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Microvascular Effects of Atrial Natriuretic Factor: Interaction With $\alpha_1$- and $\alpha_2$-Adrenoceptors

James E. Faber, David R. Gettes, and Daniel P. Gianturco

The cremaster skeletal muscle of anesthetized rats was denervated and extended with intact circulation into a tissue bath. Intravital microscopy was used to measure microvessel diameter at three different anatomical levels within the microcirculation: large distributing arterioles (control diameter = $100 \pm 7 \mu m$), large capacitance venules ($147 \pm 8 \mu m$), and small terminal arterioles ($17 \pm 1 \mu m$). Norepinephrine (NE) was added to the cremaster bath to produce intermediate reductions in diameter of large arterioles and venules (55% and 38% of maximum constriction, respectively). In the presence of NE tone, bath-added atrial natriuretic factor (ANF) produced concentration-dependent dilation of both arterioles and venules. Arteriolar IC$_{50}$ = $18 \text{ pmol}$ and IC$_{50}$ = $1.2 \times 10^{-10} \text{ M}$; venules exhibited similar sensitivity. However, the highest ANF concentration examined ($10^{-7} \text{ M}$) only reversed NE-induced tone by 70%. In a second large vessel group ANF completely reversed constriction induced by the $\alpha_1$-adrenoceptor agonist, phenylephrine, in the presence of $5 \times 10^{-7} \text{ M}$ yohimbine. However, vessels constricted with the $\alpha_2$-receptor agonist UK-14,304 (in the presence of $10^{-8} \text{ M}$ prazosin) were insensitive to ANF. A third group of terminal arterioles, which possess considerable spontaneous “intrinsic” tone, were studied in the absence of $\alpha_2$-receptor agonists. Significant dilation occurred at $>10^{-7} \text{ M}$, and the maximal response was only 25% of complete dilation with adenosine. These data indicate that ANF exhibits a high potency for reversal of $\alpha_1$-adrenoceptor-mediated constriction of large arterioles and venules. Constriction produced by $\alpha_2$-adrenoceptor occupation or by nonadrenergic “intrinsic” mechanisms appears to be insensitive to ANF. We propose that the ability of ANF to reduce microvascular resistance depends on the relative contribution of $\alpha_1$-, $\alpha_2$-, and intrinsic vasoconstrictor components to the prevailing level of smooth muscle tone. Differences in these components among regional circulations and between arterial and venous smooth muscle may contribute to the systemic hemodynamic pattern produced by ANF. (Circulation Research 1988;63:415–428)

Mammalian cardiac atrial myocytes have recently been shown to possess secretory granules that contain peptides with potent diuretic and natriuretic activities that have been termed atrial natriuretic factor (ANF) (for reviews see deBold and Cantin and Genest). The amino acid sequence of these peptides has been determined, the structure of the ANF gene defined, and peptides that mimic the action of endogenous ANF have been synthesized.1-3 Recently, it has been shown in vitro that atrial myocytes synthesize a 126-amino acid pro-ANF that is derived from a larger prepro-ANF. Pro-ANF is subsequently converted to a 28-amino acid $\alpha$-ANF that is the predominant circulating form in rats and humans.1,4 The primary stimulus for secretion of pro-ANF appears to be distension of the atria secondary to elevation in atrial pressure.5,6 It has been proposed that ANF may function importantly in body fluid and cardiovascular regulation, through actions at several different renal and cardiovascular loci to lower plasma volume and arterial and venous pressures. Although not fully understood, ANF may alter renal excretory function through effects on renal hemodynamics and tubular function, and inhibition of renin, aldosterone, and possibly vasopressin release.2

There is considerable disagreement concerning the extrarenal site and mechanism of action by which ANF lowers arterial pressure during acute administration. Although atrial peptides have been
shown in vitro to relax vascular smooth muscle of certain large central vessels (e.g., aorta and renal artery). ANF has little or no effect at physiologically relevant concentrations on smaller peripheral arteries.7-11 Furthermore, the relation of these findings to the in vivo acute hypotensive effect of ANF has not been established, and the mechanism for the hypotension is unclear. Results from in vivo studies indicate that hypotension during acute administration of ANF, even in anephric animals, is associated with a reduction in cardiac output rather than peripheral resistance.12-15 However, concomitant activation of neurohumoral reflexes may obscure a direct vasodilatory effect of ANF on resistance vessels. The reduction in cardiac output has been attributed to a decrease in preload, leading to the proposal that ANF may be a selective venodilator.13,15,16 In contrast, other evidence has led to the suggestion that ANF may instead reduce venous return by venous constriction.17 However, ANF has extremely weak inhibitory actions on constriction of large veins studied in vitro,7 which does not support either possibility.

Vessels in the microcirculation constitute important loci for regulation of many functions of the peripheral circulation. However, little is known concerning the possible effects of ANF on the functional segments of the microcirculation: the distributing arterioles (control of tissue flow and peripheral resistance), the terminal arterioles (regulation of functional capillary surface area), and the muscular venules (control of venous capacitance). The purpose of the present study was to determine whether ANF can exert direct actions at these specific vascular levels within the microcirculation. The microvasculature of skeletal muscle is a major component of total vascular resistance and capacitance, was examined using intravital microscopy. Since regulation of skeletal muscle microves sel tone appears to be dependent on both postjunctional $\alpha_1$- and $\alpha_2$-adrenoreceptors,18 and on local mechanisms,19,20 we examined whether ANF might exert selective actions on intrinsic versus adrenoceptor subtype-specific contraction of microvascular smooth muscle.

Materials and Methods

Surgical Procedures

Thirty-four male, 6- to 7-week-old Sprague-Dawley rats (mean body weight ± SEM = 164 ± 3 g) were used in these studies. Animals were anesthetized with urethane and $\alpha$-chloralose (425 and 100 mg/kg i.p.), and received supplemental anesthesia (30-40% of initial dose) approximately 130 minutes after the initial administration and at 70-minute intervals thereafter to maintain light surgical anesthesia. Experimental protocol were interrupted for at least 10 minutes after administration of anesthetic supplements before proceeding with the experiment. Rectal temperature was monitored continuously and maintained at 37 ± 0.5°C throughout the surgery and experiment. Animals breathed room air spontaneously via tracheostomy. The left carotid artery was cannulated for measurement of mean arterial pressure and heart rate (Gould Biotach, Cleveland, Ohio), which were monitored on an oscillograph (Gould) and recorded at 5-minute intervals. For all animals, the mean arterial pressure and heart rate at the beginning of the experiments were (mean ± SEM) 103 ± 2 mm Hg and 457 ± 9 beats/min, respectively; at the end of the experiments values were 100 ± 3 mm Hg and 462 ± 7 beats/min, respectively. Regression analysis revealed that these parameters did not change significantly over the 2-3.5-hour duration of the experiments.

The right cremaster muscle was prepared for in situ microvascular observation as described previously.18 The muscle with intact circulation was suspended with silk sutures over an optical port in a 40-ml tissue bath. The bath was filled from a stock reservoir containing a modified Kreb’s solution (280-290 mosm, 34 ± 0.5°C) that consisted of (mM) 25.5 NaHCO$_3$, 112.9 NaCl, 4.7 KCl, 2.5 CaCl$_2$·2H$_2$O, 1.19 KH$_2$PO$_4$, 1.19 MgSO$_4$·7H$_2$O, and 11.6 dextrose 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 tissue bath and stock solution P$_O_2$ (15-30 torr), P$_{CO_2}$ (35-45 torr), and pH (7.4 ± 0.05). Tissue bath and stock solution pH and temperature (34°C, cremaster in situ temperature) were continuously monitored via indwelling probes, and P$_O_2$ was periodically measured in each experiment (IL 113 O$_2$ analyzer).

The preparation was placed on the stage of a trinocular microscope (Nikon IC, modified, Garden City, New Jersey) and the cremaster was transilluminated. The microcirculation was viewed through a ¥10 Nikon water immersion objective, and the image was displayed via a closed-circuit camera (Cohu 4415 silicon target, Torrance, California) and stored on videotape (Sony 2600) for off-line analysis of vessel diameter. Vessel diameter (inner wall caliber) was measured either off-line with an electronic caliper from the screen of a monitor (Sony CVM 131), or in real-time with a digital image analysis system (Force Computers, FRG; Datacube, Inc, Peabody, Massachusetts). Both systems were calibrated in micron units at the end of each experiment with a stage micrometer. The video microscopic systems were accurate to ± 1 μm. Total image magnification was varied from ¥400-×1220 with a zoom projection lens (Nikon) to provide an optimal vessel image. A time reference was recorded on each video field. Video images were subjected in real-time to digital (Force-Datacube) and analog enhancement (model IV-530, FOR-A Co. Ltd, Japan) 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.

The right cremaster muscle was acutely denervated via abdominal approach by transecting the right hypogastric, genitofemoral, ilioinguinal, and lateral cutaneous nerves. These nerves constitute the primary and accessory routes for cremaster innervation in rat. Previous studies have demonstrated that a denervation less extensive than this (hypogastric transection) produced complete cremaster denervation. In that study, local lidocaine blockade of the distal cut end of the cremaster nerves at their point of entry in the cremaster did not produce additional microvessel dilation beyond that produced by hypogastric transection. In our study, denervation was used to prevent variations in nerve activity and release of norepinephrine during anesthesia and to prevent possible complications of interpretation presented by interaction of agonists and antagonists with presynaptic α-adrenoceptors. Thirty-four minutes were allowed to pass after suspension of the cremaster in the tissue bath to insure equilibration. The preparation was examined prior to the start of the protocol and judged to be acceptable if 1) mean arterial pressure and heart rate were stable, and blood pressure was ≥80 torr, 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.

**Experimental Protocol**

In each experiment, microvascular measurements were made on either a first- or second-order arteriole and its paired venule, or on an unpaired third-order arteriole. Only one vessel pair (or unpaired third-order arteriole) was studied in each animal. First-order arterioles (mean diameter ± SEM = 127 ± 16 μm, n = 6) and venules (183 ± 15 μm, n = 6), which represent the largest paired, central cremasteric arteriole and venule, were observed approximately 0.5–1.0 cm beyond their point of entrance into the bathed cremaster. Second-order arterioles (mean diameter ± SEM = 90 ± 6 μm; n = 20) and venules (133 ± 8 μm, n = 20) consisted of the first vessel pair to bifurcate to the left or right from the central vessel pair, and were observed approximately 0.3 to 0.7 cm beyond their point of bifurcation. Our previous studies have indicated that the adrenergic sensitivities of first- versus second-order vessels do not differ significantly, nor do responses to other vasoactive stimuli; in the present study sensitivity of the two vessel orders to adrenergic agonists, ANF and vasodilators were also similar. Thus, as in previous studies, we have combined results for first- and second-order vessels into one group of large arterioles and venules (i.e., distributing arterioles and capacitance venules).

Arterioles that branched from second-order arterioles at approximately right angles were designated as third-order arterioles (mean diameter ± SEM = 17 ± 1 μm, n = 13 from eight animals). Terminal arterioles were studied approximately 150–500 μm from their point of bifurcation. In five of these eight animals, responses of two vessels were recorded sequentially on videotape during the experiment by movement each minute of the objective between two image fields. Unlike large distributing arterioles or capacitance venules, terminal arterioles exhibited vasomotion (rhythmic cycles of spontaneous contraction and relaxation that are characteristic of this vessel type) during control and exposure to ANF. Instantaneous and electronically averaged diameter values for terminal arterioles were obtained with an electronic caliper by continuous measurement of vessel diameter for 30-second intervals once each minute during analysis of videotapes. The instantaneous and averaged caliper output were recorded on an oscillograph. Previous studies have established that control diameters and microvascular sensitivity to norepinephrine (NE) remain unchanged over the 2.0–3.5-hour duration of the present experiments; this was confirmed in the present study (see "Results").

**Experimental 1: Effect of ANF on adrenergic constriction of large arterioles and venules.** Twenty-six animals were studied for the effect of ANF on large arterioles and venules (one vessel pair per animal). Group 1 (n = 12 animals) consisted of animals in which vascular tone in the acutely denervated cremaster was induced by NE. After the 30–40-minute stabilization period, vessel diameter was measured for a 10-minute control period. Thereafter, adrenergic tone was set by addition to the cremaster bath of NE (mixed α₁/α₂-agonist). Drug concentrations given below represent final concentrations in the cremaster bath. The concentrations of NE (range = 3–30 × 10⁻⁸ M; mean ± SEM = 9.3 ± 2.2 × 10⁻⁸ M) and the other α-agonists used below were chosen, based on previous studies, to produce an intermediate level of arteriolar constriction (approximately 50% of maximal response). In the present studies, this constriction was established during a 10-minute measurement interval after exposure to a single drug concentration; it was sometimes necessary to add additional agonist and extend the measurement interval by 10 minutes to achieve an intermediate level of constriction. Previous studies have demonstrated that steady-state constrictor responses to intermediate concentrations of α-agonists are achieved within a 10-minute interval (usually within 5–6 minutes) after local application and are maintained for at least 40 minutes. Control experiments performed in the present study confirmed these findings (see "Results").

After achievement of an intermediate amount of constriction, the bath was changed (within 20 seconds) with re-addition of NE at the previously determined intermediate concentration. Thereafter, the steady-state response was obtained during a final 10-minute period of observation, fol-
ollowed by cumulative addition of ANF in log increments \((10^{-12} - 10^{-7} \text{ M})\) for 10-minute intervals at each concentration. Preliminary experiments indicated and the present studies confirmed that 10 minutes is sufficient time for a maximal effect to be obtained at each concentration of ANF. The bath was then changed five times over a 40-minute "wash" interval to allow removal of drugs from the tissue and reestablishment of control diameters. After a second 10-minute control period, NE was added in the same intermediate concentration used in the previous ANF concentration-response curve determination, and the response was obtained over a 10-minute interval. This was done as a control to determine if noradrenergic sensitivity had changed over the duration of the experiment.

Synthetic human ANF [102-126] was used in the present study. This peptide has equal vasorelaxant and natriuretic potency with the 28-amino acid peptide,\(^2\) which appears to be the predominant circulating form of ANF in the rat and human.\(^4,25,26\)

To determine whether significant nonspecific binding of ANF might occur to decrease the free concentration of the peptide in the cremaster bath, in five of the above experiments albumin (0.1% BSA fraction V, Sigma Chemical, St. Louis, Missouri) and Polypep (0.025%, Sigma) were added to the bath at all times during the experiment. There was no significant difference in the sensitivity (EC\(_{50}\)) for the effect of ANF on NE-induced tone in these experiments compared with the other seven animals; thus the data were pooled and albumin and Polypep were not used in subsequent experiments.

Group 2 (\(n=10\) animals) experiments were similar to Group 1 with the exception that microvascular tone was induced by selective stimulation of either \(\alpha_2\)- or \(\alpha_1\)-postjunctional adrenoceptors. Twenty to 25 minutes before the start of the control period, yohimbine \((5 \times 10^{-7} \text{ M})\) was added to the cremaster bath to block \(\alpha_2\)-adrenoceptors. After control measurements were obtained for a 5-minute interval, the selective \(\alpha_1\)-agonist phenylephrine \((\text{PE})\) was added in a concentration \((3.1 \pm 0.2 \times 10^{-6} \text{ M})\) known from previous experiments\(^18\) to produce an intermediate level of arteriolar constriction, and the steady-state response was obtained over a 10-minute interval. In several experiments the measurement period was extended to 15 minutes for both PE and UK-14,304 (UK, see below) to ensure that 10 minutes was sufficient to obtain a steady response. ANF was then cumulatively added \((10^{-11} - 10^{-7} \text{ M})\) for 10-minute intervals at each concentration. The bath was then washed (as above) over a 40-minute period, and prazosin \((10^{-8} \text{ M})\) was added 20-25 minutes before the start of a second 5-minute control period to block \(\alpha_1\)-adrenoceptors. Thereafter, the response to the selective \(\alpha_1\)-agonist was determined over a 10-minute interval, followed by successive ANF concentrations. Exposure to PE (in the presence of yohimbine) or UK (in the presence of prazosin) during the first versus second ANF concentration-response sequence was randomized among experiments.

Yohimbine and prazosin are selective, competitive antagonists of \(\alpha_1\)- and \(\alpha_2\)-adrenoceptors, respectively.\(^27\) Our previous experiments in cremaster have established that these incubation times and concentrations of yohimbine and prazosin produce specific blockade of \(\alpha_2\)- and \(\alpha_1\)-adrenoceptors, respectively, with no detectable blockade of the opposite adrenoceptor subtype.\(^18,22\) Phenylephrine and UK-14,304 are potent, highly selective agonists for \(\alpha_1\)- and \(\alpha_2\)-adrenoceptors, respectively.\(^27\)

After exposure to the final NE test concentration in the Group 1 experiments and the last ANF concentration in the Group 2 experiments, the bath was changed and either nitroprusside \((3 \times 10^{-5} \text{ M}, n=14)\) or papaverine \((3 \times 10^{-4} \text{ M}, n=12)\) was added to the bath for 5 minutes to produce maximal smooth muscle relaxation for determination of maximal diameter. In six of these animals, both nitroprusside and papaverine were added sequentially (random order) for 5-minute intervals each. In these experiments both agents dilated large arterioles to the same diameter \((104 \pm 3 \mu\text{m} \text{ for papaverine versus } 103 \pm 4 \mu\text{m} \text{ for nitroprusside})\). Thus, maximal diameters reported in "Results" represent combined responses to papaverine and nitroprusside and the average response to both agents in experiments where both were tested.

As a control for Groups 1 and 2, a group of four additional animals was studied to determine if adrenergic constriction was maintained constant over the duration of exposure to ANF. The protocol was identical to that for Group 2, with the exception that the ANF vehicle only (Krebs solution) was added at successive intervals during PE or UK constriction.

**Experiment 2: Effect of ANF on small terminal arterioles.** As observed previously\(^18,22\) the first and second order vessels studied in the above groups exhibited a modest amount of "intrinsic" tone after acute denervation (arterioles, 13-29% constriction from maximally relaxed diameter; venules, 7-12%) (see "Results"). As reported in "Results," the data from the large vessel studies suggested that ANF may have little effect on this intrinsic tone. To examine this possibility more carefully, a fourth group (eight animals) of experiments were conducted in which only small terminal arterioles with vasomotion were studied, since our previous studies\(^18,22\) and work of others\(^19\) have demonstrated that terminal arterioles possess a much higher degree of intrinsic tone than large microvessels. After a 30-40-minute stabilization period, the diameter of the selected arteriole was measured during a 5-minute control period. ANF was then cumulatively added at 5-minute intervals in approximately half-log increments. After a subsequent 40-minute wash period and return to control diameter, adenosine \((10^{-4} \text{ M})\) was added to produce smooth muscle relaxation for determination of maximal diameter and...
calculation of intrinsic tone. In several of these experiments, addition of nitroprusside or papaverine during adenosine treatment did not produce additional vasodilation.

Data Analysis

In all experiments except during wash periods vessel diameters were obtained at 1-minute intervals either on-line or during off-line analysis of videotape. Unless otherwise indicated, average values reported in the figures for diameters during control periods (no agonists or antagonists present) and average responses to ANF, vasodilators, and α-agonists and antagonists represent averages of five measurements taken at 1-minute intervals over the last 5 minutes of the test period. Control data for large arterioles and venules were analyzed with paired t tests. Analysis of variance was used as an initial test for an effect of ANF. For construction of ANF concentration-response curves, the percentage inhibition of agonist-induced tone was expressed as follows: percent inhibition = \( \frac{(D_s - D_{ag})}{D_s} \times 100 \), where \( D_s \) = the average diameter determined at each ANF concentration, \( D_{ag} \) = the average diameter during agonist alone, and \( D_c \) = the average control diameter. \(-\log\) ANF concentration-response data were made linear by double reciprocal transformation of the data. ANF IC\(_{50}\) values (ANF concentration needed to produce 50% inhibition of the agonist constriction) were calculated as a measure of the agonist sensitivity to ANF. The IC\(_{50}\) were derived from linear regression of double reciprocal plots of the concentration-response data. Concentration-response curves were obtained by standard linear regression, as was the relationship between percentage agonist constriction and sensitivity (IC\(_{50}\)) to ANF. Least-squares linear regression analysis was also performed on blood pressure and heart rate data, and on resting adrenergic constriction versus ANF sensitivity. The effect of ANF on terminal arteriolar diameter was examined with Dunnnett's procedure. Results are expressed as the mean ± SEM, with \( p<0.05 \) representing significance.

Drugs

Stock solutions of norepinephrine (Sigma) were prepared fresh daily in \( 10^{-3} \) M ascorbate saline. Phenylephrine (Sigma, dissolved in ascorbate saline), UK-14,304 (Pfizer, Kent, England; dissolved in ascorbate saline), prazosin (Pfizer, dissolved in Krebs solution), yohimbine (Sigma, dissolved in Krebs solution), nitroprusside (Sigma, dissolved in saline), and papaverine (Sigma, dissolved in distilled water) were frozen and stored at \(-20^\circ C\) for no more than 6 weeks. Stock solutions of albumin and Polypep (Sigma) were dissolved daily in Krebs solution. Synthetic human ANF [102-126] (Wy-47,663, Wyeth Laboratories, Philadelphia, Pennsylvania) was dissolved in ascorbate saline and stored at \(-20^\circ C\) for no more than 7 days. On the day of an experiment, ANF stock aliquots were thawed and diluted in Krebs solution. All drugs and successive ANF concentrations were added to the 40-ml cremaster bath in 14-40 \( \mu l \) aliquots, with the exception of yohimbine and albumin/Polypep (200 and 250 \( \mu l \) aliquots, respectively). The maximal concentration of ascorbate that was present in the cremaster bath (\( 2 \times 10^{-6} \) M) has no effect on control microvessel diameter or responses to NE. All drugs were kept on ice in a dark container throughout the experiment. In previous studies, we have found that the ascorbate added to the cremaster bath with drug aliquots is sufficient to prevent breakdown and degradation of catecholamine responses over the exposure periods (without bath change and addition of fresh NE) that were used in the present studies.

Results

Figure 1 shows results from a representative experiment that examined the response of a large arteriole (responses of the paired venule that were similar are not shown). After induction of an intermediate amount of NE constriction, ANF produced concentration-dependent dilation that partially
FIGURE 2. Grouped control data for experiment described in Figure 1. Values are mean±SEM for “n,” number of arteriole-venule pairs (and animals). C1 and C2 represent diameter during first and second control periods. NE1 and NE2, response to NE (9.3±2.2x10^-8 M) at beginning versus end of experiment. MD, maximal dilation produced by nitroprusside or papaverine. Intrinsic tone defined as difference between MD and average diameter for C1 and C2. * (**) p<0.01 (<0.001) for NE1 versus C1, for NE2 versus C2, and for MD versus average of C1 and C2.

reversed the NE-induced tone. Following a wash period and reestablishment of control diameter, the same NE concentration that was previously used produced a similar amount of constriction, indicating that the sensitivity to NE did not change over the duration of the experiment. Subsequent to the wash, comparisons of the diameter during maximal smooth muscle relaxation by nitroprusside with control diameters and with the maximal diameter achieved at the highest concentration of ANF, indicate that 1) the arteriole possessed a modest amount of “intrinsic” (i.e., nonadrenergic) tone during control conditions and 2) ANF was unable to antagonize this intrinsic component. Grouped control data for this experiment are presented in Figure 2. Similar control diameters (C1 and C2) and similar responses to the same concentration of NE (NE1 and NE2) for both arterioles and venules at the beginning versus end of the experiments indicate that intrinsic tone and noradrenergic responsiveness remained constant for the duration of the protocol. Arterioles constricted to NE (mean concentration = 9.3±2.2x10^-8 M) by 36% of control (C1). Based on previous studies, this represents a constriction of approximately 55% of the maximal NE constriction that is produced by ≥3x10^-6 M. Venules constricted (NE values, Figure 2) by 21% of control (C1), which represents approximately 38% of the maximal constriction that is produced by high NE concentrations. Arteriolar and venular diameters achieved during maximal dilation with nitroprusside or papaverine were, respectively, 13% and 12% larger than control (C1) diameters. This amount of intrinsic tone is similar to that observed in our previous studies.

ANF produced concentration-dependent dilation of large arterioles and venules that had been preconstricted with NE (Figure 3). Inhibitory concentration-response curves over a 6-log-unit range of ANF were relatively shallow. Graphical determination of IC50 values from linear regression lines indicated a similar high sensitivity of both vessel types: arteriolar and venular IC50 = 18 (50 pg/ml) and 48 (133 pg/ml) pmol, respectively. Evaluation of concentration-response data after log transformation and linear regression revealed -log EC30 values of 9.94 ±0.38 (1.2x10^-10 M) for arterioles and 9.66 ±0.87 (2.2x10^-10 M) for venules. Interestingly, the highest concentration of ANF tested (10^-7 M) only reversed 70% of the NE constriction, even though additional dilatory capacity beyond 100% remained possible as revealed by the amount of intrinsic tone in these vessels (Figure 2). These results raised two questions: 1) Is the partial reversal of NE constriction due to a selective effect of ANF on constriction produced by the action of NE at either α1- or α2-postjunctional adrenoceptors; and 2) Is intrinsic tone (non-receptor-mediated) insensitive to ANF?

Figure 4 depicts the response of a large arteriole during the protocol that was used to examine...
whether ANF has a preferential inhibitory effect on constriction produced by \( \alpha_1 \)-versus \( \alpha_2 \)-adrenoceptors. ANF completely reversed an intermediate amount of phenylephrine (\( \alpha_1 \)) constriction in a concentration-dependent manner. In contrast, \( \alpha_2 \)-adrenoceptor constriction produced by UK was unaffected by ANF. Maximal dilation of the arteriole with papaverine revealed that significant intrinsic tone was present during control conditions. When diameter during papaverine was compared with maximal dilation produced by ANF (ANFmax), it was evident that ANF had no effect on this intrinsic tone.

Control diameters during the protocol (C1 and C2) remained constant (Figure 5). Both vessel types exhibited modest intrinsic tone as revealed by the 29% increase (versus C1) in arteriolar and 7% increase in venular diameter during maximal dilation (Figure 5). Differences in diameter in this experimental group versus Group 1 (Figure 2) reflect differences between the groups in branching order and size of selected vessels: Group 1 consisted of four first-order and eight second-order pairs; Group 2 consisted of all second-order pairs. Arterioles constricted to PE (3.1 ± 0.2 \( \times 10^{-6} \) M) by 39% and to UK (1.0 ± 0.1 \( \times 10^{-6} \) M) by a significantly less amount (26%). By contrast, paired venules constricted to the same agonist dose of UK by a greater amount (20%) than they did to PE (99%, nonsignificant). Only three of the venules examined evidenced PE constriction of >5% of control (mean ± SEM = 18 ± 4%).

Large arteriolar constriction produced by the \( \alpha_1 \)-agonist PE was completely reversed in a concentration-dependent manner by ANF (IC50 = 9.0 \( \times 10^{-10} \) M) (Figure 6). A similar sensitivity to ANF was displayed by those venules that were constricted by PE (IC50 = 1.2 \( \times 10^{-9} \) M). In distinction, \( \alpha_2 \)-adrenoceptor constriction of both vessel types with UK was unaffected by ANF (Figure 6). Control experiments for the effect of ANF vehicle (Krebs solution) and time on constriction produced by PE and UK indicated that constriction to both agonists was maintained for the duration of the ANF concentration-response analysis (Figure 7). It is noteworthy that high ANF concentrations did not dilate arterioles in the PE experiments beyond 100% nor were vessels constricted with UK affected at all, although both venules and especially arterioles possessed considerable intrinsic tone evidenced by maximal dilation with nitroprusside or papaverine (Figure 5). Intrinsic tone reduced diameter during control periods by approximately 30% from the maximally dilated state in the UK experiments. However, exposure to ANF during UK constriction had no effect on diameter, indicating an absence of effect on both \( \alpha_2 \) and intrinsic tone.
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**Figure 6.** Concentration-response relationship for ANF reversal of constriction of arterioles and venules by phenylephrine or UK-14,304 (protocol shown in Figure 4). Values are mean±SEM for "n," number of arteriole-venule pairs (and animals). Venular responses for phenylephrine constriction are for three animals (seven did not constrict to phenylephrine). r and r, the linear regression coefficients for the arteriolar and venular relation, respectively. For phenylephrine constriction, the IC values (determined by geometric interpolation) for arterioles and venules respectively were 0.9 and 1.2×10⁻⁹ M.

The relation between percentage arteriolar vasoconstriction with PE or NE and the sensitivity (IC₅₀) to inhibition with ANF was examined for all experiments. ANF IC₅₀ values were obtained for each experiment by linear regression of the double reciprocal concentration-response data, and the values were regressed against percent vasoconstriction from control diameter to PE or NE. The analysis revealed no correlation between ANF sensitivity and agonist constriction over the range of 25–56% constriction (i.e., approximately 50–100% of maximal constriction) (Y = −0.01X + 10.20; r = −0.10). This is in contrast to studies by Winquist,¹¹ wherein the ability of ANF to relax methoxamine-induced tone increased 15-fold when using an EC₅₀ versus an EC₉₀ concentration of methoxamine to contract rabbit aorta. The lack of correlation between degree of agonist constriction and sensitivity to ANF for microvessels cannot be ascribed to differences in background myogenic tone among first- and second-order arterioles, since separate regression analyses of either group revealed no significant correlation. Thus, it is possible that the presence of such a correlation is peculiar to large conduit vessels and/or is only evident when comparing the effect of ANF on intermediate versus high levels (≥80%) of constriction.

As a more direct test of the question regarding the sensitivity of intrinsic tone to inhibition by ANF, small precapillary "terminal" arterioles, which characteristically possess high intrinsic tone, were studied in a separate group of animals with acute cremaster denervation and without exposure to exogenous catecholamines. ANF at concentrations as high as 4.4×10⁻⁸ M had no significant effect on control diameter (Figure 8). Only at ANF concentrations of ≥1.44×10⁻⁷ M was significant dilation produced. The maximal dilation produced by ANF only amounted to a modest 25% increase over control diameter, which was well below the 100% increase evident during complete smooth muscle relaxation with adenosine (Figure 8).

**Discussion**

The present study examined the direct effects of ANF on the microvasculature of skeletal muscle. ANF exhibited a similar high potency (IC₅₀) in the picomolar range for relaxation of distributing arterioles and muscular venules, when these microvessels were preconstricted to an intermediate level with NE. The highest ANF concentration tested (10⁻⁹ M) was only able to reverse the NE-constriction by 70%. The inhibitory action of ANF was specific for constriction mediated by α₁ but not α₂-adrenoceptors; ANF at 10⁻⁷ M completely reversed phenylephrine constriction, but had no effect on α₂-constriction with UK. Thus, the inability of ANF to fully reverse NE constriction probably reflects the insensitivity of the NE α₂ component to ANF. In contrast to the inhibitory effect of ANF on α₁-constriction, the intrinsic tone that was evident to a modest degree in large arterioles and venules but particularly prominent in terminal arte-
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Effect of atrial natriuretic factor (ANF) on diameter of terminal arterioles. Values are mean±SEM for “n,” number of arterioles in eight animals.

It should be noted that the lack of an effect of ANF on terminal arteriolar intrinsic tone is unlikely to be a consequence of direct dilation by ANF being masked by myogenic or metabolic autoregulatory vasoconstriction in response to concomitant upstream changes produced by the peptide. In the terminal arteriole experiments, the cremaster was denervated, which abolishes virtually all adrenergic tone in cremaster vessels, and no exogenous NE was administered. These considerations, together with our evidence that intrinsic tone in large arterioles is insensitive to ANF, suggest that no upstream dilation occurred during the terminal arteriole studies. Indeed, in several experiments a large second-order arteriole was visible in the image field along with the third-order arteriole; no dilation was observed in either vessel with ANF concentrations below 10^{-7} M. Therefore, it appears that the high degree of intrinsic tone and vasomotion displayed by terminal arterioles is insensitive to ANF.

Moreover, our previous evidence that adrenergic constriction of terminal arterioles may rely almost exclusively on postjunctional a_2-adrenoceptors, together with the finding in the present study that a_2-constriction of microvessels is insensitive to ANF, suggest that ANF may have little influence on adrenergic or intrinsic control of terminal arterioles. However, these data do not rule out the possibility that ANF receptors exist on the terminal arterioles, and that interactions at this level may occur between ANF and other vasoactive agents. Local selective application of ANF to terminal arterioles is needed to determine whether these vessels possess ANF receptors.

Although endogenous catecholamines of neural origin were eliminated by acute nerve transection in our study, circulating catecholamines could have an effect on the cremaster vasculature. However, our previous studies indicate that circulating catecholamine levels in this preparation are below threshold for constriction of cremaster venules and terminal arterioles and just slightly above threshold for large arterioles. Neither prazosin nor yohimbine in effective antagonist concentrations (10^{-8}-10^{-7} M) increased diameter of venules or terminal arterioles above denervated control diameters; yohimbine had no effect on large arteriolar control diameter, and prazosin only increased diameter by 8% at 10^{-7} M. This small amount of large arteriolar tone, presumably due to circulating catecholamines, would not alter our estimates of (and conclusion concerning) ANF potency against intrinsic and a-receptor subtype-selective constriction.

Results from a previous study and other results using selective a_1- and a_2-agonists (J.E. Faber, unpublished observations) suggest that adrenergic constriction is more dependent on a_1-adrenoceptors for large arterioles and on a_2-adrenoceptors for large venules. Data in the present study support this hypothesis: phenylephrine produced greater constriction of arterioles than venules, whereas the a_2-agonist UK constricted venules to a greater degree than arterioles (Figure 5). As expected such
ANF-induced elevation of cyclic GMP in cultured calcium release from intracellular stores. 45 Also, calcium entry blockers or removal of extracellular vascular smooth muscle cells is not inhibited by evidence in rat aorta that ANF inhibits NE-induced intracellular calcium stores and/or receptor-depolarization and influx of extracellular calcium antagonist. 11 This possibility is supported by In distinction, contractile mechanisms that rely on via stretch- and voltage-operated calcium channels. Myogenic tone of myogenic origin and associated with changes in membrane potential and transmembrane calcium influx. 39, 49, 50 In the present study, constriction of large arterioles and venules via a-receptor occupation was unaffected by ANF, while a-constriction was inhibited in a concentration-dependent manner. These findings are consistent with the hypothesis for a selective inhibitory action of ANF on contractile mechanisms involving release of intracellular calcium.

There are several observations, however, that may not be consistent with this hypothesis. Myogenic tone in the rabbit facial vein, which is dependent on extracellular calcium entry, 47 was inhibited by ANF. 39 However, the mechanisms responsible for myogenic tone in the rabbit facial vein (e.g., it is insensitive to calcium channel blockade 47) may not be representative of those governing myogenic tone in arterioles, which are sensitive to calcium channel antagonists. 20 Taylor and Meisheri 48 observed in rabbit aorta that ANF can inhibit calcium entry via receptor-operated channels, although at concentrations higher than vasorelaxant concentrations identified in the present study. Thus, ANF may do more than just interfere with release of calcium from intracellular stores.

It has been reported that acute constriction of vascular smooth muscle by stimulation of a-receptors involves influx of extracellular calcium, whereas stimulation of a-receptors mobilizes intracellular calcium. 37, 49, 50 In the present study, constriction of large arteries and veins via a-receptor occupation was unaffected by ANF, while a-constriction was inhibited in a concentration-dependent manner. These findings are consistent with the hypothesis for a selective inhibitory action of ANF on contractile mechanisms involving release of intracellular calcium.

The above generalization should be taken cautiously considering the heterogeneity of vascular smooth muscle both within and among different species. Such heterogeneity also extends to effects of ANF on isolated vascular smooth muscle, as demonstrated in rabbit. 7 Among arteries contracted with serotonin or NE were largely unresponsive to ANF, with the exception of myogenic contraction of rat aorta and renal arteries by norepinephrine, potassium chloride, serotonin, histamine, angiotensin II, and methoxamine (an a-receptor agonist). Contraction by many of these agonists has been associated with mobilization of intracellular calcium stores and/or receptor-operated calcium channels in certain vascular smooth muscles. 40–43 In contrast, the peptide was poorly active against K+-contractions of aorta in rabbit (but not rat) 40 and spontaneous phasic contraction of rat portal vein, which are both dependent on depolarization and transmembrane calcium influx. 39 Consistent with these findings, in the present study the modest amount of intrinsic (i.e., non–receptor-mediated) tone in large arterioles and venules and the pronounced intrinsic phasic contraction (vasomotion) of terminal arterioles were largely insensitive to ANF. Although less well understood, this type of contractile behavior of microvascular smooth muscle may be partially of myogenic origin and associated with changes in membrane potential and transmembrane calcium influx. 20, 44 Thus, it is possible that ANF, at least in physiological concentrations, may have little relaxant effect on contraction of both macrovascular and microvascular smooth muscle that is produced by depolarization and influx of extracellular calcium via stretch- and voltage-operated calcium channels. In distinction, contractile mechanisms that rely on intracellular calcium stores and/or receptor-operated calcium channels may be particularly sensitive to ANF. 11 This possibility is supported by evidence in rat aorta that ANF inhibits NE-induced calcium release from intracellular stores. 45 Also, ANF-induced elevation of cyclic GMP in cultured vascular smooth muscle cells is not inhibited by calcium entry blockers or removal of extracellular calcium. 46
striction (including NE and electrical stimulation) of either vessel type was insensitive to ANF. However, NE constriction of rat aorta was completely reversed by nanomolar concentrations of ANF. The lack of an effect of ANF on NE constriction of small cerebral arteries might be explained by the recent observations that small pial arteries may possess predominantly \( \alpha_2 \)-adrenoceptors,\(^{32} \) together with our present evidence for the lack of effect of ANF on \( \alpha_2 \)-constriction. Rat aorta is known to possess only \( \alpha_1 \)-adrenoceptors.\(^{36} \)

In small (250 \( \mu \)m diameter) renal arcuate arteries of the rat that had been constricted in vitro with NE, potassium, or serotonin, ANF produced dilation by a membrane potential-independent mechanism.\(^{8} \) However, ANF had no effect on potassium-induced constriction of similarly-sized mesenteric, femoral, cerebral and coronary vessels. Consistent with our findings, in their study ANF was unable to maximally relax NE constriction of the renal arteries; potency of ANF (\(- \log IC_{50} \) of 7.8) was approximately 100-fold lower relative to values obtained in the present study for large arterioles. The ability of ANF to inhibit NE constriction of renal arteries is consistent with the known dominance of \( \alpha_1 \)-adrenoceptors in the contraction of renal arteries and in the regulation of renal hemodynamics.\(^{36} \) However, Edwards and Weidley\(^{53} \) observed that constriction of rabbit glomerular arterioles in vitro with NE or angiotensin was insensitive to ANF at concentrations as high as \( 10^{-7} \) M. Whether this is due to the absence of ANF receptors or dominance of \( \alpha_2 \)-versus \( \alpha_1 \)-receptors on glomerular arterioles is unknown.

Recently, Proctor and Bealer\(^{54} \) examined responses of small, third-order arterioles (20–40 \( \mu \)m diameter) in the rat spinotrapezius microcirculation to a high concentration of ANF (\( 3 \times 10^{-8} \) M). In agreement with our findings for third-order arterioles, ANF had no effect on intrinsic tone. However, receptor-mediated constriction of arterioles with angiotensin II, but not vasopressin and NE, was lessened in the presence of ANF. In contrast, constriction of intestinal arterioles with angiotensin and vasopressin was lessened by pharmacological concentrations of ANF, while NE responses were unaffected. If intestinal small arterioles (and renal glomerular arterioles in the Edwards and Weidley study\(^{53} \)) have predominantly \( \alpha_2 \)- rather than \( \alpha_1 \)-adrenoceptors as we have shown for skeletal muscle,\(^{18,22} \) then the lack of an effect of ANF on NE constriction in both tissues would be expected based on our present findings of an apparent selectivity of ANF for inhibition of \( \alpha_1 \)-mediated constriction.

### Relation of Present Results to In Vivo Literature

Several in vivo studies appear at variance with the concept that \( \alpha_1 \)-adrenoceptor-mediated constriction of resistance vessels is selectively sensitive to ANF. Zukowska-Grojec et al\(^{34} \) found in pithed rats that pressor response to sympathetic stimulation or activation of (presumably) \( \alpha_1 \)-adrenoceptors (bolus NE in presence of yohimbine) were not affected by infusion of ANF at a rate sufficient to increase plasma levels to those produced by volume expansion in conscious rats. In contrast, ANF did reduce responses to activation of (presumably) \( \alpha_2 \)-adrenoceptors (bolus NE in presence of prazosin). However, in these pithed rats, ANF alone had little or no hypotensive effect which is in contrast to most studies of areflexic animals during systemic infusion (see below). In the in situ blood-perfused rat mesentery-intestine, ANF infusions had no effect on responses to sympathetic nerve stimulation or exogenous NE, despite evidence that postjunctional \( \alpha_1 \)-adrenoceptors are involved in noradrenergic regulation of this vasculature.\(^{55} \)

The reasons remain unclear for the discrepancy between our study and these in vivo studies, and for the heterogeneity of responses to ANF that have been reported for in vitro studies of different smooth muscle types. The lack of sensitivity to ANF in rat mesentery versus cremaster skeletal muscle may reflect a relative dirth of ANF receptors in mesentery.\(^{36} \) Indeed, it appears likely that the distribution of ANF receptors varies widely among different types of vascular smooth muscle.\(^{11} \) Regional differences could also arise from utilization by an agonist of different postreceptor coupling mechanisms depending on the smooth muscle type, and differences among tissue types in the ability of postreceptor mechanisms utilized by ANF to interfere with contraction. Finally differences in the smooth muscle membrane potential, and the type (e.g., \( \alpha_1 \) versus \( \alpha_2 \)) and amount of agonist-induced tone present under the experimental conditions could contribute to variability in findings.

Although several studies have examined the effect of infusion of ANF on systemic hemodynamics, the mechanisms for the hypotension remain unclear.\(^{16,56,57} \) Bolus administration of atrial extract in conscious rats lowered arterial pressure and cardiac index; heart rate and calculated total peripheral resistance (TPR) increased, presumably due to baroreflex activation.\(^{12} \) Blood flows and vascular conductances decreased to all regional circulations examined, except skeletal muscle. Given the well-known strong baroreflex influence on skeletal muscle, these data suggest that atrial peptides exert a direct vasodilatory action on this vasculature that is masked by reflex vasoconstriction, resulting in no change in resistance. This is consistent with our present findings. Other studies have provided evidence for activation of baroreflexes during ANF infusion.\(^{14,15} \) Studies by Lappe et al\(^{33} \) suggested that ANF may lower arterial pressure by relaxing venous rather than arterial smooth muscle and thereby reduce preload and cardiac output; at the same time baroreflex activation may increase regional resistance. Similar conclusions were reached by others.\(^{15,16,34} \)
The above pattern of hemodynamic responses does not resemble the effect of general vasodilators (e.g., hydralazine) or nitrates that are potent venodilators. These drugs lower mean arterial pressure by decreasing total peripheral resistance and venous return. The acute hypotensive action of ANF is not due to a direct cardio-inhibitory effect, nor solely to a renal-dependent decrease in blood volume since it persists in anephric animals. The proposal that atrial peptides may have a selective relaxant effect on venous smooth muscle, thereby decreasing venous return and thus cardiac output, was attributed to active venoconstriction and passive vascular recoil secondary to diuresis and reduced blood volume. Blood volume also decreased in anephric rats, and the authors attributed this decrease to increased capillary pressure secondary to venoconstriction. The importance of the finding that ANF may increase venous resistance and reduce venous return rests in its ability to explain the decrease in cardiac output coupled with minimal change in mean arterial pressure and an increase in TPR that is observed, especially in conscious animals, during ANF infusion. Although it is commonly assumed that venous constriction and reduced compliance will increase venous return and cardiac output, this was attributed to increased capillary pressure and cardiac output. The mechanism for the active venoconstriction is unknown but could involve baroreflexes and/or release of circulating vasocstriction hormones (although ANF appears to inhibit renin and vasopressin release). Our data suggest that ANF is able to inhibit \(\alpha_1\)-but not \(\alpha_2\)-constriction of venules in skeletal muscle. However, in a recent study and in the present study, there is both in vitro and in vivo evidence that adrenergic control of skeletal muscle venules, many veins, and general venous compliance utilizes predominantly postjunctional \(\alpha_2\)-adrenoceptors. In contrast, recent studies and the present study suggest that adrenergic control of the arterial and arteriolar circulation, with the exception of terminal arterioles, may rely predominantly on \(\alpha_1\)-adrenoceptors. Thus, based on our present evidence, ANF would be expected to have minimal inhibitory effects on adrenergic constriction of the venous circulation, but would instead interfere with adrenergic tone in resistance arterioles.

Although caution must be exercised in generalizing our present microvascular findings in skeletal muscle to other vascular beds, our results suggest a mechanism by which ANF might decrease cardiac output according to the following hypothesis. Acute infusion of ANF in physiological concentrations inhibits basal \(\alpha_1\)-mediated tone of large arterioles (in skeletal muscle and possibly elsewhere), leading to a momentary reduction in peripheral resistance and an increase in capillary pressure and flow. Overall venous adrenergic tone, which relies predominantly on \(\alpha_2\)-adrenoceptors, would be little affected. The increase in capillary pressure, together with a possible effect of ANF to increase capillary hydraulic conductivity, would increase capillary filtration and explain the persistent though attenuated reduction in plasma volume in anephric rats. Arterial pressure would momentarily decrease due to a reduction in cardiac output (secondary to a decrease in plasma volume and venous return) and the decrease in peripheral resistance. The hypotension would activate arterial baroreflexes and increase sympathetic outflow. Given the fast (seconds) nature of arterial baroreflexes, a noticeable decrease in arterial pressure and peripheral resistance may not be evident during ANF infusion at physiological concentrations. Baroreflex activation would constrict large arterioles by increasing NE activity at \(\alpha_1\)-receptors and would counteract inhibition by ANF; peripheral resistance would be maintained near normal. However, the concomitant increase in sympathetic activity to venules and veins would produce constriction via predominantly \(\alpha_2\)-adrenoceptors that would be unaffected by ANF. This could explain the increase in venous resistance observed by Trippodo et al. Consistent with this sympathetic venoconstriction hypothesis, the ANF-induced decrease in cardiac output was almost eliminated in rats subjected to chemical sympathectomy. Increased venous resistance would further decrease venous return and increase capillary pressure and filtration, favoring reduced plasma volume. These mechanisms would reduce cardiac output further. This hypothetical scheme is consistent with the observations that infusion of ANF results in a steady-state decrease in cardiac output, little effect on arterial pressure, and either no change or an increase in peripheral resistance that is dependent on baroreflex activation. The scenario is predicated, in part, on our observation of a selective inhibitory effect of ANF on \(\alpha_1\)-constriction, together with the putative differential reliance of nerve-mediated constriction of arterial and venous smooth muscle on predominantly \(\alpha_1\)-versus \(\alpha_2\)-adrenoceptors, respectively.

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