Estrogen modulates the contribution of neuropeptide Y to baseline hindlimb blood flow control in female Sprague-Dawley rats

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Jackson DN, Ellis CG, Shoemaker JK. Estrogen modulates the contribution of neuropeptide Y to baseline hindlimb blood flow control in female Sprague-Dawley rats. Am J Physiol Regul Integr Comp Physiol 298: R1351–R1357, 2010. First published March 10, 2010; doi:10.1152/ajpregu.00420.2009.—The purpose of this study was to determine the role of estrogen in neuropeptide Y (NPY) and Y1 receptor (Y1R)-mediated vascular responses in female rats. Based on earlier work from our laboratory that female rats lacked an NPY contribution to hindlimb vascular conductance relative to males, we hypothesized that estrogen modulates Y1R-mediated hindlimb blood flow control. Thus it was expected that ovariectomy would: 1) increase skeletal muscle Y1R expression, 2) decrease skeletal muscle Y2 receptor (Y2R) expression, 3) decrease peptidase activity, and/or 4) increase overall skeletal muscle NPY concentration. Separate groups of control (CTL), ovariectomized (OVX), and OVX + 17β-estradiol replacement (OVX + E2; 21-day pellet) rats were studied. Animals were anesthetized and given localized hindlimb delivery of BIBP-3226 (Y1R antagonist), while femoral artery blood flow and blood pressure were recorded. Tissue samples from the white and red vastus lateralis muscle were extracted to examine Y1R and Y2R expression, peptidase activity, and NPY concentration. We found that Y1R blockade resulted in increased baseline hindlimb blood flow and vascular conductance in OVX rats, whereas no change was noted in CTL or OVX + E2 groups (P < 0.05). This enhanced functional effect in the OVX group aligned with greater skeletal muscle Y1R expression in white vastus muscle and a substantial increase in NPY concentration in both white and red vastus muscle compared with CTL and OVX + E2 groups. There was no change in Y2R expression or peptidase activity among the groups. These data support the hypothesis that estrogen blunts Y1R activation in the rat hindlimb through an effect on Y1R expression and NPY concentration.

neuropeptide Y; dipeptidyl peptide IV; amino peptidase P; BIBP-3226; gender; sex; vascular conductance; skeletal muscle

NEUROPEPTIDE Y (NPY) is a 36-amino-acid transmitter substance released from sympathetic nerves that modulates vascular tone and blood flow to organs and tissues throughout the body (27). This neurotransmitter affects acute changes in vascular contractile state through postjunctional Y1 receptors (Y1R). In addition, NPY can elicit chronic changes in vascular morphology that appear to be mediated by Y1, Y2 (Y2R), and Y5 receptors (43). Finally, the release of sympathetic neurotransmitters [i.e., NPY and norepinephrine (NE)] is inhibited by the activation of prejunctional Y2R (40).

Historically, NPY was thought to be released primarily under conditions associated with high sympathetic discharge or high stress (44) so that its effects on vascular control would be minimal under baseline conditions. However, using a bioassay model, we have presented evidence that endogenously released NPY contributes to hindlimb vascular conductance (VC) under baseline (i.e., resting) conditions in male (13–15) but not female (13, 14) rats. The lack of baseline NPY modulation of blood flow and VC in the female hindlimb was puzzling considering both males and females demonstrate similar measures of baseline blood flow and VC. However, in these studies, baseline hindlimb flow in females was modulated predominantly by α-adrenoreceptor (AR) activation, whereas hindlimb blood flow in male rats was responsive to the endogenous synergistic action of Y1R and AR coactivation.

Also, the lack of baseline NPY-mediated effects in females was associated with decreased vascular Y1R expression and blunted NPY concentration in skeletal muscle homogenates. Thus it was hypothesized that NPY bioavailability is limited in the hindlimb of female rats. To examine the mechanism of this sexual dimorphism, our subsequent studies confirmed that female animals demonstrate 1) augmented proteolytic processing of NPY [via dipeptidyl peptidase IV (DPPIV) and aminopeptidase P (APP)], 2) augmented autoinhibitory prejunctional NPY Y2R expression, and 3) Y2R activation (14); these three elements would limit NPY bioavailability.

The current study was designed to test the hypothesis that estrogen affects NPY bioavailability and hindlimb blood control due to Y1R in female Sprague Dawley rats. Our approach was to test the functional impact of endogenous Y1R activation through the localized receptor antagonist BIBP-3226, as well as determine tissue NPY concentration, Y1R and Y2R expression, and peptidase activity in the hindlimb of female rats that were intact (CTL), ovariectomized (OVX), or supplemented with estrogen after ovariectomy (OVX + E2). The hypothesis predicted that ovariectomy would expose a relevant contribution of endogenous Y1R activation to baseline blood flow and VC in female rats as a result of: 1) increased skeletal muscle Y1R expression, 2) decreased skeletal muscle Y2R expression, 3) decreased peptidase activity, and/or 4) increased overall skeletal muscle NPY concentration. Our results confirm that estrogen affects baseline endogenous hindlimb Y1R activation, largely through enhanced skeletal muscle NPY levels and, to a smaller extent, through Y1R expression. However, we report that augmented skeletal muscle NPY levels were not related to alterations in peptidase activity or Y2R expression.

METHODS

Animals

The Council on Animal Care at the University of Western Ontario approved the experimental protocol. In total, 43 adult female age-matched Sprague-Dawley rats were used, of which 29 were ordered
Experiments

Animals recovered for 1 h following surgery. Drugs were delivered to the left hindlimb with an intra-arterial cannula (see above). After recovery, a 10-μl vehicle (0.9% saline) infusion (160 μl) was carried out followed by 10 min recovery. Baseline data were recorded for 5 min followed by the infusion of 100 μg/kg BIBP-3226 (Sigma-Aldrich) to assess the endogenous Y1R contribution to baseline blood flow and VC. This dose of BIBP-3226 was chosen based on previous work that indicated 100 μg/kg BIBP-3226 will completely block Y1R in this preparation (13, 15).

Analog signals for blood flow, blood pressures, and core temperature were sampled at 100 Hz and stored online using a Powerlab data acquisition system (ADInstruments, Colorado Springs, CO). The pulsatile ABP signal was used to calculate heart rate (HR) and mean arterial pressure (MAP). Left hindlimb VC was calculated as the ratio of Qfem/MAP. For all conditions, Qfem, VC, MAP, and HR were calculated as a 5-min stable average during the baseline period and as a 5-min average upon stability of the drug response (BIBP-3226).

The change in mean Qfem, VC, HR, and BP was compared pre- and postdrug infusion for CTL vs. OVX vs. OVX + E2 using a mixed one-way repeated-measures ANOVA (SigmaStat v3.1, 2004; Systat Software). In the event of statistical significance (P < 0.05), Tukey’s post hoc analysis was used to identify conditions that differed. Data are presented as means ± SE.

Experiment 2: Analysis of skeletal muscle Y1R and Y2R expression, peptide activity, and NPY concentration (n = 8 CTL, n = 8 OVX, and n = 8 OVX + E2 females). This experiment addressed the specific hypotheses that estrogen affected Y1R-mediated vasomotor control through changes in vascular Y1R expression, autoinhibitory prejunctional neuronal Y2R expression, and/or peptide activity. If so, then OVX rats should have greater vascular Y1R expression, decreased neuronal Y2R expression and peptide activity, and increased skeletal muscle NPY concentration compared with OVX; these effects would be reversed by estrogen replacement.

Analyses were carried out on two different skeletal muscle groups known to contain differing expression of slow-twitch oxidative (SO), fast-twitch glycolytic (FG), and fast-twitch oxidative-glycolytic (FOG) fiber types. With the animal under deep surgical anesthesia, ~70 mg of skeletal muscle samples were taken from red vastus (RV; expressing FOG > FG > SO fibers) and white vastus (WV; expressing FG > FOG) (4, 19) and were flash-frozen in liquid nitrogen. Animals were killed after tissue harvesting by an overdose of anesthetic. The same muscle tissue samples were used in the three assays carried out in this experiment. The use of skeletal muscle groups expressing differing ratios of fiber types was based on early work by others showing that blood flow to such muscles is distributed differently at rest (37) and during exercise (3, 37).

Y1R and Y2R Western Blotting

Tissue samples were homogenized in 15 vol of extraction buffer (25% glycerol, 0.42 M NaCl, 1.5 mM MgCl2, 0.2 M EDTA, 20 mM HEPES, and 10 μg/ml aprotinin; pH = 7.5) and centrifuged at 16,000 g for 20 min to collect the supernatant as the tissue extract. Sample homogenates were then stored at −70°C until the time of total protein concentration determination and electrophoresis. Total protein concentration was accomplished using the Bradford protein assay. Equal amounts of total protein (60 μg) were run on a 12% acrylamide minigel (Bio-Rad, Hercules, CA) overlaid with a 4% acrylamide stacking gel. After electrophoresis, the proteins were transferred to constant voltage in cold transfer buffer (10% running buffer, 20% methanol in ddH2O) to nitrocellulose membranes. The membranes were blocked in a 5% nonfat milk solution in Tris-buffered saline + 0.1% Tween 20 (TTBS) (80 mM Tris base, 0.5 M NaCl) overnight. Membranes were then incubated for 2 h in primary antibody specific to rat, human, or mouse NPY Y1R or Y2R (Affinity purified rabbit anti-mouse NPY Y1R and NPY Y2R IgG, catalog no. NPY Y1R11-A or NPYY2R11-A; Alpha Diagnostic International, San Antonio, TX) at
a dilution of 3 μg/ml in TTBS with 2% nonfat milk. Membranes were washed again in TTBS and then incubated in secondary antibody [goat anti-rabbit antibody conjugated to horseradish peroxidase (HRP), product no. 170–6518; Bio-Rad] in TTBS with 2% nonfat milk for 1 h. After being washed, the blots were developed using enhanced chemiluminescent Western blotting detection reagents (product no. RPN2106; Amersham) and exposed to Kodak BioMax Light film. The films were scanned and subjected to densitometric quantification (Scion Image analysis software).

**NPY Immunoassay**

NPY concentration was determined in whole muscle tissue homogenates (from each of RV and WV; see above for preparation of homogenate and total protein determination) and standards (50-μl duplicate samples) using a competitive immunoassay (Bachem Bioscience, King of Prussia, PA). All samples were incubated at room temperature for 2 h. The immunoplate was then washed five times with 300 μl of assay buffer/well. Wells were incubated at room temperature with 100 μl of streptavidin-HRP for 1 h. The immunoplate was washed again five times with 300 μl of assay buffer/well. Following washing, 100 μl of a tetramethylbenzidine peroxidase substrate solution were added to all wells. After a 40-min incubation at room temperature, the reaction was terminated by the addition of 100 μl 2 N HCl. Finally, the optical absorbance of each well was read at 450 nM (Bio-Rad Ultramark Microplate Imaging System; Bio-Rad, Hercules, CA). Absorbance measures were converted to NPY concentration by comparison with the 10-point standard curve. Results are given as a ratio of picograms NPY/μg tissue, as computed from the amount of total protein loaded per well. The assay has a minimum detectable concentration of 0.04–0.06 ng/ml or 2–3 pg/well (manufacturer’s data).

**Assay of Peptidase Activity**

Proteolytic enzyme (APP and DPPIV) activity was assessed using continuous spectrophotometric rate determination. Four grams of skeletal muscle (RV and WV) were homogenized in 20 ml of 50 mM phosphate buffer containing 5 mM EGTA (pH 7.5) and centrifuged at 10,000 g for 20 min at 4°C, after which the supernatant was aspirated and filtered through glass wool. All substrates were purchased from Sigma-Aldrich. The proteolytic enzymes DPPIV and APP are ubiquitous throughout the tissue assayed.

The basic principle of the APP assay was as follows:

\[ \text{L-leucine-}p\text{-nitroaniline} \rightarrow \text{L-leucine} + p\text{-nitroaniline} \]

The basic principle of the DPPIV assay was as follows:

\[ \text{Gly-Pro-}p\text{-nitroaniline} \rightarrow \text{Gly-Pro} + p\text{-nitroaniline} \]

Under assay conditions of 37°C in a 96-well plate, standards for p-nitroaniline (pNA, 100 μl/well; ranging from 20 to 100 nmol) were loaded in duplicate, and 100 μl of skeletal muscle samples were loaded in triplicate. Subsequently, to start the reaction, 100 μl of L-leucine-pNA (APP assay) or Gly-Pro-pNA solution (DPPIV assay) were added to each well (except blanks). The reaction was left to incubate at 37°C in a temperature-controlled plate reader (Bio-Rad Ultramark Microplate Imaging System; Bio-Rad) for 15 min, and absorbance was read at 405 nm. In this assay, one unit (1 μmol/min) of APP or DPPIV produces 1.0 μmol of pNA from L-leucine-pNA or Gly-Pro-pNA, respectively. Absorbance at 405 nm was plotted vs. nanomole pNA, and enzymatic activity was calculated in millimoles per minute per milliliter of homogenate.

**Statistics**

The effects of ovariectomy and estrogen replacement on the hemodynamic and tissue variables were assessed using a mixed one-way repeated-measures ANOVA (SigmaStat v3.11 2004; Systat Software). In the event of statistical significance (P < 0.05), Tukey’s post hoc test was used to identify conditions that differed. Data are presented as means ± SE.

**RESULTS**

**Experiment 1**

Ovariectomy with and without estrogen replacement had no affect on baseline MAP or HR, or baseline Qfem (Table 1). Similarly, BIBP-3226 did not affect MAP or HR in any of the groups.

Compared with baseline, hindlimb blood flow doubled following BIBP-3226 infusion in the O VX group (from 0.27 ± 0.1 to 0.54 ± 0.1 ml/min), with no observable change in the CTL or O VX + E2 groups (P < 0.05, Fig. 1). Compared with baseline, hindlimb VC increased in response to the Y1R antagonist BIBP-3226 (from 3.0 ± 0.6 to 5.9 ± 1.3 ml/min−1·mmHg−1; P < 0.05, Fig. 1) in the O VX group. However, this effect was not observed in the CTL or O VX + E2 groups. The heightened effect of Y1R activation in the O VX group was associated with an (~20%) increase in W V Y1R expression compared with CTL and O VX + E2 groups, but was not observed in RV skeletal muscle samples (P < 0.05, Fig. 2). There was no difference in skeletal muscle Y1R expression between CTL, O VX, and O VX + E2 groups in either RV or RV skeletal muscle.

**Experiment 2**

Compared with the CTL group, ovariectomy did not affect DPPIV activity or APP activity in either the WV or RV tissue (Table 2). In contrast, ovariectomy resulted in elevated skeletal muscle NPY concentration in both RV (~3-fold increase) and WV (~2-fold increase) compared with CTL, and this effect was reversed in O VX + E2 rats (P < 0.05, Fig. 3).

**DISCUSSION**

The main finding from this study was that ovariectomy resulted in robust endogenous Y1R-mediated regulation of hindlimb blood flow and VC, a level of control that was absent in CTL and O VX + E2 groups. The observation of Y1R-mediated vasomotor control in O VX was linked with modestly reduced vascular conductance and the presence of NPY-LI fibres in skeletal muscle with ovariectomy. This observation provides evidence that endogenous NPY-LI fibres actively participate in the vascular control of hindlimb blood flow and VC in E2-deficient rats.

**Table 1. Hemodynamic responses to the Y1 receptor antagonist BIBP-3226 in CTL, O VX, and O VX + E2 rats**

<table>
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<th>Baseline</th>
<th>BIBP-3226</th>
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<tr>
<td></td>
<td>CTL</td>
<td>O VX</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>359 ± 24</td>
<td>370 ± 7</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>90 ± 7</td>
<td>90 ± 2</td>
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Values are means ± SE. CTL, control; O VX, ovariectomized; E2, 17β-estradiol.
Augmented Y1R expression in WV and substantially elevated NPY concentration in both WV and RV muscles. Contrary to one of our hypotheses, elevated tissue levels of NPY OVX did not appear to be because of decreases in proteolytic enzyme activity or Y2R expression. Furthermore, increased Y1R expression in OVX was specific to WV tissue and was minimal relative to the observed increase in the Y1R contribution to blood flow and VC. These findings support the hypothesis that estrogen blunts Y1R activation in the hindlimb of female rats largely through an effect on NPY bioavailability and a modest decrease in Y1R expression.

The role of endogenous NPY in baseline vasomotor control has received little attention. This is due, in part, to early conclusions that this neurotransmitter was not released in measurable amounts under baseline conditions, and served primarily as a potent and prolonged vasoconstrictor reserved for stress conditions (42). However, De Potter et al. (6) reported that, when the dog splenic nerve and rat vas deferens were stimulated at frequencies ranging from 2 to 20 Hz, the ratio of NE to NPY remained the same, suggesting that NPY and NE are coreleased under baseline and stress conditions. We have confirmed that Y1R activation plays an important role in baseline vasomotor control within male rat hindlimb skeletal muscle (13, 15). The current finding that ovariectomy influenced endogenous Y1R activation in resting female animals is consistent with previous findings that intact females possess all the components for Y1R-mediated control but do not express it functionally under baseline conditions (13, 14). Thus it appears that estrogen exerts a fundamental impact on neurogenic vasomotor control through mechanisms that affect NPY bioavailability for Y1R interactions.

Estrogen and Y1R Activation

Estrogen and peptidase activity. Peptidases represent a broad range of regulatory enzymes that affect tissue remodeling and terminations of biologically important reactions. Of the many membrane-bound proteases, the two that are able to augmented Y1R expression in WV and substantially elevated NPY concentration in both WV and RV muscles.

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Table 2. DPPIV and APP activity measured in white and red vastus lateralis muscle samples from CTL, OVX, and OVX + E2 rats

<table>
<thead>
<tr>
<th></th>
<th>CTL (n = 8)</th>
<th>OVX (n = 8)</th>
<th>OVX + E2 (n = 8)</th>
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<tbody>
<tr>
<td>DPPIV activity, mmol·min⁻¹·ml of homogenate⁻¹</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WV</td>
<td>0.0028 ± 0.0001</td>
<td>0.0030 ± 0.0002</td>
<td>0.0024 ± 0.0004</td>
</tr>
<tr>
<td>RV</td>
<td>0.0045 ± 0.0005</td>
<td>0.0043 ± 0.0001</td>
<td>0.0040 ± 0.0002</td>
</tr>
<tr>
<td>APP activity, mmol·min⁻¹·ml of homogenate⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WV</td>
<td>0.0062 ± 0.0003</td>
<td>0.0060 ± 0.0004</td>
<td>0.0050 ± 0.0008</td>
</tr>
<tr>
<td>RV</td>
<td>0.0085 ± 0.0009</td>
<td>0.0084 ± 0.0005</td>
<td>0.0074 ± 0.0004</td>
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Values are means ± SE. DPPIV, dipeptidyl peptidase IV; APP, aminopeptidase P; WV, white vastus lateralis; RV, red vastus lateralis.

cleave the primary proline amino acid structure of NPY are DPPIV and APP (or X-Pro aminopeptidase). DPPIV is bound to the extracellular surface of endothelial cells, and APP is bound to the extracellular surface of smooth muscle and is anatomically positioned to modulate NPY availability for Y1R binding (24). In a simplistic model, vascular NPY is released from perivascular sympathetic nerves to act on postjunctional Y1R and prejunctional Y2R autoreceptors. Vascular smooth muscle APP terminates the action of NPY on Y1R and produces an NPY-(3–36) metabolite with high affinity for prejunctional Y1R, further reducing NPY-induced vascular effects. DPPIV will perform similar actions with primary effects on circulating NPY.

The impact of such peptidases on the regulation of skeletal muscle NPY vasomotor function is largely unknown. Furthermore, available information on the impact of the animal’s sex on peptidase activity is equivocal. For example, in isolated aortic ring segments, the enhanced NPY-induced vasoconstriction in tissue extracted from male and ovariectomized female animals vs. intact females was partially related to lower peptidase levels in the former groups (8). In humans, however, circulating levels do not appear to be different between men and women (7), although the degree to which circulating peptidases reflect membrane-bound enzymes in the neurovascular interstitial space is not known. Using receptor-specific antagonists for prejunctional Y2R (BIIE-0246), postjunctional Y1R (BIBP-3226), inhibition of DPPIV and APP, and Western blot analysis of receptor expression, we observed that our previously reported lack of endogenous baseline Y1R activation in female hindlimb vasculature (13) was strongly related to both prejunctional Y2R auto inhibition and to proteolytic processing of NPY (14). Thus it was NPY bioavailability for Y1R activation that determined sex-dependent differences in NPY-modulated vascular control in these studies.

Our ability to observe changes in VC with Y1R blockade in intact females after peptidase inhibition (14), and in ovariectomized females without peptidase inhibition (current study), suggests that ovarian hormones may influence peptidase activity. In addition, we have observed that Y1R expression varied in muscle types of the rat hindlimb, leading to analysis of peptidase activity in the glycolytic (WV) and oxidative (RV) muscles. However, within a muscle group, the activities of DPPIV and APP were similar in intact and ovariectomized females. Therefore, the current results challenge the idea that estrogen affects NPY bioavailability and Y1R activation through an increase in peptidase activity. Subsequently, these findings lead to investigation of estrogen’s potential impact on Y1R expression.

Y1R expression. To our knowledge, this is the first study to directly examine the role estrogen has on skeletal muscle Y1R expression. These data indicate that estrogen constrained Y1R expression in WV but not RV muscle. However, this effect in WV muscle was proportionately small relative to the robust blood flow/VC responses observed in OVX vs. CTL and OVX + E2 animals with Y1R blockade. Overall, the current results indicate that, once released, NPY should be available for binding to Y1R. Our previous finding that peptidase inhibition increased Y1R activation implies that females have a potent system for complete processing of NPY at the neurovascular junction, under normal conditions of release. Because Y1R had only a modest increase in expression in OVX rats, and we saw no change in peptidase activity in this cohort, it seems that the role of OVX to enhance Y1R activation mainly lies within augmented NPY release mechanisms.

Estrogen and NPY Release

In contrast to the postjunctional Y1R that elicits smooth muscle constriction, the prejunctional Y2R indirectly limits NPY’s vaso-
constrictor effect by modulating NPY release through an autoinhibitory mechanism. Thus estrogen could modulate NPY release by affecting Y1R expression. However, our results indicate that Y1R expression was not affected by OVX or OVX + E2 in either WV or RV tissue. Therefore, this mechanism could not account for enhanced Y1R activation in the OVX animals.

So far, the current data indicate that the previously observed sex differences in NPY bioavailability were due to estrogen’s impact on NPY release and Y1R expression, but not Y2R expression or peptidase activity. Notwithstanding possible changes in postreceptor sensitivity, one explanation for the current data is enhanced NPY vesicular transport/release in OVX animals resulting from changes in sympathetic nerve activity (SNA). We were not able to measure SNA in this study; however, this conclusion is consistent with accumulating evidence of sex differences in sympathetic outflow. In humans, many reports indicate that, on average, baseline SNA is lower in females compared with males (16, 22) at least until menopause after which age-related hyperadrenergic activity dominates in both genders (12, 22). One’s sex also affects baroreflex regulation of SNA discharge and neuronal recruitment strategies (32, 35).

Studies in rodents also indicate that estrogen plays a key role in baseline and reflex-mediated increases in sympathetic outflow (29, 31). Therefore, both baseline SNA and the ability to increase SNA are lower in females. The mechanism for this sex effect is not known but must involve estrogen. Estrogen receptors are situated ubiquitously throughout the central nervous system and, in the current context, are located within sympathomodulatory brain stem sites, acting directly to enhance baroreflex sensitivity (inhibition) of sympathetic outflow (29, 31). Therefore, estrogen may affect neurovascular interactions at multiple levels, both peripherally and centrally.

Potential Implications

The Y1R is implicated in acute vasoconstrictor control as well as in atherogenic growth of vascular smooth muscle (1, 26). In this regard, enhanced NPY availability in the neurovascular cleft, among other factors, may contribute to sex-related differences in risk for vascular disease. For example, postmenopausal women appear to experience more pronounced age-related vascular stiffening than men, a finding that is consistent with concurrent changes in female hormonal status (39). In addition, central arteries in men appear to be more prone to degenerative changes with age than females (39). Finally, the central arteries of prepubertal females were stiffer than matched males, a difference that was not noticeable in postpubertal children (2). Of note, the muscular brachial artery is more distensible in younger females, becoming similar to male counterparts by the fifth decade of life (38). In support of these conclusions, experimental studies have reported improved aortic elastic properties, and reduced reflected blood pressure waves, following estrogen replacement in postmenopausal women (34). These studies point to a generalized vasculoprotective role of ovarian hormones. The current results are consistent with these earlier data and provide a possible mechanism by which estrogen protects vascular tissue, that is, through an attenuated release of NPY and a decrease in deleterious NPY-mediated vascular remodeling. Understand-

ing the attenuation of NPY release in females requires additional investigation.

There were limitations to this study. In this study, a 21-day release pellet of E2 was used to supplement estrogen. Although measures of estrogen were not made in any group to verify the completeness of estrogen supplementation with this model, previous use of this approach (25) achieved physiological levels of circulating estrogen in female Sprague-Dawley rats. In addition, the complete reversal of Y1R activation at baseline by the OVX + E2 group demonstrates the impact of this supplement methodology on the major outcome variable. Thus it appears that the estrogen pellet approach was functionally intact.

It is possible that the analysis of Y1R expression in muscle tissue homogenates reflects protein from vascular and myofibril tissue. To our knowledge, the studies examining the expression of these receptors on skeletal muscle tissue vs. vascular tissue have not been reported. Nonetheless, these current and previous data show that Y1R expression in skeletal muscle homogenate is internally consistent with the dilatory response seen with BIBP-3226 infusion (13, 15), thus supporting the idea that Y1R expression is proportionate to that occurring in vascular tissue in skeletal muscle.

Perspectives and Significance

Over the past two decades, there has been growing interest in the role of sex hormones in the neural control of the circulation. This interest follows, and contributes to, the known relationship between one’s sex and vascular or cardiac health. From these perspectives, estrogen is understood to benefit cardiovascular health. One of the mechanisms by which estrogen may affect vascular control is through variations in sympathetic discharge that, in turn, affect neurotransmitter release. It is clear that one’s sex affects sympathetic discharge patterns in both humans (10, 32) and rodents (9). In recent years, the availability of Y1R-specific antagonists has revealed a significant role of NPY in vascular morphology, leading to renewed interest in the role of this neurotransmitter in cardiovascular pathology (18, 23). The current results indicate that estrogen does have a role in NPY bioavailability and that this effect appears to be related to the neural release of NPY rather than postjunctional receptor differences. Thus estrogen appears to modulate central sympathetic drive (e.g., see Ref. 30), synaptic delays, postganglionic recruitment patterns, and/or NPY release mechanisms.

In summary, this study demonstrated the important role of ovarian hormones, specifically estrogen, on the bioavailability of NPY to exert a vasoconstrictor effect on the rat hindlimb vasculature through Y1Rs. This effect was associated with heightened levels of tissue NPY and a small increase in Y1R expression that was localized to WV muscle tissue in the absence of estrogen. In contrast, the increased concentration of NPY in skeletal muscle samples was not related to a reduction in peptidase activity or downregulation of prejunctional Y2 autoreceptors. Therefore, it appears that the upregulated Y1R constrictor effect in ovarietomized animals was because of elevated levels of NPY available for receptor activation. In the absence of local mechanisms that affect NPY levels, such as peptidases and prejunctional autoinhibitory Y2Rs, it appears that estrogen diminishes the release of NPY from sympathetic varicosities. The mechanism of this effect was not addressed and could include central changes in sympathetic discharge and/or direct effects of estrogen on sympathetic neural varicosities.
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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the authors.

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