Centrally mediated effect of 17β-estradiol on parasympathetic tone in male rats

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Saleh, Tarek M., and Barry J. Connell. Centrally mediated effect of 17β-estradiol on parasympathetic tone in male rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R474–R481, 1999.—The following experiments were conducted to determine if peripherally administered estrogen has an effect on central autonomic tone and whether this change in tone results in an alteration in cardiovascular reflex control. Male Sprague-Dawley rats were anesthetized with thiobutabarbitol sodium (50 mg/kg) and instrumented to record blood pressure, heart rate, and vagal parasympathetic or renal sympathetic efferent nerve activity. Additional rats were instrumented to test the sensitivity of the cardiac baroreflex using intravenous injections of phenylephrine hydrochloride (0.025, 0.05, 0.1 mg/kg) or sodium nitroprusside (0.0025, 0.005, 0.01 mg/kg) and plotting the cardiovascular responses. Intravenous injection of estrogen (10⁻⁴, 10⁻³, and 10⁻¹ mg/kg) produced a significant increase in vagal efferent activity and in baroreflex sensitivity. The bilateral microinjection of an estrogen receptor antagonist, ICI-182,780 (1 μM, 50 nl/side) into the nucleus ambiguus blocked both the estrogen-induced increase in vagal efferent activity and baroreflex sensitivity. These results demonstrate that in male rats estrogen acts centrally to enhance baroreflex sensitivity by increasing parasympathetic efferent tone.

renal nerve; vagus nerve; ICI-182,780; nucleus ambiguus; baroreflex sensitivity

Epidemiological and experimental studies have indicated the existence of gender differences in autonomic tone (5, 18, 31). These studies also suggest that the incidence of cardiovascular disease is far less pronounced in premenopausal women compared with men, but this difference decreases with age and disappears after menopause, when cardiovascular disease becomes the leading cause of death among women (5, 31). Analysis of baroreceptor reflex sensitivity (BRS) and heart rate variability provides a measure of sympathovagal balance and serves as a risk stratifier for future cardiac arrhythmias and/or sudden cardiac death (45). Specifically, BRS is elevated in premenopausal women relative to both men and postmenopausal women (1). Interestingly, both the BRS and heart rate variability of postmenopausal women have been shown to significantly improve after estrogen replacement therapy (18). In light of this apparent cardioprotective effect, much attention has been focused on the peripheral autonomic effects of estrogen (6, 13, 28, 39), particularly as they pertain to estrogen replacement therapy after the onset of menopause (for review, see Ref. 13).

The beneficial effects of estrogen in the periphery appear to be multifactorial. For example, estrogen affects cholesterol metabolism and disposition, increases plasma levels of high-density lipoproteins (35), inhibits peroxidation of low-density lipoproteins (36), inhibits the proliferation of smooth muscle cells in the arterial wall (46), stimulates vasodilation, and suppresses the norepinephrine-induced vasoconstrictor response of coronary arteries (8, 48).

Other investigations into the effects of estrogen on peripheral autonomic tone have demonstrated that in females, estrogen increases the density and enhances the function of presynaptic α₂-adrenoceptors, resulting in a lower basal plasma norepinephrine level (11, 23) and a significant attenuation of norepinephrine-induced pressor responses (12, 20, 27) compared with men. As well, estrogen has been demonstrated to increase the rate of choline reuptake into cholinergic terminals, potentiate the activity of choline acetyltransferase (13), and increase the magnitude of the phenylephrine-induced reflex bradycardia, resulting in an enhanced BRS (37). Furthermore, epidemiological studies have indicated that premenopausal women have a lower incidence of ventricular tachycardia, ventricular fibrillation, and fatal arrhythmias after coronary artery occlusion (10, 25, 42) primarily due to an enhanced parasympathetic tone. This vagally enhanced state is beneficial when one considers such cardiovascular pathologies as myocardial infarction and heart failure, which have been shown to result in sympathetic hyperactivity and parasympathetic withdrawal (4, 13, 17, 45). Men tend to have a higher sympathetic tone and a depressed BRS compared with women under normal conditions as well as after a cardiovascular accident, contributing to an increased risk for lethal cardiac arrhythmias and sudden death (13, 40, 49). It has been postulated, however, that these gender differences in autonomic tone depend largely on a long-term exposure to estrogen (31). To date, very little research has been undertaken to determine the mechanisms involved in the short-term cardioprotective effects of estrogen administration.

Recently, our lab has shown that the direct stimulation of cervical vagal afferents in male rats for a period of 2 h resulted in an increase in plasma norepinephrine levels and a decrease in BRS (38) similar to that observed after cardiovascular pathology. Furthermore, intravenous injection of estrogen has been shown to increase BRS in a dose-dependent manner in normal male rats as well as block the depression in the BRS in vagal-stimulated male rats. In both cases, the effect of
estrogen on the BRS was shown to result from an increase in the magnitude of the reflex bradycardia response to a bolus injection of a pressor agent (37). Finally, the intravenous administration of ICI-182,780, a potent and selective estrogen receptor antagonist (19, 47), 10 min before injection of estrogen, blocked both effects (37), indicating that the enhanced reflex bradycardia was dependent on estrogen receptor activation.

In the present investigation, we will examine whether intravenously administered estrogen alters baroreflex sensitivity via a centrally mediated effect on autonomic tone. Parasympathetic and sympathetic nerve activity will be measured before and after the local microinjection of ICI-182,780 into the nucleus ambiguus, a known autonomic regulatory nucleus (24). In this way, a centrally mediated, estrogen-induced effect on autonomic tone in male rats can be demonstrated.

MATERIALS AND METHODS

All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Prince Edward Island Animal Care Committee.

General surgical procedures. Experiments were performed on a total of 48 male Sprague-Dawley rats (Charles River; Montreal, PQ, Canada) weighing 250–275 g. Rats were anesthetized with a single dose of thiobutabarbital sodium (Inactin; RBI, Natick, MA; 50 mg/kg ip), which maintained a surgical plane of anesthesia for the duration of the experiment. A polyethylene catheter (PE-50; Clay Adams, Parsippany, NJ) was inserted into the right femoral artery to monitor blood pressure and heart rate and into the right femoral vein (PE-10) for the intravenous administration of drugs. Arterial blood pressure was measured with a pressure transducer (Gould P23 ID; Cleveland, OH) connected to a Gould model 2200S polygraph. Heart rate was determined from the pulse pressure using a Gould tachograph (Biotach). An endotracheal tube was inserted, and animals were ventilated with room air on a Harvard rodent ventilator (65 strokes/min; 2.5 ml tidal volume) to facilitate respiration. Vagal parasympathetic and renal sympathetic nerve activity were determined using both a Student’s t-test and a one-way ANOVA for repeated measures. In all cases, differences were analyzed by a Student’s t-test and a one-way ANOVA for repeated measures.
Table 1. Average MAP and HR after intravenous estrogen injection and the microinjection of saline or ICI-182,780 into the nucleus ambiguus

<table>
<thead>
<tr>
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<th>Time, min</th>
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<tr>
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<td>30 Before</td>
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<tr>
<td>MAP, mmHg</td>
<td></td>
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<tr>
<td>Saline (n = 4)</td>
<td>99 ± 10</td>
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<tr>
<td>ICI-182,780 (n = 4)</td>
<td>101 ± 11</td>
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<tr>
<td>HR, beats/min</td>
<td></td>
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<tr>
<td>Saline (n = 4)</td>
<td>345 ± 18</td>
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<tr>
<td>ICI-182,780</td>
<td>355 ± 16</td>
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Data are means ± SE. Dose of estrogen, 1 × 10⁻² mg/kg. MAP, mean arterial pressure; HR, heart rate. *Significantly different from 30 min before (baseline) value (ANOVA, P < 0.05).

Results

Baseline values for mean arterial pressure (110 ± 11 mmHg; n = 48), heart rate (357 ± 19 beats/min; n = 48), parasympathetic nerve activity (22 ± 6 spikes/bin; n = 32), sympathetic nerve activity (24 ± 7 spikes/bin; n = 4), and baroreceptor sensitivity (0.52 ± 0.05 beats·mmHg⁻¹·min⁻¹; n = 12) remained unchanged during the 30-min period before experimental manipulation. The bilateral central injection of either saline (n = 36) or ICI-182,780 (n = 12) produced a very brief, transient decrease in both mean arterial pressure (12 ± 6 mmHg) and heart rate (16 ± 9 beats/min). Both parameters returned to preinjection baseline values within 60–90 s. Subsequent intravenous injection of either saline (n = 8) or any dose of 17β-estradiol (n = 16) other than the 10⁻² mg/ml dose (n = 24; see Table 1) did not alter baseline blood pressure or heart rate throughout the experimental time period.

Estrogen produces a dose-related increase in vagal parasympathetic efferent nerve activity. In the group of animals instrumented to record parasympathetic tone, mean vagal efferent nerve activity was significantly enhanced compared with preinjection values (22 ± 6 spikes/bin; n = 32) at all time points from 30 to 120 min after the intravenous injection of estrogen (10⁻⁴, 10⁻², and 10⁻¹ mg/kg; n = 4/dose; P < 0.05 for each group at 30, 60, 90, and 120 min; Fig. 1, A and B) and central microinjection of saline. The maximum changes in vagal activity at each of these time points occurred after injection of 10⁻² mg/kg estrogen (average peak activity of 109 ± 22, 159 ± 21, 174 ± 24, and 86 ± 10 spikes/bin at 30, 60, 90, and 120 min postestrogen injection; n = 4/group; P < 0.05 for each time point). For each concentration of estrogen that produced an enhanced vagal tone, the increase recovered to prestimulation levels when measured 180 min postestrogen (Fig. 1B) and remained unchanged for the duration of the experiment. Parasympathetic efferent nerve activity remained unchanged after the intravenous injection of saline or the lowest (10⁻⁵ mg/kg) and...
highest (1 mg/kg) dose of estrogen used in combination with central saline microinjections (n = 4/group; P > 0.05 at all experimental time points compared with baseline values).

Estrogen has no significant effect on renal sympathetic efferent nerve activity. In animals instrumented to record sympathetic tone before and after the central administration of saline and the intravenous injection of estrogen (10^-2 mg/kg), renal nerve activity remained unchanged from preinjection values (24 ± 7 spikes/bin) at all time points measured throughout the duration of the experiment (n = 4; P > 0.05 for all time points; data not shown). Although done in separate animals, the lack of change in sympathetic tone correlated to the time during which parasympathetic tone was optimally enhanced (Fig. 1, A and B) in animals instrumented to record vagal efferent activity.

Nucleus ambiguus mediates the estrogen-induced enhancement of the slope of the baroreflex sensitivity and parasympathetic tone. Previously in our laboratory, we have shown that a bolus intravenous injection of estrogen significantly enhances the BRS of male rats (37). This effect was primarily the result of an increase in the magnitude of the reflex bradycardia in response to the intravenous injection of the pressor agent phenylephrine. In the present investigation, estrogen (10^-2 mg/kg) injection followed by the central microinjection of saline (n = 4/group) also resulted in a significant increase in the magnitude of the phenylephrine-induced reflex bradycardia (28 ± 8 beats/min preestrogen to 56 ± 6, 66 ± 7, 38 ± 6, 51 ± 5, 56 ± 6, 57 ± 7, and 54 ± 6 beats/min at 30, 60, 90, 120, 180, 240, and 300 min, respectively; P < 0.05; Fig. 2A). As well, there was no measurable change in the phenylephrine-induced pressor response at any time point after estrogen injection (n = 4; P > 0.05 at all time points; Fig. 2A). When these phenylephrine-induced changes in mean arterial pressure and heart rate after estrogen injection (10^-2 mg/kg; n = 4) were plotted, the slopes of the regression lines (BRS) were significantly increased (0.52 ± 0.05 beats·min^-1·mmHg^-1 30 min preestrogen to 1.2 ± 0.03, 1.3 ± 0.04, 0.8 ± 0.05, 0.9 ± 0.05, 1.05 ± 0.06, 1.06 ± 0.06, and 0.95 ± 0.04 beats·min^-1·mmHg^-1 at 30, 60, 90, 120, 180, 240, and 300 min, respectively, postinjection; n = 4/group; P < 0.05 at each time point; Fig. 2B).

Conversely, intravenous injection of estrogen (10^-2 mg/kg; n = 4) did not significantly change the magnitude of the sodium nitroprusside-induced depressor response (preestrogen value of 33 ± 7 mmHg) or the reflex tachycardia (preestrogen value of 22 ± 5 beats/min) at any of the time points measured (n = 4/group; P > 0.05 for 30, 60, 90, 120, 180, 240, and 300 min postestrogen; figure not shown). Consequently, when these two variables were plotted and the slope of the regression lines determined, no significant differences between the preestrogen BRS (0.55 ± 0.05 beats·min^-1·mmHg^-1) and postestrogen BRS values were observed at any time point (n = 4/group; 0.52 ± 0.05 at 30, 0.55 ± 0.06 at 60, 0.57 ± 0.06 at 90, 0.54 ± 0.05 at 120, 0.57 ± 0.05 at 180, 0.58 ± 0.05 at 240, and 0.56 ± 0.05 beats·min^-1·mmHg^-1 at 300 min postestrogen; P > 0.05; figure not shown).

Fig. 2. Cardiovascular responses and baroreflex sensitivity after estrogen injection and saline microinjection into nucleus ambiguus. A: representative example of phenylephrine (PE; 0.1 mg/kg)-induced changes in blood pressure and heart rate (I, PE injection) measured 30 min before and 60 and 300 min after intravenous injection of estrogen (10^-2 mg/kg; ). B: changes in the slope of the regression line as an indication of baroreflex sensitivity (BRS) obtained 30 min before (-30) and at various time intervals after intravenous injection of estrogen (10^-2 mg/kg). Estrogen was given 10 min after microinjection of saline into nucleus ambiguus. *Significance [P < 0.05; analysis of covariance (ANOVA)] from the average BRS value measured 30 min (-30) before drug injection. All bars represent mean of 4 animals.

Bilateral microinjection of the estrogen receptor antagonist ICI-182,780 (1 pM; 50 nl/side) into the nucleus ambiguus in combination with an intravenous injection of saline (0.9%, 0.2 ml) produced no significant changes in vagal efferent nerve activity, the phenylephrine-induced pressor and reflex bradycardia responses and BRS when compared with preinjection values (n = 4/group; P > 0.05 for each time point for each parameter; data not shown). In addition, the combination of ICI-182,780 and intravenous estrogen (10^-2 mg/kg; n = 4) produced no significant changes in the phenylephrine-induced pressor response when experimental time points were compared with preinjection values. However, the microinjection of ICI-182,780 into the nucleus ambiguus 10 min before estrogen (10^-2 mg/kg) administration blocked the previously observed significant changes in vagal efferent nerve activity (n = 4; Fig. 3, Aa and Ab) and BRS (n = 4; Fig. 3, Ba and Bb) when measured at each time point after estrogen injection. The ICI-182,780-induced blockade of the change in the slope of the BRS was the result of an attenuated ability of estrogen to increase the magnitude of the phenylephrine-induced reflex bradycardia. The reflex bradycardia remained unchanged from preestrogen injection values (n = 4; P > 0.05 at all time points; Fig. 3Ba).

Histological verification of cannula placement. Figure 4 shows a composite diagram indicating the bilateral location of microinjection cannulas in the region of
the nucleus ambiguous obtained from all animals receiving ICI-182,780 in this investigation. Data from animals in which both microinjections of ICI-182,780 were located outside the nucleus ambiguous were not included in this study (except to confirm the effective zone for the antagonist within this nucleus). The results from such animals, as well as animals in which only a unilateral injection was made or a bilateral injection outside the nucleus ambiguous did not produce an attenuation of the estrogen-induced increase in parasympathetic tone and BRS were blocked.

Doses of estrogen between, but not including, $10^{-5}$ and $1 \text{ mg/kg}$ were effective in eliciting significant increases in parasympathetic efferent nerve activity, with a dose of $10^{-2} \text{ mg/kg}$ producing the maximal increase. Doses of estrogen $>10^{-2} \text{ mg/kg}$ produced smaller increases in parasympathetic tone, resulting in a bell-shaped dose-response curve. This finding is consistent with a previous investigation in which a similar, bell-shaped, dose-response relationship was observed between estrogen at these same doses and the BRS of male rats (37). In that investigation, the maximal effective dose of estrogen on the BRS was found to be $10^{-2} \text{ mg/kg}$, with those doses between $10^{-4}$ and $1 \text{ mg/kg}$ having a significant effect on the BRS (37). In contrast...
to previous reports on the cardiovascular effects of estrogen (8, 12, 13, 18, 35, 37, 39), our present results showed that baseline heart rate was significantly depressed after estrogen injection but only at a dose (10^{-2} mg/kg) producing a maximal effect on parasympathetic efferent activity (see Fig. 1). However, this estrogen-induced decrease in heart rate did not appear to affect the magnitude of the phenylephrine-induced reflex bradycardia, because baseline heart rate was only depressed at 60 and 90 min (see Table 1), whereas the reflex bradycardia (and hence BRS) was significantly enhanced at all time points measured.

Interestingly, our results demonstrated that administration of estrogen (10^{-2} mg/kg) after the prior microinjection of saline into the nucleus ambiguus did not affect the nitroprusside-induced depressor response or the magnitude of the reflex tachycardia. The nitroprusside-induced alteration in heart rate is mediated via an increase in cardiac sympathetic efferent nerve activity concurrent with a withdrawal of parasympathetic tone. The lack of an estrogen-induced change in the nitroprusside-evoked reflex correlates well with the observation that no significant changes in tonic sympathetic activity were observed after estrogen injection. Consistent with previous results (37), we demonstrated that intravenous estrogen (10^{-2} mg/kg) with the prior central microinjection of saline also increased the magnitude of the reflex bradycardia in response to a phenylephrine-induced rise in blood pressure. The enhanced reflex bradycardia was again independent of a change in the magnitude of the phenylephrine-induced pressor response. Therefore, it appeared that estrogen enhanced the BRS of male rats primarily by increasing parasympathetic outflow.

Of particular interest was the observation of the difference in the time course of the estrogen-induced changes in parasympathetic tone compared with that of the BRS. Vagal efferent tone was enhanced for a period of only 120 min after estrogen administration, with an optimal increase in activity occurring between 60 and 90 min. However, the estrogen-induced enhancement of the BRS appeared to be biphasic, remaining significantly different from preinjection values for the duration of the experimental time course (300 min) with peak BRS values at 60 min and between 180 and 240 min. Furthermore, our results indicate that the increase in BRS may in fact be dependent on an initial centrally mediated, estrogen-induced increase in parasympathetic tone. This is evidenced by the fact that both the elevated vagal tone and the enhanced BRS are blocked over the 300 min of testing by the prior injection of ICI-182,780 into the nucleus ambiguus. Figure 5 summarizes the relationship between the time course of the estrogen-induced (10^{-2} mg/kg) increase in vagal activity and BRS. Figure 5 clearly shows that the enhanced vagal efferent activity recovers to baseline values at around the same time that a secondary increase in BRS becomes apparent.

The nucleus ambiguus contains the majority of descending vagal preganglionic cardioinhibitory neurons (24). Estrogen receptors have been localized to the ventral medulla in the region of the nucleus ambiguus (2, 41) as well as in estrogen-containing projection neurons from the forebrain (9). Therefore, it would appear that the longer-lasting effect of estrogen on BRS is dependent on estrogen acting directly, or indirectly via an estrogenic projection pathway, on estrogen receptors in the nucleus ambiguus and increasing parasympathetic outflow. Evidence already exists to suggest that estrogen is capable of rapid, nongenomic changes in membrane excitability via the direct modulation of ion channel function (21) or via the induction of long-term potentiation (14) within the central nervous system. Also, 17β-estradiol has been described as a central nervous system “activator” that can enhance excitatory neurotransmission (15). Most recently, estrogen has been shown to bind to specific sites on neuronal membranes in the rat brain (34). Estrogen may thus have a direct, immediate effect on neuronal excitability, which might account for the short-term estrogen-induced changes in parasympathetic tone. Because it is not known what concentration of estrogen would be found locally within the nucleus ambiguus after a peripheral injection, electrophysiological investigations are underway in our laboratory to determine the direct, local effect of estrogen on the excitability of neurons within this parasympathetic preganglionic nucleus and to determine if this effect results in an increase in vagal efferent activity to the heart.

Another possible mechanism for the estrogen-mediated increase in parasympathetic tone could be via the release of neurotrophins and activation of their central receptors. It has been shown that neurons colocalize estrogen and neurotrophin receptors (44), and acute estrogen administration has been shown to significantly enhance the postsynaptic concentration of neurotrophin receptors (for review, see Ref. 3). After synaptic activity and the subsequent increase in postsynaptic intracellular calcium concentration, the calcium-dependent neurotrophin released may act as a retrograde messenger to enhance further presynaptic neurotransmitter release (3). Furthermore, neurotrophins have been implicated in the postsynaptic potentiation of excitatory neurotransmission (3). Therefore, a short-term, estrogen-dependent increase in neuronal excitabil-
ity may be maintained for a longer period of time after a neurotrophin-mediated increase in postsynaptic activity. However, in our study, no secondary increase in parasympathetic tone was observed after a return to baseline values. It therefore appears that the secondary increase in BRS is not directly related to a secondary, centrally mediated increase in parasympathetic tone.

It is possible, however, that neurotrophins may still mediate the secondary elevation of BRS at a peripheral level, because neurotrophin receptors have been localized on cardiac myocytes (16). The initial increase in parasympathetic efferent tone and subsequent postganglionic postsynaptic activity on cardiac myocytes may be adequate to activate and maintain neurotrophin release at this peripheral site. After the decline of estrogen-induced vagal efferent activity, neurotrophins released from postganglionic fibers may either enhance or maintain the presynaptic release of acetylcholine (3, 16) and thus sustain the enhanced phenylephrine-induced reflex bradycardia. This neurotrophin-induced effect would, however, be dependent on an initial estrogen-induced increase in efferent parasympathetic tone and therefore would also be blocked after the prior central microinjection of the estrogen antagonist ICI-182,780.

We cannot exclude the possibility that estrogen might be acting peripherally via genomic pathways to maintain an enhanced BRS after the decline in parasympathetic activity. Several lines of evidence suggest that steroid hormones, such as estrogen, control intracellular functions via mechanisms that activate the transcription processes via a nuclear estrogen receptor. Estrogen administration has been shown to increase the levels of choline acetyltransferase resulting in elevated circulating plasma acetylcholine levels (22, 26) as well as facilitating the high-affinity uptake of choline (30). Both of these estrogen-induced effects would result in an enhanced synaptic efficacy of acetylcholine at the sinoatrial node. This could also be responsible for a secondary long-term enhancement of the phenylephrine-induced reflex bradycardia and BRS after the recovery of the initial centrally mediated change in parasympathetic tone.

Perspectives

Several cardiovascular pathologies have been associated with serious autonomic abnormalities, characterized by enhanced sympathetic nervous system activity and parasympathetic nervous system withdrawal (29, 33). Additionally, the extent of suppression of parasympathetic tone has been correlated with the severity of heart failure and provides prognostic value to sudden cardiac death and other future clinical problems (7, 29, 40). This report demonstrates that acute estrogen administration enhances parasympathetic tone and, as previously shown, blocks the depression in baroreceptor sensitivity observed after vagal afferent stimulation (37). Taken together, these results suggest that acute estrogen administration after a cardiovascular incident may have potential therapeutic value by decreasing the imbalance in autonomic output. Evidently this would occur by a centrally mediated estrogen-induced increase in parasympathetic tone.

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