Estrogen enhancement of baroreflex sensitivity is centrally mediated

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Mohamed, Mohamed K., Mahmoud M. El-Mas, and Abdel A. Abdel-Rahman. Estrogen enhancement of baroreflex sensitivity is centrally mediated. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1030–R1037, 1999.—We have recently shown that estrogen enhances baroreceptor control of reflex bradycardia in conscious rats. The present study replicated this finding in pentobarbital sodium-anesthetized rats, and the study was extended to investigate whether this effect of estrogen is centrally or peripherally mediated. Hemodynamic responses to electrical stimulation of the central end of the aortic depressor or the vagal efferent nerve were evaluated in pentobarbital sodium-anesthetized sham-operated (SO), ovariectomized (OVX), and OVX estradiol-treated Sprague-Dawley rats. Phenylephrine (1–16 µg·kg⁻¹·iv) elicited dose-dependent pressor and bradycardic responses. Regression analysis of the baroreflex curves, relating changes in mean arterial pressure and heart rate, revealed a significantly smaller baroreflex sensitivity in OVX compared with SO anesthetized rats (−0.54 ± 0.05 and −0.91 ± 0.12 beats·min⁻¹·mmHg⁻¹, respectively; P < 0.05). Treatment of OVX rats with 17β-estradiol (E₂, 50 µg·kg⁻¹·day⁻¹ for 2 days subcutaneously) significantly enhanced baroreflex sensitivity to a level similar to that of SO rats (P < 0.05). The enhancing effect of E₂ on the baroreflex-mediated bradycardia, observed in conscious and anesthetized rats, seems to be selective because the baroreflex-mediated tachycardic responses measured in a separate group of conscious rats were not altered by ovariectomy or E₂ administration. Electrical stimulation of the aortic nerve elicited frequency-dependent depressor and bradycardic responses that were significantly smaller in OVX compared with SO values (P < 0.05). Treatment of OVX rats with E₂ restored the hemodynamic responses to aortic stimulation to near SO levels. On the other hand, hemodynamic responses to vagal stimulation were not affected by OVX or treatment with E₂. These findings suggest that enhancement of reflex bradycardia by estrogen is centrally mediated and involves interaction with central projections of the aortic nerve.

ovariectomy; aortic nerve stimulation; 17β-estradiol

Epidemiologic studies have shown that the incidence of coronary heart disease is relatively low among premenopausal women and exhibits a sharp rise with the occurrence of menopause (35). The increased risk of coronary heart disease in young women with bilateral oophorectomy (28) and the beneficial effect of estrogen replacement therapy in postmenopausal women (29) further support a cardiovascular protective role for estrogen. Several factors have been suggested to explain the cardiovascular protective role of estrogen. Kushwaha and Hazzard (18) showed that estrogen has a beneficial effect on plasma lipoprotein, decreasing low-density lipoprotein cholesterol and increasing high-density lipoprotein cholesterol. The protective effect of estrogen may also relate to its vasodilatory properties. It has been shown that estrogen enhances vasodilation by enhancing endothelial nitric oxide activity (34) and inhibiting voltage-gated Ca²⁺ channels (37).

The role of defective cardiovascular autonomic regulation in cardiac mortality has been documented both experimentally and clinically (15, 25, 32). In effect, reported findings provided indirect evidence to support a facilitatory role for ovarian hormones in the autonomic regulation of cardiovascular function. This view is supported by the finding that hormone replacement therapy produces favorable effects on the cardiovascular autonomic regulation in postmenopausal women (12) and that oral contraceptive users exhibit greater heart rate (HR) responses during behavioral stressors (10). In a recent study from our laboratory (8), we provided the first experimental evidence that implicated estrogen in the modulation of the baroreceptor control of HR. In that study (8), we investigated the effects of ovariectomy and estrogen treatment on reflex bradycardic responses to peripherally mediated pressor responses in conscious rats. The results showed that ovariectomy caused a significant attenuation of baroreflex HR responses, which suggests that endogenous estrogen enhances baroreflex sensitivity (BRS) (8). Further support to this notion was the finding that 17β-estradiol (E₂) restores baroreflex gain in ovariectomized (OVX) rats to levels comparable with those obtained in sham-operated (SO) rats (8). Similar findings of baroreflex enhancement by estrogen have been demonstrated in OVX (11) and male rats (23). The mechanism, however, by which estrogen modulates baroreflex function has not been investigated.

The main objective of the present study was to test the hypothesis that estrogen acts centrally to enhance BRS. To this end, we investigated the effect of estrogen on hemodynamic responses to electrical stimulation of the central end of the aortic depressor nerve in pentobarbital sodium-anesthetized rats. Baroreflex-mediated decreases in HR in response to increments in blood pressure (BP) evoked by bolus intravenous doses of phenylephrine were evaluated, and changes in baroreflex sensitivity were correlated to changes in hemodynamic responses to aortic and vagal stimulation. Because the part of the current study that involved aortic and vagal nerve stimulation was undertaken, for technical reasons, in anesthetized rats, we felt it important that our previous findings in conscious rats (8) be
replicated in pentobarbital sodium-anesthetized rats. Changes in mean arterial pressure (MAP) and HR evoked by aortic stimulation at different frequencies were compared in SO, OVX, and OVX estradiol-treated (OVX-E₂) rats. The OVX rat was used as a model for surgical menopause (2, 8). Furthermore, the possibility that peripheral mechanisms contribute to estradiol-mediated facilitation of baroreflex function was investigated by evaluating hemodynamic responses (MAP and HR) to electrical stimulation of the vagal efferents in the three rat preparations. Finally, the study was extended to investigate whether estrogen influences baroreflex-mediated tachycardic responses tested by sodium nitroprusside. This study was undertaken in a separate group of conscious rats to complement our previous findings in conscious rats, which demonstrated enhancement of baroreflex-mediated bradycardia by E₂ (8).

MATERIALS AND METHODS

A total of 69 female Sprague-Dawley rats (9–10 wk old; Charles River, Raleigh, NC) were employed in this study.

Ovariectomy

Two weeks before intravascular cannulation, bilateral ovariectomy was performed as described in previous studies including our own (2, 8). Briefly, the rat was anesthetized using methohexital (brevital sodium; 50 mg/kg ip). The lower part of the back was shaved and a single 2- to 3-cm incision was made in the skin to expose the back muscles. A small 1- to 2-cm incision was made in the muscles overlying the ovaries on both sides, and the ovaries were isolated, tied off with sterile suture, and removed. The muscles and the skin were sutured separately, and the rats were allowed to recover for ~2 wk before the time of the experiment. Sham operation was performed by exposing the ovaries without isolation. After ovariectomy or sham operation, each rat received a subcutaneous injection of buprenorphine hydrochloride (Buprenex; 30 µg/kg) to control pain and an intramuscular injection of 50,000 U/kg of penicillin G benzathine and penicillin G procaine in an aqueous suspension (Durapen). Rats were housed in separate cages.

Intravascular Cannulation

On the day of the experiment, the rats were prepared for measurement of BP according to our previous studies (5, 7, 8). Briefly, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip). Polyethylene-50 catheters filled with heparinized saline (200 U/ml) were introduced via the left femoral artery and vein to the abdominal aorta and inferior vena cava, respectively, for measurements of BP and intravenous injection of drugs, respectively. The arterial catheter was connected to a Gould-Statham pressure transducer, and BP was displayed on a Grass polygraph. HR was computed from BP wave forms by a Grass tachograph and displayed on another channel of the polygraph. Body temperature was maintained at 37°C using an overhead lamp.

For BP measurement in conscious rats, the catheters were tunnelled subcutaneously and exteriorized at the back of the neck between the scapulae. The catheters were flushed with heparin (200 U/ml) and plugged with stainless steel pins. Incisions were closed with surgical clips and swabbed with povidone-iodine solution. Each rat received a subcutaneous injection of the analgesic buprenorphine hydrochloride (0.3 µg/rat) and an intramuscular injection of 60,000 U of penicillin G benzathine and penicillin G procaine in an aqueous suspension. Rats were housed in separate cages. The experiment started 48 h later. Experiments were performed in strict accordance with institutional animal care and use guidelines.

Aortic Nerve Stimulation

Electrical stimulation of the aortic depressor nerve was performed as described elsewhere (9, 38). Briefly, the aortic depressor nerve was identified at its junction with the superior laryngeal nerve, carefully separated from the adjacent cervical sympathetic nerve, and sectioned as low in the neck as possible. Other arterial baroafferent nerves (right aortic and both carotid sinus nerves) were left intact. It has been shown that left aortic nerve stimulation elicits similar hemodynamic responses in the presence and absence of other baroaffere(nes (9). The aortic nerve and the electrode were covered with mineral oil. The central end of the cut left aortic depressor nerve was stimulated electrically for 10 s using a stainless steel bipolar electrode. Electrical stimulation with square-wave pulses of 0.2 ms was delivered to the animal using a Grass stimulator (model S48) through an isolation unit. The stimulus intensity was 30 V, and the stimulation frequencies were 1, 2, 4, 8, 16, and 32 Hz. These stimulation parameters activated both A and C fibers of the aortic nerve in the rat (1).

Vagal Nerve Stimulation

Both vagi were cut, and the peripheral end of the right vagus (38) was stimulated using the same stimulation parameters mentioned above with the aortic depressor nerve.

Experimental Protocols

Effect of estrogen on reflex bradycardia. Three groups of female rats (SO, OVX, and OVX-E₂; n = 6–10 each) were used in this study to investigate the effect of estrogen on baroreceptor control of reflex bradycardia. Forty-eight hours before baroreflex testing, E₂ (50 µg/kg, dissolved in sesame oil) or equal volume of vehicle was injected subcutaneously in single daily doses for two consecutive days (8). On the day of the experiment, intravascular cannulations were performed under pentobarbital sodium anesthesia as mentioned above. A period of 30 min was allowed at the beginning of the experiment for stabilization of BP and HR. A dose-response curve of the responses of BP and HR to phenylephrine was constructed in all rats by intravenous injection of randomized doses of phenylephrine hydrochloride (1, 2, 4, 8, or 16 µg/kg) at 5-min intervals. Phenylephrine was dissolved in saline, and the injection volume was kept constant at 0.05 ml/100 g body wt with a flush volume of ~0.1 ml saline. The MAP (diastolic + one-third pulse pressure) and HR values before and after phenylephrine administration were measured, and the peak changes in both variables were used for construction of the baroreflex curves.

Effect of estrogen on hemodynamic responses to aortic nerve stimulation. This experiment was performed to determine whether estrogen-mediated enhancement of BRS involves a central mechanism. In the same groups of anesthetized rats used in the phenylephrine experiment, hemodynamic responses (BP and HR) to electrical stimulation of aortic depressor nerve were evaluated. After baroreflex testing with phenylephrine, the central end of left aortic depressor nerve was electrically stimulated (1–32 Hz, 0.2 ms duration, supramaximal voltage for 10 s) at 5-min intervals. Decreases in MAP and HR were measured, and frequency-depressor and frequency-bradycardic response curves were compared in the three groups of rats.
Effect of estrogen on hemodynamic responses to vagal stimulation. This experiment was performed to determine whether the effect of estrogen on baroreflex function involves peripheral mechanisms. Separate three groups of female rats (SO, OVX, and OVX-E2; n = 7–9 each) were used in this experiment. Thirty minutes after bilateral vagotomy, the peripheral end of the right vagus nerve was electrically stimulated using the same stimulation parameters used for the aortic nerve.

Effect of estrogen on reflex tachycardia. The effect of OVX and estrogen treatment on baroreceptor control of reflex tachycardia was investigated in three separate groups of conscious female rats (SO, OVX, and OVX-E2; n = 5 or 6 each). A dose-response curve of the reductions in BP produced by sodium nitroprusside (1, 2, 4, 8, or 16 µg/kg at 5-min intervals) was constructed along with the reflex tachycardic responses. Changes in MAP and HR were measured and used for the construction of the baroreflex curves. This additional experiment did not involve nerve stimulation, and therefore was undertaken in conscious rats to complement our previous study, which demonstrated E2 enhancement of baroreflex-mediated bradycardia in the same rat model (8). It must be remembered that the ability of estrogen to enhance the baroreceptor control of reflex bradycardia was equally demonstrated in conscious (8) and anesthetized rats.

Drugs
Phenylephrine hydrochloride, sodium nitroprusside, E2, pentobarbital sodium (Sigma, St. Louis, MO), methohexital sodium (Eli Lilly, Indianapolis, IN), Buprenex (Richtit & Coleman, Richmond, VA), and Durapen (Vedco, Overland Park, KS) were purchased from commercial vendors.

Statistical Analysis
Values are expressed as means ± SE. The relationship between increases in MAP evoked by phenylephrine and associated decreases in HR was assessed by regression analysis for individual animals as described in our previous studies (5, 6). The regression coefficient (slope of the regression line), expressed as beats per minute per millimeters of mercury, was taken as an index of baroreceptor reflex sensitivity. The results of estrogen on hemodynamic responses to aortic or vagal stimulation were evaluated by comparison of the slopes of the regression lines of the frequency-ΔMAP and frequency-ΔHR relationships for individual rats. The repeated-measures ANOVA followed by a Newman-Keuls post hoc analysis was used for multiple comparisons, and the level of significance was set at P < 0.05.

RESULTS
Effect of Estrogen on Reflex Bradycardia

The baseline values of MAP (114 ± 3, 110 ± 4, and 105 ± 4 mmHg) and HR (338 ± 12, 350 ± 15, and 343 ± 11 beats/min) of anesthetized SO, OVX, and OVX-E2 rats, respectively, were similar. The increases in MAP evoked by intravenous bolus administration of phenylephrine (1–16 µg/kg) and the associated baroreflex-mediated bradycardic responses in pentobarbital sodium-anesthetized SO, OVX, and OVX-E2 rats are shown in Figs. 1 and 2. Phenylephrine elicited dose-dependent pressor responses of similar magnitudes in all groups of rats, indicating that estrogen had no effect on pressor responses to phenylephrine (Fig. 1A). On the other hand, the baroreflex-mediated bradycardic responses were significantly attenuated in OVX compared with SO rats (P < 0.05; Fig. 1B). Comparison of the baroreflex curves, relating bradycardic responses to phenylephrine-induced increases in MAP, revealed that OVX caused an upward shift in the baroreflex curve (Fig. 2) and a significant reduction in the slope of the regression line that represents the baroreflex sensitivity (P < 0.05; Fig. 2, inset); i.e., for a comparable rise in MAP there was a significantly smaller bradycardic response in OVX compared to SO rats (P < 0.05; Fig. 2). The correlation coefficients of the regression lines were highly significant (P < 0.001) and ranged from 0.93 to 0.99.

Treatment with E2 (50 µg·kg⁻¹·day⁻¹ for 2 consecutive days) enhanced bradycardic responses to phenylephrine (Fig. 1B) and shifted the baroreflex curve toward that of the SO rats (Fig. 2). The regression coefficient (BRS) of OVX-E2 rats was increased to levels that were significantly higher than the corresponding OVX value and similar to the SO value (P < 0.05; Fig. 2, inset).
**Effect of Estrogen on Reflex Tachycardia**

The baseline values of MAP (115 ± 6, 118 ± 3, and 119 ± 3 mmHg) and HR (372 ± 13, 400 ± 11, and 398 ± 12 beats/min) of conscious SO, OVX, and OVX-E2 rats, respectively, were similar. The baroreflex curves relating changes in MAP evoked by nitroprusside (1–16 µg/kg) and the associated changes in baroreflex-mediated tachycardic responses in conscious SO, OVX, and OVX-E2 rats are shown in Fig. 3. Nitroprusside elicited dose-dependent depressor responses of similar magnitudes in all groups of rats (Fig. 3). Compared with SO rats, ovariectomy had no effect on the reflex tachycardic responses to nitroprusside, as indicated by the similar slopes of the regression lines in the two groups of rats (Fig. 3). Treatment of OVX rats with E₂ (50 µg·kg⁻¹·day⁻¹ for 2 consecutive days) resulted in significant reductions in reflex bradycardic responses to phenylephrine-evoked rises in BP as well as depressor and bradycardic responses to electrical stimulation of the central end of the aortic depressor nerve. Treatment of OVX rats with E₂ restored the baroreflex sensitivity and aortic stimulation-mediated hemodynamic responses to near-SO levels. Electrical stimulation of efferent vagal nerve produced frequency-dependent depressor and bradycardic responses that were not altered by ovariectomy or E₂ treatment, suggesting that enhancement of baroreceptor control of hemodynamic responses to aortic and vagal nerve stimulation involves (P < 0.05). Treatment with E₂ (50 µg·kg⁻¹·day⁻¹ for 2 consecutive days) resulted in significant enhancements in the depressor responses to aortic nerve stimulation (P < 0.05; Fig. 4A). The frequency-related decreases in MAP in SO and OVX-E₂ rats were not statistically different (Fig. 4A). The bradycardic responses to aortic nerve stimulation were also significantly enhanced in OVX-E₂ compared with OVX rats (P < 0.05; Fig. 4B). The slope of regression line relating the stimulation frequency and decreases in MAP (Fig. 5A) and HR (Fig. 5B) were significantly decreased after ovariectomy and restored to SO levels after treatment with E₂ (P < 0.05).

Electrical stimulation of the vagal efferent nerve elicited frequency-related decreases in MAP (Fig. 6A) and HR (Fig. 6B). These hemodynamic responses were similar in SO, OVX, and OVX-E₂ rats (Fig. 6). The slopes of regression lines relating the stimulation frequency and decreases in MAP (Fig. 7A) or HR (Fig. 7B) were also similar in all groups of rats.

**DISCUSSION**

The current study presents evidence that implicates central neural pathways of the baroreceptor reflex arc in estrogen-mediated facilitation of baroreceptor control of reflex bradycardia. This notion is supported by the findings that bilateral ovariectomy resulted in significant reductions in reflex bradycardic responses to phenylephrine-evoked rises in BP as well as depressor and bradycardic responses to electrical stimulation of the central end of the aortic depressor nerve. Treatment of OVX rats with E₂ restored the baroreflex sensitivity and aortic stimulation-mediated hemodynamic responses to near-SO levels. Electrical stimulation of efferent vagal nerve produced frequency-dependent depressor and bradycardic responses that were not altered by ovariectomy or E₂ treatment, suggesting that enhancement of baroreceptor control of hemodynamic responses to aortic and vagal nerve stimulation involves (P < 0.05).
reflex bradycardia by estrogen is not peripherally mediated. Finally, the lack of effect of estrogen on baroreflex-mediated tachycardic responses suggests differential modulation by estrogen of baroreceptor control of HR in female rats.

Recent findings from our laboratory (8) suggest a favorable role for estrogen in the modulation of reflex bradycardia in conscious rats. In that study (8), ovariectomy caused a significant attenuation of baroreflex HR responses, and treatment of OVX rats with E2 restored baroreflex responsiveness to levels comparable with those obtained in SO rats (8). A similar facilitatory effect of estrogen on reflex bradycardia has been demonstrated (11, 23). In the present study, we tested the hypothesis that estrogen-induced facilitation of baroreflex function is centrally mediated. The objective of the study was accomplished by investigating the effect of ovariectomy with and without E2 treatment on depressor and bradycardic responses to electrical stimulation of the central end of the aortic depressor nerve and the peripheral end of the vagal efferent nerve. It has been shown that the rat aortic depressor nerve contains mainly baroreceptor sensory afferents (24), and its electrical stimulation provides a pure baroreceptor input into the baroreflex arc. Baroreflex curves relating increases in BP evoked by phenylephrine and reciprocal changes in HR were also constructed, and the slopes of the regression lines were taken as a measure of BRS (5, 16).

The present finding that the reflex bradycardic responses to peripherally mediated increases in BP were reduced in OVX rats [a model of surgical menopause in young women (2)] and restored to SO levels after estrogen treatment supports the hypothesis that estrogen facilitates the baroreflex control of heart rate (8, 11, 23). Furthermore, this finding in experimental animals is in agreement with the clinical observations, which suggested a favorable effect of ovarian hormones on cardiovascular autonomic regulation in women (10, 12). The present finding that E2 treatment had no effect on baseline BP, even though it enhanced the BRS, agrees with the findings of previous studies (2, 8) that reported no changes in BP after ovariectomy or short- and long-term E2 treatment. Notably, an enhanced BRS serves as a safeguard against potential perturbations in BP, but it may not necessarily elicit a change (decrease) in baseline BP. In effect, Huikuri et al. (12) showed that the enhanced BRS in men compared with women is associated with a comparable baseline BP in the two sexes. Our own findings showed that the reduction in BRS evoked by selective denervation of aortic (5) or carotid sinus (6) baroreceptors is not associated with chronic rises in BP. Given that cardio-

**Fig. 4.** Depressor (A) and bradycardic (B) responses to electrical stimulation (1–32 Hz, 0.2 ms duration, supramaximal voltage for 10 s) of central end of aortic depressor nerve in pentobarbital sodium-anesthetized SO and OVX rats treated with vehicle or E2 (50 µg·kg$^{-1}$·day$^{-1}$ for 2 days, dissolved in sesame oil). Values are means ± SE; n for each group shown in parentheses. *,#P < 0.05 vs. SO and OVX values, respectively.

**Fig. 5.** Bar graphs showing slopes of frequency-depressor (A) and frequency-bradycardic (B) response curves to electrical stimulation (1–32 Hz, 0.2 ms duration, supramaximal voltage for 10 s) of central end of aortic depressor nerve in pentobarbital sodium-anesthetized SO and OVX rats treated with vehicle or E2 (50 µg·kg$^{-1}$·day$^{-1}$ for 2 days, dissolved in sesame oil). Values are means ± SE. *,#P < 0.05 vs. SO and OVX values, respectively. Veh, vehicle.
pulmonary baroreceptors play a minor role in HR responses measured by the Oxford method (16) and that the aortic nerve of the rat consists exclusively of baroreceptor afferents (21), it is conceivable that the BRS measured in the present study reflected changes in the responsiveness of arterial baroreceptor afferents. It is notable that estrogen enhancement of BRS does not seem to be influenced by anesthesia. The enhancement of BRS in anesthetized rats in the present study was comparable to that obtained in conscious rats in our previous study (8). The findings of this experiment suggested that the anesthetized rat preparation used to investigate the influence of estrogen on baroreflex responses to aortic nerve stimulation is appropriate for testing the stated hypothesis. However, ovariectomy also reduced circulating progesterone, which makes it difficult to ascertain whether the deficit in estrogen or progesterone accounts for the reduced BRS in OVX rats. To support the tested hypothesis, E2 was injected subcutaneously to OVX rats in a dose regimen that caused enhancement of BRS in our previous study (8). Our own findings showed that subcutaneous implantation of Silastic tubing containing a dose of E2 similar to that used in the present study produced plasma estrogen concentrations in OVX rats (20–30 pg/ml; unpublished observation) similar to physiological levels (2). The present finding that the reflex tachycardic responses were not influenced by ovariectomy or estrogen administration may suggest a differential effect of estrogen on reflex bradycardic (facilitation) and tachycardic (no effect) responses. The reasons for these differential actions of E2 remain to be investigated.

The present study presents evidence that implicates the central projections of the baroafferent nerves in the modulatory effect of estrogen on baroreflex function. This notion is supported by the finding that bilateral ovariectomy, which drastically reduces circulating estrogen levels (2), caused significant reductions in the reflex depressor and bradycardic responses to electrical stimulation of the central end of the aortic depressor nerve. The administration of E2, but not the vehicle, to OVX rats resulted in significant increases in BRS and restored it to near-SO levels. To rule out a role for estrogen interaction with neurotransmitter release and/or the receptors at the target organ level, we investigated the effects of ovariectomy with and without estrogen on bradycardic and depressor responses elicited by stimulation of the peripheral end of the vagus nerve terminals. The findings demonstrated that the hemodynamic responses to vagal nerve stimulation were not altered under these conditions. These findings argue against a modulatory role for estrogen on cardiac muscarinic receptors or on the synthesis or release of acetylcholine from parasympathetic nerve terminals.

Fig. 6. Depressor (A) and bradycardic (B) responses to electrical stimulation (1–32 Hz, 0.2 ms duration, supramaximal voltage for 10 s) of peripheral end of vagal nerve in pentobarbital sodium-anesthetized SO and OVX rats treated with vehicle or E2 (50 µg·kg⁻¹·day⁻¹ for 2 days, dissolved in sesame oil). Values are means ± SE; n for each group shown in parentheses.

Fig. 7. Bar graphs showing slopes of frequency-depressor (A) and frequency-bradycardic (B) response curves to electrical stimulation (1–32 Hz, 0.2 ms duration, supramaximal voltage for 10 s) of peripheral end of vagal nerve in pentobarbital sodium-anesthetized SO and OVX rats treated with vehicle or E2 (50 µg·kg⁻¹·day⁻¹ for 2 days, dissolved in sesame oil). Values are means ± SE.
Interestingly, reported findings suggest a facilitatory role for estrogen on cholinergic neurotransmission in the central nervous system. O'Malley et al. (22) reported that ovariectomy reduces the activity of choline acetyltransferase and neuronal acetylcholine content in rat cerebral cortex and that these effects are reversed after administration of E2. Furthermore, E2 enhances the activity of high-affinity choline uptake carrier (22), a rate-limiting step for acetylcholine synthesis (4). Little information is available, however, concerning the effect of estrogen on peripheral cholinergic neurotransmission. In one study, Max (20) showed that ovariectomy has no effect on choline acetyltransferase activity in the rat extensor digitorum longus muscle. It is possible, therefore, that estrogen may modulate cholinergic neurotransmission in central (22) but not peripheral (20) tissues. Taken together, the present findings may suggest that estrogen acts within the central nervous system to modulate baroreflex function through interaction with neuronal pathways involved in the central processing of baroreceptor information. This notion is supported by the recent conclusion reported by He et al. (11), who showed that administration of E2 to OVX rats enhances baroreflex-mediated control of HR and sympathetic neural activity (both renal and splanchnic) tested by phenylephrine. It is notable, however, that the evidence presented in the current study to support a central mechanism for estrogen-BRS interaction is not unequivocal. The fact that the cut central end of the left aortic nerve was stimulated, whereas the right aortic nerve and the carotid sinus baroafferents were left intact, raises the possibility that estrogen may have acted peripherally to modulate baroreflex function. Furthermore, the role of direct vascular effects of estrogen in BP control, in addition to its modulatory effect on reflex bradycardia, should not be overlooked. For example, estrogen has been shown to elicit decreases in vascular resistance and BP, at least partly via enhancing endothelial nitric oxide activity and inhibiting voltage-gated Ca2+ channels (33, 34, 37).

The conclusion that estrogen acts centrally to enhance baroreflexes may be supported by the finding of Simery et al. (27), who identified estrogen receptor mRNA-containing neurons in the nucleus of the solitary tract (NTS), the first central synapse of arterial baroafferents within the baroreflex loop (31). It has been shown that neurons from the NTS project into medullary vagal centers such as the dorsal motor nucleus of the vagus and the nucleus ambiguous (17). Therefore, estrogen may influence the latter areas at least indirectly through its action within the NTS. Similarly, estrogen receptor mRNA-containing neurons exist in the caudal ventrolateral medulla (27), a brainstem area that is involved in the central processing of baroreceptor information (3, 19). The possibility should be considered, therefore, that estrogen may enhance baroreflexes by altering the functional aspects of one or more sites within the baroreflex arc. In effect, evidence has been presented that supports facilitatory and inhibitory roles for estrogen on central glutamatergic (36) and GABAergic (14) neurotransmission, respectively. Both types of neurotransmissions are essential for the modulation of central baroreflexes (19). Furthermore, estrogen has been shown to cause a significant increase in the density of central α2-adrenoceptors (13), which are known to facilitate baroreflex function (19, 30). Other studies, however, reported variable effects for estrogen on the binding activity of α2-adrenoceptors (26). Further studies are needed, however, to determine the exact central mechanism involved in baroreflex enhancement by estrogen.

In summary, the present study highlights the importance of the central nervous system in estrogen-induced facilitation of the reflex bradycardic responses. Depressor and bradycardic responses to aortic nerve stimulation were significantly attenuated in OVX rats and were restored to control levels after treatment with estrogen. These findings suggest that estrogen interacts with neural pathways involved in central processing of baroreceptor information. The ability of estrogen to enhance baroreflex function does not involve the target organs as suggested by the lack of an effect of ovariectomy and estrogen administration on hemodynamic responses to efferent vagal stimulation. Nonetheless, because the contralateral aortic depressor and the carotid sinus nerves were left intact, contribution of the peripheral baroreceptors to estrogen action on baroreflexes may not be ruled out.

Perspectives

Reported clinical and experimental findings have suggested a protective role for estrogen against cardiovascular diseases. A growing body of evidence suggests that the incidence of cardiovascular events is greater in men and postmenopausal women compared with premenopausal women. Furthermore, the prevalence of cardiovascular disorders is less in postmenopausal women receiving estrogen therapy. The cardiovascular protective properties of estrogen have been accounted for by its vasodilatory and beneficial effects on the lipid profile. The finding in the present study that estrogen exerts a favorable effect on the baroreflex control of heart rate in sexually mature female rats presents new insight into the cardiovascular protective actions of estrogen. Notably, the baroreflex HR response to changes in BP reflects the capacity of reflex autonomic regulation. In effect, it has long been established that defective cardiovascular autonomic control plays an important role in cardiac mortality and that diminished baroreflexes accompany and may contribute to the development of hypertension in humans and several other models of experimental hypertension. Although it is not clear whether the diminished baroreflex function is the cause or effect of hypertension, the latter is widely accepted as a leading cause in the pathophysiology of serious heart diseases, including atherosclerosis and angina pectoris. The demonstration in the present study, therefore, that pertains to the favorable effect of estrogen on the baroreflex control of HR adds a new dimension toward the understanding of the mechanism of estrogen-mediated cardioprotection.
REFERENCES