Preservation of Venular but Not Arteriolar Smooth Muscle $\alpha$-Adrenoceptor Sensitivity During Reduced Blood Flow

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To compare arteriolar versus venular smooth muscle sensitivity to myogenic and metabolic inhibition during reduced blood pressure and flow, we measured the diameter of first-order venules (diameter, 230 $\mu$m) and arterioles (diameter, 156 $\mu$m) of the denervated, blood-perfused rat cremaster skeletal muscle that was suspended in a tissue bath. Sensitivity was determined for bath-added norepinephrine in the presence of yohimbine or prazosin to produce $\alpha_1$- and $\alpha_2$-adrenoceptor constriction, respectively, and for KCI to examine non-receptor-mediated sensitivity. To reduce venular pressure and flow, vasopressin, which constricts cremaster arterioles but not venules, was applied locally at a maximally effective concentration. This arteriolar constriction had no effect on venular sensitivity to $\alpha_1$-adrenoceptor and KCI-mediated constriction. Venular sensitivity ($-\log M EC_{50}$) to $\alpha_2$ and to KCI activation was 6.20±0.10 and 1.20±0.04 in the absence and 6.34±0.09 and 1.30±0.03 in the presence of arteriolar constriction, respectively. Venular sensitivity to $\alpha_2$ activation was actually greater during arteriolar constriction (6.25±0.11 in the absence of constriction versus 7.06±0.13 in the presence of constriction, $p<0.001$). In a second series, the effect of reduced cremaster perfusion pressure and flow on both arteriolar and venular sensitivity was examined by mechanically lowering cremaster inflow. Reduction of first-order arteriolar and venular flow by 82–85% attenuated arteriolar $\alpha_1$ and abolished $\alpha_2$ sensitivity but had no effect on venular adrenergic sensitivity; KCI sensitivity was increased. These data indicate that, in contrast to arteriolar smooth muscle, venular smooth muscle $\alpha$-adrenoceptor sensitivity is preserved during reduced pressure and flow and, thus, is little affected by metabolic and myogenic regulation. The selective depressant effect on arteriolar adrenergic but not KCI constriction suggests that myogenic/metabolic inhibition of arterioles is receptor specific. (Circulation Research 1991;69:1215–1225)

Reduced intravascular pressure and blood flow, acting by myogenic and metabolic mechanisms, respectively, attenuate contraction of arterial smooth muscle. These local mechanisms modulate neurally derived adrenergic tone of resistance vessels and are central to autoregulation of blood flow and tissue oxygenation.1,2 Several studies have demonstrated that small arterioles are more sensitive to metabolic and myogenic inhibitory signals than larger resistance vessels.3–11 However, the cellular mechanisms that underlie metabolic and myogenic inhibition of adrenergic contraction remain unclear. Using skeletal muscle as a microvascular model, we have found evidence for a selective distribution12 and innervation13 of $\alpha$-adrenoceptors, with large arterioles and small arteries served by both $\alpha_1$-adrenoceptors (dominant) and $\alpha_2$-adrenoceptors and small arterioles dependent on $\alpha_2$-adrenoceptors. This differential receptor influence, together with a greater sensitivity of $\alpha_2$- than $\alpha_1$-adrenergic constriction of arterioles to metabolic6–8 and myogenic inhibition14, may underlie the preferential local modulation of adrenergic tone in small arterioles when compared with larger resistance arterioles and arteries.

In comparison with the responses of precapillary vessels, the effects of increased metabolic and myogenic inhibitory signals on venular sensitivity to constrictor stimuli and on the regulation of capacitance and venous return have received less attention (see References 1 and 2). Examinations of the effects of
low tissue perfusion produced by hemorrhage, though a disturbance with complicated systemic effects, have suggested that venous smooth muscle may be less sensitive to vasodilator metabolites when compared with arterioles and arteries. In rat skeletal muscle, adrenergic constriction of venules, in contrast to arterioles, exhibits little or no myogenic inhibition during reduced transmural pressure. In that study, venules also behaved passively during increases in transmural pressure, suggesting that skeletal muscle venules may evidence little response to myogenic stimuli. These data are consistent with other reports demonstrating the absence of venular contractile responses to stretch. Thus, venular contractile mechanisms may be less susceptible to local metabolic and myogenic inhibition than resistance vessels.

The purpose of the present study was to test this hypothesis by determining the influence of local reduction of blood flow and perfusion pressure on venular sensitivity to constrictor stimuli. We examined the sensitivity of rat cremaster first-order venules and arterioles to \( \alpha_1 \) and \( \alpha_2 \)-adrenergic-induced and KCl-induced constriction during 1) selective maximal arteriolar constriction produced with local vasopressin application and 2) reduction in cremaster inflow created by stenosis of the supplying iliac artery. The results demonstrate a remarkable preservation or even enhanced venular sensitivity to adrenergic agonists during low-flow conditions that, in contrast, greatly inhibit arteriolar responsiveness. These distinct differences in the sensitivity of the resistance and capacitance vascular segments to local regulation may be important in maximizing autoregulation of oxygen delivery by resistance vessels while, at the same time, minimizing local interference with reflex regulation of venous return to the heart.

Materials and Methods

Surgical Procedures

Fifty-eight male, 6–7-week-old Sprague-Dawley rats (mean±SEM body weight, 164±2 g) were anesthetized with urethane and \( \alpha \)-chloralose (425 and 100 mg/kg i.m., respectively). Detailed descriptions of the methods used in this study are described elsewhere. All rats, the mean arterial pressure (left carotid cannula) was 101±2 mm Hg at the beginning of the experiments and 99±2 mm Hg at the end. The right cremaster muscle with intact circulation was suspended over an optical port in a 40-ml tissue bath filled with a modified Krebs’ solution. Nitrogen and CO\(_2\) were bubbled through both the tissue bath and the Krebs’ stock reservoir to provide mixing and to maintain tissue bath and stock solution PO\(_2\) (5–15 mm Hg), PCO\(_2\) (35–45 mm Hg), and pH (7.4±0.05). Bath PO\(_2\) was maintained lower than the normal value (20–30 mm Hg) for this preparation. This was done to minimize a possible contribution of bath oxygen to cremaster tissue PO\(_2\) during experiments designed to reduce vascular delivery of oxygen by arteriolar constriction or by arterial stenosis (see below). Tissue bath and stock solution pH and temperature were continuously monitored, and PO\(_2\) was periodically measured in each experiment. The microcirculation was viewed at \( \times400–1,220 \) magnification, and vessel diameter (inner wall caliper) was measured with a videomicroscopic digital image analysis system. Transillumination (420–600 nM band-pass) was confined to 10–20-second intervals once per minute during control periods and experimental interventions.

The right cremaster was acutely denervated by nerve transection to prevent endogenous release of norepinephrine (NE) and changes in release produced by interaction of agonists and antagonists with presynaptic \( \alpha \)-adrenoceptors. Propranolol (1 \( \mu \)M) was present in the cremaster bath at all times to block \( \beta \)-adrenoceptors. Thirty to 40 minutes was allowed to pass after suspension of the cremaster in the tissue bath to ensure equilibration. The preparation was examined before the start of the protocol and judged to be acceptable if 1) mean arterial pressure was stable and \( \geq 80 \) mm Hg, 2) terminal arterioles in the area of study exhibited vasomotion, and 3) no venous stasis, leukocyte adhesion, or petechial hemorrhages existed in the area of study during control periods. Experiments were terminated if these criteria could not be maintained.

Experimental Protocol

Microvascular measurements were made on the cremaster first-order arteriole (1A) and its paired venule (1V), and only one vessel pair was studied in each rat. 1As (control diameter=156±3 \( \mu \)m, \( n=58 \)) and 1Vs (control diameter=230±5 \( \mu \)m, \( n=58 \)) were observed \(-0.5–1.0 \) cm beyond their point of entrance into the bathed cremaster.

Arginine vasopressin (AVP, Quad Pharmaceuticals, Indianapolis, Ind.) was used to produce selective arteriolar constriction and reduce cremaster blood flow and oxygen delivery. A preliminary experiment was done to document the selectivity of AVP for constriction of 1A but not 1V (\( n=8 \) rats and 1A/1V pairs). Twenty minutes before the initial 5-minute control period, phentolamine (0.1 \( \mu \)M, CIBA-GEIGY, Summit, N.J.) and propranolol (1 \( \mu \)M) were added to the bath to block \( \alpha \)- and \( \beta \)-adrenoceptors. The selectivity of these antagonists has been determined previously; they were present to ensure that the AVP constriction did not involve indirect activation of adrenoceptors, since AVP can amplify adrenoceptor sensitivity of vascular smooth muscle. Response at each AVP concentration was obtained over a 10-minute interval, which was sufficient to produce a maximal sustained effect. After obtaining the response to the first three concentrations of AVP, within 20 seconds the bath was changed (to ensure stability of bath composition), and AVP, propranolol, and phentolamine were reintroduced; the last three AVP concentrations were then tested. Thereafter, the bath was changed four
times over a 30-minute “wash” interval to reestablish control diameters. After a second 5-minute control period, the concentration of AVP that had produced ~50% of the maximal response (EC\textsubscript{50}) was tested again over a 10-minute period to determine if sensitivity to AVP had changed by the end of the experiment. If necessary, the concentration was increased at 10-minute intervals until a 50% response was obtained. The bath was changed, and nitroprusside (NP, 3 x 10^{-5} M) was added for 10 minutes to produce complete smooth muscle relaxation for determination of maximal diameter.

**Experiment 1: Effect of reduction of cremaster flow by arteriolar constriction with AVP on venular \( \alpha \)-adrenoceptor and KCl sensitivity.** Thirty-one rats were studied in this experiment. In group 1 (\( n = 7 \)), the effect of arteriolar constriction on venular \( \alpha \)-adrenoceptor sensitivity was examined. Twenty to 25 minutes before the start of the first control period (\( C_1 \), Figure 2, top panel), yohimbine (10^{-6} M) was added to the cremaster bath to block \( \alpha \)-receptors and to select for NE activation of \( \alpha \)-receptors. Yohimbine (or prazosin, see below) remained in the cremaster bath throughout the protocol. After a control interval, AVP was applied in a concentration (3,000 microunits/ml) determined in the above preliminary study to produce maximal arteriolar but not venular constriction. After the steady-state AVP response was obtained over a 10–15-minute period, NE was cumulatively added in approximately half-log increments for 5-minute intervals. After a 30-minute wash period and a second control period (\( C_2 \)), AVP (3,000 microunits/ml) was again added. Fifteen minutes later, NE was retested at a concentration (3 x 10^{-7} M) that produced an approximately half-maximal venule constriction during the first concentration–response curve (CRC) to detect any change in adrenergic sensitivity over time. Ten minutes later, the bath was changed and NP was added to obtain maximal diameter. A separate group of six rats was studied as a control for group 1 to determine venule \( \alpha \)-adrenergic sensitivity (yohimbine was present throughout) in the absence of arteriolar constriction with AVP. As is commonly done, NE was used in conjunction with high concentrations of adrenoceptor antagonists to allow examination of adrenoceptor stimulation with the endogenous ligand.

In group 2 (\( n = 6 \)), the effect of arteriolar AVP constriction on venular sensitivity to \( \alpha \)-adrenoceptor stimulation was examined. The protocol was the same as for group 1 except that prazosin (10^{-7} M) was added in place of yohimbine to block \( \alpha \)-adrenoceptors. A separate control group (\( n = 7 \)) was studied to determine 1V \( \alpha \)-sensitivity (prazosin was present throughout) in the absence of arteriolar AVP constriction.

In group 3, KCl was used to examine the effect of arteriolar constriction with AVP on non–receptor-mediated constriction of venules. The protocol was similar to that for \( \alpha \)-adrenergic constriction, except control (no AVP) and experimental (AVP) curves were obtained in the same experiment. A KCl cumulative CRC was obtained by changing the tissue bath and adding different Krebs’ solutions with KCl substituted in equimolar amounts for NaCl. The Krebs solutions were maintained at standard bath conditions: pH 7.40, PO\textsubscript{2}=5–15 mm Hg, and 34.0°C. Phentolamine (50 \( \mu \)M) and propranolol (1 \( \mu \)M) were present at all times to block adrenoceptors. The tissue was exposed to each concentration of KCl-Krebs for 15 minutes, with diameter measurements taken over the last 5-minute interval. After a 30-minute wash period, a second CRC was obtained in the presence of 3,000 microunits/ml AVP as described above for adrenergic constriction. Whether AVP was present during the first or second CRC was randomized. After a second wash period, maximal diameter was obtained with NP.

**Experiment 2: Effect of reduction of cremaster flow by arterial stenosis on venular and arteriolar sensitivity.** In a second series of experiments (\( n = 15 \)), pressure and flow to the cremaster were reduced by stenosis of the right common iliac artery. This permitted alteration of metabolic and myogenic conditions in both the venular and arteriolar circuits by a nonpharmacological method. A pneumatic micro-occluder was placed around the iliac artery, with a Doppler flow probe located proximal to the occluder. A “control” NE CRC was obtained (in the presence of prazosin or yohimbine) as in the previous protocol. After a 30-minute wash period and a second control period, stenosis was adjusted to lower flow in the cremaster 1A and 1V while causing minimal change in diameter from the initial control (\( C_2 \)) diameter; a decrease in iliac artery flow velocity of ~75% was required to achieve this result. At the onset of stenosis, the 1A generally dilated, whereupon stenosis was increased until the diameter passively declined back to control. Stenosis had no effect on arterial pressure. After a 15–20-minute interval and a 5-minute control period during reduced flow, a second NE \( \alpha \)- or \( \alpha \)-adrenoceptor CRC was obtained. The stenosis was then reversed, the bath was changed, and NP was applied to determine maximal diameter. In three preliminary experiments, volume flows in the 1A and 1V were determined during stenosis and during AVP. Stenosis sufficient to reduce iliac artery velocity by 75% reduced 1A and 1V flow by 85±2% and 83±13%, respectively. Maximal arteriolar constriction with 3,000 microunits/ml AVP reduced 1A and 1V flow by 85±2% and 82±8%, respectively. Microvessel volume flow was determined by measurement of center-line red blood cell velocity with an optical velocimeter and by the equation \( Q_r = (V/1.6) \times (\pi D^2)/4 \), where \( Q_r \) is the total blood flow, \( V \) is the center-line red blood cell velocity, and \( D \) is the inside diameter of the vessel.

**Data Analysis**

In all experiments except during wash periods, vessel diameters were obtained at 1-minute intervals. Unless otherwise indicated, average values reported in figures and tables for diameters during control
periods represent averages of five measurements taken at 1-minute intervals. Values reported for agonist responses represent averages of two measurements taken at 1-minute intervals during the last 2-minute interval before a change in drug concentration. Agonist responses are expressed as percent of control diameter or as a percent of maximal response to NE, KCl, or AVP: response = (D_c - D_d)/(D_c - D_m)×100, where D_c is the control diameter, D_d is the diameter produced by x concentration of agonist, and D_m is the smallest steady-state diameter reached for each agonist response curve. In the stenosis protocol, responses are normalized to the maximal response produced during the control CRC. Data were analyzed with paired and grouped t tests where appropriate. Analysis of variance and Dunn-Bonferroni procedures were used when data were compared among more than two groups, and -log EC_{50} values were calculated as a measure of agonist sensitivity. EC_{50} values were derived from nonlinear least-squares regression analysis. Results are expressed as mean±SEM, with p<0.05 representing significance. Unless otherwise indicated, drugs were obtained from Sigma Chemical Co., St. Louis, Mo., and dissolved in saline, 10^{-3} M ascorbate saline (NE), or Krebs’ solution. All drugs were added to the 40-ml cremaster bath in 14–40-μl aliquots.

Results

Selective Arteriolar Constriction by AVP

AVP produced concentration-dependent constriction of arterioles (EC_{50}, 123±1 micromicrons/ml) but had no effect on venules (Figure 1). Diameters during C_l (before AVP CRC) versus C_2 (after CRC and 30-minute bath wash) did not differ for arterioles (143±6 versus 150±2 μm) or venules (199±9 versus 196±9 μm). The maximal response to AVP (3,000 micromicrons/ml) reduced arteriolar diameter to 50±3 μm. AVP (500 micromicrons/ml), when retested at the end of the experiment, reduced arteriolar diameter to a value (74 μm) similar to the diameter (64 μm) obtained for the same concentration during the CRC. Relative to C_l, NP produced significant (p<0.01, n=6) dilation of 1A (156±3 μm) but not 1V (196±10 μm). A second-order venule (n=8) was also simultaneously examined in each experiment; like 1Vs, second-order venules were unaffected by AVP (data not shown). Based on this preliminary study, a high concentration of AVP (3,000 micromicrons/ml) was used in subsequent experiments to produce selective, maximal constriction of arterioles. This permitted examination of the effect of reduced pressure and flow, in the absence of a change in venule baseline diameter, on venular reactivity.

Effect of Arteriolar AVP Constriction on Venular Reactivity

Figure 2 (top panel) shows results from a representative experiment. Control experiments were conducted in the absence of AVP. Maximal arteriolar constriction with AVP had no effect on a_{1}-adrenoceptor sensitivity of venules (Figure 2, bottom panel), and a_{2}-adrenergic sensitivity was actually increased (Figure 3, top panel). These results suggested that venular sensitivity to NE stimulation during reduced intravascular pressure and flow is preserved (a_{1}) or enhanced (a_{2}). To determine if these findings are specific to a_{2}-adrenoceptor constriction or are evident as well for “direct activation” of the contractile apparatus, KCl was used as a receptor-independent, depolarizing stimulus. Like adrenoceptor stimulation, KCl constriction of venules was preserved in the presence of AVP (Figure 3, bottom panel).

Baseline data for the adrenergic sensitivity–AVP experiments are presented in Table 1. For the venules, there was no difference in control diameters within a given group, nor between control versus AVP a_{1} or a_{2} groups. In the presence of high AVP, maximal responses to the highest NE concentration (NE_{MR} in Table 1) were augmented compared with the control groups. Maximal responses in the presence of AVP were also enhanced when the data were analyzed as percent of control (p=0.04, a_{1} 1V group; p=0.0001, a_{2} 1V group). The greater maximal response to NE during AVP means that the a_{1} and a_{2} CRC during AVP would be shifted slightly more to the right if responses were given in absolute change in diameter; however, normalization aids statistical analysis by excluding variance that arises because of different control diameters that vary among animals. Relative to the initial NE (NE_{1}) CRC, there was no change in response to an intermediate concentration of NE when tested at the end (NE_{2}) of the experiments (Table 1, NE_{2} versus NE_{1}); however, a_{2} sensitivity declined modestly at the end of the experiments. Venules had no intrinsic tone, as indicated by the failure of NP to dilate venules beyond C_l diameters. In the KCl experiments, venule diameters during C_l (240±12 μm), during the control period after steady state reached with AVP (C_{AVP}) (254±12 μm), and during C_l (241±11 μm) were not significantly different. The maximal constriction produced

Figure 1. Graph showing effect of vasopressin (AVP) on large arterioles and venules. Values are mean±SEM for the number of vessels (n); one arteriole and one venule were studied in each rat. Arteriolar responses are normalized as percent of maximal response to AVP obtained for each rat; venular responses are normalized as percent of control (before AVP) diameter. EC_{50} for arterioles is 123±1 micromicrons/ml.
by the highest concentration of KCl was similar during control (184±11 μm) versus AVP (179±13 μm). Diameter during NP (252±15 μm) was not significantly different from C1. These baseline data validate the comparison of CRCs between the control and AVP groups.

Baseline arteriolar data are given in Table 1. Consistent with the effect on venules, in the presence of AVP a small amount of additional arteriolar constrictions occurred with exposure to NE, as reflected in the slightly greater NE(50) (Table 1) during AVP versus control (also significant for α2; p=0.03 when normalized as percent of control). All but one arteriolar group tended to dilate to NP (Table 1), although only one of these groups exhibited statistically significant intrinsic tone. In the KCl experiments, arteriolar diameters during C1 (160±7 μm) and C2 (145±9 μm) were not significantly different. Diameter in the presence of AVP alone (C0AVP) was 38±2 μm. Unlike venules, the maximal arteriolar constrictions produced by the highest concentration of KCl was greater (p<0.05) during AVP (20±4 μm) versus control (43±5 μm). Diameter during NP (175±6 μm) was significantly different from C1, indicating the presence of intrinsic tone.

Effect of Arterial Stenosis on Venular and Arteriolar Sensitivity

Experiments were conducted with stenosis of the iliac artery to provide a nonpharmacological (i.e.,
non-AVP) method to reduce cremaster pressure and flow. It is important to note that this design also allowed a direct comparison of the effect on large arteriolar versus large venular reactivity in the same experiment. Stenosis significantly attenuated arteriolar sensitivity to $\alpha_1$ stimulation and completely abolished $\alpha_2$-mediated arteriolar constriction (Figures 4 and 5). In contrast, venular sensitivity for both adrenoceptor populations was unaffected by stenosis (Figures 4 and 5). Stenosis also had no effect on KCl sensitivity of venules, and in contrast to adrenoceptor stimulation, arteriolar sensitivity to KCl was enhanced during stenosis (Figure 6).

Baseline data for the stenosis experiments are given in Table 2. Control diameters of venules and arterioles within a group were not significantly different ($C_1$ versus $C_2$). Stenosis had no effect on resting venule diameter, although arteriolar diameter during stenosis was slightly smaller in the $C_1$ group. Maximal responses to agonists in the absence and presence of stenosis (MR$C_1$ and MR$ST$, respectively, on Table 2) were not significantly different within any group; although as seen in Figure 5, during stenosis, arterioles exhibited no response to $\alpha_2$ stimulation. Arteriole but not venule groups exhibited intrinsic tone, as evidenced by dilation to NP relative to initial control diameters ($C_1$).

**Discussion**

Our laboratory has shown that local autoregulatory mechanisms modulate $\alpha_1$- and $\alpha_2$-adrenergic constriction of arterioles differently. $\alpha_2$-Adrenoceptor--

### Table 1. Baseline Diameters and the Effect of Arteriolar Constriction With Vasopressin on Venular Adrenergic Sensitivity

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<th>$C_{AVP}$</th>
<th>$C_2$</th>
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Values are mean±SEM for the number of vessels ($n$). See Figure 2 for protocol. $C_1$, first control period; $C_{AVP}$, control period after steady state reached with 3,000 microunits/ml vasopressin (AVP); $C_2$, control period after bath wash; NE$\text{MR}$, maximal response to norepinephrine (NE) attained during concentration–response curve; NE$C_1$, and NE$C_2$, constrictor responses to $3 \times 10^{-3}$ M NE during the concentration–response curve (NE$C_1$) and to the same concentration tested at the end of the experiment (NE$C_2$); NP, $3 \times 10^{-3}$ M nitroprusside.

$\dagger p<0.05$ vs. $\alpha_1$ control value for NE$\text{MR}$; $\ddagger p<0.01$ vs. corresponding value for NE$C_1$; $\S p<0.001$ vs. $\alpha_2$ control value for NE$\text{MR}$; $\|$ $p<0.05$ vs. corresponding value for NE$C_1$; $|| p<0.05$ vs. corresponding value for $C_1$.

![Figure 4](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.69.5.1220/-/DC1/fig4.png)

**Figure 4.** Recording of an experiment examining the effect of reduced blood flow produced by arterial stenosis on venular (IV) vs. arteriolar (IA) smooth muscle sensitivity to $\alpha_1$- and $\alpha_2$-adrenoceptor (\(\alpha_2\) shown here, with prazosin present) and KCl-induced constriction. $\Delta B$, bath change; NE, norepinephrine; NP, nitroprusside. Protocol is similar to experiment 1 in "Materials and Methods." $C_0$, $C_1$, and $C_2$ are the control periods before the generation of the first concentration–response curve, arterial stenosis, and a second concentration–response curve conducted in the presence of stenosis, respectively. NP was tested to determine the intrinsic vascular tone during control.
mediated constriction of arterioles exhibits much greater susceptibility than \( \alpha_1 \) constriction to inhibition by acidosis,\(^6\) reduced oxygen delivery or increased demand,\(^7,8\) and decreased transmural pressure.\(^14\) The greater sensitivity of \( \alpha_2 \) constriction of arterioles may relate to the reliance of \( \alpha_2 \) constriction on \( \text{Ca}^{2+} \) influx via ligand-gated, dihydropyridine-sensitive \( \text{Ca}^{2+} \) channels\(^21\) and an inhibitory action of these metabolic and myogenic stimuli on \( \text{Ca}^{2+} \) influx.\(^7,8,14\) Muscular venules, like arterioles, possess both \( \alpha_1 \) and \( \alpha_2 \)-adrenoceptors.\(^12\) However, unlike arterioles, \( \alpha_2 \) constriction of venules is not sensitive to inhibition by calcium channel antagonists at comparable concentrations,\(^21\) and venules exhibit little myogenic behavior.\(^14\) Thus, we hypothesized that, compared with arterioles, both \( \alpha_1 \) and \( \alpha_2 \) constriction of venules might be less susceptible to inhibition during reduced blood flow and perfusion pressure. Consistent with this hypothesis, in the present study reduction of venular flow by \( \sim \)80% with either precapillary constriction by AVP or iliac artery stenosis had no effect on venular \( \alpha_1 \) or \( \alpha_2 \)-adrenergic constriction. However, consistent with our previous studies, iliac stenosis decreased arteriolar \( \alpha_1 \) and abolished \( \alpha_2 \)-adrenoceptor sensitivity. Thus, under the present experimental conditions, adrenergic constriction of venules, unlike arterioles, was not susceptible to inhibition by local autoregulatory mechanisms.

To gain insight into the mechanism for this differential sensitivity between arterioles and venules and adrenoceptor types, we also examined the effect of reduced flow on KCl-induced constriction. Elevating extracellular potassium produces direct (i.e., non-receptor-mediated) activation of smooth muscle by depolarization and augmentation of voltage-sensitive \( \text{Ca}^{2+} \) channel conductance.\(^22\) Like adrenergic sensitivity, venule KCl sensitivity was unaffected during reduced flow by AVP or stenosis. However, while stenosis attenuated arteriolar adrenoceptor responsiveness, sensitivity of arterioles to KCl was actually increased. The observation that arteriole sensitivity to KCl was enhanced while adrenoceptor sensitivity was strongly attenuated suggests that metabolic and myogenic signals may exert their inhibitory actions on adrenoceptor coupling in arterioles at a level proximal to the contractile proteins.

**Methodological Considerations**

A key assumption in this study is that the procedures used were able to reduce cremaster blood flow and intravascular pressure sufficiently to produce a metabolic and myogenic disturbance within the venular (IV) environment that was comparable to that obtained in the arteriolar (1A) environment. Several
observations support this assumption. Vasopressin was used to pharmacologically lower flow and pressure in the venules by selectively constricting precapillary vessels. Flow was also mechanically reduced by stenosis of the iliac artery to allow comparison of arteriolar versus venular sensitivity to reduced pressure–flow and to circumvent potential pharmacological complications of AVP (see below). Importantly, flows in the 1A and 1V were decreased equally by 80–85% for both vessels in both protocols. This suggests that the metabolic disturbance in the 1V environment was at least comparable to that in the 1A and may have been greater, given the location of the 1V downstream from the 1A. Bath PO₂ was reduced to ~50% of normal for this tissue to ensure that bath oxygen would not circumvent the reduction in vascular oxygen delivery produced by the interventions. We have no direct evidence for the degree of metabolic disturbance produced by the hypoperfusion protocols. Other studies using venous stenosis (which avoids decreases in pressure) indicate that a reduction of blood flow of this magnitude to resting skeletal muscle would produce significant metabolic inhibition of arteriolar smooth muscle.²⁷

We did not measure 1V pressure and can only estimate the magnitude of the decrease in either protocol. However, pressure in the 1V of the cremaster averages 7–8 mm Hg.²³ In a study by House and Johnson,²⁴ a reduction in cat sartorius flow of 80% was found to cause a 50% decrease in pressure of similarly sized 1Vs. Also, constriction of the cremaster vasculature with an intermediate concentration of NE (0.1 μM) lowered 1V pressure by 23% (from 7.4 to 5.7 mm Hg). In that study,²³ the 1V was also constricted by NE, which would produce less of a pressure drop in the 1V than with AVP. Thus, it seems likely that the interventions used in the present study decreased venular pressure significantly. Our observation that 1V diameter did not change when venular flow (and presumably pressure) was decreased by either AVP or iliac stenosis is completely consistent with the behavior of in situ venules reported by several other groups,²⁵,²⁶ perhaps because the walls of skeletal muscle venules might be prevented from passively collapsing by the surrounding tissue.

![Graph showing the effect of arterial stenosis on venular (1V) vs. arteriolar (1A) responses to KCl-induced constriction. Values are mean±SEM for the number of vessels (n); one arteriole and one venule were studied in each rat. Responses are normalized to the maximal response obtained in the control concentration-response curve.](image)

**FIGURE 6.** Graphs showing the effect of arterial stenosis on venular (1V) vs. arteriolar (1A) responses to KCl-induced constriction. Values are mean±SEM for the number of vessels (n); one arteriole and one venule were studied in each rat. Responses are normalized to the maximal response obtained in the control concentration-response curve.

**TABLE 2.** Baseline Diameters for Large Arterioles and Venules and the Effect of Iliac Artery Stenosis on Microvessel Sensitivity

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>C₁</th>
<th>C₂</th>
<th>MRc</th>
<th>MRst</th>
<th>NP</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>C₁</td>
<td>C₂</td>
<td>MRc</td>
<td>MRst</td>
<td>NP</td>
</tr>
<tr>
<td>Venule</td>
<td></td>
<td>C₁</td>
<td>C₂</td>
<td>MRc</td>
<td>MRst</td>
<td>NP</td>
</tr>
<tr>
<td>a₁</td>
<td>5</td>
<td>243±11</td>
<td>245±11</td>
<td>237±10</td>
<td>120±9</td>
<td>132±8</td>
</tr>
<tr>
<td>a₂</td>
<td>5</td>
<td>245±11</td>
<td>243±13</td>
<td>246±15</td>
<td>161±10</td>
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<td>205±17</td>
<td>203±18</td>
<td>151±10</td>
<td>146±17</td>
</tr>
<tr>
<td>Arteriole</td>
<td></td>
<td>C₁</td>
<td>C₂</td>
<td>MRc</td>
<td>MRst</td>
<td>NP</td>
</tr>
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<td>162±10</td>
<td>133±9⁴</td>
<td>155±6</td>
<td>55±3</td>
<td>55±4</td>
</tr>
<tr>
<td>a₂</td>
<td>5</td>
<td>150±7</td>
<td>136±11</td>
<td>146±13</td>
<td>74±6</td>
<td>...</td>
</tr>
<tr>
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<td>5</td>
<td>132±12</td>
<td>138±9</td>
<td>139±9</td>
<td>47±3</td>
<td>35±2</td>
</tr>
</tbody>
</table>

Values are mean±SEM for the number of vessels (n). See Figure 4 for protocol. C₁ and C₂, first and second control periods, respectively; Cst, control period after stenosis; MRc and MRst, maximal response (MR) during control (C) concentration–response curve and during stenosis (ST); NP, 3x10⁻³ M nitroprusside.

* p<0.01 and † p<0.05 vs. corresponding value for C₁.
AVP is known to augment responsiveness of vascular smooth muscle to catecholamines, although whether this effect is different for α1 or α2 constriction is not known. The lack of an effect of AVP on skeletal muscle venules has been noted previously, perhaps owing to an absence of AVP receptors coupled to contractile mechanisms. However, in certain other tissues, such as splanchic organs, venules are very sensitive to AVP. It is unlikely that any interaction of AVP with adrenergic mechanisms could have occurred to account for the lack of venule inhibition, since KCl responses were also not inhibited during AVP. More important, a similar lack of inhibition of venular adrenoceptor sensitivity in the presence of clear arteriolar inhibition was observed when flow was reduced by iliac artery stenosis. However, the conditions produced in the IV by AVP versus stenosis were not identical, as evidenced by differences in α2 sensitivity relative to control during AVP (α2 sensitivity increased) and stenosis (α2 sensitivity remained unchanged). The basis for this difference is not apparent. However, the marked flow reduction produced by stenosis would be expected to cause dilation of terminal arterioles, while AVP strongly constricts the entire precapillary network. Despite these differences, the similar finding of selective preservation of venular adrenoceptor sensitivity obtained with two different methods of flow reduction suggests that adrenergic constriction of venules is resistant to local inhibition.

Relevance to Other Findings

With the exception of some specialized tissues (e.g., bat wing), venules exhibit little or no basal tone or myogenic constriction to increases in transmural pressure (see Reference 14). There is also evidence that venular smooth muscle may be less sensitive to metabolic inhibition. Pressure-independent reduction of cremaster blood flow by 30% via iliac vein stenosis had no effect on venular adrenergic sensitivity, whereas arteriolar α2 stimulation was depressed. Also, acidosis reduced α2-adrenoceptor sensitivity (EC50) of arterioles but had no significant effect on venules. In vitro data for large veins support these findings, and in vivo studies indicate that venous smooth muscle (capacitance response) is less sensitive than arterial to adrenergic inhibition during decreased flow, decreased PO2, and increased osmolarity.

The mechanisms whereby venular but not arteriolar smooth muscle retains sensitivity to adrenergic stimulation during reduced pressure–flow remain speculative. Venular smooth muscle cells may have a relative dirth of stretch-activated cation channels, rendering them less susceptible to myogenic inhibition. Differences in cellular metabolism and mechanisms of excitation–contraction coupling could exist and minimize venular sensitivity to metabolic inhibitory signals such as low oxygen and pH. It is also possible that, relative to arterioles, venules may normally be in a hyperpolarized state, since, unlike arterioles, venules exhibit little basal tone. Because reduced pressure and tissue oxygen both hyperpolarize smooth muscle of resistance arteries, but not venular adrenoceptor reactivity may, in turn, be inhibited during reduced pressure–flow. The preservation of venular and arteriolar constriction to KCl suggests that the smooth muscle contractile apparatus, per se, is not inhibited by local metabolic or myogenic signals. Venular sensitivity to KCl, like adrenergic sensitivity, was unaffected by reduced flow, whereas sensitivity to KCl was actually increased. The mechanism for this difference is unknown.

It is well known that α-adrenoceptors are coupled to ligand-gated Ca2+ channels. Occupation of α2-adrenoceptors results in influx of extracellular Ca2+, whereas α1-receptor stimulation promotes both Ca2+ influx and release from intracellular stores. Previous studies from our laboratory have shown that acidosis selectively attenuates α2 constriction of arterioles by possibly inhibiting Ca2+ influx with much less effect on venular α2 constriction. Interestingly, unlike arterioles, venular α2 constriction was insensitive to inhibition by calcium channel antagonists. Thus, different α-adrenoceptor coupling mechanisms or modes of action of local inhibitory signals between arteriolar and venular smooth muscle may confer differential sensitivity of arterioles and venules to local inhibitory signals.

Physiological Relevance

Compared with resistance vessels, capacitance vessel smooth muscle is required to function in a low pressure and low oxygen environment that normally experiences further reductions in pressure and metabolic disturbances during precapillary constriction and/or increased tissue metabolic rate. Since the venous circulation is downstream from arterioles and represents a small portion of vascular resistance, sensitivity of capacitance vessels to metabolic and myogenic controls would have little consequence for autoregulation of tissue blood flow, oxygen tension, or capillary pressure and would actually interfere with reflex regulation of venous return. Differential sensitivity of adrenergic constriction of resistance vessels and precapillary sphincters, but not capacitance vessels, to local myogenic and metabolic modulation would allow neural–local adjustments in vascular resistance to be optimized to achieve control of both arterial pressure and local tissue oxygen, while maintaining independent reflex control of venous return. For example, during normal physiological increases in precapillary resistance and decreased tissue flow (e.g., sympatheoexcitation), the lack of inhibition of venular adrenoceptor sensitivity by the attendant reduction in venule pressure and flow would preserve reflex venoconstriction and control of venous return.

The potential significance of capacitance vessel resistance to local inhibition is also evident during pathophysiological conditions that reduce oxygen de-
livery, such as hemorrhagic hypotension and hypoxia. For example, Mellander and Lewis\textsuperscript{9,10} found that during hemorrhagic hypotension reflex adrenergic constriction of precapillary sphincters in cat hind limb was inhibited first, followed in time by inhibition of resistance vessels. This is consistent with our evidence\textsuperscript{6–8,12,13} that α\textsubscript{2}-adrenoceptors are dominant on precapillary sphincter vessels and very sensitive to local inhibition, whereas larger resistance vessels have a greater reliance on α\textsubscript{1}-adrenoceptors, which are less sensitive to local inhibition. However, capacitance vessel responsiveness to sympathetic nerve stimulation was preserved much longer than for resistance vessels during hemorrhage.\textsuperscript{9,10} Sympathetic constriction of resistance vessels would lower capillary pressure and favor increased capillary reabsorption to help restore circulating volume. As a short-term response in hypotension, sustained venular constriction reinforces this compensatory mechanism by facilitating venous return. During prolonged hypotension, however, a maintained increase in postcapillary resistance would favor a decrease in the precapillary/postcapillary resistance ratio and, combined with the progressive depression in resistance vessel sensitivity to adrenergic constriction, would favor increased filtration and exacerbate the situation. This may be one of the contributing factors to the irreversible phase of hemorrhagic shock.\textsuperscript{16}

To summarize, reductions in microvascular pressure and flow by precapillary constriction with AVP or by upstream arterial stenosis had no effect on the sensitivity of venular smooth muscle to adrenergic or KCl-induced constriction. In contrast, arterial stenosis markedly attenuated α\textsubscript{1} and abolished α\textsubscript{2} constriction of arterioles. Arteriolar sensitivity to KCl, however, was actually enhanced during stenosis. These data suggest that a fundamental difference exists in the sensitivity of α-adrenoceptor constriction of resistance and capacitance vessel smooth muscle to inhibition by metabolic and myogenic control mechanisms. This difference may be important in maximizing autoregulation of oxygen delivery by precapillary sphincters and resistance vessels, while at the same time minimizing local interference with reflex regulation of venous return.

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