Effect of Acidosis on Contraction of Microvascular Smooth Muscle by \(\alpha_1\)- and \(\alpha_2\)-Adrenoceptors

Implications for Neural and Metabolic Regulation

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Our previous studies have identified that adrenergic regulation of large arterioles and venules in skeletal muscle uses both postsynaptic \(\alpha_1\)- and \(\alpha_2\)-adrenoceptors, whereas terminal arterioles appear to be subserved primarily by \(\alpha_2\)-receptors. Adrenergic constriction of terminal arterioles is known to be particularly susceptible to inhibition by increased tissue metabolic rate. The purpose of this study was to examine the influence of tissue acidosis on \(\alpha_1\)- and \(\alpha_2\)-adrenoceptor constriction of skeletal muscle microvessels to determine if this differential receptor distribution might have significance in neural-metabolic interactions. Intravital microscopy of rat cremaster skeletal muscle was used to obtain concentration-response curves (diameter changes) of large distributing arterioles (mean diameter, 100 \(\mu\)m), small precapillary arterioles (20 \(\mu\)m), and capacitance venules (150 \(\mu\)m) for addition to the tissue bath of \(\alpha\)-adrenergic agonists during normal pH (7.4) and during tissue bath acidosis (pH 7.1) produced by increasing bath P\(\text{CO}_2\). The following \(\alpha\)-agonists were used: phenylephrine (\(\alpha_1\)), B-HT 933 (\(\alpha_2\)), and norepinephrine (mixed \(\alpha_1/\alpha_2\)). Acidosis had no effect on baseline diameter of the three vessel types, indicating a lack of effect on “intrinsic tone.” Acidosis also had no effect on large microvessel sensitivity to phenylephrine but markedly reduced responses to B-HT 933. Acidosis had no effect on large arteriolar and venular sensitivity to norepinephrine but markedly decreased (\(\times 300\)) small precapillary arteriolar sensitivity. These data suggest that 1) \(\alpha_1\)- but not \(\alpha_2\)-adrenoceptor-mediated constriction of microvessels may be selectively sensitive to modest reductions in tissue pH, and 2) the prevalence of \(\alpha_2\)-receptors on terminal arterioles and the marked sensitivity of \(\alpha_2\) constriction to tissue acidosis may contribute to the particular susceptibility of neural constriction at this level of the microcirculation to metabolic inhibition. (Circulation Research 1990;66:1643–1657)

Adrenergic constriction of arterioles, particularly small terminal arterioles 10–30 \(\mu\)m in diameter, is opposed by increased tissue metabolic rate or decreased blood flow. During continuous nerve stimulation, large arterioles and small arteries in various tissues, including skeletal muscle, generally exhibit maintained constriction, whereas terminal arterioles evidence a much smaller sustained constriction after the initial peak response. The mechanism responsible for this “sympathetic escape” may involve inhibition of neural constriction by vasodilator metabolic “feedback” signals released during reduced flow and oxygen delivery. For example, increasing tissue oxygen tension inhibits escape, and the development of muscle hypoxia is resisted by an increase in functional capillary density before an increase in overall tissue blood flow. The latter study also suggests that terminal arterioles may be more sensitive to metabolic control signals associated with changes in tissue oxygenation. Attenuation of neural constriction by local metabolic mechanisms may be important to balance oxygen supply with oxygen demand. But the mechanisms by which metabolic signals attenuate neural constriction of microvessels—particularly terminal arterioles—remain unclear.

Based on sensitivity of selective agonists and antagonists, we recently observed in skeletal muscle that...
adrenergic constriction of microvascular smooth muscle uses both postjunctional \( \alpha_1 \) - and \( \alpha_2 \)-adrenoceptors.\(^5,^6\) Large arterioles and muscular venules possess both \( \alpha_1 \) - and \( \alpha_2 \)-adrenoceptors, whereas terminal arterioles appear to have only \( \alpha_2 \)-adrenoceptors. Studies from our laboratory\(^7\) suggest that this apparent heterogenous receptor distribution may have functional significance. Only \( \alpha_2 \)-adrenoceptors mediate constriction of large resistance arterioles during direct nerve stimulation, even though both adrenoceptor subtypes are present. In contrast, neural constriction of small arterioles, which are also innervated, is subserved by postjunctional \( \alpha_2 \)-receprors.

Given the greater sensitivity of terminal arteriole adrenergic constriction to metabolic inhibition\(^1,^4\) and our evidence for a differential reliance of large versus small arterioles on \( \alpha_1 \) - and \( \alpha_2 \)-adrenoceptors, respectively, we wondered whether a difference may exist in the sensitivity of \( \alpha_1 \) and \( \alpha_2 \) constriction to metabolic feedback signals such as CO\(_2\)/H\(^+\).

There is no agreement in the literature concerning this question. No difference was detected in the effect of systemic acidosis on pressor responses to \( \alpha_1 \) - and \( \alpha_2 \)-adrenoergic agonists in pithed rats.\(^8\) In other studies, systemic acidosis reportedly attenuated \( \alpha_2 \)-mediated and potentiated \( \alpha_2 \)-mediated responses.\(^9,^10\) In contrast, acidosis decreased \( \alpha_2 \) but not \( \alpha_1 \) constriction of canine metatarsal vein.\(^11\) Surprisingly, no studies have examined the direct effect of local tissue acidosis on \( \alpha_2 \)-adrenoceptor sensitivity in the microcirculation. This question is important because metabolic control signals such as CO\(_2\)/H\(^+\) function as major local regulators of microvascular smooth muscle tone. Thus, the purpose of this study was to determine the effect of a reduction in pH on \( \alpha_1 \) versus \( \alpha_2 \)-mediated constriction of skeletal muscle microvessels.

**Materials and Methods**

**Surgical Procedures**

Experiments were performed on sixty 6- to 7-week-old male Sprague-Dawley rats (mean body wt, 163±1 g) that were housed in a controlled environment (23±1°C) with food and water ad libitum. The animals were anesthetized with urethane and \( \alpha \)-chloralose (425 and 100 mg/kg, i.p.). Anesthetic supplements (20-40% of initial dose) were administered as needed to maintain light surgical anesthesia and were generally given during periods when data were not collected (i.e., “wash” periods); otherwise, the protocol was interrupted for at least 10 minutes after administration of anesthesia. Rectal temperature was maintained at 37°±0.5°C. Animals breathed room air spontaneously by tracheostomy. The left carotid artery was cannulated for measurement of mean arterial pressure and heart rate, which were displayed on an oscillograph.

The right cremaster muscle was acutely denervated via abdominal approach by transecting the right lateral cutaneous, iliohypogastric, ilioinguinal, and genitofemoral nerves. This produces complete denervation of the cremaster but has no effect on the sensitivity of the microvasculature to topically applied vasoactive agents.\(^12\) Denervation prevented variations in nerve activity and release of norepinephrine during anesthesia and prevented possible complications of interpretation presented by interaction of agonists with presynaptic \( \alpha_2 \)-adrenoceptors. The cremaster muscle was prepared for in situ microvascular observation as described previously.\(^5\) The muscle with intact circulation was suspended over an optical port in a 40-ml tissue bath. The bath was filled from a stock reservoir containing a modified Krebs’ solution (280–290 mosm, 34±0.5°C) that consisted of (mM) NaHCO\(_3\) 19.9, NaCl 118.5, KCl 4.7, CaCl\(_2\) · 2H\(_2\)O 2.55, KH\(_2\)PO\(_4\) 1.19, MgSO\(_4\) · 7H\(_2\)O 1.19, and dextrose 11.6 dissolved in demineralized water. Nitrogen and CO\(_2\) were bubbled through both the tissue bath and the Krebs’ stock reservoir to provide mixing and to maintain PO\(_2\) (15–30 mm Hg), Pco\(_2\) (35–45 mm Hg), and pH (7.4±0.05 or 7.1±0.05, achieved by varying the rate at which CO\(_2\) was bubbled through the solution). Based on the composition of the bicarbonate buffer, a Pco\(_2\) of 80 mm Hg is required to achieve a pH of 7.1. Tissue bath and stock solution pH were continuously monitored, and tissue bath temperature was maintained at the normal cremaster in situ temperature (34°C). Tissue bath and stock solution Pco\(_2\) was monitored by an indwelling oxygen macroelectrode (IL 113, Allied Instrumentation Laboratory, Lexington, Massachusetts).

The preparation was placed on the stage of a trinocular microscope (Nikon IC, modified, Garden City, New Jersey), and the cremaster was transilluminated (heat-filtered 20-W quartz-halogen). The microcirculation was viewed through a ×10 Nikon water-immersion objective (0.22 na). The image was displayed by a silicon target camera (model 4415, Cohu, Torrance, California) on a high-resolution monitor and stored on videotape. Vessel wall diameter (inner wall caliber) was measured either off-line with an electronic caliper\(^5\) from the monitor screen or in real-time with a digital image-analysis system (Force Computers, Munich, FRG, and Datascope, Inc., Peabody, Massachusetts). Both systems were calibrated in micron units at the end of each experiment with a stage micrometer. The video microscopic measurement system was accurate to ±1 μm. Total image magnification was varied from ×400 to ×1,220 with a zoom projection lens (Nikon) to provide an optimal vessel image. Video images were subjected in real-time to analog enhancement (model IV-530, FOR-A Co., Ltd., Tokyo, Japan) before digital enhancement to improve vessel wall contrast and contour for measurement of vessel diameter. In some experiments, a green filter (VG-9) was positioned in the illumination axis to further enhance microvessel contrast with the surrounding tissue. A time reference accurate to 0.01 second was recorded on each video field.

The preparation was allowed to equilibrate for approximately 30 minutes and was considered acceptable
if 1) mean arterial pressure and heart rate were stable, and blood pressure was \( \geq 80 \text{ mm Hg} \); 2) terminal arterioles in the area of study exhibited vasomotion (spontaneous rhythmic cycles of constriction and dilation at 5–50 cycles/min); and 3) no venous stasis, leukocyte adhesion, or petechial hemorrhages existed in the area of study. Surgical procedures required approximately 2 hours, and the subsequent experimental protocols were 2–3 hours in duration.

**Experimental Protocol**

**Experiment 1: Effect of acidosis on \( \alpha \)-adrenergic constriction of large arterioles and venules.** Microvascular measurements were made on either a first- or second-order arteriole and its paired venule, which were selected on the basis of anatomical position and image clarity. One vessel pair was studied in each animal. First-order arterioles (diameter, 128±5 \( \mu \text{m} \), \( n=26 \)) and venules (169±6 \( \mu \text{m} \), \( n=26 \)), which represent the largest central vessel pair, were observed approximately 0.5–1.0 cm beyond their point of entrance into the bathed cremaster. Second-order arterioles (82±5 \( \mu \text{m} \), \( n=22 \)) and venules (117±8 \( \mu \text{m} \), \( n=21 \)), defined as the first vessel pair to bifurcate from the central vessel pair, were observed approximately 0.3–0.7 cm beyond their point of bifurcation. Previous studies in this laboratory have determined that the adrenergic sensitivity of first- and second-order vessels does not differ significantly\(^5\); this was confirmed in the present study. Thus, the results for first- and second-order vessels have been combined and reported as two groups, represented as large arterioles and venules. The cremaster bath Krebs' solution contained at all times propranolol (10\(-6\) M), for blockade of \( \beta \)-adrenergic receptors, and cocaine (4×10\(-6\) M) and normetanephrine (10\(-5\) M), for blockade of neuronal and nonneuronal catechol-amine uptake mechanisms, respectively.\(^5,6\) Drug concentrations expressed here and elsewhere represent final bath concentrations.

The effect of tissue acidosis on \( \alpha \)-adrenoceptor-mediated constriction of large arterioles and venules was evaluated with concentration-response curves at pH 7.4 and 7.1 for the selective \( \alpha_2 \)-adrenoceptor agonist B-HT 933 (group 1, \( n=14 \)), the selective \( \alpha_1 \)-agonist phenylephrine (group 2, \( n=14 \)), and the mixed \( \alpha_1/\alpha_2 \)-agonist norepinephrine (group 3, \( n=10 \)). Only one agonist was evaluated per experiment. The protocol (Figure 1) was similar for all three groups. After the 30-minute stabilization period at the desired pH, vessel diameter was measured for a 5-minute control period (C1, Figure 1). A concentration-response curve (CRC1) was constructed by stepwise, cumulative addition of agonist to the cremaster bath. The concentration was increased in approximately half-log increments every 5 minutes. Previous studies\(^7\) indicated and present studies confirmed that 3-minute intervals were sufficient to obtain the maxi-
mal, steady-state response to a given concentration of these agonists when they were applied in approximately half-log increments starting from less than or equal to threshold concentrations. After completion of CRC1, the cremaster bath was changed three times over 10 minutes with Krebs’ solution that had been equilibrated to the pH desired for the second concentration-response curve (CRC2). The flow rate of CO2 was adjusted to maintain the desired new pH in the cremaster bath. An additional 15–20 minutes were allowed to pass before beginning CRC2 in order to permit vessels to return to control diameter.5,6 This 25–30-minute interval (“wash” period, Figure 1) ensured that the equilibration time at the new pH equaled the equilibration time before generation of CRC1. After a 5-minute control period (C0), CRC2 was obtained as before at the new pH. Whether CRC1 or CRC2 was obtained at pH 7.4 or 7.1 was randomized. Agonist effects were reversed during a second 25–30-minute wash period at pH 7.4. After return to control diameter (C0), a concentration of the agonist that produced an intermediate amount of constriction at pH 7.4 (EC50) was added, and the response was obtained over a 10-minute interval to determine if any alteration in preparation sensitivity had occurred over time.5,6,13 Maximal adrenergic constriction was then determined by a 5-minute exposure to a high dose of norepinephrine (10−5 M) previously determined to produce maximal large vessel constriction in this preparation.5 All concentration-response data were normalized to this maximal response.

The cremaster bath was again washed (two changes over 5 minutes, pH 7.4) and either nitroprusside (3×10−3 M) or papaverine (3×10−4 M) was added to the bath for 5 minutes to produce complete smooth muscle relaxation for determination of maximal diameter. Both vasodilators produce comparable maximal dilation in this preparation.13

Additional animals were studied as controls for group 1 (B-HT 933, n=4) and group 2 (phenylephrine, n=6) in order to determine if sensitivity of α2- or α1-mediated constriction decreased over the 2–3-hour duration of the protocols. Although the EC50 test dose performed near the end of all experiments provided a control for any change in sensitivity, we also evaluated two successive complete concentration-response curves generated without any intervening change in tissue pH. The protocol used for these control studies was identical to that described above, with the exception that pH was maintained at 7.4 throughout the entire experiment. Previous studies indicated no change in the norepinephrine concentration-response relation during a protocol of similar duration.5,13

**Experiment 2: Effect of acidosis on α-adrenergic constriction of small arterioles.** Twelve animals were studied for the effect of pH on small (“terminal”) third-order arteriolar sensitivity to norepinephrine. Arterioles that branched from second-order arterioles at approximately right angles and with diameters ≤50% of the parent arteriole were designated as third-order arterioles. These arterioles (diameter, 21±3 μm) were studied approximately 150–500 μm from their point of bifurcation. One arteriole was studied in each animal. These arterioles generally exhibited vasomotion (rhythmic cycles of spontaneous contraction and relaxation) during control and agonist treatments. Instantaneous and electronically averaged diameter values were obtained with an electronic caliper4 by continuous measurement of vessel diameter for 20-second intervals once each minute during off-line analysis of videotapes. The instantaneous and averaged caliper output were recorded on an oscillograph. The experimental protocol was similar to that described above and depicted in Figure 1 with the exception that only one concentration-response curve was generated in each experiment. After a 30-minute stabilization period and 10-minute control period at pH 7.4 (C0), the cremaster bath pH was maintained at either 7.4 or 7.1 for 15 minutes (C1) (randomized among experiments), and then a concentration-response curve was obtained for norepinephrine. After a 30-minute wash period and a 10-minute control period at pH 7.4, a concentration of norepinephrine that produces intermediate constriction at pH 7.4 (EC50) was applied for 10 minutes. In those experiments in which bath pH was maintained at 7.4 throughout, this EC50 response near the end of the protocol allowed detection of any change in adrenergic sensitivity over the duration of the experiment. In those experiments in which the concentration-response curve was obtained at pH 7.1, this later EC50 response served as an indication of within-experiment effect of pH 7.1 on small arteriolar response to norepinephrine. Maximal vasoconstriction of third-order arterioles was determined by a 5-minute period with bath norepinephrine concentration at 3×10−6 M.5 All concentration-response data were normalized to this maximal constriction. The bath was then changed twice over a 5-minute interval, and papaverine was added to obtain maximal arteriolar diameter.

**Data Analysis**

Measurements of vessel diameter were obtained at 1-minute intervals throughout the experiment, except during wash or equilibration periods. Measurements were made either on-line or during off-line analysis of videotape. Control diameter values represent the average of five measurements taken at 1-minute intervals over the last 5 minutes of the control period (no drugs). Unless otherwise indicated, values for agonist responses represent average diameter during the last 2 minutes of the test period. For construction of concentration-response curves, agonist responses are expressed as a percentage of the maximal response to norepinephrine: Response=(D−D0)/(Dmax−D0)×100, where D is the control diameter, D0 is the diameter produced by x concentration of agonist, and Dmax is the diameter obtained during maximal constriction with norepinephrine 1×10−5 M (large arterioles...
and venules) or 3 x 10^{-6} M (small arterioles). Concentration-response curves and EC_{50} values (agonist concentration needed to produce 50% of the maximal response) were derived from nonlinear, least-squares, sigmoid regression analysis of the concentration-response data. Based on the normal distribution of log concentrations, -log EC_{50} values were calculated for statistical comparison of agonist sensitivity.

Data were analyzed with paired and grouped t tests where appropriate. Analysis of variance and the Dunn-Bonferroni procedure were used for multiple comparisons. Least-squares linear regression analysis was performed on blood pressure and heart rate data. Results are expressed as the mean±SEM, with p<0.05 representing significance.

**Drugs**

All agonists were prepared daily in 10^{-3} M ascorbate saline. Propranolol HCl, cocaine HCl, and nitroprusside were dissolved in saline, normetanephrine bitartrate in ascorbate saline, and papaverine in distilled water; aliquots were frozen ≤ 6 weeks and thawed on the day of the experiment. Drugs were added to the 40-ml cremaster bath as 27–90-μl aliquots. The maximal cumulative concentration of ascorbate in the bath (8 x 10^{-6} M) has no effect on control microvessel diameter or responses to agonists.\(^5\,13\) The ascorbate added to the cremaster bath with drug aliquots is sufficient to prevent breakdown and degradation of catecholamine and B-HT 933 responses over the exposure periods that were used in the present studies.\(^5\,13\) Drugs were kept on ice in a dark container throughout the experiment. All drugs were obtained from Sigma Chemical (St. Louis, Missouri) with the exception of B-HT 933, which was donated by Thomae GmbH (Biberach an der Riss, FRG).

**Results**

**Effect of Acidosis on Responses of Large Arterioles and Venules to \(\alpha\)-Adrenergic Agonists**

**Control data.** Figure 1 shows a representative experiment that examined the effect of acidosis (pH 7.1) on \(\alpha_1\)-adrenergic receptor-mediated constriction induced by phenylephrine. This same protocol was used to examine the effect of acidosis on large microvessel constriction mediated by the selective \(\alpha_2\)-adrenergic agonist B-HT 933 and the mixed \(\alpha_1/\alpha_2\)-adrenergic agonist norepinephrine. Acidosis, as used here, refers to the pH of the tissue bath rather than the pH of the vascular wall interstitium, where pH may be greater than bath pH. Because CO\(_2\) is highly soluble in water and cell membranes, however, the increase in tissue bath P\(_{CO_2}\) will likely be followed by at least a directionally similar increase in the P\(_{CO_2}\) of cremaster interstitial fluid and a tendency toward acidosis in the vascular smooth muscle intracellular space.

Acidosis (pH 7.1) had no effect on baseline diameter. Arterioles were 102±5 μm at pH 7.4 and 106±5 μm at pH 7.1 (n=38); venules were 141±7 μm at pH 7.4 and 140±7 μm at pH 7.1 (n=37). Thus, baseline diameters of arterioles and venules during the three successive control periods (C\(_1\), C\(_2\), and C\(_3\)) were compared as one indication of preparation stability. These baseline diameters did not differ significantly (Figure 2). The maximal constriction elicited with a high concentration of norepinephrine (10^{-5} M) was similar among the large arterioles and venules for the three agonist groups (Figure 2) and was comparable to previous studies in this laboratory that did not involve changes in cremaster bath pH.\(^3\) Thus, exposure of microvessels to pH 7.1 had no effect on maximal vasoconstrictor responses obtained upon return of the cremaster bath to pH 7.4. The amount of basal (control) tone present in the vessels was indicated by comparison of nitroprusside- or papaverine-induced maximal dilation to the average diameter during the third control period (Figure 2). Maximal arteriolar diameter for the phenylephrine, B-HT 933, and norepinephrine groups was 21%, 17%, and 21% greater than control, respectively. These data, taken with the finding that control diameters did not change, indicate that a similar amount of basal smooth muscle tone was present in the arterioles in all three agonist groups, and that this tone was sustained over the duration of the experimental protocol. No statistically significant tone could be demonstrated in the venules. The finding that pH 7.1 had no effect on baseline diameter (see above) indicates that acidosis of pH 7.1 does not influence the intrinsic smooth muscle tone in these large microvessels.

Comparison of constriction to an intermediate concentration of agonist early in the experiment versus at the end of the protocol revealed a significant decrease in arteriolar sensitivity to phenylephrine (Figure 3). Subsequent control studies in which two full concentration-response curves were generated at pH 7.4 also revealed a significant reduction in the arteriolar, but not venular, sensitivity to phenylephrine (Figure 4, Table 1). No such decline in B-HT 933 or norepinephrine sensitivity was observed in the present study (Figures 3 and 4, Table 1) or in previous studies with norepinephrine.\(^5\) The apparent desensitization of phenylephrine-mediated arteriolar constriction (Figure 3) does not appear to be due to prior exposure of the tissue to pH 7.1 because the same desensitization is indicated in these control experiments in which the pH was maintained at 7.4 throughout the duration of the experiment (Figure 4, Table 1). This decreased sensitivity raised concerns regarding a possible influence of protocol duration and previous exposure of the tissue to high phenylephrine concentrations (during CRC\(_1\)) on phenylephrine responses obtained during CRC\(_2\). Desensitization of rabbit aorta to the \(\alpha_1\)-adrenergic receptor-mediated constrictor effects of epinephrine has also been observed.\(^14\) Thus, the effect of local tissue acidosis on large microvessel responses to the various agonists are presented for groupwise comparisons of only the first concentration-response curve (either pH 7.4 or 7.1) obtained in each experiment. However, results
with analysis of the effect of pH on the two within-experiment concentration-response curves were similar (not shown).

**Figure 2.** Control diameter ($C_1$, $C_2$, and $C_3$) during experiments for protocol shown in Figure 1. NE, maximal constriction to $10^{-5}$ M norepinephrine. Average (percent of $C_3$) maximal constriction given above the NE columns. Maximal diameter during nitroprusside (NP) or papaverine (PPV). Intrinsic tone indicated by blackened area and compared with $C_3$ for significance. Significance by analysis of variance and Bonferroni paired t test. Control diameters remained constant; maximal NE constriction and basal tone were comparable among the three agonist groups.

**Figure 3.** Response of large arterioles and venules to an intermediate dose of $\alpha$-adrenoceptor agonist tested at end of experiments (PE$_2$, BHT$_2$, NE$_2$) versus response obtained for same dose of agonist during concentration-response curve generated at pH 7.4 (PE$_1$, BHT$_1$, NE$_1$). PE, phenylephrine; BHT, B-HT 933; NE, norepinephrine. Response to agonists normalized to maximal response to high concentration of NE ($10^{-5}$ M). Average test dose of agonists (in moles): PE, $4.3 \times 10^{-8}$±$0.71 \times 10^{-8}$; B-HT 933, $2.7 \times 10^{-5}$±$0.54 \times 10^{-5}$; NE, $1.5 \times 10^{-7}$±$0.33 \times 10^{-7}$. Significance by paired t test. Only arteriolar response to PE showed evidence of desensitization.

**Effect of acidosis on $\alpha$-adrenoceptor sensitivity.** Figure 5 shows the effect of acidosis on large microvessel responses to the $\alpha$-adrenergic agonists. The data represent comparison of the first concentration-response curve of each experiment only. Maximal response and EC$_{50}$ values are presented in Table 2. Reduction of bath pH to 7.1 produced a significant dextral displacement of the concentration-response curve for $\alpha_1$-adrenoceptor–mediated constriction of both arterioles and venules. Acidosis did not affect constriction mediated by $\alpha_2$-adrenoceptors or mixed $\alpha_1/\alpha_2$ constriction with norepinephrine. As shown in Table 2, pH 7.1 significantly reduced (30-fold) B-HT 933 sensitivity ($-\log$ EC$_{50}$) of arterioles, but not venules; maximal responses were significantly reduced for both vessel types.

Relative to norepinephrine and phenylephrine, B-HT 933 gave significantly smaller maximal responses for both arterioles and venules (Table 2). This agrees with previous data from our laboratory.$^5$ It should be noted that because B-HT 933 does not produce 100% constriction, estimates of EC$_{50}$ values were made by normalizing the B-HT 933 concentration-response data against the response obtained in each experiment to the highest concentration of B-HT 933 ($3 \times 10^{-4}$ M) at pH 7.4. Hence, the B-HT 933 EC$_{50}$ value represents the concentration needed to produce 50% of the maximal constriction that could be induced by B-HT 933 at pH 7.4. This allows more accurate quantitative comparisons of $-\log$ EC$_{50}$ values for determination of pH effect on B-HT 933 sensitivity. But it should also be noted that, again to facilitate comparisons, the B-HT 933 concentration-response curve (Figures 4 and 5) and maximal response data
(Tables 1 and 2) are expressed as a percent of the maximal constriction induced in these vessels by a high concentration of norepinephrine (10^{-5} M).

**Effect of Acidosis on Terminal Arteriolar Responses to Norepinephrine**

Our previous results indicate that adrenergic constriction of small, third-order terminal arterioles is subserved predominantly by \( \alpha_2 \)-adrenoceptors.5–7 The results of the experiments described above for large arterioles and venules suggest that \( \alpha_2 \)-mediated constriction is significantly attenuated by pH 7.1, whereas constriction mediated solely by \( \alpha_1 \)-adrenoceptors or by norepinephrine acting on a mixed \( \alpha_1/\alpha_2 \)-receptor population is not altered. In a separate group, we studied the effect of pH 7.1 on norepinephrine-induced constriction of terminal arterioles. Venules were not compared in these studies because most third-order venules are devoid of smooth muscle.1 Local tissue acidosis (pH 7.1) profoundly shifted to the right (300-fold) the concentration-response relation of third-order arterioles for norepinephrine (Figure 6). Maximal response (normalized to constriction produced by 3x10^{-6} M norepinephrine at end of experiment [see "Materials and Methods"] and −log EC_{50} values of these terminal arterioles at pH 7.4 were 86±7 and 8.89±0.66, respectively; at pH 7.1 they were 40±13 and 6.55±0.56, respectively (p<0.025 for both −log EC_{50} and maximal response). This marked reduction in norepinephrine-mediated constriction of small arterioles at pH 7.1 contrasts with the lack of effect of acidosis on norepinephrine-

![Figure 4](https://circres.ahajournals.org/)

**Figure 4.** Control experiments in which two successive concentration-response curves (CRCs) were obtained for large arterioles and venules at pH 7.4 for B-HT 933 and phenylephrine (PE). Values normalized as a percent of maximal response to norepinephrine (NE) (10^{-5} M). Consistent with Figure 3, arteriolar response to PE showed evidence of desensitization. Significance by analysis of variance and paired Bonferroni t test. Additional statistical analysis in Table 1. Previous studies indicate no significant difference in two consecutive CRCs for large arterioles and venules constricted with NE.5,13

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<th>Table 1. Effect of Protocol Duration on Responses of Large Arterioles and Venules to Selective ( \alpha )-Adrenoergic Agonists</th>
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Values expressed as mean±SEM.

Phenylephrine −log EC_{50} derived from nonlinear regression analysis of concentration-response data normalized to norepinephrine 10^{-5} M; maximal response expressed as percent of response to this high norepinephrine dose. Because B-HT 933 does not produce maximal constriction, −log EC_{50} for B-HT 933 was estimated by nonlinear regression analysis of concentration-response data normalized to highest concentration of B-HT 933 (3x10^{-4}) (see text for details). CRC\(_1\) and CRC\(_2\), first and second concentration-response curves; both at pH 7.4. 

*\( p<0.05 \) for CRC\(_1\) versus CRC\(_2\). Significance by analysis of variance and Bonferroni.
mediated constriction of large microvessels (Figure 5) and is consistent with predominantly α1-receptors mediating the response of terminal arterioles to norepinephrine.\textsuperscript{5–7}

Consistent with the large vessel experiments, pH 7.1 had no effect on baseline diameter; control diameters did not change over the duration of the protocol; and papaverine-induced maximal dilation revealed the presence of significant resting (intrinsic) smooth muscle tone (Figure 7). The intrinsic tone present in these small arterioles was substantially greater than that in the large arterioles (Figure 2). In those experiments in which the concentration-response curve was determined at pH 7.4, reexposure to an intermediate concentration of norepinephrine (EC\textsubscript{50}) at the end of the protocol served as an indication of any effect of protocol duration on adrenergic sensitivity. As shown by the first two bars in Figure 8, there was no decline in sensitivity of the small arterioles at the end of the experiment (NE\textsubscript{b} vs. NE\textsubscript{e}). In those experiments in which the concentration-response curve was obtained at pH 7.1, constriction at the end of the protocol with an intermediate concentration of norepinephrine at pH 7.4 served as a within-experiment indication of the effect of pH on small arteriolar norepinephrine-mediated constriction. Consistent with the full concentration-response data (Figure 6), acidosis significantly but reversibly reduced norepinephrine-induced constriction (Figure 8).

For all animals studied (both large and small vessel experiments, \(n=60\)), the mean arterial pressure and heart rate at the beginning of the experiments were (mean±SEM) 99±2 mm Hg and 445±5 beats/min, respectively; at the end of the experiments, values were 100±2 mm Hg and 460±6 beats/min, respectively. Regression analysis indicated no significant change in these parameters over the duration of the experiments.

**Discussion**

The major finding of the present study was that CO\textsubscript{2}-induced acidosis (bath pH 7.1) selectively inhib-
ited \(\alpha_2\)-but not \(\alpha_1\)-adrenoceptor-mediated constriction of skeletal muscle microvessels. Large venular and especially large arteriolar constriction produced by a selective \(\alpha_2\)-adrenoceptor agonist (B-HT 933) was significantly reduced (30-fold, arterioles) at pH 7.1 (Figure 5). Adrenergic constriction induced in these large vessels by a selective \(\alpha_1\)-adrenoceptor agonist (phenylephrine) or by the mixed \(\alpha_1/\alpha_2\)-agonist norepinephrine was not affected. In contrast, norepinephrine contractile sensitivity of terminal arterioles, which depends primarily on \(\alpha_2\) stimulation,\(^5,7\) was profoundly reduced (300-fold) by acidosis. Because moderate increases in skeletal muscle activity can decrease tissue pH to 6.8,\(^15\) the level of acidosis that selectively decreased \(\alpha_1\)-mediated constriction of microvessels in this study was within physiological range.

An increase in PCO\(_2\) is accompanied by an increase in hydrogen-ion concentration and, thus, acidosis. Because pH was altered in this study by adjusting CO\(_2\), it is possible that the observed effects are in part due to direct action of CO\(_2\) independent of H\(^+\) effects. In conditions in which acidosis results from elevation of CO\(_2\), it is difficult to separate the effects of CO\(_2\) per se, from the effects of H\(^+\), and the present study does not include experiments designed for that purpose. Importantly, tissue acidosis can occur physiologically from accumulation of CO\(_2\) subsequent to reduction in tissue blood flow, or by increased production of CO\(_2\) during increased metabolic rate. Thus, manipulation of tissue pH by eleva-

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<th>Table 2. Effect of Acidosis on (\alpha)-Adrenoceptor–Mediated Constriction of Large Arterioles and Venules</th>
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<td><strong>Group</strong></td>
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<td>B-HT 933</td>
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Values expressed as mean±SEM.

Phenylephrine and norepinephrine \(-\log\text{EC}_{50}\) data derived from nonlinear regression analysis of concentration-response data normalized to norepinephrine \(10^{-5}\) M. Because B-HT 933 does not produce maximal constriction, \(-\log\text{EC}_{50}\) for B-HT 933 was graphically estimated from analysis of concentration-response data normalized to highest concentration B-HT 933 \(3\times10^{-4}\) M at pH 7.4 (see text for details). One experiment was excluded from calculation of B-HT 933 pH 7.1 \text{EC}_{50} value because it showed no response with acidosis. Maximal response for all data expressed as a percent of response to \(10^{-4}\) M norepinephrine.

\(p<0.05\) for results at pH 7.1 versus 7.4.

\(p<0.05\) for B-HT 933 versus norepinephrine at pH 7.4.

\(p<0.05\) for B-HT 933 versus PE at pH 7.4. Significance by analysis of variance and Bonferroni.
arteriolar constriction is mediated primarily by $\alpha_2$-adrenoceptors. Large arterioles and venules have both $\alpha_1$- and $\alpha_2$-receptors. Thus, our conclusion that $\alpha_2$- but not $\alpha_1$-mediated constriction of these large segments is attenuated by pH 7.1 is not complicated by a possible longitudinal gradient because this comparison is made at a single level (arteriole and paired venule) within the microcirculation. Therefore, it is reasonable to conclude that the strong sensitivity of small arteriolar norepinephrine-mediated constriction to acidosis results from the predominant reliance of this vessel segment on $\alpha_2$-receptors and the selective sensitivity of $\alpha_2$-mediated constriction to pH 7.1. Norepinephrine-mediated constriction of large arterioles is maintained during pH 7.1 because of the large $\alpha_1$ component of adrenergic constriction at this level and the insensitivity of $\alpha_1$-mediated constriction to acidosis. In addition, the lack of effect of pH 7.1 on the significant basal tone of either small or large arterioles suggests that any longitudinal PCO$_2$ gradient in our preparation was not significant enough to inhibit intrinsic tone in these small vessels.

It has been observed in several studies$^{17}$ of whole animal or regional vascular beds that $\alpha_2$-antagonists preferentially block pressor responses to exogenous (blood-borne) norepinephrine, whereas $\alpha_1$-antagonists preferentially inhibit responses to sympathetic nerve stimulation. Thus, it was proposed that postjunctional $\alpha_1$-receptors are located mainly near the neuroeffector junction and respond primarily to neurally released norepinephrine, whereas $\alpha_2$-receptors are located extrajunctionally and respond to circulating catecholamines.$^{17}$ Recent studies, however,$^{17,19}$ do not support this distinct anatomic receptor distribution. Evidence in canine hind limb$^{18,19}$ and rat tail artery$^{17}$ indicates that both $\alpha_1$- and $\alpha_2$-receptors respond to neural stimulation and, therefore, both may be located close to nerve varicosities. In canine saphenous vein, which also possesses both receptor types, it appears that $\alpha_2$-receptors are preferentially activated by neurally released norepinephrine.$^{17}$ Thus, it has been demonstrated in a variety of preparations in which $\alpha_1$- and $\alpha_2$-receptors coexist that either subtype may be preferentially innervated, or that both may be innervated. A recent study from our laboratory$^7$ using direct sympathetic stimulation of rat cremaster microcirculation indicates that $\alpha_1$-receptors are preferentially innervated on large arterioles that possess both receptor types. In contrast, neural constriction of small arterioles is mediated by $\alpha_2$-receptors. Thus, the present study, which indicates a selective sensitivity of $\alpha_2$-mediated constriction to acidosis, suggests that the heterogeneous distribution of $\alpha$-receptor subtypes across skeletal muscle microcirculation has functional importance for the integration of neural and metabolic control mechanisms.

A possible limitation of studies of the intact microcirculation concerns interpretation of terminal arteriolar responses to vasoactive agents applied to the whole microvascular bed. For example, large arteriolar constriction would be expected to decrease "downstream" small terminal arteriolar flow and

![Figure 8. Response of small arterioles to an intermediate (approximately EC$_{50}$) concentration of norepinephrine (NE) tested at pH 7.4 at end of experiments (NE$_2$) versus response obtained for same dose during concentration-response curve (CRC) (NE$_1$). Left panel: CRC determined at pH 7.4. EC$_{50}$ concentration chosen from CRC obtained in each experiment (mean±SEM: 2.6×10$^{-4}$±0.45×10$^{-4}$ M). Right panel: CRC determined at pH 7.1. NE concentration tested at end was always 1×10$^{-4}$ M, a concentration arbitrarily chosen before initiating studies. Significance by paired t test. *p<0.05.](image)
intravascular pressure. This could favor both metabolic and myogenic dilation and altered wall stress of terminal arterioles, and affect their sensitivity to vasoactive agents. Also, $\alpha_2$ constriction is more susceptible than $\alpha_1$ constriction to myogenic inhibition by reduced transmural pressure. But because acidosis did not affect norepinephrine-mediated constriction of large arterioles or venules in our preparation, and because pH 7.1 had no effect on resting tone of large or terminal arterioles (Figures 2 and 7), mechanical forces and metabolic factors experienced by the terminal arterioles during norepinephrine constriction should be similar at both pH 7.4 and 7.1. Thus, our interpretation of the effect of acidosis on adrenergic constriction of terminal arterioles cannot be explained by differences in wall stress or length-tension considerations at bath pH 7.4 versus 7.1.

It is also unlikely that the differential effect of pH on $\alpha_1$ and $\alpha_2$ constriction of large arterioles and venules is a consequence of differences in downstream terminal arteriolar responses to phenylephrine and B-HT 933. In other studies, concentration-response analysis of phenylephrine, B-HT 933, and UK14304 (an $\alpha_2$-agonist) indicated that small arteriolar responsiveness to phenylephrine is 25-fold lower than large arteriolar responses along the same vessel tree in the same animal, and appears to be largely a passive response to upstream constriction (i.e., low slope, bell-shaped curve). With $\alpha_2$-agonists, the opposite was observed, with 34-fold greater sensitivity of small versus large arterioles and evidence for active constriction of both vessel types (JE Faber, unpublished data, 1988). These observations are consistent with primarily $\alpha_2$-receptors on terminal arterioles. Thus, in the present study, phenylephrine should have produced minimal active constriction of terminal arterioles regardless of bath pH. However, addition to the bath of norepinephrine or B-HT 933 would likely have different effects on terminal arterioles depending on whether pH was 7.4 or 7.1. At pH 7.4, terminal arterioles would predictably constrict to these agonists to a greater degree than at pH 7.1 (Figure 5). Consequently, intravascular pressure and wall stress would be less for large arterioles and greater for large venules at pH 7.1 than at 7.4. This mechanical effect alone would favor a leftward shift of the large arteriolar curve and a rightward shift of the venular curve for these agonists at pH 7.1. This would in fact lessen the magnitude of any decrease in large arteriolar B-HT 933 sensitivity due to acidosis, but could artifically contribute to or produce a venular rightward shift at pH 7.1. But because no such shifts were evident for norepinephrine-mediated constriction of large arterioles, it is unlikely that the effect of acidosis on $\alpha_2$ constriction of large arterioles arises from downstream versus upstream mechanical differences. The decrease in venular sensitivity to B-HT 933 at pH 7.1, however, could have been produced indirectly by such a mechanism. Thus, compared with arterioles, acidosis may have less of an effect on $\alpha_2$-adrenergic constriction of venular smooth muscle. This is supported in the present study by an absence of effect of pH 7.1 on the venular EC$_{50}$ response to B-HT 933 (Table 2). A smaller sensitivity of constriction of venous smooth muscle to acidosis has also been reported previously.

In the denervated cremaster, both the large distributing arterioles and small terminal arterioles had significant basal tone during control as estimated by the extent of dilation to nitroprusside or papaverine (Figures 2 and 7). Comparison of Figures 7 and 2 indicates that the terminal arterioles had considerably greater basal tone. Tissue bath acidosis (pH 7.1) had no effect on this basal tone in either large arterioles or small terminal arterioles, which is surprising in light of several in vivo pump-perfused studies that show a generally augmented blood flow upon injection of acid solutions. It is possible that this difference is due to the use of acids rather than $\text{CO}_2$, or to a greater acidotic stimulus in the in vivo studies. It is also possible, however, that dilation in the in vivo preparations resulted from the effect of acidosis to decrease neural transmission and norepinephrine release, thus reducing tonic sympathetic activity in these neurally intact beds. The cremaster preparation in the present study is denervated. Indeed, Zooster et al show that when the sympathetic innervation of dog hind limb is removed or pharmacologically inhibited, flow increases less during infusion of acid solution than during infusion of the same solution in sympathetically intact preparations.

Under denervated conditions, basal tone in large and small terminal arterioles may be primarily dependent on intrinsic mechanisms. The absence of an effect of acidosis on the resting tone of large or terminal arterioles suggests that modest increases in tissue $\text{CO}_2/\text{H}^+$ (at least pH 7.1) do not contribute to metabolic control through inhibition of intrinsic tone, but may instead depend on selective inhibition of extrinsic $\alpha_2$-neurogenic tone. Thus, adrenergic constriction is attenuated at pH 7.1 by a selective effect on $\alpha_2$-mediated constriction with no effect on $\alpha_1$-mediated or basal tone. It is possible that metabolic feedback signals other than $\text{CO}_2/\text{H}^+$, or greater acidosis, may attenuate $\alpha_1$-mediated or intrinsic tone, thus contributing to attenuation of adrenergic constriction or general dilation during conditions of decreased flow or increased metabolic rate.

Our results demonstrate that acidosis selectively decreases $\alpha_2$-adrenoceptor–mediated constriction of skeletal muscle microvessels. These results are supported by evidence from a number of isolated large vessel studies. Edvinsson and Sercombe reported that pH 7.1 had no effect on resting basal diameter but reduced norepinephrine constriction of feline middle cerebral arteries. Feline middle cerebral arteries predominantly have $\alpha_2$-receptors. Norepinephrine-induced constriction of rabbit aorta ( $\alpha_2$-adrenoceptors) was not affected by pH 7.1 but was reduced at pH 6.8. Curro and Greenberg observed that $\alpha_2$- but not $\alpha_1$-mediated constriction of isolated canine metatarsal vein was reduced at pH 6.8. In a
recent study\textsuperscript{30} published after our results first appeared in abstract form,\textsuperscript{31} acidosis (pH 7.0) markedly reduced norepinephrine constriction in feline middle cerebral artery (a\textsubscript{r}), whereas norepinephrine constriction of rabbit pulmonary artery (a\textsubscript{r}) was not affected. These in vitro data agree with our findings in the microcirculation that acidosis selectively inhibits a2-adrenoceptor–mediated constriction. We hypothesize that local modulation of adrenergic control mechanisms by a CO\textsubscript{2}/H\textsuperscript{+} metabolic feedback signal may be conferred on a specific segment of the microcirculation, or in certain tissues themselves, by the relative contribution of a\textsubscript{r} versus a2-adrenoceptors to adrenergic regulation of that segment or tissue.

Our data and the in vitro findings cited above appear at variance with pithed animal studies in which systemic acidosis either had no selective effect on a\textsubscript{r} or a2-adrenoceptor–mediated constriction\textsuperscript{8} or attenuated a\textsubscript{r}-mediated responses and potentiated a2-mediated responses.\textsuperscript{9,10} However, interpretation of pressor responses to vasoactive agents in pithed animal experiments can be complicated by differences in effects on the heart and vasculature as well as secondary neural, humoral, and local effects produced by low baseline arterial pressure. Additionally, the systemic acidosis used in these studies likely alters a number of physiological systems that affect heart and vascular smooth muscle, which could complicate any comparisons with the direct effect of pH on smooth muscle.

In contrast with the present findings, a previous study of cremaster microcirculation\textsuperscript{32} observed a 10-fold reduction in large arteriolar sensitivity to norepinephrine when tissue bath conditions were set to pH=6.9 and P\textsubscript{CO2}=65 mmHg. Comparison of these findings with our data is complicated by the fact that the cremaster preparation was not denervated in the former study,\textsuperscript{32} so there was potential for action of endogenously released norepinephrine on presynaptic a2-receptors, and effects of acidosis on nerve activity and presynaptic release.\textsuperscript{25} Additionally, the greater degree of acidosis in the former study may have been sufficient to produce inhibition of norepinephrine constriction of large arterioles.

The mechanism responsible for the particular sensitivity of a2-mediated constriction to acidosis is unclear. It has been demonstrated that a large a\textsubscript{r} but no a\textsubscript{r}-receptor reserve exists in the arterial circulation of the pithed rat.\textsuperscript{33} If a similar difference in receptor reserve exists for cremaster arterioles, and if acidosis decreases the number of both functional a\textsubscript{r}- and a2-adrenoceptors, then the a\textsubscript{r} response, but not the a\textsubscript{r} response, might be more sensitive to acidosis. However, our data do not support the concept that a possible difference in a\textsubscript{r} versus a2-adrenoceptor reserve in cremaster microcirculation contributes to the selective sensitivity of a\textsubscript{r} versus a2-mediated constriction to acidosis. According to receptor theory,\textsuperscript{34} any intervention that decreases the number of functionally coupled receptors would result in a parallel, rightward shift in the concentration-response curve of a receptor population that has a reserve. There was no shift in either the large arteriolar or venular response to phenylephrine or norepinephrine at pH 7.1 (Figure 6). This suggests that pH 7.1 has no effect on the number of functional a\textsubscript{r}-adrenoceptors, and that the effect of pH to reduce a2-mediated constriction results from selective interaction with the a2-adrenoceptor or with postreceptor mechanisms resulting from a2-receptor occupation.

Acidosis could reduce a\textsubscript{r} responses by selectively decreasing a\textsubscript{r}-receptor affinity or number. It has been demonstrated that a reduction in pH from 8.0 to 6.8 reduces affinity of porcine brain a2-adrenergic receptors for the agonists epinephrine, norepinephrine, and UK14304.\textsuperscript{35} a2-Adrenoceptors may contain peptide or amino acid bonds that are labile and subject to acid hydrolysis.\textsuperscript{11} Thus, it is possible that pH 7.1 in our experiments could reduce a2-adrenoceptor–mediated constriction by changing the conformation of the a2 but not the a\textsubscript{r}-receptor and thereby selectively reduce a2-receptor affinity.

Acidosis could also alter postreceptor signaling mechanisms unique to a\textsubscript{r} contraction. It has been demonstrated in a number of in vitro studies of large veins and in vivo pressure-flow studies that activation of a\textsubscript{r}-adrenoceptors with full a\textsubscript{r}-agonists (e.g., phenylephrine and norepinephrine) relies on both influx and release of intracellular calcium to produce contraction, whereas a2-receptors may rely solely on influx of extracellular calcium.\textsuperscript{36} The mechanisms responsible for the receptor-specific Ca\textsuperscript{2+} influx appear to be different.\textsuperscript{36} There is evidence that acidosis may inhibit influx of calcium into nerve terminals,\textsuperscript{25} myocardial cells,\textsuperscript{37} and vascular smooth muscle.\textsuperscript{38–41} The entire extracellular calcium concentration-response curve for isolated arterial tissue\textsuperscript{41} is shifted to the right by acidosis. Krafte and Kass\textsuperscript{37} report that acidosis decreases calcium current through L-type channels in rat ventricular cells. Elevated extracellular calcium inhibits the vasodilator effects of acidosis on pial arteries\textsuperscript{39} and in perfused dog hind limb and gastrocnemius muscle.\textsuperscript{40} The authors of this latter study suggest that there may be competition between calcium and hydrogen ions for binding at one or more cellular sites such as the sarcoplasmic reticulum or contractile proteins of vascular smooth muscle. Because acidosis did not affect a\textsubscript{r}-mediated constriction in our study, it seems unlikely that calcium and hydrogen ions compete at intracellular binding sites common to both a\textsubscript{r}- and a2-receptor contractile mechanisms. It is possible, however, that the selective effect of acidosis to inhibit a\textsupscript{r}-mediated constriction may result from competition between hydrogen and calcium at a plasma membrane calcium channel, with subsequent reduction in calcium influx as suggested by Krafte and Kass.\textsuperscript{37} Data from our laboratory\textsuperscript{42} indicate that a2-adrenoceptors on skeletal muscle (cremaster) microvessels are more dependent than a\textsubscript{r}-receptors on influx of extracellular calcium for constriction. Thus, acidosis may inhibit a\textsubscript{r} constriction by H\textsuperscript{+} inhibition of calcium influx but, in the case of a full a\textsubscript{r}-agonist (phenylephrine) or mixed a\textsubscript{r}/a2-agonist
(norepinephrine), no inhibitory effect of pH 7.1 is seen because these agonists can produce constriction by release of intracellular calcium. Further studies are needed to determine the mechanism by which modest acidosis selectively inhibits \( \alpha \)-adrenergic constriction of microvascular smooth muscle.

The major site of vascular resistance is usually considered to be the small arterioles (<100 \( \mu \)m). However, large arterioles and even small arteries significantly contribute to total vascular resistance in skeletal muscles.\(^1\) For example, microvascular pressure measurements in rat cremaster, rat spinotrapezius, and cat tenuissimus muscles show that 60–70% of the total vascular resistance can be attributed to vessels proximal to small arterioles (e.g., larger than 3A); 15–20% of the network resistance resides in small arterioles; and the remaining fraction is confined to the capillaries and venules.\(^{45,44}\) Thus, in muscles such as cremaster, where a substantial amount of arterial pressure is dissipated in vessels proximal to third-order arterioles, large arterioles and small arteries contribute a greater fraction of the total network resistance in these vascular beds than do the small arterioles. In these tissues, dilation of the large vessels can substantially increase total blood flow, whereas dilation of small arterioles has much less effect on overall bed resistance and flow. But the small arterioles regulate capillary flow and, therefore, distribution of available flow within the tissue.

Integration of neural and metabolic regulation of microvascular smooth muscle is not well understood. In particular, it is not known why adrenergic constriction of the smallest arterioles is more sensitive to metabolic regulation. Our data suggest that modest tissue acidosis may preferentially attenuate norepinephrine constricting of terminal arterioles versus large arterioles because of inherent differences in \( \alpha \)-adrenoceptor distribution, innervation, and sensitivity to acidosis. Based on these data, we propose the following model for the interaction of \( \text{CO}_2/\text{H}^+ \) (and possibly other metabolic feedback signals, i.e., tissue \( \text{PO}_2 \), adenosine, osmolality) with \( \alpha \)-receptors on vascular smooth muscle of skeletal muscle microvessels (Figure 9): According to this model, large arteriolar constriction relies on a mixed adrenoceptor population, but preferentially on \( \alpha_1 \)-receptors, whereas the terminal arterioles have predominantly \( \alpha_2 \)-receptors.\(^{5–7}\)

The fine lines depict the adrenergic nerve net that invests these vessels. Vasodilator metabolites such as \( \text{CO}_2/\text{H}^+ \) increase in skeletal muscle during increased skeletal muscle activity or decreased oxygen availability. Changes in the interstitial concentration of these metabolites “feedback” by diffusion to the microvasculature where they oppose adrenergic constriction. Certain metabolites or metabolite concentrations may also directly inhibit vascular smooth muscle contractility. A predominance of \( \alpha_1 \)-receptors, preferential innervation of \( \alpha_1 \)-receptors, and minimal sensitivity of \( \alpha_1 \)-receptor-mediated constriction to acidosis could preserve adrenergic control of large arterioles during modest reductions in tissue pH. In contrast, dominance of terminal arterioles by \( \alpha_2 \)-receptors that are innervated and a particular sensitivity of \( \alpha_2 \)-mediated constriction to \( \text{CO}_2/\text{H}^+ \) (and possibly other metabolites) may make adrenergic constriction of these precapillary arterioles especially susceptible to metabolic inhibition during “mismatches” in oxygen supply and demand. Such a hierarchy might provide a control system that minimizes loss of reflex control of resistance and capacitance vessels, while maximizing the ability of local metabolic factors to override neural control of terminal arterioles and ensure adequate capillary flow during alterations in blood supply (e.g., during hypotension or hypoxemia) or tissue oxygen demand (e.g., during increased tissue metabolic rate). The prevalence of \( \alpha_2 \)-receptors on terminal arterioles may be responsible, in part, for the greater sensitivity of adrenergic constriction of terminal arterioles to metabolic regulation.

To summarize, modest reduction of tissue pH within the physiological range for active skeletal muscle\(^{15}\) significantly decreased \( \alpha_2 \)-mediated constriction of large venules and especially of large arterioles. Constriction of these vessels by \( \alpha_1 \)-adrenoceptors was
not affected, nor was large arteriolar and venular constriction with norepinephrine. In contrast, norepinephrine constriction of small terminal arterioles, which is largely dependent on α2-receptors, was markedly reduced by acidosis. These data suggest that increases in local CO2/H+ concentration produced by altered parenchymal tissue metabolic rate, oxygen delivery, or blood pH may directly influence adrenergic responsiveness of microvessels by a selective action on α2-mediated constriction.

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KEY WORDS • α-adrenergic receptors • vascular smooth muscle • microcirculation • norepinephrine • phenylephrine • hydrogen ion • B-HT 933
Effect of acidosis on contraction of microvascular smooth muscle by alpha 1- and alpha 2-adrenoceptors. Implications for neural and metabolic regulation.

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