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Sympathetic regulation of ovarian functions under chronic estradiol treatment in rats

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ABSTRACT

Activation of the sympathetic nerve to the ovary (superior ovarian nerve: SON) decreases ovarian blood flow and estradiol secretion in rats in the estrous phase. The present study examined the effects of long-term estradiol treatment on the sympathetic regulation of both ovarian blood flow and estradiol secretion. Non-pregnant Wistar rats received sustained subcutaneous estradiol (5 μg/day) or saline for 4 weeks. Chronic estradiol treatment did not affect ovarian blood flow at rest, while changed the basal ovarian estradiol secretion rate, i.e., narrow ranges (4–34 pg/min) in estradiol-treated rats, versus wide ranges (3–192 pg/min) in saline-treated rats of different estrous cycles. SON was electrically stimulated at different frequencies (2, 5 and 20 Hz). Ovarian blood flow was decreased by SON stimulation in a stimulus frequency-dependent manner in both saline- and estradiol-treated rats, but the threshold was shifted from 2 Hz to 5 Hz after chronic estradiol treatment. Ovarian estradiol secretion rate was not significantly changed by SON stimulation at any frequency in saline-treated rats, while it was markedly decreased by SON stimulation at high frequencies (5 and 20 Hz) in estradiol-treated rats. In conclusion, chronic estradiol treatment augments sympathetic inhibition of ovarian estradiol secretion perhaps by inhibiting the hypothalamic–pituitary–ovarian axis.

1. Introduction

Ovarian estradiol secretion is hormonally controlled in a cyclic manner by a hierarchical feedback mechanism consisting of the hypothalamic–pituitary–ovarian axis (Ojeda, 2012). In addition to this hormonal regulation of the ovary, recent studies in rats have shown that the ovarian autonomic innervation, particularly sympathetic nerve, regulates ovarian hormonal secretion in addition to ovarian blood flow (see reviews by Uchida, 2015, Uchida and Kagitani, 2015).

Histological studies showed that rat ovaries receive dense adrenergic innervation, which is derived from two routes: 1) the ovarian nerve plexus (ONP) along the ovarian artery and 2) the superior ovarian nerve (SON) in the suspensory ligament (Baljet and Drukker, 1979; Lawrence and Burden, 1980). Both sets of fibers terminate in a dense perivascular plexus around arteries, but those from SON terminate by abutting on steroidogenic interstitial cells (Lawrence and Burden, 1980). Our recent studies on the rats on the day of estrous phase showed that stimulation of either SON or ONP reduces blood flow through the ovary, while stimulation of SON, but not of ONP reduces estradiol secretion (Kagitani et al., 2008). These ovarian vascular and estradiol secretory responses to SON activation are mediated by α1- and α2-adrenoceptors, respectively (Kagitani et al., 2011). These results indicate that the ovarian estradiol secretion is directly inhibited by SON via activation of α2-adrenoceptors rather than via indirect influence of reduced ovarian blood flow. Furthermore, inhibitory effects of SON on the ovarian estradiol secretion have been shown to be activated in anesthetized rats by physical stress, such as noxious mechanical stimulation of hindpaw (Uchida et al., 2012). Therefore, the sympathetic control system of ovarian estradiol secretion may function in response to external and internal environmental changes (Stener-Victorin et al., 2003; Chávez-Genaro et al., 2007; Uchida et al., 2012).

Clinically, ovarian hormones, including estradiol, are administered for long-term in medical therapy for dysmenorrhea associated with endometriosis or for contraception (Hacker et al., 2010; Whalen and Rose, 2011). This administration is intended for suppressing the hypothalamic–pituitary–ovarian axis via negative feedback effects. Estradiol administration is also used for the treatment of climacteric symptoms and prevention of chronic disease in elderly women (Gruber et al., 2002; Rozenberg et al., 2013). In many animal studies, the effects of long-term estradiol treatment in the ovariectomized condition have been examined on different organs (Chataigneau and Schini-Kerth, 2005; Zoubina et al., 2001; Keiler et al., 2013). Although it is important to understand the association between ovarian functions and long-term estradiol administration, only a few studies have investigated the effect of estradiol treatment on the ovary itself. Kasturi et al. (2009) reported morphological changes in ovaries after long-term estradiol treatment. However, there have been no reports on the effects of long-term estradiol treatment on estradiol secretion from the ovary,

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sympathetic control system of ovarian estradiol secretion, or on the ovarian blood flow, which supports ovarian functions.

The present study aimed to clarify whether sympathetic regulation of ovarian functions, including ovarian blood flow and ovarian estradiol secretion, is affected by long-term estradiol treatment. To ascertain this, we used long-term estradiol-treated acyclic rats, and compared the changes in response to SON activation in ovarian blood flow and estradiol secretion between these rats and saline-treated normal cyclic rats.

2. Materials and methods

2.1. Animals

The experiments were performed on 12 female adult Wistar rats (5–6 months old). All animal experiments were conducted according to the Guidelines for Animal Experimentation prepared by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

2.2. Sustained subcutaneous infusion of estradiol

The rats were divided into two groups: 1) a saline-treated group ($n = 6$); and 2) an estradiol-treated group ($n = 6$). The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and an osmotic mini-pump (Alzet, model #2004, 0.25 μl/h, 28 days duration) containing either water-soluble 17β-estradiol (Sigma, Missouri, USA) (17β-estradiol: 0.84 mg/ml) or saline was inserted into a subcutaneous pocket via a small incision over the shoulders (Fig. 1A). Osmotic minipumps provide constant drug release rates (Herrlich et al., 2012; Theeuwes and Yum, 1976). The dose and duration of 17β-estradiol were 5 μg/day and 28 days, respectively. The wound was sutured with cotton thread and the rats were returned to their cage. After awakening, the animals were housed at an ambient temperature of 22 °C ± 1 °C and fed laboratory food with water ad libitum.

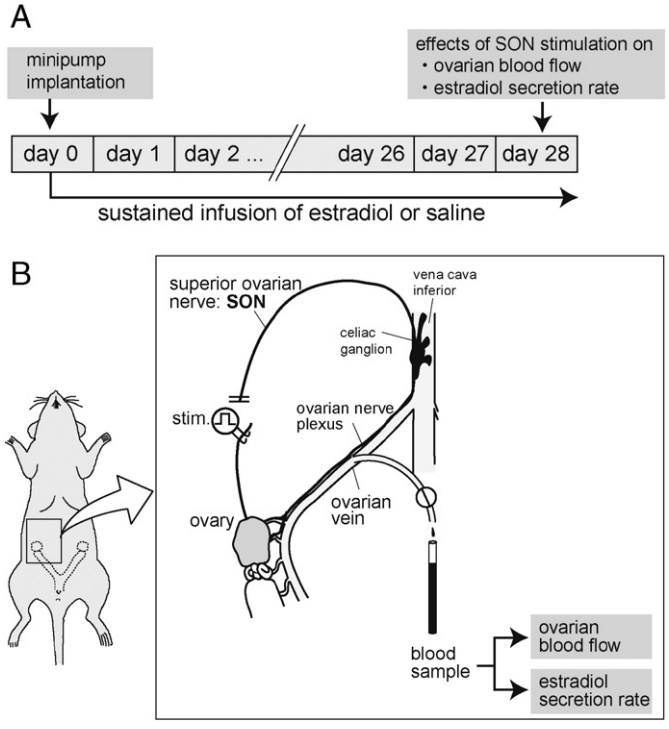

Fig. 1. Schematic diagram of the experimental procedures. A: Diagram showing the time course of the present experiments. B: Illustration showing the experimental procedures for collecting ovarian venous blood to measure ovarian blood flow and estradiol secretion rate and for stimulating superior ovarian nerve (SON).

Before implanting a mini-pump, all animals demonstrated a regular 5-day estrous cycle, established by examining daily vaginal smears. In the saline-treated rats, who received mini-pumps containing saline, the estrous cycle was maintained. Of the six saline-treated rats, one showed estrous phase and the others showed diestrous phase on the day of experiment. In all six rats receiving mini-pump containing estradiol, the estrous cycle was disrupted by around 10 days after implantation, as reported previously (Brawer et al., 1986). The acyclic conditions continued in estradiol-treated rats till the day of experiment.

2.3. General surgery and anesthesia

Twenty-eight days after the mini-pump implantation, changes in both ovarian blood flow and estradiol secretion rates in response to electrical stimulation of SON were examined under general anesthesia (Fig. 1A). The rats were anesthetized with urethane (1.1 g/kg, i.p.); the trachea was cannulated, and respiration was artificially maintained using a 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 continuously recorded through a cannula in the 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., i.p., or s.c., every 1–2 h.

2.4. Measurement of ovarian blood flow and estradiol secretion rate

2.4.1. Collection of blood samples

Ovarian venous and systemic arterial blood samples were collected to determine the ovarian estradiol secretion rates and ovarian blood flow, as described previously (Kagitani et al., 2008). In brief, 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 (Fig. 1B). The uterine vein, at anastomosis with ovarian vein, and other veins connected to the right ovarian vein were occluded. The animals were continuously infused with a heparin sodium solution (200 IU/kg/h, Ajinomoto Co. Ltd., Tokyo, Japan) to insure free blood flow through the tubing, and ovarian venous blood samples were collected into the hematocrit tubes by capillary action and venous pressure. As blood was taken from the ovarian vein, a solution of 4% Ficoll PM70 was administered at a rate of 2 ml/h to the jugular vein by an infusion pump (TE-331S, 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, two drops (about 40 μl) of blood were removed before collecting each sample. Blood samples were collected every 5–10 min. Each 60–70 μl sample of blood was collected into heparinized hematocrit capillary tubes (Drummond Scientific Company, Pennsylvania, USA). Once all the samples of ovarian venous blood had been collected, a sample of systemic arterial blood (about 140 μl) was collected into the hematocrit capillary tubes through a catheter inserted into the right femoral artery.

2.4.2. Determination of ovarian blood flow

Blood volume (μl) was determined for each ovarian venous blood sample and divided by the collection time (min) to determine the ovarian venous blood flow rate (μl/min).