Y1- and α1-receptor control of basal hindlimb vascular tone

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Submitted 19 December 2003; accepted in final form 17 March 2004

Jackson, Dwayne N., Earl G. Noble, and J. Kevin Shoemaker. Y1- and α1-receptor control of basal hindlimb vascular tone. Am J Physiol Regul Integr Comp Physiol 287: R228–R233, 2004.—The role of endogenous Y1-receptor activation on skeletal muscle vasculature under baseline conditions is currently debated and no in vivo studies have been performed to address this issue. Therefore, this study was designed to address the effect of Y1-receptor and/or α1-adrenoceptor antagonism on basal hindlimb vascular conductance in male Sprague-Dawley rats in vivo. Left hindlimb vascular conductance, carotid artery mean arterial pressure, and heart rate were measured during low volume infusion of N2-(diphenylacetyl)-L-N-[4-(4-hydroxyphenyl)methyl]-L-arginine amide (BIBP3226; 100 μg/kg), prazosin (20 μg/kg), and combined blockade to the left hindlimb. Vascular conductance increased 1.5 ± 0.5 μl min⁻¹ mmHg⁻¹ with BIBP3226 infusion, 1.7 ± 0.5 μl min⁻¹ mmHg⁻¹ with prazosin infusion, and 4.8 ± 1.0 μl min⁻¹ mmHg⁻¹ with combined blockade (P < 0.05). Interestingly, systolic vascular conductance increased in all three conditions, but diastolic vascular conductance only increased in the two conditions where BIBP3226 was present. These data indicate that Y1-receptor activation plays an important role in the regulation of vascular conductance in the resting rat hindlimb. Furthermore, this effect was of the same magnitude as the α1-adrenoceptor contribution. The differential flow profiles following α1-blockade with and without Y1-receptor blockade supports local differences in receptor distribution.

neuropeptide Y; norepinephrine; vascular conductance; BIBP3226

THE SYMPATHETIC branch of the autonomic nervous system is an essential modulator of the peripheral circulation and blood pressure (BP). Several reports indicate an association between essential hypertension and augmented sympathetic nerve activity and its link to the increase in vascular resistance (19) and wall thickness (7). These combined effects of sympathetic outflow appear to include the dual actions of the sympathetic neurotransmitters norepinephrine (NE) and neuropeptide Y (NPY) acting on postjunctional α- and Y-receptors, respectively.

NPY, a 36-amino-acid residue, is costored with NE within peripheral sympathetic nerves that supply blood vessels. Nerve fibers that contain NPY are more abundant around resistance vessels and become denser with decreasing vessel size (35). It has been established that the receptor responsible for the vasoconstrictor effects of NPY in cat skeletal muscle is the NPY-Y1 receptor (9). NPY has been implicated in hypertension (22) with high circulating levels in both men and women with the disease (38). Moreover, the mitogenic potential of NPY has been established (29, 34, 40, 41). Nonetheless, it is reasonable to expect that Y1-receptor activation must occur chronically under baseline conditions if it is to factor importantly in long-term BP regulation and/or mitogenic effects. However, chronic basal Y1-receptor activation has not been demonstrated with certainty.

The relationship between basal Y1-receptor activation and BP regulation is debated. Evidence from Y1-receptor knockout mice that display normal basal BP (26) suggests that NPY does not play a critical role in BP maintenance. Moreover, infusion of the Y1-receptor antagonist N2-(diphenylacetyl)-L-N-[4-(4-hydroxyphenyl)methyl]-L-arginine amide (BIBP3226) does not affect basal BP in normotensive and spontaneously hypertensive rats (which have elevated plasma NPY levels) (39). Consequently, interest in the role of NPY as a regulator of baseline BP has declined, whereas its role during shock and/or chronic stress (e.g., sepsis, hemorrhage, and cold stress) has been expressed (30, 43). This evidence has contributed to the concept that the release of NE and NPY is differentially regulated (1, 3, 17) with NE released at lower nerve activity and NPY released only under high neuronal stimulation frequencies. In this model, it is proposed that NE is contained and released from small dense-cored vesicles (SDCV), whereas NPY is contained within large DCV. This hypothesis predicts that NE primarily controls vascular smooth muscle tone during basal conditions, whereas NPY should not, its release only occurring during periods of high stress. Pharmacological and physiological studies support the differential release of NPY and NE (16, 17).

However, data from a series of studies by De Potter et al. (4–6) bring into question differential release of NE and NPY. This group has tested several different vascular beds innervated by sympathetic nerves with differing ratios of large DCV and SDCV, including the isolated perfused sheep spleen, dog spleen, and rat vas deferens (the latter containing primarily SDCV) (4, 5). They have reported that at nerve stimulation frequencies ranging from 2 to 20 Hz, the ratio of NE to NPY remains constant (5). From this perspective, both NPY and NE should contribute to baseline vascular tone.

Thus the goal of the present study was to test the hypothesis that Y1-receptor activation contributes importantly to baseline vascular tone. This hypothesis was addressed by examining the effect of selective Y1-receptor and/or α1-adrenoceptor antagonism on basal hindlimb vascular conductance in male Sprague-Dawley rats in vivo. In this study, skeletal muscle vasculature was investigated because it has a high level of basal tone and a large range of response to changes in physiological state that contribute to BP control both at rest and during stress. The results indicate that Y1-receptor activation contributes importantly to baseline hindlimb vascular tone.

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Table 1. Heart rate and blood pressure responses associated with each condition

<table>
<thead>
<tr>
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<th>BIBP3226</th>
<th>Prazosin</th>
<th>BIBP3226 + Prazosin</th>
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</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>351 ± 19</td>
<td>328 ± 13</td>
<td>332 ± 13</td>
</tr>
<tr>
<td>Drug</td>
<td>354 ± 18</td>
<td>324 ± 13</td>
<td>327 ± 9</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>103 ± 5</td>
<td>103 ± 3</td>
<td>106 ± 3</td>
</tr>
<tr>
<td>Baseline</td>
<td>104 ± 6</td>
<td>87 ± 4†</td>
<td>85 ± 4†</td>
</tr>
</tbody>
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Values are means ± SE. BIBP3226, N²-(diphenylacetyl)-N²-(4-hydroxyphenyl) methyl-L-arginine amide. †P < 0.05, significant difference from baseline; †P < 0.05, significant difference from BIBP3226 condition.

**METHODS**

The Council on Animal Care at the University of Western Ontario approved the experimental protocol.

**Animals.** Twenty-three adult male Sprague-Dawley rats (body wt 256–426 g; Charles River Laboratories, Wilmington, MA) were used in this study. Rats were housed in a temperature (24°C) and light (12-h cycle)-controlled room in standard Plexiglas cages, fed rat chow (Prolab Rat, Mouse, and Hamster 3000 Diet), and were allowed to eat and drink water ad libitum. Before surgery, the animals were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (Inactin; 100 mg/kg; Sigma-Aldrich). Internal body (rectal) temperature was monitored continuously and was maintained at 37 ± 0.5°C with the use of a thermally controlled water-perfused heating pad.

**Surgery.** A tracheal cannula was inserted to facilitate spontaneous respiration, and end-tidal CO₂ measures were made from expired air at periodic intervals throughout the experiment. The left common carotid artery was cannulated with polyethylene (PE-50) tubing to permit arterial BP recording from the amplified signal (model ML118 PowerLab Quad Bridge Amplifier; ADInstruments, Colorado Springs, CO) of a pressure transducer (model MTL844; ADInstruments).

Through a midline abdominal incision, the gut was carefully moved aside and covered with sterile gauze moistened with sterile saline (0.9% NaCl). With the use of cotton swabs, a small portion of the descending aorta and right iliac artery was cleaned of fat and exposed. The right iliac artery was cannulated (PE-50), and the tubing was advanced to the bifurcation of the aorta. This cannula was used for arterial BP recordings and evaluation of drug effects.

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Femoral artery blood flow (Q_{fem}) was measured with the use of a flowmeter (model TS420 Perivascular Flowmeter Module; Transonic Systems, Ithaca, NY) and Transonic flow probe (0.7 SB) positioned ∼3 mm distal to the femoral triangle. Special care was taken to ensure that surrounding nerves and vessels were not damaged during this procedure. Innocuous water-soluble gel was spread over the entire opened area of the hindlimb to maintain hydration of exposed tissue and quality of arterial flow signal.

**Experimental protocol.** Animals recovered for 1 h after surgery; however, BP and HR were stable and at normal levels within 30 min. After recovery, a vehicle (0.9% saline) infusion (160 μl) was carried out, followed by a 10-min recovery. Baseline data were recorded for 5 min, followed by one of three bolus drug infusions (carried out on 3 separate groups of animals). These included the following: 1) 100 μg/kg BIBP3226, a specific NPY Y₁-receptor antagonist (Sigma-Aldrich); 2) 20 μg/kg prazosin (specific α₁-adrenoceptor antagonist; Sigma-Aldrich); and 3) combined 100 μg/kg BIBP3226 + 20 μg/kg prazosin. The volume of each drug infusion was held constant at 160 μl. Previous pilot work conducted in our laboratory (not presented) confirmed that 100 μg/kg BIBP3226 provided the maximal dilator response in this preparation. Our preliminary data also established that 20 μg/kg prazosin caused the least systemic effect (i.e., drop in BP) for the greatest increase in hindlimb blood flow. Furthermore, it was confirmed that the drug doses utilized completely blocked the Y₁- and α₁-receptors in our model. Specifically, the agonist-induced decrease in vascular conductance (60 ± 13% with 100 nM NPY and 49 ± 6% with 3 μM NE; n = 3 animals) was completely abolished by a subsequent dose of the respective antagonist (100 μg/kg BIBP3226 or 20 μg/kg prazosin). Moreover, after receptor blockade, an ensuing infusion of the corresponding agonist (either 100 nM NPY or 3 μM NE) had no effect on hindlimb vascular conductance.

**Results**

Animals exhibited normal MAP (103–106 mmHg) and HR (328–351 beats/min) levels during the baseline period and there were no intercondition differences among these variables (Table 1). Furthermore, end-tidal CO₂ in all conditions remained in the normal range throughout the experiment (38–42 mmHg). Vehicle infusion did not affect any of the measured variables in any of the conditions. Baseline vascular conductance levels for the various groups were not different before any of the pharmacological treatments (Table 2).

**Effect of Y₁-receptor antagonism.** MAP and HR were unaffected by BIBP3226 infusion (Table 1; n = 7). Compared with baseline, mean Q_{fem} and hindlimb vascular conductance increased by 160 ± 60 μl/min and 1.5 ± 0.5 μl/min•mmHg⁻¹, respectively, after BIBP3226 infusion (Fig. 1; P < 0.05). Compared with baseline, systolic blood flow (240 ± 87 μl/min), and systolic vascular conductance (2.3 ± 0.7 μl/min•mmHg⁻¹) increased after BIBP3226 infusion (Fig. 2; P < 0.05). Diastolic blood flow and vascular conductance also increased from baseline by 120 ± 47 μl/min and 1.2 ± 0.4 μl/min•mmHg⁻¹, respectively (Fig. 2; P < 0.05). The percent change (%Δ) in hindlimb vascular conductance was 72 ± 19%.

**Effect of α₁-receptor antagonism.** MAP decreased with prazosin infusion from 103 ± 3 to 87 ± 3 mmHg and HR was unaffected (Table 1). After prazosin infusion (n = 8), Q_{fem} and vascular conductance values were measured before each pharmacological treatment (Table 2).

Table 2. Baseline values of hindlimb vascular conductance before three pharmacological treatments

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<tr>
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<th>BIBP3226</th>
<th>Prazosin</th>
<th>BIBP3226 + Prazosin</th>
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<tbody>
<tr>
<td>Mean</td>
<td>2.8 ± 0.7</td>
<td>1.8 ± 0.5</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Systolic</td>
<td>4.5 ± 0.8</td>
<td>4.6 ± 1.1</td>
<td>6.5 ± 1.4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>1.9 ± 0.8</td>
<td>0.4 ± 0.4</td>
<td>0.5 ± 0.5</td>
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Values are means ± SE and measured in microliters per minute per mmHg.
hindlimb vascular conductance increased by 130 ± 49 µl/min and 1.7 ± 0.5 µl·min⁻¹·mmHg⁻¹, respectively (Fig. 1; P < 0.05). The %Δ in hindlimb vascular conductance was 131 ± 32% [not significant (NS) vs. BIBP3226 condition]. Compared with baseline, systolic blood flow and systolic vascular conductance were elevated after prazosin infusion (406 ± 95 µl/min and 5.3 ± 1.0 µl·min⁻¹·mmHg⁻¹, respectively). This increase was greater than that observed in the
BIBP3226 infused group ($P < 0.05$; Fig. 2). There was no observable increase in diastolic blood flow or diastolic vascular conductance with prazosin infusion.

Effect of combined $Y_1$- and $\alpha_1$-receptor antagonism. MAP decreased from $106 \pm 3$ to $85 \pm 4$ mmHg ($P < 0.05$) and HR was unaffected by combined $Y_1$- and $\alpha_1$-receptor antagonism (see Table 1). $Q_{\text{tot}}$ and hindlimb vascular conductance increased by $381 \pm 99$ $\mu$L/min and $4.8 \pm 1.0$ $\mu$L·min$^{-1}$·mmHg$^{-1}$, respectively (Fig. 1; $P < 0.05$). The %Δ in hindlimb vascular conductance with combined blockade was $315 \pm 156\%$ (NS vs. BIBP3226 or prazosin).

Combined blockade resulted in an elevation in systolic blood flow and systolic vascular conductance from baseline ($765 \pm 139$ $\mu$L/min and $10.1 \pm 1.3$ $\mu$L·min$^{-1}$·mmHg$^{-1}$, respectively). The increase in systolic blood flow was higher than that of the BIBP3226 condition, and the increase in systolic vascular conductance was greater than the BIBP3226 and prazosin conditions (Fig. 2; $n = 8$; $P < 0.05$).

The infusion of combined BIBP3226 + prazosin resulted in an increase in diastolic blood flow and diastolic vascular conductance above baseline ($218 \pm 79$ $\mu$L/min and $2.4 \pm 0.9$ $\mu$L·min$^{-1}$·mmHg$^{-1}$, respectively). This increase was similar to that observed in the BIBP3226 condition but was different from the prazosin condition (Fig. 2; $P < 0.05$).

**DISCUSSION**

The primary and novel observation from this study was the increase in baseline hindlimb blood flow and vascular conductance after $Y_1$-receptor blockade. Furthermore, the contribution of $Y_1$-receptors to mean blood flow and vascular conductance was similar to that of $\alpha_1$-adrenoceptors. These findings are in support of the work by De Potter et al. (4, 5) that NPY and NE are coreleased from peripheral sympathetic nerves under baseline conditions.

As indicated above, there is debate over the role of NPY in the regulation of baseline vascular tone. NPY knockout models (26) and systemic $Y_1$-receptor blockade studies (39) have not observed any appreciable effect of NPY on basal BP. Nonetheless, the current data indicate an important role of $Y_1$-receptor activation on baseline vascular tone in skeletal muscle. A possible explanation for these apparently conflicting results is that neither NPY receptors nor the control of systemic BP are homogenously distributed. In support of this, observations from in vitro studies indicate that samples of small artery taken from human skeletal muscle were more reactive to NPY than those from mesenteric beds (27, 28). It is noteworthy that, in humans, it is the visceral organs that contribute most to systemic BP regulation (31). If so, then NPY effects on baseline local vascular tone may be limited to skeletal muscle beds and microvascular flow distribution with little overall impact on systemic BP.

An unexpected observation of the current study was that the increase in mean vascular conductance was similar after BIBP3226 or prazosin treatment, but this was achieved by different flow response profiles in the two conditions. There was an increase in systolic conductance from baseline in both treatments. However, diastolic conductance increased after the BIBP3226 treatment but not after prazosin. Furthermore, diastolic and systolic conductance increased when both drugs were given simultaneously. This pattern may be explained by the differential distribution of $\alpha_1$-adrenoceptors and $Y_1$-receptors. Both in vitro (11) and in vivo (9, 15) studies have shown that NPY promotes only a weak constriction in large blood vessels and the preferential site of action occurs on the small arterioles (<25 μm). In addition, intravital microscopy studies demonstrate that adrenergic regulation of large arterioles and venules in skeletal muscle involves both $\alpha_1$- and $\alpha_2$-adrenoceptors with higher concentrations of $\alpha_2$-receptors in small (25 μm) arterioles (10). Thus $Y_1$-receptor blockade should lead to preferential dilation of the smaller arterioles leading to an increase in both systolic and diastolic conductance. Alternatively, the effects of prazosin should be localized primarily on larger arterioles and feed arteries with special effects on systolic conductance because the persisting resistance in the downstream arterioles would limit low-pressure flow. Therefore, in addition to important contributions to baseline vascular conductance in skeletal muscle, $Y_1$-receptors also appear to exert important regulatory influence over the distribution of microvascular flow with contributions to diastolic vascular resistance. Experimental support for this hypothesis and examination of the implications of this control feature for oxygen transport, BP regulation, and microvascular remodeling remain to be determined.

On the basis of currently available data, it is generally understood that NPY exerts its effect on vasomotor tone by modulating the effects of NE (8, 12, 33, 37). This experiment was not designed to address specifically the synergy between $Y_1$-receptor and $\alpha_1$-adrenoceptor activation. However, the potential for heterogeneous spatial distribution of $Y_1$- and $\alpha_1$-receptor types may make it difficult to assess the interaction between the vasomotor effects of NPY and NE in skeletal muscle microvascular networks. Statistically, the differences between the combined blockade trial and the independent effects of BIBP3226 or prazosin were simply additives for each of the mean systolic or diastolic conductance data. It is noteworthy that the diastolic conductance response tended to indicate that such an interaction might exist ($P = 0.08$; combined blockade vs. BIBP3226 alone); however, sample size calculations indicated that at least 32 animals would be required to observe such an effect. Overall, it is proposed that more specific experimental designs will be required to address whether differential distribution of $Y_1$ versus $\alpha_1$-receptors minimize the potential for interactive postreceptor effects in skeletal muscle vessels.

In the present study, there was no HR response with prazosin-induced hypotension, an observation that is consistent with previous examinations (18, 20, 25). Assuming normal baroreflex control, a significant drop in BP should elicit reflex tachycardia. The explanation for this contradictory response likely relates to the impact of prazosin on intracellular levels of cGMP at cholinergic receptor sites in the heart (13).

A potential limitation of this study is that barbiturate anesthetics may increase the systemic adrenergic activity (36). Such an effect might cause an overestimation of normal NPY effects on baseline vascular tone. However, studies in awake and anesthetized animals suggest that this class of anesthetics (including Inactin) has little effect on basal organ vascular conductance (2). Furthermore, Matsukawa and Ninomiya (21) observed that sympathetic levels did not vary between the first and fourth hour after barbiturate (pentobarbital) anesthesia and that these levels were not different from awake rest levels. Our
experiments commenced 1 h postsurgery and were completed (including surgical time) before the 4-h limit observed in this previous study (21). In addition, Matsukawa and Ninomiya confirmed that the relative increase in sympathetic outflow after 4 h of anesthesia was related to the depth of anesthesia; when a supplemental dose (5–10 mg/kg) of pentobarbital was administered, the previously observed elevation in renal sympathetic nerve activity was inhibited (21). Inactin (used in the current study) provides a stable plane of anesthesia for up to 9 h (32). To examine directly whether the type of anesthesia affected Y1-receptor-dependent vascular control, additional studies were performed in three male Sprague-Dawley rats using α-chloralose (80 mg/kg) and urethane (500 mg/kg). In these additional animals BIBP3226 infusion resulted in a 1.9 ± 0.7 µL·min⁻¹·mmHg⁻¹ increase in hindlimb vascular conductance, similar to the current findings. These data provide additional confidence that the anesthetic used here did not confound the validity of the current observations.

It is possible that the drop in MAP associated with prazosin infusion may have decreased myogenic vascular tone in this experiment and subsequently led to increased vascular conductance (14) independent of hindlimb receptor blockade. This may affect conclusions regarding both the potential for interactive roles by Y1- and α1-receptor on mean hindlimb conductance and for the differential effects on systolic versus diastolic conductance. However, we do not believe that this affected the conclusions of this study because with combined infusion the increase in Q(T)(a) and vascular conductance was markedly greater than with prazosin alone despite a similar reduction in MAP. Moreover, myogenic contributions are unlikely to have affected the differential effects on systolic versus diastolic conductance because, with the double blockade, there was a similar drop in BP as with prazosin alone and both systolic and diastolic vascular conductance increased.

A major assumption of the current study is that the activation of Y1-receptors was due to NPY released from sympathetic neuronal varicosities. Certainly, these neurons contain NPY (35). However, other sources of NPY exist, and these may have impacted on the current analysis. For example, in rats, NPY is released from the adrenal medulla (23) as well as from aggre-


gation of blood platelets (24). However, the contribution of the adrenal medulla to circulating plasma NPY has been shown to be insignificant in sham-operated rats (42). Furthermore, adrenergic demedullation fails to affect baseline NPY levels (23, 42). Moreover, it has been shown that platelet-derived NPY release is only observed in vivo during high stress and is negligible under basal conditions (42).

In conclusion, these data provide evidence that Y1-receptor activation contributes to the maintenance of basal hindlimb vascular tone in the intact rat. In addition, the effect of baseline endogenous Y1-receptor activation on mean vascular conductance was similar to basal α1-adrenoceptor effects. Finally, the observations suggest that activation of Y1- and α1-receptors leads to spatial differences in microvascular control in skeletal muscle.

GRANTS

This study was supported by The Natural Science and Engineering Research Council of Canada (NSERC) and The Academic Development Fund from the University of Western Ontario (to J. K. Shoemaker) and The Heart and Stroke Foundation (HSF) of Ontario Grant T5036 (to Earl G. Noble). D. N. Jackson was the recipient of a NSERC Post-Graduate Scholarship and HSF of Canada/Canadian Institutes of Health Research Institute of Gender and Health Doctoral Research Award.

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