hand, ovarian blood flow is decreased by stimulation of either the SON or the ONP. These results suggest that activation of autonomic nerves to the ovary produces vasoconstriction and inhibition of estradiol secretion, independently. In addition, we showed that the reduction of estradiol secretion rate during SON stimulation is blocked by an alpha 2-adrenoceptor antagonist, but is not influenced by an alpha 1-adrenoceptor antagonist or a beta-adrenoceptor antagonist (Kagitani et al., 2011). On the other hand, the reduction of ovarian blood flow during SON stimulation is blocked by an alpha 1-adrenoceptor antagonist, but is not influenced by an alpha 2-adrenoceptor antagonist or a beta-adrenoceptor antagonist. These results indicate that decreases in ovarian estradiol secretion and ovarian blood flow in response to SON stimulation is caused by activation of alpha 2-adrenoceptors and alpha 1-adrenoceptors, respectively.

In the present study, we aimed to clarify whether the inhibitory effect of SON on estradiol secretion is a secondary response to inhibitory effect of sympathetic nerve on testosterone, a precursor of estradiol. In order to accomplish this, we investigated the effects of electrical stimulation of the SON and the ONP on the secretion rate of testosterone from the ovary, using the same procedures of our previous study on autonomic regulation of the ovarian estradiol secretion rate (Kagitani et al., 2008). In addition, we examined the role of alpha adrenoceptors, especially of

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http://dx.doi.org/10.1016/j.autneu.2013.10.007

alpha 2-subtype, in the control of the ovarian testosterone secretion rate by nerve stimulation. Finally, differences in the secretion rate of testosterone and estradiol from the ovary were compared.

2. Materials and methods

2.1. Animals

Adult virgin female Wistar rats (3–6 months old; 160–205 g body weight), were maintained in a 12 h:12 h light–dark schedule. Rat chow and water were provided ad libitum. Twenty-four animals with a regular 5-day estrous cycle, established by examining daily vaginal smears, were used on the day of estrus. All procedures were approved by the Animal Committee of the Tokyo Metropolitan Institute of Gerontology.

All 24 rats were used for blood sampling to measure testosterone secretion. In 4 rats, estradiol secretion was also measured in addition to testosterone secretion. Of 24 rats, 11 were used for the data of basal level of testosterone secretion before nerve stimulation. Of 24 rats, 18 were used for SON stimulation, and other 6 were used for ONP stimulation. Of 18 rats used for SON stimulation, 5 were used for alpha 2-adrenoceptor blockade, and 6 were used for alpha 1-adrenoceptor blockade. In all 24 rats, blood pressure was recorded.

2.2. Surgical procedures

Rats were anesthetized with urethane (1.1 g/kg, i.p.). The trachea was cannulated and respiration was maintained using an artificial respirator (model 683, Harvard, Holliston, Massachusetts, USA). End-tidal CO_2 concentration was monitored using a gas monitor (Microcap, Oridion Medical, Jerusalem, Israel), and was maintained at 3–4% by controlling respiratory volume and frequency. Systemic blood pressure was recorded continuously through a cannula in a common carotid artery with a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan). The jugular vein was cannulated for infusion of necessary solutions. The animal was immobilized by administration of gallamine triethiodide (20 mg/kg, i.v., as required, Sigma, Missouri, USA). The core body temperature was monitored in the rectum, and was maintained at around 37.5 °C using a body temperature control system containing a thermostatically-regulated DC current heating pad and an infrared lamp (ATB-1100, Nihon Kohden, Tokyo, Japan). During the surgery, urethane (10% of the dose used for initial anesthesia) was administered i.v. every 1–2 h.

2.3. Collecting blood samples and measurement of testosterone

Samples of ovarian venous and systemic arterial blood were collected to determine the secretion rate of testosterone from the ovary, as described previously (Kagitani et al., 2008). Following a midline laparotomy, a polyethylene catheter (external diameter, 0.61 mm; internal diameter, 0.28 mm; Natsume Co. Ltd., Tokyo, Japan) was inserted into the right ovarian vein. The uterine vein, at the anastomosis with the ovarian vein, and other veins connected to the right ovarian vein were occluded. The animals were infused continuously with a heparin sodium solution (200 IU/kg/h, Ajinomoto Co. Ltd., Tokyo, Japan) to insure the free flow of blood through the tubing, and ovarian venous blood samples were collected into hematocrit tubes by capillary action and venous pressure. As blood sample was taken from the ovarian vein, a solution of 4% Ficoll 70 was administered at a rate of 2 ml/h to the jugular vein by an infusion pump (STC-521, Terumo, Tokyo, Japan). When samples were not being collected, the ovarian venous blood was shunted into the right femoral vein through a catheter. Since the dead space volume was approximately 30 μl with this method, 2 drops (about 40 μl) of blood were removed before collecting each sample. Normally, 4 samples were taken from each rat. The first and second samples were taken at 20 min and at 5 min before nerve stimulation, respectively. The third sample was taken during nerve stimulation. The final sample was taken at 5 min after the end of nerve stimulation. In 2 rats, two samples (5 min before and during nerve stimulation) were taken. Each 60–70 μl sample of blood was collected into heparinized hematocrit capillary tubes (Drummond Scientific Company, Pennsylvania, USA). Once all samples of ovarian venous blood had been collected, a sample of systemic arterial blood (about 140 μl) was collected into hematocrit capillary tubes through a catheter inserted into the right femoral artery. The plasma volume (μl) was determined for each ovarian venous blood sample and divided by the collection time (min) to give the ovarian venous plasma flow rate (μl/min). The secretion rate of testosterone was calculated from the absolute testosterone concentration of the ovarian venous plasma (the systemic arterial testosterone concentration was subtracted from the ovarian venous testosterone concentration) and the ovarian venous plasma flow rate.

After the blood samples were centrifuged for 5 min at 11,000 rpm, plasma samples were collected and ethylene diamine tetra-acetate disodium was added (1–2 mg/ml plasma). The samples were frozen and stored at −80 °C until testosterone measurements were performed. Plasma testosterone levels were measured by enzyme immunoassay, utilizing a testosterone EIA kit (Cayman Chemical Co., Michigan, USA). In a part of blood samples taken from rats that received SON stimulation, plasma estradiol concentration in addition to plasma testosterone concentration was also measured by enzyme immunoassay, utilizing an estradiol (17β-estradiol) EIA kit (Cayman Chemical Co., Michigan, USA). We used plasma samples diluted 15–50 times or 2–6 times with EIA buffer for the assay of testosterone or estradiol, respectively, and calculated the original concentration of each hormone.

2.4. Stimulation of the superior ovarian nerve (SON) and ovarian nerve plexus (ONP)

In 18 rats, the right SON running along the suspensory ligament together with the ligament was cut at approximately 20 mm from the ovary. In 6 other rats, the right ONP running along the ovarian artery was cut at approximately 20 mm from the ovary. The peripheral end was placed on a bipolar platinum-iridium wire stimulating electrode, and the nerve was covered with warm liquid paraffin. Electrical square pulse stimulation 0.5 ms wide, 20 Hz, 20 V (for SON) or 5 V (for ONP) was applied to these nerves for 5 min. In this study, all stimulations were of 5 min duration to allow time for the sampling of ovarian venous blood during the stimulus period.

2.5. Drug administration

Prazosin (0.1 mg/kg, prazosin hydrochloride; Sigma, USA), or yohimbine (1 mg/kg, yohimbine hydrochloride; Wako, Japan) was administered intravenously, to block alpha 1-, or alpha 2-adrenoceptors, respectively. These blockers were administered between collecting the first and second samples (about 17 min before nerve stimulation).

2.6. Data analysis

Data are expressed as means ± SEM. Statistical comparisons were carried out by means of one-way repeated-measures ANOVA followed by Dunnett's multiple comparison test, one-way factorial ANOVA followed by Dunn's multiple comparison test, or paired *t*-test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Secretion rate of testosterone from the ovary under resting conditions

The mean concentration of testosterone in the ovarian venous plasma under resting conditions before nerve stimulation was 1.05 ± 0.15 ng/ml ($n = 11$, Fig. 1). Testosterone concentrations in systemic arterial plasma and systemic venous plasma were approximately one third of that