Estrogen in the Paraventricular Nucleus Attenuates L-Glutamate–Induced Increases in Mean Arterial Pressure Through Estrogen Receptor β and NO

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Abstract—Estrogen (E2) acts in the brain to decrease blood pressure (BP) responses to psychological stress. A likely site for the effects of E2 is the hypothalamic paraventricular nucleus (PVN), an important regulator of autonomic functions. We studied the effects of E2 in the PVN on BP and heart rate (HR) responses to L-glutamate injections into the PVN of male urethane-anesthetized rats. Microinjections of L-glutamate (50 nmol) into the PVN increased BP by 14±2.5 mm Hg and HR by 30±5.6 bpm. Microinjections of E2 (0.1, 1, and 10 pmol) into the PVN 30 minutes before L-glutamate dose-dependently attenuated the pressor response by 25%, 34%, and 59%, respectively, but did not affect HR. We determined that E2 receptor (ER) β mediates the effect of E2, because activation of ERβ with diarylpropionitrile (50 pmol) attenuated the response by 57%, whereas activation of ERα with propyl-pyrazole-triol (20 pmol) had no effect. Furthermore, inhibition of ERβ with R,R-tetrahydrochorysene (50 pmol) blocked the effect of E2, but inhibition of ERα with methyl-piperidino-pyrazole (1 nmol) did not. Finally, we found that the effect of E2 is mediated by NO, because the NO synthase (NOS) inhibitor, N⁵-nitro-L-arginine methyl ester (2 nmol), the neuronal NOS inhibitor, 7-nitroindazole sodium salt (0.1 pmol), and the endothelial NOS inhibitor, N⁵-(1-iminoethyl)-L-ornithine (200 pmol) blocked the effect of E2. The effect was partially blocked with the γ-aminobutyric acidα receptor inhibitor bicuculline. Our results demonstrate that E2 in the PVN attenuates the L-glutamate–induced pressor response and that this effect is mediated by ERβ, NO produced by neuronal NO synthase and eNOS, and partly by γ-aminobutyric acid. (Hypertension. 2006;48:1130-1136.)

Key Words: 17β-estradiol ■ blood pressure ■ estrogen receptor ■ nitric oxide synthase ■ autonomic nervous system

The increased incidence of cardiovascular disease in postmenopausal women has been attributed to the loss of estrogen (E2).¹,² and it was observed that hormone replacement therapy confers cardiovascular benefits in these women.¹–³ In 1993, the Women’s Health Initiative trial began recruiting >16 000 postmenopausal women to assess the benefits and risks of hormone replacement therapy on the incidence of heart disease, cancers, and fractures.⁴ Surprisingly, the trial was terminated in 2002 because of increased risks of breast cancer and stroke.⁵ Although the Women’s Health Initiative trial has been criticized for its design and conclusions,⁶,⁷ the trial illustrates the need to better understand the effects of E2 on the cardiovascular system and on general physiology to develop specific therapies that will contribute to cardiovascular health in women and men.

E2 provides cardiovascular benefits in response to stress. E2 decreases sympathetic activity⁶ and blood pressure (BP) responses to mental stress in postmenopausal women.⁵,⁸ We and others have shown that E2 attenuates BP and HR responses to restraint stress⁹,¹⁰ and cage switch¹⁰ in ovariectomized (OVX) rats. E2 in the brain also modulates cardiovascular function as microinjections of E2 into the parabrachial nucleus (PBN),¹¹ and the nucleus of the solitary tract¹² of male rats decrease resting BP and HR and increase baroreceptor sensitivity. These data demonstrate that E2 modulates cardiovascular responses to stress and that the brain is an important site of E2 action.

The PVN of the hypothalamus regulates neuroendocrine and autonomic functions.¹³ E2 attenuates the numbers of activated neurons in the PVN of OVX rats induced by footshock¹⁴ and immobilization,¹⁵ and blocking endogenous E2 in the PVN decreases the amount of restraint stress-induced corticosterone release in cycling female rats.¹⁶ These data suggest that E2 acts in the PVN to affect homeostatic processes, but the effects of E2 in the PVN on cardiovascular function have not been investigated.

NO is an important regulator of sympathetic activity. NO in the PVN tonically inhibits sympathetic output to the periphery, and inhibition of NO release here increases sympathetic output.¹⁷–¹⁹ We have shown that NO in the brain mediates the effect of E2 on BP responses to restraint stress in OVX rats.⁹ Furthermore, NO levels increase in response to
restRAINT/immobilization stress in the hypothalamus and brain stem of E2-treated OVX rats and in the PVN of E2-treated male rats. E2 can act on NO in the PVN, because neurons here express neuronal NO synthase (nNOS) and E2 receptor β (ERβ). We have shown recently that, in the PVN of rat hypothalamic slice cultures, E2 alters the expression of nNOS and eNOS via ERβ. These results revealed a relationship between E2 and NO in the PVN, but the functional significance of this relationship is unknown. We hypothesize that E2 affects NO release in the PVN to modulate cardiovascular function.

γ-Aminobutyric acid (GABA) also plays an important role in regulating autonomic activity. In the PVN, GABA inhibits sympathetic output and mediates the inhibition of sympathetic output by NO. GABA has also been shown to mediate the effects of E2 on cardiovascular function in the PBN. These studies suggest that GABA may also mediate the cardiovascular effects of E2 in the PVN.

The brain is an important, yet often overlooked, target of the actions of E2 on cardiovascular function. Although the cardiovascular effects of E2 in a small number of brain stem autonomic nuclei have been studied, the effects of E2 in the PVN are unknown. The goals of this study were to investigate the acute effects of E2 microinjections into the PVN on resting BP and HR and on l-glutamate–induced increases in BP and HR. We also studied the roles of ERs in the effects of E2 using agents selective for ERα and ERβ. Finally, we investigated the roles of NO and GABA in the effects of E2 using agents selective for nNOS, eNOS, and the GABA_A receptor.

Methods

Animals

Male Sprague–Dawley rats (250 to 350 g) were purchased from the Biological Sciences Animal Center, University of Alberta. Rats were housed at 21°C in a 12:12-hour light–dark cycle and were fed ad libitum. All of the experimental procedures were approved by Health Sciences Laboratory Animal Services at the University of Alberta.

Pharmacological Agents

The pharmacological agents used in this study included the ER antagonist ICI 182,780 (Tocris), the ER agonists diarylpropionitrile (DPN) (Tocris) and propyl-pyrazole-triol (PPT) (Tocris), which are 70-fold more selective for β than α and 1000-fold more selective for α than β, respectively. The pure ERβ antagonist and methyl-piperidino-pyrazole (MP) (Tocris), the ER antagonist that is 200-fold more selective for α than β. Concentrations of these agents were chosen based on the relative effective potencies of each compound in relation to the concentration of E2 used, as demonstrated by dose–response experiments performed in human endometrial cancer (HEC-1) cells. Other pharmacological agents used included the NO inhibitor N-nitro-1-arginine methyl ester (L-NAME) (Sigma), the selective eNOS inhibitor N-(1-Iminoethyl)-L-ornithine (L-NIO) (Tocris), the selective NOS inhibitor 7-nitroindazole sodium salt (7-NiNa) (A.G. Scientific), and the GABA_A receptor antagonist bicuculline (Sigma). Effective concentrations of L-NAME, 7-NiNA, L-NIO, and bicuculline were chosen based on a previous microinjection study from this laboratory.

Drugs were freshly diluted in saline, except for MPP, which was diluted in water, from stock solutions prepared in ethanol or DMSO. The maximal amount of ethanol or DMSO present in solutions was 1%, except in the case of MPP, where 10% DMSO was present. Vehicle injections contained concentrations of ethanol or DMSO equal to those present in each drug-containing solution.

Surgical Procedures

Rats were anesthetized with intraperitoneal injections of urethane (1.75 g/kg, Sigma), and body temperature was maintained at 37°C with a heating pad. The left femoral artery was cannulated to measure mean arterial pressure (MAP) and HR as described previously. MAP and HR were recorded and allowed to stabilize for 20 to 30 minutes before starting each experiment. A guide cannula was then lowered into the PVN according to the coordinates 6.8 mm anterior, 0.1 mm lateral, and 2.2 mm ventral to the interaural 0, as described previously, and unilateral microinjections of solutions (100 µL) were made over 1 minute.

Experimental Protocols

Each animal was used in only 1 experiment and received a total of 2 microinjections into the PVN. The first injection varied with each experiment and is described below. MAP and HR were then measured for 30 minutes to determine the effects of each pharmacological agent alone on resting MAP and HR. Thirty minutes after the first injection, L-glutamate (50 nmol) was injected into the PVN. MAP and HR were measured for an additional 30 minutes to determine the effects of the pharmacological agent(s), delivered in the first injection, on the l-glutamate–induced increases in MAP and HR.

Effects of Vehicle and E2 on l-Glutamate–Induced Increases in MAP and HR

Animals received an injection of vehicle (saline or water; n=15) or E2 (0.1, 1, and 10 pmol; n=8 for each group) into the PVN.

Role of ERs in the Effect of E2 on the l-Glutamate–Induced Increase in MAP

Animals received an injection of one of the following: ICI 182,780 (10 pmol; n=6), DPN (5, 50, or 100 pmol; n=6 for each group), PPT (10 or 100 pmol; n=7 for each group), MPP (1000 pmol; n=8), or R,R-THC (50 pmol; n=5) into the PVN. Other animals received an injection of E2 (10 pmol) in combination with one of the following: ICI 182,780 (10 pmol; n=7), MPP (1000 pmol; n=8), or R,R-THC (5 and 50 pmol; n=5 for both groups).

Role of NO and the GABA_A Receptor in the Effect of E2 on the l-Glutamate–Induced Increase in MAP

Animals received an injection of one of the following: L-NAME (2000 pmol; n=7), 7-NiNa (0.1 pmol; n=7), L-NIO (200 pmol; n=9), or bicuculline (200 pmol; n=8) into the PVN. Other animals received an injection of E2 (10 pmol) in combination with one of the following: L-NAME (2000 pmol; n=6), 7-NiNa (0.1 pmol; n=6), L-NIO (200 pmol; n=8), or bicuculline (200 pmol; n=7).

Verification of Injection Sites

At the end of each experiment 1% Evan’s Blue (Sigma) was injected into the PVN. Brains were removed and fixed in ice-cold 4% paraformaldehyde for 2 days. Brains were frozen, and serial coronal sections (50 µm) were stained with neutral red (0.5%, Fisher Scientific). The locations of the injection site were determined with light microscopy, and only rats with injection sites located within the PVN were included in the data analysis.

Analyses and Statistics

Baseline MAP and HR were calculated by averaging values for 5 minutes before the first injection. Peak changes in MAP or HR were determined by subtracting baseline values from the peak value reached within 30 minutes after injection. The area under the curve (AUC) was determined to measure amplitude over time by calculating the area between baseline and each amplitude value for 20 minutes after injection. All of the data are presented as mean±SEM. Significant differences were determined by 1-way or 2-way ANOVA.
followed by the posthoc Tukey test and were considered significant when \( P < 0.05 \).

**Results**

Microinjection of E2 into the PVN Attenuates the L-Glutamate–Induced Pressor Response

Microinjections of vehicle or E2 (0.1 to 10 pmol, 100 nL) into the PVN had no effects on baseline MAP (83 ± 2.2 mm Hg) or HR (375 ± 5.0 bpm; data not shown). Microinjections of L-glutamate (50 nmol, 100 nL) 30 minutes after microinjection of saline significantly increased MAP and HR by 14 ± 2.5 mm Hg and 30 ± 5.6 bpm (Figure 1A, 1C, and 1E). L-Glutamate also increased the AUC for the MAP and HR responses (71 ± 33.6 mm Hg x S and 146 ± 67.6 bpm x S; Figure 1D and 1F). Microinjections of E2 (0.1, 1, and 10 pmol) into the PVN 30 minutes before L-glutamate dose-dependently attenuated the increases in MAP by up to 59% with 10 pmol E2. The AUC for the MAP response was also attenuated with 10 pmol E2 (from 71 ± 33.6 for saline to −39 ± 14.9 mm Hg x S; Figure 1B through 1D). E2 had no effect on the increases in HR or AUC for the HR response (Figure 1E and 1F). Figure 2A through 2C include schematic representations of serial coronal sections of the PVN showing the injection sites in 15 saline-treated and 10 E2 (10 pmol)-treated rats.

E2 Attenuates the L-Glutamate–Induced Pressor Response via ERβ

The ER antagonist ICI 182,780 (5 pmol) was coinjected with E2 into the PVN 30 minutes before L-glutamate and was

**Figure 1.** Representative MAP traces after injection of (A) saline or (B) E2 (10 pmol) into the PVN followed by L-glutamate (L-glu) 30 minutes later. Effect of L-glutamate injection into the PVN after E2 injection (0 to 10 pmol) on (C) the change in MAP, (D) AUC for the MAP response, (E) change in HR, and (F) AUC for the HR response. Data represent mean ± SEM. n = 8. *P < 0.05.
found to block the effects of E2 on MAP and AUC for the MAP response (Figure 3A and 3B). Microinjection of the ERα agonist PPT (10 and 20 pmol) before L-glutamate had no effect on the increases in MAP and the AUC for the MAP response (Figure 3C and 3D) or on the increases in HR and the AUC for the HR response (data not shown). When the ERα antagonist MPP (1000 pmol) was coinjected with E2 into the PVN, the effect of E2 on MAP or the AUC for the MAP response was not affected (Figure 3C and 3D). Microinjection of the ERβ agonist DPN (5, 50, and 100 pmol), before L-glutamate, attenuated the increases in MAP by up to 57% with 50 pmol DPN and attenuated the increases in AUC for the MAP responses (from 71 ± 33.6 for saline to −40 ± 27.0 mm Hg × S for 50 pmol DPN; Figure 3E and 3F). DPN had no effect on the increase in HR (data not shown). When the ERβ antagonist R,R-THC (5 and 50 pmol) was coinjected with E2, the effects of E2 on MAP and the AUC for the MAP response were blocked (Figure 3E and 3F). Microinjections of ICI 182,780 (5 pmol), DPN (100 pmol), PPT (20 pmol), R,R-THC (50 pmol), or MPP (1000 pmol) alone had no effects on baseline MAP and HR.

**NO Mediates the Attenuation of the L-Glutamate–Induced Pressor Response by E2**

The nonselective NOS inhibitor l-NAME (2000 pmol) was coinjected with E2 (10 pmol) into the PVN 30 minutes before L-glutamate and was found to block the effects of E2 on MAP and AUC for the MAP response (Figure 4A and 4B). The nNOS inhibitor 7-NiNa (0.1 pmol) and the eNOS inhibitor L-NIO (200 pmol) were then each coinjected with E2 (10 pmol); both blocked the attenuation of E2 of the increases in MAP and the AUC for the MAP response (Figure 4C through 4F). Microinjection of L-NAME (2000 pmol) alone had no affect on baseline HR but induced an increase in MAP (13 ± 3.6 mm Hg) that returned to baseline within 5 to 10 minutes. Microinjection of 7-NiNa (0.1 pmol) or l-NIO (200 pmol) alone had no effects on baseline MAP or HR.

**GABAA Receptors Are Involved in the Pathway by Which E2 Attenuates the l-Glutamate–Induced Pressor Response**

The GABAA receptor antagonist bicuculline (200 pmol) was coinjected with E2 into the PVN 30 minutes before L-glutamate and produced a strong trend to block the effect of E2 on the
increase in MAP (E2 alone; 5.75 ± 0.9 versus E2 plus bicuculline; 10.2 ± 1.5 mm Hg; \( P = 0.183 \)) and in the AUC for the MAP response (E2 alone; 38 ± 14.9 versus E2 plus bicuculline; 23 ± 21.3 mm Hg x S; \( P = 0.073 \); Figure 5A and 5B). Microinjection of bicuculline (200 pmol) alone induced increases in MAP (11 ± 2.3 mm Hg) and HR (28 ± 4.8 bpm) that returned to baseline within 15 to 20 minutes.

**Discussion**

In the present study, we investigated the effects of E2 in the PVN on resting MAP and HR and on L-glutamate–induced increases in MAP and HR. We show that E2 has no effect on resting MAP or HR but attenuates the L-glutamate–induced increase in MAP. Using pharmacological agents selective for ER\(\alpha\) and ER\(\beta\), we also determined that the effect of E2 is mediated by ER\(\beta\). Furthermore, we found that E2 acts on NO produced by nNOS and eNOS in the PVN to attenuate the L-glutamate–induced pressor response. Finally, we show that GABA partially mediates the effect of E2 in the PVN.

Our study is the first to investigate the effects of E2 in the PVN on MAP and HR and was carried out in male rats for several reasons. First, we wanted to avoid confounding effects of differing levels of circulating E2 found in cycling females. Second, the expression profiles of ER\(\alpha\) and ER\(\beta\) in the brain and, more specifically, in the PVN are identical in intact male and female rats.29 Third, previous investigations on the acute effects of E2 on cardiovascular function in other autonomic nuclei were performed in male rats.11,12 We found that E2 (0.1 to 10 pmol) has no effect on resting MAP or HR. Similarly, resting BP is unaffected by microinjections of E2 into the nucleus ambiguus13 and insular cortex,29 and resting HR is unaffected by microinjections into the rostral ventrolateral medulla (RVLM)12 and insular cortex.29 In contrast, microinjections of E2 into the PBN and the nucleus of the solitary tract decrease resting BP and HR.11,12 These data suggest that the effects of E2 on BP and HR in central autonomic nuclei are regionally specific. Because neurons in the PVN of anesthetized animals are quiescent30,31 and electrical lesions to the PVN do not affect resting BP and HR in conscious rats,32 we postulate that resting BP and HR are unchanged by E2 in the PVN, because these neurons are less active than neurons of brain stem centers in regulating acute changes in BP during rest.

We tested our hypothesis that E2 modulates BP and HR responses to excitatory stimulation of the PVN in anesthetized rats by investigating the effects of E2 in the PVN on the L-glutamate–induced increases in MAP and HR. Microinjections of L-glutamate (50 nmol, 100 nL) into the PVN increased MAP, HR, and the AUC for the MAP response (E2 alone; \(-38±14.9\) versus E2 plus bicuculline; \(23±21.3\) mmHg x S; \( P = 0.073 \); Figure 5A and 5B). Microinjection of bicuculline (200 pmol) alone induced increases in MAP (11±2.3 mm Hg) and HR (28±4.8 bpm) that returned to baseline within 15 to 20 minutes.

**Figure 4.** Role of NO in E2’s attenuation of the L-glutamate–induced increase in MAP (A) and AUC for the MAP response (B) using the nonselective NOS inhibitor L-NAME. Role of nNOS on MAP and AUC for the MAP response (C and D) using the selective nNOS inhibitor 7-NiNa. Role of eNOS on MAP and AUC for the MAP response (E and F) using the selective eNOS inhibitor L-NIO. All concentrations are picomoles. Data represented as mean±SEM. n=6. *\( P < 0.05 \).

**Figure 5.** Role of GABA in E2’s attenuation of the L-glutamate–induced increase in MAP (A) and AUC for the MAP response (B) using the selective GABA\(\alpha\) receptor antagonist bicuculline (Bic). All concentrations are picomoles. Data represented as mean±SEM. n=6. *\( P < 0.05 \).
where E2 supplementation attenuated BP but not HR responses. Because the effect of E2 on MAP was observed only after the PVN was excited by L-glutamate, our results suggest that E2 acts in the PVN to restore cardiovascular homeostasis in response to perturbations. In support of our findings, others have found that inhibition of PVN neurons with baroreceptor inputs was observed only when these neurons were excited with excitatory amino acids. To confirm our hypothesis, however, it will be important to perform these experiments in conscious animals.

We show that E2 injected into the PVN 30 minutes before L-glutamate attenuates the pressor response. The rapidity of the effect of E2 indicates that it is likely mediated by a nongenomic signaling mechanism, further supporting the idea that E2 acts in the PVN to quickly restore cardiovascular homeostasis in response to perturbations. Because rapid nongenomic actions of E2 can be ER dependent or ER independent, we investigated the role of ERs in the effect of E2. We confirmed that the effect of E2 is mediated by ERs in the PVN, because the nonselective ER antagonist ICI 182,780 inhibited the attenuation of the pressor response by E2. Using the ERβ agonist DPN and the ERβ antagonist R,R-THC, we further determined that activation of ERβ is required for E2 to attenuate the L-glutamate–induced pressor response. We also show that ERα is not involved in the effect of E2, because the ERs agonist, PPT, and the ERα antagonist, MPP, had no effects on the pressor response. Interestingly, we found that 100 pmol of DPN was less effective at attenuating the L-glutamate–induced effects than 50 pmol. This type of biphasic response has been described previously for E2, including the regulation of nNOS activity in cerebellar tissue.

We have shown previously that activation of ERβ alters eNOS and nNOS expression in the PVN of rat hypothalamic slice cultures. Activation of ERβ in the PVN also attenuates restraint stress-induced increases in corticotropin, corticosterone plasma levels, and c-FOS expression in the PVN of gonadectomized male rats. These studies show that activation of ERβ in the PVN mediates genomic and nongenomic effects. Our study is the first to demonstrate that activation of ERβ in the PVN plays an important role in the rapid modulation of BP responses to excitatory stimulation. Furthermore, neuronal projections from the PVN to the RVLM, 50% of which express ERβ, centrally regulate autonomic function, suggesting that this is a likely pathway through which ERβ activation in the PVN modulates cardiovascular function.

Because NO plays an inhibitory role on sympathetic output in the PVN and we have shown that E2 alters NOS expression in the PVN, we hypothesized that attenuation of E2 of the L-glutamate–induced pressor response is mediated by NO. Indeed, we found that inhibition of NO with the nonselective NOS inhibitor L-NAME blocked the effects of E2. We further show, using selective NOS inhibitors, that NO produced by eNOS and nNOS mediates the effects of E2. Similarly, our laboratory has shown that NO produced by eNOS and nNOS in the PVN mediates adrenomedullin–induced decreases in BP. These current findings further support the hypothesis from our recent in vitro study that E2 produced by eNOS in blood vessels within the PVN can act on neighboring neurons to influence autonomic pathways.

GABA in the PVN inhibits sympathetic output and mediates NO-induced inhibition of sympathetic activity. Therefore, we investigated the role of the GABAA receptor in the attenuation of the pressor response by E2. Using the GABAA receptor antagonist, bicuculline, we found that GABAA receptors are involved in mediating the effect of E2. Together with our NOS inhibitor data, our results suggest that E2 acts in the PVN to attenuate the pressor response through the actions of NO, some of which are likely mediated by GABA in the PVN.

**Perspectives**

In this study we have shown that E2 in the PVN of male rats rapidly attenuates the L-glutamate–induced pressor response by activating ERβ to recruit NO and GABA. Together with our previous study, which demonstrated that E2 acts on NO in the brain to attenuate BP responses to psychological stress in OVX rats, these findings lead us to hypothesize that E2 mediates its beneficial cardiovascular effects by acting within the PVN to restrain BP responses to stimuli that increase arterial pressure. These results demonstrate that the brain is an important target for the effects of E2 on cardiovascular function and contribute to our understanding of how E2 provides protection against cardiovascular disease.

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**Disclosures**

None.

**References**


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