Role of 17β-estradiol in the modulation of baroreflex sensitivity in male rats

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Saleh, Tarek M., and Barry J. Connell. Role of 17β-estradiol in the modulation of baroreflex sensitivity in male rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R770–R778, 1998.—Female mammals have an enhanced baroreflex sensitivity compared with their male counterparts, leading researchers to speculate that estrogen modulates autonomic tone. Therefore, this study tests the hypothesis that exogenous estrogen can enhance the baroreflex sensitivity of male rats. Male Sprague-Dawley rats anesthetized with thiobutabarbital sodium (50 mg/kg) were instrumented to measure blood pressure and heart rate and for the intravenous injection of drugs. The baroreflex was tested using intravenous injections of phenylephrine (0.025, 0.05, and 0.1 mg/kg), and the cardiovascular responses were plotted to obtain a measure of the sensitivity of the cardiac baroreflex. Intravenous injection of estrogen produced dose-related increases in the baroreflex sensitivity due to an increase in the magnitude of the reflex bradycardia. In a separate group of animals, stimulation of the vagus nerve for 2 h resulted in a decrease in baroreflex sensitivity. This effect was blocked when estrogen (1 × 10⁻² mg/kg) was administered immediately before the end of stimulation. In conclusion, intravenous injection of estrogen in male rats significantly enhanced baroreflex sensitivity and blocked the attenuation in the baroreflex sensitivity observed after vagal stimulation.

visceral afferents; sympathetic; parasympathetic; ICI-182,780

RECENTLY, MUCH ATTENTION has focused on the cardioprotective effects of estrogen, particularly as they pertain to hormone therapy replacement regimes after the onset of menopause in females (for review, see Ref. 9). During this time, many beneficial effects of estrogen on the peripheral vasculature have been identified (5); however, the mechanism by which estrogen acts has not been fully elucidated.

Most notable and relevant to the current investigation has been the effect of estrogen on modulating autonomic tone. Evidence has been provided to show that estrogen increases the density and enhances the function of presynaptic α₂-adrenoceptors, resulting in a significant attenuation of norepinephrine-induced responses (14, 23). For this reason, it has been suggested that women have a lower basal plasma level of norepinephrine compared with men (17). In addition, exposure to a psychological stressor results in a smaller increase in plasma norepinephrine, producing a blunted pressor response in women compared with men (17).

Clinically, plasma norepinephrine levels measured shortly after cardiovascular pathologies, such as myocardial infarction, are significantly elevated (3, 11, 37). This increase in plasma norepinephrine is most likely a result of cardiac spillover and thus indicative of a hyperactive sympathetic response to cardiac pathology (42). Recently, Schwartz and colleagues (33) have developed an experimental model involving coronary artery occlusion in the conscious dog. These researchers have provided substantial evidence correlating a decrease in the sensitivity of the baroreflex with an increase in both plasma norepinephrine and mortality after an arrhythmic event. Subsequent studies by the same investigators have shown that, after coronary artery occlusion, the abnormal activity of ischemic ventricular muscle fibers produces an increase in vagal afferent traffic, thereby resulting in a hyperactive sympathetic output (32, 33). These effects on sympathetic tone and baroreflex sensitivity were completely blocked by vagotomy but were unaffected by selective barodenervation or after blockade of the cardiac sympathetic ganglion (for review, see Ref. 3). This evidence suggests that vagal afferents play a major role in mediating these pathologically induced effects.

Epidemiological studies have shown that a significant proportion of men with higher than normal sympathetic tone and depressed baroreflex sensitivity after an initial myocardial infarction are at an increased risk for lethal cardiac arrhythmias and sudden death (37). However, in females, a lower incidence of ventricular tachycardias, ventricular fibrillation, and fatal arrhythmias after coronary artery occlusion are observed (20, 35), primarily due to an augmentation of parasympathetic tone (for review, see Ref. 9). In addition, epidemiological studies suggest that females tend to have a lower risk for sudden cardiac death (7). These findings suggest that females have an enhanced parasympathetic tone, which exerts protective and antifibrillatory effects after a cardiovascular insult (9).

Evidence has been provided to suggest that premenopausal women have a higher baroreflex sensitivity compared with men and postmenopausal women (2). Furthermore, the baroreflex sensitivity of postmenopausal women has been shown to significantly improve after replacement therapy with estrogen (12). These studies suggest that estrogen may modulate autonomic tone as a means of returning the baroreflex sensitivity of postmenopausal women to premenopausal values. Recently, our laboratory has shown that after the direct stimulation of vagal afferents in male rats for a period of 2 h, a significant increase in plasma norepinephrine and decrease in baroreflex sensitivity results (31). In the present investigation, we hypothesize that exog-
nous injections of 17β-estradiol 3-sulfate (water-soluble form; Sigma) were administered at doses of 1 × 10⁻⁴, 1 × 10⁻³, 1 × 10⁻², 1 × 10⁻¹, 2 × 10⁻¹, and 1.0 mg/kg (injection volume = 0.2 ml; n = 4/dose). An additional four animals were administered saline (vehicle; 0.9%) intravenously. The baroreflex was tested with phenylephrine 30 min before and every 30 min after, estrogen injection for 5 h. A graph relating the dose of estrogen to the average slope of the baroreflex sensitivity plot was constructed. From this plot, the dose of estrogen that produced the greatest changes in cardiovascular parameters and consequently baroreflex sensitivity was then used in the antagonist and vagal stimulation protocols that follow.

To determine if the observed effects of estrogen on the baroreflex sensitivity were mediated by an action on estrogen receptors, the selective and reversible estrogen receptor antagonist ICI-182,780 (Tocris, Bollwinn, MO) was used. The dose of ICI-182,780 (5 mg/kg in 0.9% saline and 0.03% ethanol) used in these experiments was found to be the most effective in completely blocking the effect of the optimal dose of estrogen (1 × 10⁻² mg/kg). Therefore, in a separate group of animals (n = 4) instrumented as above, ICI-182,780 was administered 10 min before estrogen injection, and the baroreflex was tested 30 min before and 30, 60, 90, and 120 min postestrogen injection. Control groups of animals were given ICI-182,780 followed by saline (n = 4) and saline followed by saline injection (n = 4).

Vagal stimulation. In 16 separate animals, the left vagus nerve was isolated through a midline cervical incision and was placed on stainless steel electrodes that were fixed in place with dental impression material (Ash Temple). The vagus nerve was crushed distal to the stimulating electrodes, permitting activation of visceral afferents only. The stimulus intensity (0.5–2 mA) used to activate vagal afferents was determined using a 5-s train of pulses (50 Hz and 2-ms pulse duration; 1 s on-1 s off cycle) to produce a maximal reflex decrease in heart rate (range from 70 to 110 beats/min) and blood pressure (30–40 mmHg). The 1 s on-1 s off cycle of 2-h duration was chosen to prevent the baroreflex adaptation observed with sustained visceral activation (16, 30, 31). Animals would subsequently receive either saline (0.9%) and saline (0.9%) or estrogen (0.01 mg/kg; n = 4), ICI-182,780 (5 mg/kg) and estrogen (0.01 mg/kg; n = 4), or ICI-182,780 (5 mg/kg) and saline (0.9% n = 4). Estrogen or vehicle was administered immediately before termination of the vagal stimulation, and the ICI-182,780 or vehicle was administered 10 min before estrogen or vehicle injections. The cardiac baroreflex was then tested with phenylephrine 30 min before and 30, 60, 90, and 120 min after the 2 h of vagal stimulation.

Data analysis. All data are presented as means ± SE and were analyzed by a one-way ANOVA for repeated measures followed by a Student-Newman-Keuls post hoc analysis. Statistical comparison for multiple regressions was done using analysis of covariance. Differences were considered significant at P ≤ 0.05.

RESULTS

The baroreflex sensitivities for all nonstimulated and stimulated animals (n = 56) before drug treatment or nerve stimulation were not significantly different from each other (average slope = 0.59 ± 0.05; P > 0.05). Furthermore, there were no significant changes in the baseline values for mean arterial pressure or heart rate after the intravenous injection of any dose of 17β-estradiol, ICI-182,780, or saline (P > 0.05). For example, Table 1 shows the absolute mean arterial pressures and heart rates after the intravenous injection of estrogen.

Estrogen enhances the reflex bradycardia and consequently the slope of the baroreflex sensitivity. Intravenous injections of phenylephrine evoked dose-depen-

<table>
<thead>
<tr>
<th>Time</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
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<tr>
<td>30 min Before</td>
<td>105 ± 12</td>
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<td>30 min After</td>
<td>107 ± 12</td>
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<td>60 min After</td>
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<td>101 ± 13</td>
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<td>120 min After</td>
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<td>300 min After</td>
<td>103 ± 11</td>
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Data are means ± SE. Estrogen was administered in a dose of 1 × 10⁻² mg/kg. MAP, mean arterial pressure; HR, heart rate.
dent increases in blood pressure that were accompanied by reflexive decreases in heart rate. Injection of phenylephrine (0.1 mg/kg) 30 min before estrogen injection produced an average increase in mean arterial pressure of 32 ± 7 mmHg, which elicited an average reflex decrease in heart rate of 19.5 ± 5 beats/min (Fig. 1A).

After intravenous injection of estrogen (1 × 10^{-2} mg/kg), the baroreflex was tested at 30-min intervals over 5 h. The phenylephrine-induced pressor response was not significantly different from the prestimulated response at any of the postestrogen time points tested. The average change in mean arterial pressure for all time points was 33 ± 8 (P > 0.05). In contrast, the mean reflex bradycardia was significantly increased (for example, 98 ± 11 beats/min at 30 min postestrogen injection; P < 0.05; Fig. 1A) compared with the preestrogen injection value measured at each time point over the 5-h time course (Fig. 1A).

In all animals receiving estrogen or saline, the slope of the baroreflex sensitivity plot 30 min before drug injection was 0.62 ± 0.05 (n = 28). For saline-injected control animals, the baroreflex slope remained unchanged from this value (P > 0.05) for the duration of the experimental time course (5 h postdrug injection; Fig. 1B). In addition, estrogen had no significant effect on the slope of the baroreflex sensitivity at either the lowest (1 × 10^{-4} mg/kg) or highest dose (1.0 mg/kg; P > 0.05; Fig. 1B) throughout the experimental time course. The remaining doses of estrogen significantly enhanced the slope of the baroreflex sensitivity at 30 min postestrogen injection (P < 0.05; Fig. 1B), and this effect was maintained for the duration of the experimental time course (P < 0.05; Fig. 1B). The slope of the baroreflex sensitivity plot for the dose at estrogen that produced the greatest effect (1 × 10^{-2} mg/kg) was 1.75 ± 0.06 at 30 min postinjection. The baroreflex sensitivity remained significantly enhanced at these four doses of estrogen for 5 h postinjection (Fig. 1B).

Estrogen-induced effects are blocked by ICI-182,780. The estrogen receptor antagonist ICI-182,780 was used to confirm that the estrogen-induced effects were in fact mediated via an estrogen receptor. When ICI-182,780 (5 mg/kg) was injected 10 min before either estrogen or saline, the average increase in mean arterial pressure evoked by phenylephrine (0.1 mg/kg) was 34 ± 7 and 29 ± 9 mmHg, respectively (P > 0.05). The magnitudes of the reflex bradycardia (20 ± 6 and 22 ± 7 beats/min, respectively; P > 0.05; Fig. 2A) at 30 min postestrogen injection were not significantly different from each other or prestimulated values. The estrogen-induced enhancement of the reflex bradycardia had recovered 120 min post-ICI-182,780 injection.

The baroreflex sensitivities measured at 30 min after administration of either ICI-182,780 and saline (0.58 ± 0.05) or ICI-182,780 and estrogen (0.61 ± 0.06) were not significantly different from prestimulated values (30 min preinjection; 0.59 ± 0.05 and 0.60 ± 0.06, respectively; P > 0.05; Fig. 2B) or from values measured at 60 or 90 min (data not shown; P > 0.05) postestrogen injection. The antagonistic effect of ICI-182,780 on the estrogen-induced enhancement of the baroreflex sensitivity was no longer present 120 min postestrogen injection.
postestrogen injection (Fig. 2B). At this time, testing of the baroreflex with phenylephrine resulted in a significant enhancement of the baroreflex sensitivity (0.71 ± 0.05; P < 0.05) in response to estrogen injection (Fig. 2, A and B).

Estrogen blocks the decreased baroreflex sensitivity resulting from vagal stimulation. In all animals undergoing left cervical vagal stimulation (1 s on-2 s off cycle), both the change in mean arterial pressure (38 ± 6 mmHg) and heart rate (55 ± 11 beats/min), which were significantly lower than baseline values (P < 0.05), remained at these values for the duration of the stimulation. On termination of the vagal stimulation, mean arterial pressure and heart rate returned to prestimulated levels (97 ± 12 mmHg and 362 ± 11 beats/min, respectively; an average increase in mean arterial pressure of 22 ± 6 mmHg and heart rate of 60 ± 15 beats/min; P > 0.05) within 5 min. In control animals (n = 4), intravenous injection of saline (0.9%) at 10 min and immediately before termination of the vagal stimulation did not significantly alter arterial pressure or heart rate. However, testing of the baroreflex 30 min poststimulation resulted in an average increase in mean arterial pressure (81 ± 9 mmHg; Fig. 3, A and Ba) that was significantly (P < 0.05) greater than that observed before stimulation (40 ± 8 mmHg; Fig. 3, A and Ba). In contrast, the reflex bradycardia at 30 min after stimulation (25 ± 6; Fig. 3, A and Bb) in response to this enhanced pressor response was not significantly different from that observed before the stimulation (15.9 ± 7; P > 0.05; Fig. 3, A and Bb). As a result, the slope of the baroreflex sensitivity was significantly decreased (0.30 ± 0.01; P < 0.05; Fig. 3, Ca and Cb) compared with that measured before vagal stimulation (0.50 ± 0.05). The baroreflex sensitivity recovered to prestimulated values after 2 h (P < 0.05). These findings are consistent with those reported previously by Saleh and Connell (31).

In four animals, saline was injected 10 min before estrogen (1 × 10⁻² mg/kg), which was injected immediately before termination of the vagal stimulation. Testing of the baroreflex 30 min poststimulation produced an increase in mean arterial pressure (82 ± 10 mmHg; Fig. 4, A and Ba) that was not significantly different from that observed in vagal-stimulated, saline-injected controls (P < 0.05) but significantly greater than prestimulated values (P < 0.05). The reflex bradycardia (31 ± 6 beats/min) was however significantly enhanced compared with both prestimulated and saline-injected controls (P < 0.05; Fig. 4, A and Bb). Determination of the baroreflex slope indicated no significant change from that observed in these animals before vagal stimulation (0.58 ± 0.05; P > 0.05; Fig. 4, Ca and Cb). Both the enhanced pressor response and reflex bradycardia recovered to prestimulated values 2 h after the termination of the vagal stimulation (P > 0.05; Fig. 4, Ca and Cb).

In four animals, ICI-182,780 (5 mg/kg) was injected 10 min before estrogen (1 × 10⁻² mg/kg) administration, which was injected immediately before termination of the vagal stimulation. Testing of the baroreflex 30 min later resulted in a significant increase in mean arterial pressure of 20 ± 6 mmHg (P < 0.05) but no significant change from that observed in vagal-stimulated, saline-injected controls (P > 0.05; Fig. 4, A and Bb). Determination of the baroreflex slope indicated no significant change from that observed in these animals before vagal stimulation (0.41 ± 0.05; P > 0.05; Fig. 4, Ca and Cb).
arterial pressure of 70 ± 8 mmHg (P < 0.05; Fig. 5, A and Ba) accompanied by a reflex decrease in heart rate of 25 ± 5 beats/min (P > 0.05; Fig. 5, A and Bb). The baroreflex sensitivity plot at 30 min demonstrated a significant decrease in the slope of the regression line (0.31 ± 0.03) compared with the prestimulated value (P > 0.05; Fig. 5, Ca and Cb). Both the enhanced pressor response and the decreased baroreflex sensitivity recovered to prestimulated values 2 h after the termination of the vagal stimulation (P > 0.05; Fig. 5, Ca and Cb).

Finally, in four animals, ICI-182,780 (5 mg/kg) was injected 10 min before saline (0.9%) administration, which was injected immediately before termination of the vagal stimulation. The phenylephrine (0.1 mg/kg)-induced pressor response and the slope of the baroreflex sensitivity plot were significantly altered in an identical fashion (data not shown) to that observed after ICI-182,780 and estrogen injection at 30 min poststimulation (P < 0.05). Again, the pressor response and slope of the regression line returned to prestimulated values 2 h after the termination of the vagal stimulation (P > 0.05). ICI-182,780 did not affect the magnitude of the reflex bradycardia at any time point (P > 0.05).

**DISCUSSION**

Our results demonstrated that the intravenous injection of estrogen in male rats enhanced baroreflex sensitivity. This effect was caused by an increase in the magnitude of the reflex bradycardia in response to a phenylephrine-induced rise in blood pressure. This effect was most appreciated when estrogen injection...
was combined with cervical vagal stimulation. We have shown in previous reports (30, 31), as well as in the present investigation, that, after phasic stimulation of the vagus nerve for 2 h, an attenuated baroreflex sensitivity resulted. This attenuation was caused by an increase in the magnitude of the pressor response associated with phenylephrine injection. Under these conditions, the magnitude of the reflex bradycardia remained unchanged regardless of the magnitude of the pressor response or dose of phenylephrine injected. In the present investigation, animals were given estrogen before termination of the vagal stimulation. Subsequent testing of the baroreflex resulted in an enhanced pressor response (A and Ba) and reflex bradycardia (A and Bb) after vagal stimulation and intravenous estrogen (1 × 10⁻² mg/kg) injection. Average changes in MAP and HR to increasing doses of PE before, after, and during recovery from vagal stimulation and estrogen injection are shown (n = 4; Ba and Bb). These pressor (MAP) and bradycardia (HR) responses are plotted together on the graph of baroreflex function (Ca), and the slope of each regression line (before, after, and recovery) is plotted in the form of a histogram (Cb). *Significance from prestimulated (before) values (P < 0.05; ANCOVA).

Doses of estrogen between 1 × 10⁻⁴ and 1 mg/kg were effective in eliciting a significant increase in the magnitude of the reflex bradycardia and consequently baroreflex sensitivity. Doses of estrogen >1 × 10⁻² mg/kg produced smaller changes in baroreflex sensitivity compared with the optimal dose (1 × 10⁻² mg/kg), resulting in a bell-shaped dose-response graph. This finding was consistent with other research on the effects of estrogen at both low and high doses. For example, the beneficial effects of estrogen on the peripheral vasculature were attenuated when plasma estrogen levels were below or above a physiological range (4). Furthermore, therapeutically induced hyperestrogenemia, which may result when high doses of estrogen are administered in the
treatment of prostrate cancer in men (26, 39) or when oral contraception is used in women (22), has been suggested as a cardiovascular risk factor.

The results of this study are the first to demonstrate in male rats the effects of estrogen on the reflex bradycardia response to a transient increase in arterial pressure. Classically, changes in the magnitude of the reflex bradycardia evoked by phenylephrine have been used as an indication of changes in parasympathetic tone (27). Research on the effects of estrogen on parasympathetic tone has been done primarily in human female subjects or in female rats. These results have indicated that the plasma acetylcholine level was higher in females than in males (21) and was significantly reduced after ovariectomy (25). Furthermore, it was demonstrated that estrogen potentiated the activity of choline acetyltransferase (15). Muneta and colleagues (24) demonstrated that baroreflex sensitivity was higher in females than in males and was significantly reduced after ovariectomy but not castration. Finally, Du and colleagues (9) suggested that, in females, estrogen exerts cardioprotective and antifibrillatory effects via an augmentation of parasympathetic tone. These lines of evidence support a role for an estrogen-mediated enhancement of parasympathetic tone in females.

The effects of estrogen on sympathetic tone are inconsistent. In in vitro studies, estrogen used in supraphysiological concentrations was found to inhibit the activity of tyrosine hydroxylase, a rate-limiting enzyme in the synthesis of catecholamines (18). How-
ever, the inactive isomer 17α-estradiol as well as several glucocorticoids also produced these effects (19). Furthermore, evidence exists that estrogen increased the density and enhanced the function of presynaptic α2-adrenoceptors (14, 23). Experimental studies in the rat have demonstrated that the inhibition of norepinephrine secretion, mediated by presynaptic α2-adrenoceptors, was more potent in females than in males under both normal and pathophysiological conditions (8). This accounted for a reduction in the release of norepinephrine and a less pronounced sympathetic response to nerve stimulation as demonstrated in the isolated and perfused hearts of female rats (8). In contrast to these findings, we did not observe any significant effect of estrogen on the phenylephrine-induced pressor response after vagal stimulation or under normal conditions.

The cardiovascular responses observed 30 min after vagal stimulation and estrogen administration demonstrated that the enhanced baroreflex sensitivity was mediated by an increase in the magnitude of the reflex bradycardia (see Fig. 4, A and Bb). This would suggest that estrogen increased parasympathetic tone. However, because neither plasma catecholamine levels nor sympathetic nerve activity was measured in these animals, we could not rule out the possibility of an estrogen-induced inhibition of sympathetic tone. This seemed unlikely, however, given the consistency of the phenylephrine-induced pressor response before and after vagal stimulation (Fig. 4, A and Ba). Also, preliminary results from our laboratory indicate that intravenously injected estrogen did not significantly effect baseline sympathetic tone over a period of 1 h, as measured by renal nerve recording (unpublished observations).

Subsequent experiments in our laboratory may involve monitoring renal sympathetic nerve activity after combined estrogen injection and vagal stimulation.

The peripheral effects of estrogen have been well documented. Estrogen has been shown to have a vasodilatory effect on the vasculature (6). It is known that estrogen affected cholesterol metabolism and disposition, increased plasma levels of high-density lipoproteins (5), and inhibited peroxidation of lipoproteins (29). Estrogen also inhibited atherosclerotic plaque formation and the proliferation of smooth muscle cells in arterial walls (1, 28, 38). Estrogen stimulated vasodilation and suppressed the sympathetically mediated vasoconstrictor response of coronary arteries (6, 41). In our study, none of the drugs injected had any immediate or long-term effect on baseline blood pressure and heart rate. We cannot conclude from the results presented in the current study if estrogen was acting centrally to modulate autonomic tone or peripherally to produce the observed effects on the reflex bradycardia.

In conclusion, the results presented here support a role for estrogen in enhancing the baroreflex sensitivity in male rats by increasing the magnitude of the reflex bradycardia in response to a phenylephrine-induced increase in blood pressure. In addition, we have demonstrated that estrogen blocked the attenuation in the slope of the baroreflex sensitivity previously observed after 2 h of vagal stimulation (31). Experimental evidence now indicates that estrogen acts centrally to modulate parasympathetic tone (9) via estrogen receptors located in autonomic preganglionic nuclei (34, 36).

Previous experiments in our laboratory have provided evidence to support a role for the parabrachial nucleus of the pons in mediating the decrease in baroreflex sensitivity observed after vagal stimulation (31). Therefore, it is tempting to speculate that estrogen may be acting in the parabrachial nucleus to modulate autonomic output and, as a result, block the decrease in baroreflex sensitivity. Future experiments may investigate the role of estrogen in the modulation of autonomic function via central cardiovascular preganglionic nuclei in both the male and female rat.

Perspectives

Measurement of baroreflex sensitivity has already gained acceptance as a diagnostic tool for determining changes in the state of the autonomic nervous system after cardiovascular pathology. Evidence has been presented in the literature which strongly supports the hypothesis that if an individual’s baroreflex sensitivity is significantly depressed, his/her susceptibility to sudden death is increased (for review, see Ref. 3). Therefore, research into therapeutic treatments that can enhance baroreflex sensitivity, such as that demonstrated with estrogen in the present report, is of clinical relevance.

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