Endothelial modulation of neural sympathetic vascular tone in canine skeletal muscle

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King-VanVlack, C. E., S. E. Curtis, J. D. Mewburn, S. M. Cain, and C. K. Chapler. Endothelial modulation of neural sympathetic vascular tone in canine skeletal muscle. J. Appl. Physiol. 85(4): 1362–1367, 1998.—The effect of nitric oxide synthase (NOS) inhibition and endothelin-A (ETₐ) receptor blockade on neural sympathetic control of vascular tone in the gastrocnemius muscle was examined in anesthetized dogs under conditions of constant flow. Muscle perfusion pressure (MPP) was measured before and after NOS inhibition (N-nitro-l-arginine methyl ester; L-NAME) and ETₐ receptor blockade [cyclo-(d-Trp-d-Asp-Pro-D-Val-Leu); BQ-123]. Zero and maximum sympathetic nerve activities were achieved by sciatic nerve cold block and stimulation, respectively. In group 1 (n = 6), MPP was measured 1) before nerve cold block, 2) during nerve cold block, and 3) during nerve stimulation. Measurements under these conditions were repeated after L-NAME and then BQ-123. The same protocol was followed in group 2 (n = 6) except that the order of L-NAME and BQ-123 was reversed. MPP and muscle vascular resistance (MVR) increased after L-NAME and then decreased to control values after BQ-123. MVR decreased after BQ-123 alone and, with the addition of L-NAME, increased to a level not different from that observed during the control period. MVR fell during nerve cold block. This response was not affected by administration of L-NAME followed by BQ-123, but it was attenuated by administration of BQ-123 before L-NAME. The constrictor response during sympathetic nerve stimulation was enhanced by L-NAME; no further effect was observed with BQ-123, nor was the response affected when BQ-123 was given first. These findings indicate that endothelin contributes to 1) basal vascular tone in skeletal muscle and 2) the increase in skeletal muscle vascular resistance after NOS inhibition. Finally, nitric oxide “buffers” the degree of constriction in skeletal muscle vasculature during maximal sympathetic stimulation.

Endothelin; nitric oxide; vascular resistance

RESTING VASCULAR TONE represents the integrated consequence of an interplay between local (endothelial, myogenic, metabolic) and central (neural, hormonal) regulatory systems. Our studies (4, 14, 34) and those of others (16, 39) have shown that inhibition of the endothelial vasodilator substance nitric oxide (NO) results in a substantial elevation of muscle vascular resistance in anesthetized dogs. In contrast, removal of the endothelium in vivo was associated with only a small increase (4) or no change in muscle vascular resistance (13). These findings suggested that the mechanism(s) responsible for the large increase in skeletal muscle vascular resistance after NOS synthase (NOS) inhibition were more complex than just the loss of the vasodilatory action of NO and may involve the increased release or production of a vasoconstrictor substance. Some evidence supports this possibility. For example, in vitro studies have demonstrated that 1) the production of the vasoconstrictor endothelin-1 (ET-1) was inhibited by NO in porcine aorta (1), 2) ET-1 and NO are reciprocally regulated in proliferating bovine aortic endothelial cells (8), and 3) the production of guanosine 3',5'-cyclic monophosphate by NO under conditions of increased shear stress suppressed ET-1 release from human umbilical vein endothelial cells (20). Furthermore, the increase in total peripheral resistance after NOS inhibition was blunted in anesthetized rats after blockade of endothelin receptors (27, 28). These findings suggest that NO exerts a tonic inhibition on endothelin production that is removed by NOS inhibition. The elevated vascular tone after NOS inhibition could also be the result of increased sympathetic tone and/or increased responsiveness of vascular smooth muscle to sympathetic activation. In support of this theory, ganglionic blockade prevented the pressor response to NOS inhibition in anesthetized dogs (32) and rats (21, 27, 36), and the constrictor response of excised rat tail arterial strips to norepinephrine administration or sympathetic field stimulation was increased after either endothelial removal or NOS inhibition (27, 29).

We hypothesized that NO acts to modulate vascular tone by preventing the production and/or the constrictor action of endothelin and that NO may alter the constrictor response to neural sympathetic activity in resistance vessels of in vivo skeletal muscle at rest. These hypotheses were tested by using the in situ canine gastrocnemius muscle preparation in which the impact of NOS inhibition and endothelin-A (ETₐ) receptor blockade on vascular tone was assessed over a wide range of sympathetic neural activity.

METHODS

Mongrel dogs (n = 12) were anesthetized with pentobarbital sodium (32 mg/kg iv) with an additional 65 mg (iv) given hourly. The animals were intubated, ventilated to maintain arterial Pco₂ between 30 and 35 Torr, and paralyzed with succinylcholine chloride (30 mg/kg im, 0.1 mg/min iv). A catheter was placed in the left brachial artery for measurement of mean arterial pressure and for withdrawal of arterial blood samples. The left gastrocnemius muscle was cleared of overlying tissue, and the arterial and venous flows were measured over the popliteal vessels. The animals were heparinized (1,000 U/kg iv), and a catheter containing an electromagnetic flow probe was inserted into the popliteal vein for measurement of venous outflow; a second channel in the outflow path allowed for in situ zero-flow calibration without
occluding venous outflow from the muscle. Venous outflow was returned to the animal via a reservoir attached to a catheter in the right femoral vein. The left popliteal artery was ligated and catheterized with a two-channel catheter; one line allowed for autoperfusion of the muscle with blood from the right femoral artery while the other line contained an in-line pump. Once the initial autoperfusion flow rate was determined, the muscle was pump perfused at constant flow equal to the initial autoperfusion rate. The sciatic nerve, which carries >90% of the sympathetic fibers to the gastrocnemius vasculature (3), was cleared for a distance of ~5 cm and placed in a cooling probe that was used to reversibly block sympathetic nerve traffic. A Peltier element served to cool the nerve to ~2.5°C; the technical aspects of the probe have previously been described (18). A stimulating electrode was placed on the nerve distal to the cooling probe. The femoral nerve was sectioned to eliminate any other sources of neural sympathetic activation.

Once surgical preparations were complete, a 30-min period was allowed for stabilization of cardiovascular and metabolic parameters. In one group of six animals, the protocol consisted of a 30-min control period, a 30-min period of systemic NOS inhibition by using N-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg iv), and a 45-min period of combined NOS inhibition and ETA-receptor blockade in the gastrocnemius muscle vasculature with [cyclo(o-Trp-d-Asp-Pro-d-Val-Leu); BQ-123; 2 × 10⁻⁸ mol · kg⁻¹ · muscle wt⁻¹ · min⁻¹] ia. In a second group of six animals, the order of NOS inhibition and ETA-receptor blockade was reversed such that the control period was followed by a 45-min period of ETA-receptor blockade and then a 30-min period of combined ETA-receptor blockade and NOS inhibition. The effectiveness of NOS inhibition in our preparation has been established in earlier experiments in our laboratory (12) and that of ETA-receptor blockade by ourselves (15) and others (37). In each experimental period, muscle perfusion pressure, which was used as an index of changes in muscle vascular resistance, was measured at rest and then during zero (nerve cold block) and maximum sympathetic nerve activity (stimulation at 40 V, 2-ms duration, 10 Hz) (19). Muscle perfusion pressure measured before and after nerve cold block was a reflection of the level of basal (resting) sympathetic tone present in the preparation. The nerve cooling and stimulation procedure lasted ~8–10 min. No motor effects were observed in the paralyzed muscle during nerve stimulation.

All values are reported as means ± SE. Differences between experimental periods within each group were assessed by using a single repeated-measures ANOVA. Multiple comparisons between specific mean values within each group were performed by using paired t-tests with Bonferroni (Dunn) critical values (33). This multiple-comparison procedure controls the overall type 1 error across multiple comparisons but in doing so is extremely stringent, making it more difficult to detect true differences in the treatment means. Accordingly, the overall level of accepted significance was set at P < 0.10, resulting in a Bonferroni-adjusted level, per comparison, of P < 0.033. No between-group comparisons were performed.

RESULTS

Basal muscle vascular resistance and perfusion pressure increased significantly after NOS inhibition with L-NAME (Table 1). Subsequent blockade of ETA receptors with BQ-123 resulted in significant decreases in both vascular resistance and perfusion pressure to values that were not different from those observed during the control period. When BQ-123 was administered first, basal muscle vascular resistance and perfusion pressure fell significantly from control levels (Table 2). Subsequent NOS inhibition resulted in a significant rise in both muscle perfusion pressure and muscle vascular resistance to values that were not different from those observed during the control period.

The changes in muscle vascular resistance that occurred during nerve cold block and maximal sympathetic activation during control, NOS inhibition, and ETA-receptor blockade are shown in Fig. 1. These data reflect the changes in resistance from basal levels (Table 1) that occurred in both groups at each period of the protocol during the nerve-cold block and stimulation procedures. The magnitude of the decrease in muscle vascular resistance that occurred during nerve cold block in the control period was not affected by NOS inhibition alone or in combination with ETA-receptor blockade (bottom). In contrast, the increase in muscle vascular resistance during maximal sympathetic activation was significantly greater after NOS inhibition compared with control (top). Subsequent blockade of ETA receptors did not affect this enhanced pressor response.

The changes in muscle vascular resistance that occurred during nerve cold block and maximal sympathetic activation when ETA-receptor blockade preceded NOS inhibition are shown in Fig. 2. The decrease in muscle vascular resistance observed during nerve cold block in the control period was not affected by

Table 1. Resting vascular resistance and perfusion pressure in skeletal muscle during NOS inhibition and subsequent ETA-receptor blockade

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NOS Inhibition</th>
<th>NOS + ETA Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance, PRU</td>
<td>1.46 ± 0.16</td>
<td>2.46 ± 0.29†</td>
<td>1.64 ± 0.18</td>
</tr>
<tr>
<td>Perfusion pressure, mmHg</td>
<td>119 ± 11</td>
<td>176 ± 15†</td>
<td>129 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE. NOS, nitric oxide synthase; ETA, endothelin-A; L-NAME, N-nitro-L-arginine methyl ester; PRU, peripheral resistance units in mmHg · ml⁻¹ · min⁻¹ · kg⁻¹. *Significant difference from control value, P < 0.033. †Significant difference from L-NAME + BQ-123 value, P < 0.033.

Table 2. Resting vascular resistance and perfusion pressure in skeletal muscle during ETA-receptor blockade and subsequent NOS inhibition

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ETA Blockade</th>
<th>ETA + NOS Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance, PRU</td>
<td>0.83 ± 0.10</td>
<td>0.62 ± 0.09*</td>
<td>0.98 ± 0.09†</td>
</tr>
<tr>
<td>Perfusion pressure, mmHg</td>
<td>109 ± 6</td>
<td>88 ± 7†</td>
<td>136 ± 12†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference from control value, P < 0.033. †Significant difference from BQ-123 value, P < 0.033.
**DISCUSSION**

The noteworthy findings of this study were that 1) resting muscle vascular resistance was reduced after ETA-receptor blockade alone, 2) the increase in resting muscle vascular resistance after NOS inhibition was abolished after blockade of ETA receptors, and 3) the pressor response to maximum sympathetic stimulation after NOS inhibition was significantly increased and ETA-receptor blockade did not affect this augmentation.

The increase in basal (resting) muscle vascular resistance after NOS inhibition is a well-documented finding (14, 16, 34, 39) and could be the result of 1) increased sympathetic tone and/or increased responsiveness of vascular smooth muscle to sympathetic activation, 2) removal of basal NO dilation, and/or 3) unmasking of a tonic endothelial-mediated constriction and/or increased release of an endothelial constrictor agent. Our experimental approach, which used the in situ gastrocnemius muscle, provided insight into the mechanism(s) underlying this response.

Our data do not support the contention that increased sympathetic neural tone is responsible for the increase in resting muscle vascular resistance after NOS inhibition. In our experimental model, if sympathetic outflow to skeletal muscle had increased after NOS inhibition, then the decrease in muscle vascular resistance during nerve cold block would have been greater after L-NAME. This was not the case; therefore, we concluded that NOS inhibition had no effect on basal sympathetic tone in skeletal muscle vasculature. Others have demonstrated that NOS inhibition may increase central sympathetic activation because the pressor response after NOS inhibition was reduced by ganglionic blockade in anesthetized dogs (32), anesthetized rats (21, 29, 36), and pithed rats (36). Furthermore, elevated catecholamine levels have been measured in anesthetized dogs (7) and conscious rats (40) after NOS inhibition. Zanchi et al. (40) showed that the plasma levels of neuropeptide Y, a neurotransmitter that is coreleased with norepinephrine, were unchanged despite elevated norepinephrine and epinephrine levels after NOS inhibition in conscious rats. These authors concluded that L-NAME crossed the blood-brain barrier to inhibit brain NOS with subsequent central activation of the sympathetic nervous system (40). In our experiments, the increased mean arterial pressure after NOS inhibition may have elicited a baroreceptor reflex that could have offset any direct central effect of L-NAME to increase sympathetic activation. This possibility is supported by the finding that lumbar sympathetic nerve discharge was reduced after NOS inhibition in anesthetized rats (11).

Plasma levels of endothelin, which average 10–20 pg/ml in both anesthetized (6, 23) and awake dogs (23), might suggest that endothelin exerts a chronic constrictor influence on vascular tone. The findings of the present study support this premise because resting skeletal muscle vascular resistance was significantly reduced (~25%) after ETA-receptor blockade. Similar
findings of a decrease in basal vascular resistance after \( \text{ET}_A \)-receptor blockade with BQ-123 have been reported for the forearm vasculature in awake human subjects (10). Additional studies have demonstrated that total peripheral resistance and forearm vascular resistance decreased ~20% in awake human subjects after \( \text{ET}_A \) plus endothelin-B (\( \text{ET}_{A+B} \))-receptor blockade with TAK-044 (9), whereas, in anesthetized pigs, pulmonary, renal, splenic, and intestinal resistance fell 28, 26, 60, and 34%, respectively, with the \( \text{ET}_{A+B} \)-receptor antagonist bosentan (38). Others reported that basal systemic hemodynamic variables (i.e., cardiac output, mean arterial pressure) were unaffected by \( \text{ET}_{A+B} \)-receptor blockade with bosentan in both anesthetized dogs (30) and anesthetized rats (29) or by \( \text{ET}_A \)-receptor blockade with BQ-123 in anesthetized rats (29). It seems clear that whole body hemodynamic variables do not necessarily reflect the contribution of ET-1 to basal vascular tone of individual peripheral vascular beds.

Our findings showed that the increase in basal skeletal muscle vascular resistance observed after NOS inhibition was abolished when \( \text{ET}_A \) receptors were blocked after NOS inhibition (Table 1). Furthermore, when \( \text{ET}_A \) receptors were blocked first, basal muscle vascular resistance did not increase above control levels with NOS inhibition (Table 2). Results from experiments in whole animals have indicated that endothelin was only partially responsible for the increase in total peripheral resistance after NOS inhibition. With respect to the latter, Richard et al. (29) found that the 25% increase in mean arterial pressure in anesthetized rats after \( \text{l-N} \)-NAME was significantly blunted but not abolished by \( \text{ET}_{A+B} \)-receptor blockade with bosentan or \( \text{ET}_A \)-receptor blockade with BQ-123. Furthermore, Nafriali et al. (27) showed that the pressor response to \( \text{l-N} \)-NAME in anesthetized rats was only partially reversed with inhibition of endothelin production with the use of phosphoramidon. The difference between the observations in skeletal muscle and the whole animal supports the concept of regional variation in the regulation of vascular tone by NO (35).

We believe that increased endothelin production contributed to the increase in muscle vascular resistance after NOS inhibition because 1) the sizable increase in muscle vascular resistance with NOS inhibition was reversed with \( \text{ET}_A \)-receptor blockade and 2) the depressor effect observed in muscle vascular resistance with \( \text{ET}_A \)-receptor blockade alone was less than that observed when \( \text{ET}_A \)-receptor blockade was given after NOS inhibition. This conclusion is consistent with the findings of others that plasma endothelin levels were elevated in the anesthetized dog after NOS inhibition (30) and that endothelin production was inhibited in vitro by NO in porcine aortic (1), bovine aortic (8), and human umbilical vein (20) endothelial cells. Another finding was that the fall in muscle vascular resistance during nerve cold block was abolished during NOS inhibition when BQ-123 was given first (Fig. 2), but no such effect was observed with the reverse-order presentation of these antagonists (Fig. 1). The nerve-cold-block procedure allowed us to assess the contribution of sympathetic neural tone to the basal vascular resistance in skeletal muscle during control and during NOS inhibition and/or \( \text{ET}_A \)-receptor blockade. Accordingly, this observation suggested that there was little, if any, sympathetic contribution to skeletal muscle vascular tone during NOS inhibition when preceded by \( \text{ET}_A \)-receptor blockade. We are unaware of any evidence nor do our own results provide any insight into the mechanism(s) underlying this finding.

The third notable finding in our study was that the pressor response to sympathetic nerve stimulation was enhanced after NOS inhibition. \( \text{ET}_A \)-receptor blockade did not affect this response, suggesting that the augmentation was associated with other mechanisms related to NOS inhibition. Three alternative mechanisms for this finding might include 1) a smaller initial vessel diameter at the onset of sympathetic stimulation, 2) the absence of NO production induced by increased shear stress during sympathetic stimulation, and/or 3) the absence of NO production induced by \( \alpha_2 \)-adrenergic-receptor stimulation. Our data argue against the first possibility that the enhanced resistance changes during sympathetic nerve stimulation were due to a smaller initial internal vessel diameter in the absence of NO production (22). When vascular resistance was returned to control levels after \( \text{ET}_A \)-receptor blockade (Table 1) or when the increase in muscle vascular resistance with NOS inhibition was prevented by prior \( \text{ET}_A \)-receptor blockade (Table 2), the same enhanced constrictor response to sympathetic nerve stimulation occurred. In answer to the second possibility, under the experimental conditions of constant flow used in the present study, shear stress would be increased during sympathetic nerve stimulation (2). Increased shear stress results in an increased production of NO (5, 17), which would be expected to partially or totally offset the extent of vasoconstriction during sympathetic nerve stimulation observed in the control period. This buffering effect would be eliminated after NOS inhibition with a resultant enhanced pressor response to sympathetic nerve stimulation. Finally, with respect to the third possibility, an enhanced constrictor response to sympathetic stimulation during NOS inhibition has also been demonstrated in rat tail artery segments (28, 31), renal vascular bed in anesthetized dogs (7), and pithed rats (25). Interaction between the sympathetic and NO systems may occur by activation of \( \alpha_2 \)-receptors on endothelial cells by norepinephrine with the subsequent activation of NO production (24–26). If this occurred, the constrictor response to sympathetic nerve stimulation during the control period in the present experiments would have been offset to some degree by \( \alpha_2 \)-adrenergic-receptor activation of NO production. This buffering effect would have been eliminated after NOS inhibition. The exact nature and extent of these mechanisms in our experimental model remain unknown at this time.

To summarize, we used the combination of in vivo reversible nerve cooling and stimulation in conjunction with pharmacological inhibition to examine the effect of NOS inhibition and \( \text{ET}_A \)-receptor blockade on skeletal...
muscle vascular tone at zero, resting, and maximum sympathetic activation. The results demonstrated that endothelin contributed to resting vascular tone in skeletal muscle and that increased endothelin release was partially responsible for the rise in muscle vascular resistance with NOS inhibition at rest. Finally, the enhanced constrictor response to maximal sympathetic activation during NOS inhibition appeared to be due to the disruption of a specific interaction between NO and endothelin. These data indicated that NO release during maximal sympathetic activation acts to offset the degree of sympathetic induced vasoconstriction under resting conditions in skeletal muscle vasculature in vivo. We conclude that NO acts to modulate skeletal muscle vascular tone by both its inhibitory action on the production of endothelin and dilatory “buffering” of sympathetic vasoconstriction.

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