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SHORT COMMUNICATION

The role of alpha adrenoceptors in the vascular and estradiol secretory responses to stimulation of the superior ovarian nerve

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Abstract Electrical stimulation of the superior ovarian nerve in rats reduces both the plasma flow rate of ovarian venous blood (ovarian blood flow) and the ovarian estradiol secretion rate. Here, we examined the possible roles of alpha-adrenoceptors in these processes. The reduction of the plasma flow rate was blocked by an alpha 1- (prazosin), but not by an alpha 2- (yohimbine) adrenoceptor blocker. In contrast, the reduction of the estradiol secretion rate was blocked by yohimbine but not by prazosin. We conclude that ovarian vascular and estradiol secretory responses to superior ovarian nerve activation are mediated by alpha 1- and alpha 2-adrenoceptors, respectively.

Keywords Superior ovarian nerve · Ovarian estradiol secretion · Ovarian blood flow · Alpha-adrenoceptors · Rat

Introduction

The ovary receives autonomic nerves [1–5]. The adrenergic component of this innervation reaches the ovary via the superior ovarian nerve, located in the suspensory ligament, and via the ovarian nerve plexus which is distributed around the ovarian artery. In the rat, both sets of fibers terminate in dense perivascular plexus around arteries, but those from the superior ovarian nerve terminate abutting on steroidogenic interstitial cells [6]. Stimulation of the extrinsic nerves to the ovary reduces blood flow through the ovary, while stimulation of the superior ovarian nerve, but not of the ovarian nerve plexus, is known to reduce estrogen output [7].

Examples of adrenergic innervation of endocrine secretory structures are found in the kidney [8] and pineal gland [9], where activation of beta-adrenoceptors increases the secretion of renin and melatonin, respectively, and in the pancreas, where stimulation of alpha 2-adrenoceptors results in the inhibition of insulin secretion [10–12]. In contrast, sympathetic vasoconstriction is usually mediated by alpha 1-adrenoceptors [13]. Since stimulation of the superior ovarian nerve inhibits ovarian estradiol secretion and ovarian blood flow, it is highly likely that alpha adrenoceptors may mediate such ovarian responses. Therefore, in the current study, we investigate the possible role of alpha 1- and alpha 2-adrenoceptors in the vascular and endocrine responses to stimulation of the superior ovarian nerve. Furthermore, we have also investigated the role of beta adrenoceptors in the ovarian response to nerve stimulation.

Materials and methods

Adult virgin female Wistar rats (4–8 months old), of 175–205 g body weight, were kept in a room with a 12 h:12 h light–dark schedule. Rat chow and water were provided ad libitum. Fifteen 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.

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All experimental conditions, and techniques for anesthesia, collecting and measuring ovarian venous estradiol secretion rate, and stimulating the superior ovarian nerve, were the same as those previously described [7]. In brief, rats were anesthetized with urethane (initially 1.1 g/kg, i.p. and supplemented with 0.1 g/kg when necessary thorough a 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 approx. 37.5°C. Systemic mean arterial pressure was measured through a common carotid artery. Ovarian venous blood samples were collected through a catheter in the right ovarian vein. Animals 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.

The peripheral cut end of the right superior ovarian nerve was electrically stimulated at supra-maximal intensity for C-fibers (0.5 ms, 20 V, 20 Hz, 5 min). Four samples were taken from each rat. The first sample was taken at 20 min and the second 5 min before nerve stimulation. Between these two samples (about 17 min before nerve stimulation); prazosin (0.1 mg/kg, prazosin hydrochloride; Sigma, USA), yohimbine (1 mg/kg, yohimbine hydrochloride; Wako, Japan), or propranolol (1 mg/kg, propranolol hydrochloride; Sigma) was administered intravenously to block alpha 1-, alpha 2-adrenoceptors, or beta-adrenoceptors, respectively. During the nerve stimulation, the third sample was taken. The final blood sample was taken 5 min after the end of nerve stimulation. Once all samples of ovarian venous blood 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, MI, 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. During the nerve stimulation, in order to avoid entering the ovarian venous blood to systemic circulation, ovarian venous blood was collected during the nerve stimulation (from the onset of nerve stimulation to the end of the stimulation). The dead space volume from the catheter was removed (taking about 1 min). Owing to this blood sampling procedure, changes in ovarian venous estradiol concentration during nerve stimulation would not affect estradiol concentration in systemic arterial plasma. We confirmed the stability of estradiol concentration in systemic arterial plasma between samples collected both at the beginning and at the end of experiment [7]. When the nerve was not stimulated, the ovarian venous plasma flow rate, the ovarian venous estradiol concentration, and the estradiol secretion rate from the ovary were all stable when compared between 5 samples for 35 min [7].

All values are given as mean ± SEM. Statistical comparisons were carried out by means of a Friedman test followed by Dunn's multiple comparison test, paired t test. A p value of < 0.05 was considered to be statistically significant.

Results

The administration of prazosin (alpha 1-adrenoceptor blockade) significantly reduced the basal level of mean arterial pressure from 88.7 ± 6.7 to 60.9 ± 5.1 mmHg ($n = 6$). However, the basal values of ovarian plasma flow rate (40.2 ± 6.3 μl/min), estradiol concentration of ovarian venous plasma (0.131 ± 0.014 pg/μl), and the secretion rate of estradiol (2.57 ± 0.63 pg/min) remained unchanged following the administration of prazosin (Fig. 1). Following the administration of prazosin, repetitive electrical stimulation of the superior ovarian nerve did not result in changes in either ovarian plasma flow rate or mean arterial pressure. However, estradiol concentration in ovarian venous plasma and the secretion rate of estradiol from the ovary both decreased during stimulation (Fig. 2a) but returned to pre-stimulus basal levels 5 min following the end of stimulation. During nerve stimulation, the estradiol concentration in ovarian venous plasma and the secretion rate of estradiol were significantly reduced from 0.146 ± 0.014 to 0.120 ± 0.009 pg/μl (82 ± 5% of the pre-stimulus values), and from 3.25 ± 0.44 to 2.28 ± 0.25 pg/min (71 ± 8% of the pre-stimulus values, respectively (Fig. 2ab, ac).

Yohimbine (alpha 2-adrenoceptor blockade) administration significantly reduced the basal level of mean arterial pressure from 109.8 ± 6.8 to 81.3 ± 2.4 mmHg ($n = 4$). However, the basal values of ovarian plasma flow rate, estradiol concentration of ovarian venous plasma, and the secretion rate of estradiol remained unchanged following the administration of yohimbine (Fig. 1). Following yohimbine administration, repetitive electrical stimulation of the superior ovarian nerve result in reduced ovarian plasma flow rate, but did not change the estradiol concentration in ovarian venous plasma, the secretion rate of estradiol from the ovary, or the mean arterial pressure during stimulation (Fig. 2b). Reduced plasma flow rates returned to the pre-stimulus basal level 5 min after the end

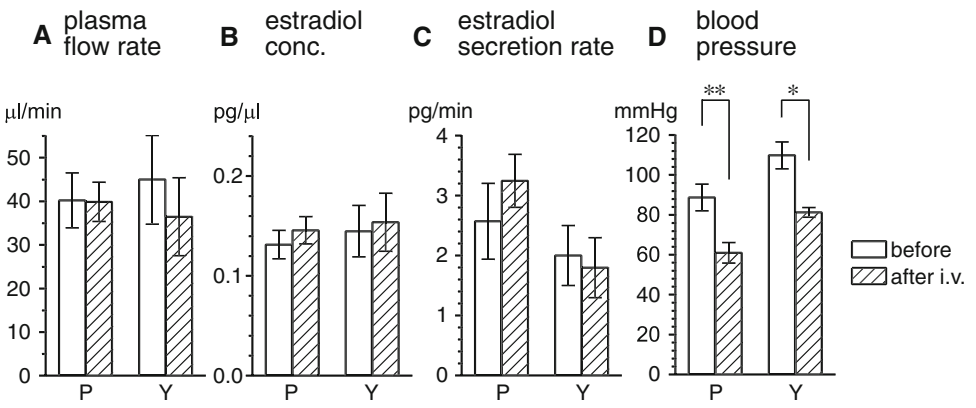

Fig. 1 Basal levels of ovarian venous plasma flow rate (**a**), estradiol concentration in ovarian venous plasma (**b**), the secretion rate of estradiol from the ovary (**c**), and mean arterial pressure (**d**), before (*white columns*) and after (*hatched columns*) the administration of prazosin (*P*) ($n = 6$) or yohimbine (*Y*) ($n = 4$). $*p < 0.05$ and $**p < 0.01$ indicate values that were significantly different to values obtained before the administration of each alpha-adrenoceptor antagonist, as determined by the paired *t* test. Each *column* and *vertical bar* show mean ± SEM

of stimulation. During nerve stimulation, the ovarian plasma flow rate significantly decreased from 28.4 ± 8.0 to 20.8 ± 5.0 μl/min (76 ± 5% of the pre-stimulus values) (Fig. 2ba). Nerve stimulation had a tendency to increase the estradiol concentration in ovarian venous plasma, but the response was not statistically significant (Fig. 2bb). One rat used to study the effect of yohimbine exhibited quite high (about 10 times higher) estradiol concentration in ovarian venous plasma. We did not include this rat in the data presented in Figs. 1 and 2, but the general effects of yohimbine on basal levels and nerve stimulation-induced responses were the same in this particular rat as the other 4 rats described above.

We tested the effect of propranolol (beta-adrenoceptor blockade) in 4 rats. Following the administration of propranolol, repetitive electrical stimulation of the superior ovarian nerve reduced ovarian venous plasma flow rate and the secretion rate of estradiol to 74 ± 7 and 58 ± 16% of pre-stimulus values, respectively. Propranolol did not alter the basal levels of any of the parameters measured.

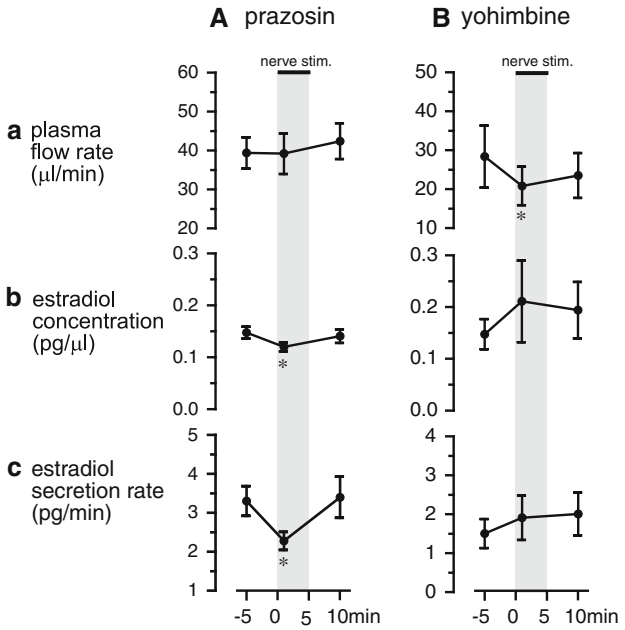

Fig. 2 Effects of prazosin (**a**) and yohimbine (**b**) on the superior ovarian nerve stimulation-induced responses of ovarian venous plasma flow rate (*a*), ovarian venous estradiol concentration in ovarian venous plasma (*b*), secretion rate of estradiol from the ovary (*c*). Data are expressed as mean ± SEM (**a** $n = 6$, **b** $n = 4$). $*p < 0.05$ compared with pre-stimulus control values using a Friedman test followed by Dunn's multiple comparison test. Stimulation for 5 min (0–5 min) was applied as indicated by annotation of the *upper portion* of each *gray bar*

Discussion

Our previous study showed that superior ovarian nerve stimulation decreased both plasma flow rate of ovarian venous blood (ovarian blood flow) and ovarian estradiol secretion rate in anesthetized rats [7]. Our present study demonstrated that these two events induced by stimulation of the superior ovarian nerve stimulation were caused by the activation of different alpha adrenoceptors subtypes. The reduction in ovarian estradiol secretion in response to stimulation of the superior ovarian nerve was due to the activation of alpha 2-adrenoceptors, while the reduction in ovarian venous plasma flow rate was due to the activation of alpha 1-adrenoceptors (Fig. 3). In control experiments without drugs in our previous study [7], electrical stimulation of the superior ovarian nerve resulted in reductions in ovarian venous plasma flow rate, ovarian venous estradiol concentration, and estradiol secretion rate from the ovary, reaching 76 ± 3, 83 ± 6, and 53 ± 6% of pre-stimulus values, respectively. We will discuss the present

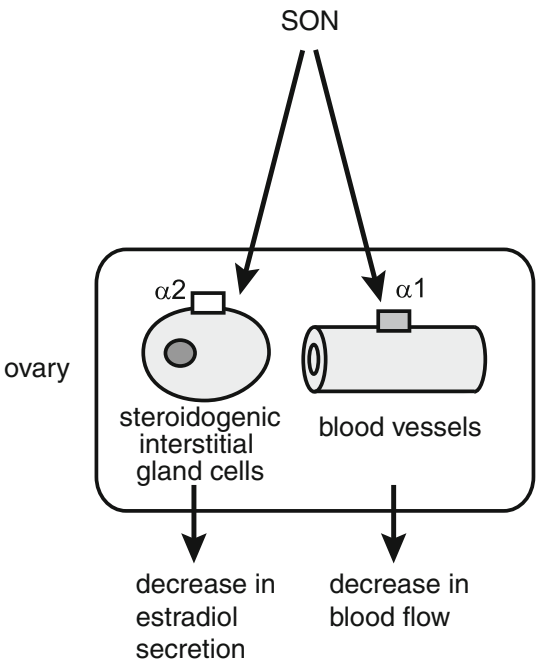

Fig. 3 Schematic diagram of a possible mechanism for the inhibition of ovarian blood flow and estradiol secretion by superior ovarian nerve (*SON*) activation of alpha 1-adrenoceptors and alpha 2-adrenoceptors, respectively

results of ovarian responses after alpha adrenoceptor antagonists (the present results), by comparing the ovarian responses of the control experiments without drugs, in the following sessions.

In the present study, we observed that blocking alpha 2-adrenoceptors by the i.v. administration of yohimbine resulted in the reduced rate of ovarian plasma flow being maintained while the reduced rate of ovarian estradiol secretion was abolished. The degree of decrease in ovarian plasma flow rate during nerve stimulation after administration of yohimbine (76 ± 5%) was similar to those responses without drugs (76 ± 3%). These results suggest that the activation of ovarian alpha 2-adrenoceptors is responsible for reducing the rate of ovarian estradiol secretion, but not in reducing the rate of ovarian plasma flow by nerve stimulation. Although yohimbine was administered systemically, the site of action of yohimbine was expected to be in the ovary because the responses of ovarian estradiol secretion are evoked by the stimulation of peripheral cut end of the nerve.

An earlier in vivo study in rats reported that the topical application of the beta 2-adrenoceptor agonist (fenoterol) increased ovarian estradiol secretion [14]. However, these authors did not examine the possible role of alpha adrenoceptors in relation to ovarian estradiol secretion. In our present study, after administration of the alpha 2-adrenoceptor antagonist, yohimbine, the nerve stimulation did not increase ovarian estradiol secretion rate. Furthermore, we confirmed that the beta-adrenoceptor antagonist, propranolol, had no effect on the nerve stimulation-induced reduction of ovarian estradiol secretion rate. The degree of decrease in estradiol secretion rate during nerve stimulation after administration of propranolol (58 ± 16%) was not significantly different from the control responses without drugs (53 ± 6%). Therefore, the beta adrenoceptor-mediated increase mechanism of ovarian estradiol secretion rate appears to be only a minor aspect in the regulation of ovarian estradiol secretion by the superior ovarian nerve. Our results indicate that noradrenaline released from adrenergic nerve terminals in the ovary predominantly activate alpha 2-adrenoceptors which, in turn, suppress estradiol secretion rate from the ovary.

Alpha 1-adrenoceptor immunoreactivity has been detected in the blood vessel walls as well as some other cells in the rat ovary [15]. Our study showed that reduced rates of ovarian plasma flow in response to stimulation of the superior ovarian nerve could be abolished by treatment with the alpha 1-adrenoceptor antagonist prazosin. This suggests the involvement of alpha 1-adrenoceptors in ovarian adrenergic vasoconstrictor activity, as in other organs [13]. On the other hand, the reduced rate of ovarian estradiol secretion in response to stimulation of the superior ovarian nerve was not changed by treatment with the alpha 1-adrenoceptor antagonist, prazosin. The degree of decrease in estradiol secretion rate during nerve stimulation after administration of prazosin (71 ± 8%) was not significantly different from the control responses without drugs (53 ± 6%). This, therefore, indicates that the alpha 1-adrenoceptor is not involved in the regulation of ovarian estradiol secretion via the nervous system.

To achieve a correct calculation of the estradiol secretion rate, systemic arterial blood samples should be taken at the same time or immediately after the collection of each ovarian venous sample. However, repeated collections of systemic arterial blood worsen the rats' condition, and make it difficult to observe correct physiological responses. Thus, in the present experiments, we obtained a systemic arterial blood sample after all samples of ovarian venous blood had been collected. The arterial plasma estradiol concentration was used for calculation of ovarian estradiol secretion rate for all ovarian venous samples in that animal. Therefore, it is important to remember that those ovarian estradiol secretion rates are estimated values. In the ovarian venous blood sampling procedure of the present experiments, the ovarian venous blood during nerve stimulation does not enter into systemic circulation. Furthermore, we confirmed the stability of estradiol concentration in systemic arterial plasma between samples collected both at the beginning and at the end of experiment [7]. Therefore, the estimated values of ovarian estradiol secretion rate may not be much different from the true value.

In the present study, i.v. administration of prazosin and yohimbine significantly reduced the basal level of systemic blood pressure but did not affect resting basal levels of ovarian plasma flow rate. In order to carry out our study, the superior ovarian nerve was severed for stimulation, while the ovarian nerve plexus was kept intact. It is possible that the ovarian vessel may have been dilated because the tone of the ovarian vessel produced by the ovarian nerve plexus was released following the blockade of alpha 1- and 2-adrenoceptors on the ovarian blood vessels. Thus, the ovarian blood flow might have been maintained even though systemic blood pressure has decreased. On the other hand, the basal secretion rate of ovarian estradiol remained unchanged following the administration of these blockers. This suggests that alpha 1- and 2-adrenoceptors did not contribute to the basal secretion of ovarian estradiol in the present experimental conditions.

In our present study, we made two significant discoveries. Firstly, the administration of prazosin maintained a reduced rate of ovarian estradiol secretion via stimulation of the superior ovarian nerve, but abolished the reduced rate of ovarian plasma flow. Secondly, the administration of yohimbine maintained a reduced rate of ovarian plasma flow, but abolished the reduced rate of estradiol secretion. These results suggest that the reduction in ovarian estradiol secretion was due to the direct inhibitory effects of the superior ovarian nerve on ovarian estradiol synthesis via the stimulation of alpha 2-adrenoceptors rather than the indirect influence of reduced ovarian blood flow.

Insufficient blood flow to the ovary and low ovarian estradiol secretion caused by the hyperactivation of sympathetic nerves to the ovary may be a possible cause for ovarian dysfunction such as anovulation [16–18]. The present results may therefore extend our knowledge concerning the neural regulation of ovarian function and provide a new perspective for pharmacological intervention in women with ovarian dysfunction.

In conclusion, we have demonstrated that ovarian blood flow and ovarian estradiol secretion are regulated by superior ovarian nerve activation of alpha 1-adrenoceptors and alpha 2-adrenoceptors, respectively.

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References

1. Burden HW (1972) Adrenergic innervation in ovaries of the rat and guinea pig. Am J Anat 133:455–461
2. Burden HW (1985) The adrenergic innervation of mammalian ovaries. In: Ben-Jonathan N, Bahr JM, Weiner RI (eds) Serono symposia publications from Raven Press. Catecholamines as hormone regulators, vol 18. Raven Press, New York, pp 261–278
3. Baljet B, Drukker J (1979) The extrinsic innervation of the abdominal organs in the female rat. Acta Anat (Basel) 104:243–267
4. Owman C, Stjernquist M (1988) Origin, distribution, and functional aspects of aminergic and peptidergic nerves in the male and female reproductive tracts. In: Björklund A, Hökfelt T, Owman C (eds) Handbook of chemical neuroanatomy. The peripheral nervous system, vol 6. Elsevier, Amsterdam, pp 445–544
5. Traurig HH, Papka RE (1993) Autonomic efferent and visceral sensory innervation of the female reproductive system: special reference to the functional roles of nerves in reproductive organs. In: Maggi CA (ed) Nervous control of the urogenital system. Harwood, Switzerland, pp 103–141
6. Lawrence IE Jr, Burden HW (1980) The origin of the extrinsic adrenergic innervation to the rat ovary. Anat Rec 196:51–59
7. Kagitani F, Uchida S, Hotta H (2008) Effects of electrical stimulation of the superior ovarian nerve and the ovarian plexus nerve on the ovarian estradiol secretion rate in rats. J Physiol Sci 58:133–138
8. Ganong WF, Barbieri C (1982) Neuroendocrine components in the regulation of renin secretion. In: Ganong WF, Martini L (eds) Frontiers in neuroendocrinology, vol 7. Raven Press, New York, pp 231–262
9. Reiter RJ (1982) Neuroendocrine effects of the pineal gland and of melatonin. In: Ganong WF, Martini L (eds) Frontiers in neuroendocrinology, vol 7. Raven Press, New York, pp 287–316
10. Porte D Jr, Girardier L, Seydoux J, Kanazawa Y, Posternak J (1973) Neural regulation of insulin secretion in the dog. J Clin Invest 52:210–214
11. Metz SA, Halter JB, Robertson RP (1978) Induction of defective insulin secretion and impaired glucose tolerance by clonidine. Selective stimulation of metabolic alpha-adrenergic pathways. Diabetes 27:554–562
12. Nakaki T, Nakadate T, Ishii K, Kato R (1981) Postsynaptic alpha-2 adrenergic receptors in isolated rat islets of Langerhans: inhibition of insulin release and cyclic 3′:5′-adenosine monophosphate accumulation. J Pharmacol Exp Ther 216:607–612
13. Yamamoto R, Kawasaki H, Takasaki K (1984) Postsynaptic alpha-adrenoreceptor populations in several vascular systems of the anaesthetized rat. J Auton Pharmacol 4:231–239
14. Zsolnai B, Varga B, Horváth E (1982) Increase of ovarian progesterone secretion by beta 2-adrenergic stimulation in oestrous rats. Acta Endocrinol (Copenh) 101:268–272
15. Itoh MT, Ishizuka B (2005) Alpha1-Adrenergic receptor in rat ovary: presence and localization. Mol Cell Endocrinol 240:58–63
16. Lara HE, Ferruz JL, Luza S, Bustamante DA, Borges Y, Ojeda SR (1993) Activation of ovarian sympathetic nerves in polycystic ovary syndrome. Endocrinology 133:2690–2695
17. Zackrisson U, Mikuni M, Peterson MC, Nilsson B, Janson PO, Brännström M (2000) Evidence for the involvement of blood flow-related mechanisms in the ovulatory process of the rat. Hum Reprod 15:264–272
18. Sverrisdóttir YB, Mogren T, Kataoka J, Janson PO, Stener-Victorin E (2008) Is polycystic ovary syndrome associated with high sympathetic nerve activity and size at birth? Am J Physiol Endocrinol Metab 294:E576–E581