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Endothelial and neuronal nitric oxide synthases variably modulate the estrogen-mediated control of blood pressure and cardiovascular autonomic control

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Summary

We have previously shown that long-term estrogen (E₂) replacement lowers blood pressure (BP) and improves the cardiovascular autonomic control in ovariectomized (OVX) rats. In this study, we investigated whether constitutive and/or inducible nitric oxide synthase (NOS) modulate these E₂ effects. We evaluated changes in BP, myocardial contractility index (dP/dt_{max}), and power spectral indices of hemodynamic variability following selective inhibition of eNOS [N⁵-(1-iminoethyl)-L-ornithine; L-NIO], nNOS (N^ω-propyl-L-arginine; NPLA), or iNOS (1400W) in telemetered OVX rats treated for 16 weeks with (OVXE₂) or without (control, OVXC) E₂. OVXE₂ rats exhibited: (i) reduced BP, and increased dP/dt_{max}, (ii) cardiac parasympathetic dominance as reflected by the reduced low-frequency (LF, 0.25–0.75 Hz)/high-frequency (HF, 0.75–3 Hz) ratio of interbeat intervals (IBI_{LF/HF}), and (iii) reduced LF oscillations of systolic BP, suggesting a reduced vasomotor sympathetic tone. eNOS inhibition (L-NIO, 20 mg/kg i.p.) elicited a shorter-lived pressor response in OVXE₂, than in OVXC, rats along with reductions in dP/dt_{max} and increases in the spectral index of spontaneous baroreflex sensitivity (index α). NPLA (1 mg/kg i.p.) reduced BP and increased IBI_{LF/HF} ratio in OVXE₂, but not OVXC rats. The iNOS inhibitor 1400W (5 mg/kg i.p.) caused no hemodynamic changes in OVXC or OVXE₂ rats. Overall, constitutive NOS isoforms exert restraining tonic modulatory BP effects, which encompass eNOS-mediated reduction and nNOS-mediated elevation in BP in OVXE₂ rats. Baroreflex facilitation, and dP/dt_{max} reductions might account for the shorter pressor action of L-NIO in E₂-treated, compared with untreated, OVX rats.

Keywords

Blood pressure; cardiac autonomic control; spontaneous baroreflex sensitivity; nitric oxide synthases; ovariectomy; estrogen

Introduction

Abnormalities in the autonomic control of cardiovascular functions and heart rate variability (HRV) contribute to cardiovascular morbidity and mortality (1,2). Current evidence suggests a causal role for improved autonomic activity in the cardiovascular protective effect of estrogen. For example, central autonomic modulation has been implicated in the estrogen-evoked facilitation of the arterial baroreflex function (3–5) and HRV (6). The favorable

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effect of female gonadal hormones on HRV is further supported by the observation that premenopausal women exhibit higher HRV than postmenopausal women or middle-aged men (7,8). Clinical studies demonstrate opposite effects for estrogen on sympathetic (inhibition) and parasympathetic activity (enhancement), which contribute to the favorable effect of the hormone on autonomic function (6,9). In a previous study, we showed that the detrimental effects of long-term OVX on cardiac autonomic and baroreflex control, compared with sham-operated (SO) rat values, are circumvented by estrogen supplementation (10). Importantly, the concept that estrogen favorably modulates cardiac functions has been challenged because hormone replacement therapy failed to modify (11) or worsened the HRV (12) profile. Such discrepancies might relate to differences in the hormonal regimens (preparation, dose and route of administration) and average age of the population enrolled in these studies.

The NOS/NO signaling influences the cardiovascular autonomic control and its modulation by ovarian hormones. For example, Heaton et al. (13) showed that NO facilitates the cardiomotor vagal activity via interaction with the pre-/post-ganglionic junction. Also, nNOS-derived NO inhibits the heart rate response to sympathetic nerve stimulation (14). Paradoxically, a sympathoexcitatory effect for the nNOS-derived NO develops in some pathological conditions such as heart failure (15). NO plays a contributory role in the estrogen-evoked parasympathetically-mediated vasodilatation in the submandibular glands of female rats (16). In OVX rats, the reduced NO release from the skeletal muscle, caused by estrogen deficiency, contributes to augmented sympathetically-mediated vasoconstriction during muscle contraction (17). Apart from its interaction with estrogen, NO derived from the myocardial PI3K/Akt/eNOS and iNOS pathway also facilitates the increased cardiac parasympathetic dominance and hypotension caused by ethanol in female rats (18).

The current study is a follow-up to our previous report (10), in which we documented the ability of estrogen to lower BP and to circumvent the detrimental changes in the cardiac autonomic control caused by the depletion of ovarian hormones. Here, pharmacological studies were undertaken to evaluate the roles of constitutive and inducible NOS in the favorable cardiovascular and autonomic effects of estrogen. Telemetered OVX rats treated with E₂ (OVXE₂) for 16 weeks, and their untreated controls (OVXC), as described in our previous study (10), were utilized to investigate the effect of selective inhibition of constitutive or inducible NOS on BP, dP/dt_{max}, and spectral indices of hemodynamic variability and baroreflex activity.

Results

Hemodynamic and autonomic effects caused by estrogen replacement

The hemodynamic and autonomic parameters obtained at the conclusion of the 16-week period that followed OVX and hormone replacement are shown in Table 1. Compared with pair-fed OVXC rats, OVXE₂ rats exhibited reduced BP, IBI_{LF/HF} ratio, and SBP_{LF} (Table 1). On the other hand, +dP/dt_{max} was increased in OVXE₂ compared with OVXC rats (Table 1). No differences in HR or spontaneous baroreflex activity (index α) were observed (Table 1). The plasma estrogen levels measured in OVXE₂ and OVXC rats, at the end of the study (week 16), were 58±4 and 4±1 pg/ml, respectively (P<0.05).

Hemodynamic effects of selective NOS inhibitors in OVXE₂ and OVXC rats

Figures 1 and 2 illustrate the effect of selective inhibition of eNOS, nNOS, or iNOS on MAP, HR and dP/dt_{max} responses in OVXE₂ and OVX rats. Compared with the corresponding saline values, the eNOS inhibitor L-NIO (20 mg/kg; i.p.) caused significant (P<0.05) increases in BP in OVXC and OVXE₂ rats (Fig 2A). The peak pressor effect of L-

NIO was observed at approximately 40 min, and was of similar magnitude (30 ± 3 vs. 30 ± 4 mmHg) in the OVXE₂ and OVXC groups (Fig. 1A). However, the pressor effect of L-NIO in OVXE₂ rats lasted for 80 min after which the MAP returned to levels similar to those caused by saline injection (Fig. 1A). By contrast, following a slight decline after 80 min, the pressor effect of L-NIO in OVXC rats remained significantly ($P < 0.05$) higher than corresponding values in the L-NIO treated OVXE₂ rats throughout the 5-hr observation period (Fig. 1A). In the two groups of rats, L-NIO caused similar, short-lived decreases in HR (Fig. 1B). On the other hand, the inhibition of nNOS with NPLA (1 mg/kg) caused significant decreases in MAP in OVXE₂ rats in contrast to no effect in OVXC rats (Fig. 1C). No changes in MAP were caused by the iNOS inhibitor 1400W (5 mg/kg, Fig. 1E) in either rat group. $dP/dt_{\max}$ was decreased by L-NIO in OVXE₂, but not in OVXC, rats (Fig. 2A). NPLA (Fig. 2B) or 1400W (Fig. 2C) produced no changes in $dP/dt_{\max}$ in the two rat groups.

Cardiovascular autonomic effects of selective NOS inhibitors in OVX and OVXE2 rats

Changes in spectral indices of the cardiovascular autonomic control caused by the selective NOS inhibitors in OVXC and OVXE₂ rats are shown in figures 3–5. Compared with saline, eNOS inhibition (L-NIO) had no effect on the $IBI_{LF/HF}$ ratio (sympathovagal balance index) or SBP_{LF} (vasomotor sympathetic activity index) in OVXE₂ rats (Figs. 3A, 4A). However, in OVXC rats, L-NIO significantly ($P < 0.05$) reduced both parameters during the first 2 hr of the study (Figs. 3A, 4A). nNOS inhibition (NPLA) increased $IBI_{LF/HF}$ in OVXE₂, but not in OVXC, rats (Fig. 3B), and had no effect on SBP_{LF} in either rat group (Fig. 4B). The spectral indices of spontaneous baroreflex sensitivity, $HF\alpha$ (Fig. 5A) and $LF\alpha$ (Fig. 5B), were increased by L-NIO in OVXC and OVXE₂ rats, but the duration of the increases was substantially longer in OVXE₂ rats (~ 4 vs. ~ 1 hr) (Fig. 5A, B). Further, the spontaneous baroreflex sensitivity in OVXC or OVXE₂ rats remained unaltered after treatment with NPLA (Fig. 5C, D) or the iNOS inhibitor 1400W (Fig. 5E, F).

Materials and Methods

Female Sprague-Dawley rats (9–10 weeks; 190–225 g) were purchased from Harlan Farms (Indianapolis, IN). Rats were housed individually and allowed free access to water and Purina chow and were maintained on a 12-12-h light-dark cycle with light off at 4:00 p.m. and room temperature maintained at $22 \pm 1^\circ\text{C}$. The methods and experimental protocols were approved by the institutional animal care and use committee and carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Telemetry transmitter implantation and ovariectomy

Rats were anesthetized with a mixture of ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.). The method used for implantation of the telemetry transmitter (Data Sciences Int., St. Paul, MN) was detailed in our previous studies (10,19). Bilateral OVX was performed at the same time of telemetry transmitter implantation as in our previous studies (20,21). The ovaries were isolated, tied off with sterile suture and removed.

Estrogen replacement

Subcutaneous silicone tubing (10 mm length, 1.57 mm inner diameter $\times$ 3.18 mm outer diameter, Silastic®, Dow Corning) filled with approximately 25 mg of 17β -estradiol-3-benzoate [1,3,5(10)-estratriene-3,17 β -diol-3-benzoate] were used as described elsewhere (10). For control OVX rats, empty silicone tubings were used. Tubings were sealed with medical adhesive type A (Silastic®, Dow Corning), gas sterilized, and implanted subcutaneously at the back of the neck in rats anesthetized with isoflurane. The tubings were implanted 3 weeks after transmitter implantation and OVX operation.

Measurement of plasma estrogen

The radioimmunoassay assay (Diagnostic Systems Laboratories, Inc., Webster, TX), employed in previous studies (10), was used for the measurement of plasma estrogen.

Hemodynamic effects of selective inhibitors of constitutive or inducible NOS

Rats were fed a standard Lieber-DiCarli high protein liquid diet (Dyets Inc., Bethlehem, PA) for one week before implantation of the telemetry device. The diet was provided daily to rats 30 min prior to the start of the dark cycle. Two groups of rats (OVXC, $n=6$; OVXE₂, $n=7$) were pair-fed to allow similar nutrient and fluid consumption as in previous studies (20,21). Fresh diets were prepared every other day, and stored in the refrigerator until dispensed. Rats were maintained on liquid diet for 16 weeks. At the conclusion of 16-week period described in our previous study (10), each rat in the OVXC or OVXE₂ group received 4 different i.p. injections at 3-day intervals: (i) saline (1 ml/kg), (ii) the eNOS inhibitor L-NIO (20 mg/kg), (iii) the nNOS inhibitor NPLA (1 mg/kg), or (iv) the iNOS inhibitor 1400W (5 mg/kg). All injections were made at 9:00 am, and hemodynamic monitoring continued for 5 hr. The chosen doses of the NOS inhibitors were based on published reports (22–24). At the conclusion of the study, blood samples were collected from the rats for the measurement of plasma estrogen. The collected blood was centrifuged at 5000 rpm for 5 min and plasma was aspirated and stored at -80°C till analyzed.

Data acquisition and analysis

Individual rat cages were placed on the tops of the radio receivers, and data were acquired by data acquisition system (Dataquest A.R.T. 4.0, Data Sciences Int.). BP waveforms were sampled at a rate of 1000 Hz for 20 sec every 10 min. Changes in hemodynamic parameters from baseline values evoked by various drug treatments in pair-fed OVX and OVXE₂ rats were averaged in 20-min blocks (i.e. the average of 2 successive measurements) for analysis as in our previous studies (19,25). Baseline hemodynamic values were taken as the average of the 40-min period that preceded saline or drug administration. The interbeat interval (IBI) was calculated from BP waveforms. The maximum rate of rise of BP waveform was computed by Data Sciences software and served as an estimate of left ventricular contractility (dP/dt_{max}) (10,18).

Spectral analysis of hemodynamic variability

Changes in sympathetic and vagal activities were determined by the frequency domain analysis of IBI and SBP data series as reported (25–27). The Data Sciences software (Dataquest A.R.T. 4.0) employs the periodogram function of the rectangular window for direct transformation of data points into power spectral density graphs. The Data were interpolated to obtain equally spaced samples with an effective sampling frequency of 10 Hz (0.1 s). A second-order quadratic was employed to fit a smooth curve to the existing data points and produce a smoother visual representation of data. Spectra were integrated into 2 specific frequency bands, LF (0.25–0.75 Hz) and HF (0.75–3 Hz) bands. The HF bands reflect the cardiac parasympathetic component while the LF bands reflect the sympathetic component. Therefore, the computed ratio of LF/HF ($IBI_{\text{LF/HF}}$) reflects the sympathovagal balance of the heart (25,26). The LF spectral band of systolic BP (SBP_{LF}) was also measured as index of the vasomotor sympathetic tone (25,26). The IBI and SBP spectral data were also used for the computation of spontaneous BRS (index α). The latter is defined as the square root of the ratio of IBI and SBP powers at LF ($LF\alpha$) and HF ($HF\alpha$) as in our previous studies (10,25). Parameters of hemodynamic variability were averaged every 60 min.

Drugs

N^ω-propyl-L-arginine (Tocris Bioscience, Ellisville, MO), N⁵-(1-iminoethyl)-L-ornithine (Biotium Inc., Hayward, CA), 17β-estradiol, 1400W (Sigma Chemical Co., St. Louis, MO), Ketaject (ketamine), Xylaject (xylazine) (Phoenix Pharmaceuticals Inc., St Joseph, MI), Toradol (ketorolac tromethamine, Abbott Labs, Chicago, IL), and Durapen (Penicillin G benzathine and penicillin G procaine, Vedco Inc., Overland Park, KS) were purchased from commercial vendors.

Statistical analysis

All values are expressed as means±S.E.M. The mean arterial pressure (MAP) was calculated as the diastolic pressure + 1/3 pulse pressure (systolic-diastolic pressures). The unpaired Student's t-test was used to compare baseline hemodynamic data in OVXC and OVXE₂ groups. The repeated measures two-way analysis of variance (ANOVA) followed by a Newman-Keuls post-hoc test was used to analyze the effects of NOS inhibitors on hemodynamics.

Discussion

In a recent study, we reported that long-term estrogen replacement in telemetered OVX rats, a model of surgical menopause (28), lowers BP and favorably impacts the cardiovascular autonomic control (10). The present pharmacological study employed the same model system to identify the roles of constitutive and inducible NOS in the BP and autonomic effects of estrogen. The most important findings of this study are: (i) the inhibition of eNOS (L-NIO) and nNOS (NPLA) increased and decreased BP, respectively, suggesting counterbalancing tonic effects of the two constitutive NOS isoforms on the BP regulating mechanisms whereby eNOS promotes reduction while nNOS promotes elevation in BP in OVXE₂ rats. (ii) L-NIO produced a shorter-lived pressor response in OVXE₂, than in OVXC, rats due probably to the concomitant facilitation of baroreflex activity, and the reductions in myocardial contractility. (iii) Spectral analysis also suggests that the longer-lasting pressor effect of L-NIO in OVXC rats elicited feedback reduction in vasomotor sympathetic activity, and facilitated cardiac vagal dominance.

In agreement with our previous report (10), the present study highlights a key role for the cardiovascular autonomic control in the modest hypotensive response caused by long-term E₂ replacement in OVX rats. This is because the frequency domain analysis of hemodynamic variability showed that OVXE₂ rats exhibited reduced $IBI_{LF/HF}$ ratio and SBP_{LF} , which suggest cardiac vagal dominance and vasomotor sympathoinhibition, respectively. Evidence suggests an important role for NOS isoforms in the cardiovascular actions of E₂. For example, E₂ increases the expression/activity of eNOS, and decreases the expression of caveolin-1, an allosteric inhibitor of NOS activity, in some central sites (29,30). The upregulation of brain eNOS activity has been shown to reduce BP and renal sympathetic nerve activity (31). Unlike eNOS, contradictory reports are available concerning the effect of E₂ on central nNOS expression (29,32) whose activation is believed to cause hypertension and renal sympathoexcitation (33). In the latter study, selective NOS inhibition implicated nNOS, but not eNOS or iNOS, in the NO-evoked increases in BP and renal sympathetic activity. In addition to its central role, peripheral NOS also plays fundamental role in cardiovascular and autonomic control (13,14,18) as well as in the estrogen-dependent biological effects (16,17).

With that said, the current study sought pharmacological evidence to implicate constitutive and/or inducible NOS in the favorable cardiovascular and autonomic effects of E₂ in a model of surgical menopause. The elevation in BP mediated by eNOS inhibition (L-NIO) is

consistent with the role of NO in the tonic reduction of BP. Similar pressor effects were demonstrated in reported studies after nonselective NOS inhibition (34,35) or selective eNOS inhibition (36,37). Our finding, however, that L-NIO produced a relatively longer-lasting pressor effect in OVXC (5 hr) compared with OVXE₂ rats (80 min) was interesting. This may be explained by at least two observations of the current study. First, the reductions in the myocardial contractility index $dP/dt_{\max}$, which positively correlates with stroke volume and cardiac output (38), seen in L-NIO-treated OVXE₂ is a potential contributor to the diminished magnitude and duration of the pressor response in these rats. Second, spectral analysis of hemodynamic variability revealed that L-NIO produced a prolonged facilitation of the spontaneous baroreflex responsiveness (index α) in OVXE₂ rats in contrast to a short-lived effect in OVXC rats (Fig. 5A, B). Notably, the arterial baroreflex system is an important homeostatic mechanism for hemodynamic stabilization and BP control (39). Recent studies have shown that acute or chronic stimulation of baroreceptor afferents causes prolonged reductions in BP (40,41). It is tempting to speculate that the baroreflex facilitation seen in L-NIO-treated OVXE₂ rats might constitute a counter-regulatory mechanism that acted to offset the concomitant elevation in BP. This view might be supported by the observation that the pressor response elicited by NOS inhibition is abolished by baroreceptor and vagal denervation (42). This interesting possibility will be investigated in future studies via studying the cardiovascular responses to NOS inhibition in OVXE₂ rats after pharmacologic (ganglionic autonomic or vagal blockade) or surgical (sinoaortic denervation) interruption of the baroreflex pathway.

Despite the potential roles of cardiovascular autonomic control, and the reduced myocardial contractility index in the differential BP response to L-NIO in OVXC and OVXE₂ rats, it is important to elaborate more on the discrepancy in the duration of the pressor effect caused by eNOS inhibition (L-NIO) in OVXC and OVXE₂ rats. Because evidence from our laboratory and others (43,44) suggests that estrogen replacement in OVX rats positively correlates with eNOS protein expression and activity, we anticipated that pharmacologic eNOS inhibition would elicit greater pressor response in OVXE₂ than in OVX rats. It is conceivable, however, to propose that the E₂ enhancement of NOS expression/activity might have limited the eNOS inhibitory capacity of L-NIO. It is known that pharmacologic NOS inhibition is achieved via competition of the NOS inhibitor with L-arginine for a common site on the NOS molecule (45,46). Therefore, it is possible that the selected L-NIO dose, which adequately inhibited eNOS in reported studies (23), might not be sufficient to fully inhibit the upregulated eNOS in the OVXE₂ rats, and resulted in the lesser and shorter-lived pressor response in these rats. It remains to be determined if a higher dose of L-NIO would elicit greater pressor responses in OVXE₂ rats.

In sharp contrast with the effect of L-NIO, nNOS inhibition (NPLA) significantly reduced BP in OVXE₂ rats (Fig. 1C). The estrogen-dependence of the hypotensive effect of NPLA is confirmed by the inability of NPLA to alter BP in OVXC rats (Fig. 1C). Since estrogen replacement per se caused modest reduction in BP in OVXE₂, compared with OVXC, rats, the ability of NPLA to uncover additional decreases in BP in these rats may suggest that the tonic pressor activity of nNOS hinders the development of the full BP lowering effect of estrogen. Importantly, the hypotensive action of NPLA in OVXE₂ rats cannot be accounted for by changes in myocardial contractility, cardiovascular autonomic control or baroreflex activity because none of these functions was altered in NPLA-treated OVXE₂ rats. The only obvious autonomic effect for nNOS inhibition (NPLA) was the remarkable and sustained increase in the $IBI_{LF/HF}$ ratio, the spectral index of the cardiac sympathovagal balance (Fig. 3A), which implies an increased sympathetic dominance of the heart. Evidently, this cardiac sympathoexcitatory effect would constitute a baroreflex-dependent consequence rather than the cause of the associated NPLA-evoked hypotension in OVXE₂ rats. Importantly, these

novel findings support the sympathoexcitatory role for nNOS observed in other model systems in reported studies (15).

The mechanism by which nNOS inhibition increases the cardiac sympathetic activity is not clear. As discussed above, it is highly likely that this effect was a baroreflex response to the reduction in BP rather than due to nNOS inhibition. In fact, nNOS regulates heart function in health and in disease states. For example, the nNOS-derived NO delays the development of heart failure after myocardial infarction via suppressing xanthine oxidoreductase activity and associated oxidative stress (47). The latter is responsible for β -adrenergic hyporesponsiveness, inhibition of cardiac excitation-contraction coupling, depressed responsiveness to activator calcium, and hypertrophy of cardiomyocytes in infarcted nNOS knockout mice (48–50). Further, nNOS-derived NO increases sympathetic activity, and BP via interaction with arterial baroreceptors or other central pathways (51). Obviously, the inhibition of nNOS (NPLA) would be expected to produce the opposite effects. Therefore, additional studies are needed to explain the cardiovascular and autonomic effects of NPLA observed in OVXE₂ rats in this study.

In conclusion, the present study elucidated the roles of NOS isoforms in the BP and autonomic effects of estrogen replacement in OVX rats. Because L-NIO increased and NPLA decreased BP in OVXE₂ rats, our data suggest that the constitutive NOS isoforms exert contrasting tonic modulatory effects on BP, which encompass eNOS-mediated reduction and nNOS-mediated elevation in BP in OVXE₂ rats. The facilitation of arterial baroreflexes and inhibition of myocardial contractility might be responsible for the relatively shorter-lived pressor effect of L-NIO in OVXE₂ compared with OVXC rats. The inhibition of nNOS (NPLA) in OVXE₂ rats shifts the cardiac sympathovagal balance towards sympathetic dominance perhaps as a baroreflex response to the hypotensive response. Together, the findings suggest that the two constitutive NOS isoforms exert differential modulation of the hemodynamic and autonomic effects of estrogen in ovariectomized rats. Further, the findings yield new insight into the role of nNOS as a potential therapeutic target for lowering BP in hypertensive postmenopausal women who are receiving estrogen replacement therapy.

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Figure 1.

Changes in mean arterial pressure (MAP, left panels) or heart rate (HR, right panels) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg i.p., panels A and B), nNOS (NPLA, 1 mg/kg i.p., panels C and D), or iNOS (1400W, 5 mg/kg i.p., panels E and F) or equal volume of saline (0.9% NaCl) in telemetered ovariectomized (OVX), or estrogen-replaced OVX (OVXE2) rats. *P<0.05 vs. saline values; +P<0.05 vs. L-NIO (OVX) values.

Figure 2.

Changes in myocardial contractility index ($+dP/dt_{max}$) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg i.p., panel A), nNOS (NPLA, 1 mg/kg i.p., panel B), or iNOS (1400W, 5 mg/kg i.p., panel C) or equal volume of saline (0.9% NaCl) in telemetered ovariectomized (OVX), or estrogen-replaced OVX (OVXE2) rats. * $P < 0.05$ vs. saline values; + $P < 0.05$ vs. L-NIO (OVX) values.

Figure 3.

Changes in the LF/HF ratio of IBI evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg i.p., panel A), nNOS (NPLA, 1 mg/kg i.p., panel B), or iNOS (1400W, 5 mg/kg i.p., panel C) or equal volume of saline (0.9% NaCl) in telemetered ovariectomized (OVX), or estrogen-replaced OVX (OVXE2) rats. * $P < 0.05$ vs. saline values; + $P < 0.05$ vs. L-NIO (OVX) values.

Figure 4.

Changes in low-frequency SBP spectral density (SBP-LF, 0.25–0.75 Hz) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg i.p., panel A), nNOS (NPLA, 1 mg/kg i.p., panel B), or iNOS (1400W, 5 mg/kg i.p., panel C) or equal volume of saline (0.9% NaCl) in telemetered ovariectomized (OVX), or estrogen-replaced OVX (OVXE2) rats. * $P < 0.05$ vs. saline values; + $P < 0.05$ vs. L-NIO (OVX) values.

Figure 5.

Changes in index α , the spectral index of spontaneous baroreflex gain, in the high-frequency (HF, left panels) and low-frequency (LF, right panels) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg i.p., panels A and B), nNOS (NPLA, 1 mg/kg i.p., panels C and D), or iNOS (1400W, 5 mg/kg i.p., panels E and F) or equal volume of saline (0.9% NaCl) in telemetered ovariectomized (OVX), or estrogen-replaced OVX (OVXE2) rats. * $P < 0.05$ vs. saline values; + $P < 0.05$ vs. L-NIO (OVX) values.

Table 1

Average baseline levels of mean arterial pressure (MAP), heart rate (HR), myocardial contractility ($dP/dt_{\max}$), and spectral powers of hemodynamic variability at low (0.25–0.75 Hz) and high (0.75–3 Hz) frequency ranges, and spontaneous baroreflex sensitivity (Index α).

Parameter	OVX	OVXE ₂
MAP (mmHg)	102.1±1.2	96.0±1.4 *
HR (beats/min)	352.5±2.8	358.5±7.2
$dP/dt_{\max}$ (mmHg/sec)	1456±79	1782±59 *
IBI _{LF/HF}	2.47±0.28	1.55±0.37 *
SBP-LF (mmHg ² /Hz)	0.16±0.01	0.11±0.01 *
Index α (HF, sec/mmHg)	1.75±0.11	1.40±0.18
Index α (LF, sec/mmHg)	0.65±0.06	0.68±0.11

Values are means±SEM of 6–7 observations. MAP, mean arterial pressure; HR, heart rate; IBI, interbeat interval; SBP, systolic blood pressure.

* P<0.05 vs. OVX values (unpaired Student's t-test).