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Short communication

Mechanism of physical stress-induced inhibition of ovarian estradiol secretion in anesthetized rats

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ABSTRACT

This study examined the site of main integration center in the physical stress-induced inhibition of ovarian estradiol secretion because of ovarian sympathetic nerve (superior ovarian nerve: SON) activation in anesthetized rats. In central nervous system-intact rats, electrical stimulation of the tibial afferent nerve at 10 V increased the efferent activity of the SON by 39 ± 13% and reduced the ovarian secretion of estradiol by 34 ± 7%. These responses were observed in decerebrate rats but were abolished in spinal rats. Thus, the main integration center for this ovarian hormonal response is located in the brain stem.

1. Introduction

Stress can cause changes in hormonal secretion, such as glucocorticoids, thyroid hormones, and sex hormones (Martí and Armario 1998; Pacak and McCarty 2010). It is well known that the hypothalamus acts as the main integration center for these stress-induced hormonal responses (Kovács 2013; Herman et al. 2016). Stress is also known to cause a reduction in estradiol secretion from the ovary and exerts an inhibitory effect upon female reproductive function (Goncharov et al. 1979; Toufexis et al. 2014). Again, the hypothalamus is considered to play a pivotal role in these stress responses in ovarian function (Ferin 2010; Genazzani et al. 2010).

In a recent study, we demonstrated that physical stress (noxious mechanical stimulation of a hindpaw) led to a reduction in estradiol secretion from the ovary in anesthetized rats (Uchida et al. 2012; Uchida 2015; Uchida and Kagitani 2015). We further showed that the physical stress-induced inhibition of ovarian estradiol secretion was caused through the ovarian sympathetic nerve (superior ovarian nerve: SON) because denervation of the SON abolished the response. Although it is speculated that the hypothalamus acts as an integrative center for this physical stress-induced inhibition of ovarian estradiol secretion, final proof is yet to be elucidated. Thus, the present study aimed to use decerebrate rats to investigate whether the hypothalamus is essential for the physical stress-induced inhibition of ovarian estradiol secretion.

2. Methods

Adult virgin female Wistar rats (3–10 months of age; 160–230 g in body weight) were maintained under a 12 h:12 h light:dark schedule. Rat chow and water were provided ad libitum. Overall, 40 rats with a regular 5-day estrous cycle, established by examining daily vaginal smears, were used on the day of estrus. The study was conducted with the approval and in accordance with the guidelines for animal experimentation prepared by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

All experimental conditions and techniques for anesthesia, recording efferent nerve activity from the SON, collecting and measuring ovarian venous estradiol secretion rate, and stimulating the tibial nerve, were the same as those previously described (Uchida et al. 1999; Kagitani et al. 2008; Uchida et al. 2012). In brief, rats were anesthetized with urethane (initially 1.1 g/kg, i.p. and supplemented with 0.1 g/kg when necessary thorough the jugular vein). The trachea was cannulated and respiration was maintained using an artificial respirator. End-tidal CO_2 concentrations were kept at 3%–4%. Rectal temperature was maintained at approximately 37.5 °C. Systemic mean arterial pressure was measured via the common carotid artery.

In nine rats, efferent nerve discharges of the left or right SON were recorded (Fig. 1A). The SON was cut at the point before it joins the suspensory ligament. The nerve was covered with warm liquid paraffin and the proximal cut segment was placed on a bipolar platinum iridium wire electrode. Mass action potentials were amplified (S-0476, Nihon Kohden) with a 0.01 s time constant, audibly monitored through a speaker, visually displayed on a digital oscilloscope (DS-5312, Iwatsu, Tokyo), and digitized (micro 1401, Cambridge Electronic Design, UK). Nerve discharges were counted in 1-min intervals using software (Spike 2 software, Cambridge Electronic Design, UK). The stimulus artifacts that arise from repetitive stimulation of the tibial afferent nerves were removed by the software (Spike 2) which continuously blocked out a

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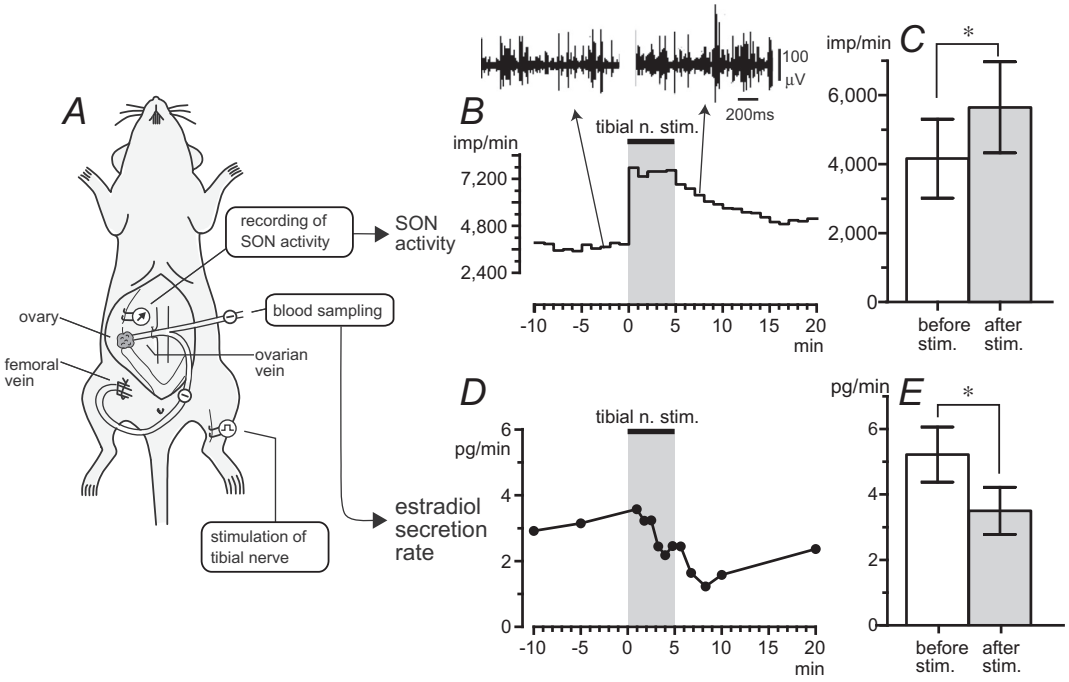

Fig. 1. The effect of tibial nerve stimulation at 10 V upon SON efferent activity and estradiol secretion rate from the ovary in CNS-intact rats. A: Schematic diagram of the experimental procedures. B: Typical responses of SON efferent activity. Upper traces: sample recordings of SON activity. Lower trances: recordings of SON activity expressed as a histogram of impulses per 1 min. C: Graphs summarizing the responses of SON activity ($n = 5$). Levels of nerve activity before (− 10 to 0 min) and after (0 to 10 min) stimulation were averaged for each rat. Activity of SON either the left ($n = 4$) or right ($n = 1$) side was recorded. D: Sample response of the ovarian estradiol secretion rate. E: Graphs summarizing the responses of estradiol secretion rate from the ovary ($n = 7$). Estradiol secretion rate before (− 10 to − 5 min) and after (5–10 min) were averaged for each rat. Each column and vertical bar represents the mean ± SEM. *$P < 0.05$; compared with values before stimulation using the paired *t*-test.

1 ms period synchronous with the stimulation.

In 31 rats, ovarian venous blood samples were intermittently collected through a catheter in the right ovarian vein (Fig. 1A). The rats were infused continuously with a heparin sodium solution to ensure the free flow of blood through the tubing, and ovarian venous blood samples were collected into hematocrit tubes while noting the time of collection. The plasma volume (μl) determined for each blood sample was divided by the collection time (min) to give the ovarian venous plasma flow rate (μl/min). When samples were not being collected, the ovarian venous blood was shunted into the right femoral vein through a catheter. Once all ovarian venous blood samples had been collected, we obtained a sample of systemic arterial blood via a catheter in the right femoral artery. Plasma estradiol (17β-estradiol) concentration was measured by enzyme immunoassay (Cayman Chemical CO., Michigan, USA). The secretion rate of estradiol from the ovary was calculated from differences in the estradiol concentration between ovarian venous plasma and systemic arterial blood plasma, and from the flow rate of ovarian venous plasma.

The left tibial nerve was dissected free from the surrounding tissues and cut. The central cut end segment of the nerve was then placed on bipolar platinum iridium wire electrodes for electrical stimulation (Fig. 1A). The nerve was stimulated at supra-maximal intensity for group IV fibers (0.5 ms, 10 V, 10 Hz, 5 min) by a digital electrical stimulator (SEN-3301, Nihon Kohden, Tokyo). In parts of experiments, the stimulus parameters were set at 0.2 V (supra-threshold for groups I + II fibers but subthreshold for groups III and IV fibers, at 50–100 Hz), and 1.0 V (supra-threshold for groups I, II and III fibers but subthreshold for groups IV fibers, at 10–20 Hz) (Uchida et al. 2008).

Decerebration was performed by transection between the hypothalamus and midbrain in 11 rats in a manner which was similar to the method described previously (Osaka et al. 1989). The rats were mounted on a stereotaxic instrument (SR-5R-HT, Narishige) in a prone position, and portions of the parietal bone were removed bilaterally. With an L-shaped spatula, the brain was cut at a portion 4-mm rostral from the inter aural line (Fig. 2A) (Paxinos and Watson 2009). Particular care was taken not to injure the mid-sagittal sinus. After the end of experiments, the completeness of the transection was verified histologically.

Full transection of the spinal cord was performed at the second cervical level (C2) in nine anesthetized rats (Fig. 2A). Mean arterial pressure after spinalization was maintained at around 50 mmHg by injection of 4% Ficoll 70 (Amersham Biosciences, Uppsala, Sweden).

For the experiments of ovarian venous blood sampling, all three procedures, i.e., central nervous system (CNS)-intact, decerebrate and spinal were conducted in the different animals. For the experiments of recording the SON activity, one, two, or three different procedures were conducted in each rat, in the order of CNS-intact, decerebrate, spinal. The rat was kept in resting condition for > 30 min after the different surgical procedure was finished.

Data are expressed as means ± SEM. Statistical comparisons were carried out using one-way ANOVA followed by the Dunnett's multiple comparison test, the paired *t*-test, and the Kruskal–Wallis tests. A *P*-value of < 0.05 was considered to be statistically significant.

3. Results

3.1. The responses of SON activity and estradiol secretion to tibial nerve stimulation in CNS-intact rats

The effect of 5 min of tibial afferent nerve stimulation at 10 V upon SON efferent nerve activity (five rats) and estradiol secretion (seven rats) from the ovary was examined in CNS-intact rats. Fig. 1B and D shows typical responses while Fig. 1C and E show summarized graphs.

The efferent nerve activity of the SON, which was spontaneously active, was elevated during tibial nerve stimulation at 10 V (Fig. 1B). Although SON activity started to gradually decrease after the end of stimulation, it remained elevated for up to 15 min after the end of stimulation (Fig. 1B). Levels of nerve activity before (− 10 to 0 min)