Selective Antagonism of Hormone-Induced Vasoconstriction by Synthetic Atrial Natriuretic Factor in the Rat Microcirculation

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Synthetic atrial natriuretic factor (ANF) was either added to suffusate solutions (30 nM) or infused into the jugular vein (0.1 nanomol/min/100 g) of anesthetized rats. Steady-state blood flow was calculated from arteriolar diameter and red blood cell velocity measurements using video microscopy in the intestinal or skeletal muscle microcirculation. Arterioles demonstrated spontaneous vasomotor tone by dilating to topical adenosine, but topical or intravenous ANF did not cause vasodilation. Either angiotensin, norepinephrine, or vasopressin was added to the suffusates in the presence or absence of a cyclooxygenase inhibitor (30 μM, meclofenamate or indomethacin) because each agonist is known to stimulate vasoactive prostanoid synthesis. In the intestine, angiotensin (500 nM) caused 40 ± 2% blood flow decreases during intravenous saline but only 23 ± 6% during intravenous ANF. Angiotensin (162 nM) and a cyclooxygenase inhibitor caused 19 ± 4% blood flow decreases but only 8 ± 5% decreases with cyclooxygenase inhibitor and topical ANF. In contrast, norepinephrine (2–5 μM) caused vasoconstriction that was not altered by topical or intravenous ANF, either alone or in combination with cyclooxygenase inhibitors. In the spinotrapezius muscle, angiotensin (1–2 nM) plus a cyclooxygenase inhibitor caused 40–60% blood flow decreases but only 20–30% decreases during intravenous or topical ANF. Topical or intravenous ANF did not alter the vasoconstriction evoked by arginine vasopressin (0.5–1.0 nM) or by norepinephrine (40–230 nM). Thus, 1) supraphysiologic concentrations of ANF produced no direct vasodilation in the intestinal or skeletal muscle microcirculation; 2) there was a regional difference in sensitivity to topically-applied vasoconstrictor hormones between the two tissues; 3) ANF reduced, but did not eliminate, the vasoconstriction caused by angiotensin by a mechanism that did not involve cyclooxygenase products; and 4) ANF did not alter the vasoconstriction caused by norepinephrine or arginine vasopressin. (Circulation Research 1987;61:42-49)

Crued atrial extracts or synthetic atrial natriuretic factor (ANF) relax isolated vascular smooth muscle that is precontracted with a variety of agonists. ANF causes relaxation by binding to high-affinity receptors and increasing cyclic guanosine 3'-phosphoric acid (cGMP) through an endothelium-independent mechanism. Unfortunately, this well-defined mechanism in vitro does not accurately predict the hemodynamic effects of ANF in vivo. Systemically administered ANF causes regional perfusion to increase, decrease, or not change, which could indicate reflex compensatory changes in regional vascular resistance or regional and segmental differences in vascular sensitivity to ANF. If ANF does have a direct vascular effect in the peripheral tissues, the arterioles are probably a site of action, but responses of small arterioles to ANF have not been previously studied in intact tissues. Whole-organ blood-flow changes in response to vasoactive substances may not necessarily be extrapolated to the microcirculation because of regional and segmental differences in vascular reactivity. Thus, direct observation of arteriolar responses in intact tissues may provide new insight into the peripheral vascular actions of ANF.

Materials and Methods
Male Sprague-Dawley rats (120–300 g) were anesthetized with 13% urethane and 1% chloralose (1.2 ml/100 g i.p.). Supplemental doses were administered when necessary (0.1 ml/100 g i.v.). The trachea, carotid artery, and jugular vein were cannulated. Respiration was spontaneous on room air. Rectal temperature was continuously monitored (43°TA YSI, Yellow Springs, Ohio) and maintained at 36–38°C with a heat lamp. Arterial blood Po2, Pco2, and pH were periodically measured on a Radiometer (Copenhagen) analyzer and were typically >70 mm Hg, <40 mm Hg, and 7.40–7.45, respectively. Carotid arterial blood pressure was continuously monitored with a Gould-Statham P23ID transducer (Oxnard, Calif.).

Microcirculatory Preparations
In separate groups of animals, the intestinal or skeletal muscle microcirculation was studied. The intes-
tine was prepared with a slight modification of a published technique. The mucosal and serosal surfaces were each suffused with bicarbonate-buffered Ringer's solutions comprising (in mM) NaCl 137.9, KCl 4.7, CaCl₂ 2.0, MgSO₄·7H₂O 1.2, and NaHCO₃ 20. Ascorbic acid (0.5 mM) was added as an antioxidant. Solutions were equilibrated with a gaseous mixture of 5% CO₂, 0.5% O₂, and the balance N₂ in glass chambers enclosed in water jackets at 37° C. Tissue temperature was continuously monitored (LN 3207, YSI) and maintained at 36–37° C by varying the flow rates of the heated solutions that suffused the mucosa (average, 5–7 ml/min) and serosa (average, 1–3 ml/min). Isoproterenol (Sigma Chemical Co., St. Louis) was added to the serosal suffusate (<0.05 μM) to suppress spontaneous intestinal motility; the threshold concentration for causing vasodilation is 1 μM. In 10–25% of the preparations, motility could not be suppressed, and the experiment was terminated since accurate measurements were impossible.

The spinotrapezius muscle microcirculation was prepared with a slight modification of a published technique. Throughout the surgery, the tissue was suffused with a bicarbonate-buffered Ringer's solution identical to that described above.

Both preparations were transilluminated with white light and observed with long working-distance (4–6 mm) Leitz L20X (Ruckleigh, N.J.) or L32X objectives, a long-working-distance Leitz L-11 condensor, and either a Leitz Diavert or a Leitz Laborlux microscope equipped with a trinocular head and discussion bridge (Leitz 24875-512). The optics were aligned in accordance with Kohler's principle to remove the contrast-reducing flare of scattered light from outside the visual field. The discussion bridge provided coincident images to a Panasonic 3230 (Seacaucus, N.J.) color television camera (newview tube) and a rotating prism velocimeter. The television signal was conveyed to a For-A LV-550 (Tokyo) video micrometer and high resolution Panasonic CT 1920V color monitor. Technical details are provided in several recent studies from this lab.

Arteriolar diameter was continuously measured with the video micrometer, and red blood cell velocity was continuously measured with the rotating prism velocimeter. Arterial blood pressure, arteriolar diameter, red cell velocity, and tissue temperature were continuously recorded on a Gould polygraph. The hemodynamic variables were digitized every 5 seconds by a Commodore (Palo Alto, Calif.) microcomputer. Blood flow was calculated on-line from the product of a constant, arteriolar diameter and red blood cell velocity and was recorded on a DEC-writer. Each hemodynamic variable was averaged over a 30–60-second interval every 2–6 minutes.

Handling and Storage of Synthetic Atrial Natriuretic Factor (ANF)

Purified crystalline [Leu]ANF-26 (rANF, Ang 101-Tyr 126) was obtained as a gift from Merck Sharp & Dohme Research Laboratories. The peptide was dissolved in 0.9% NaCl and stored in 4-ml aliquots (3 μM) in plastic syringes at −11° C. In some experiments, an aliquot was thawed and infused into the jugular vein at a rate of 0.1 nmol/min/100 g with a Sage syringe pump for 10–30 minutes. In other experiments, an aliquot was thawed and diluted with bicarbonate-buffered Ringer's (30 nM). This solution was heated to 37° C and equilibrated with various gas mixtures in water-jacketed glass chambers, then suffused over the intestine (3–7 ml/min) or spinotrapezius muscle (5–7 ml/min). Unused solutions were refrigerated and discarded after 24 hours.

Experimental Protocols

After a 30–60-minute postsurgery stabilization period, a first order arteriole in the jejunal submucosal microcirculation (resting diameter, 40–70 μm) or a third order arteriole in the spinotrapezius muscle microcirculation (resting diameter, 20–40 μm) was tested for vasomotor tone with the topical application of 5–500 μM adenosine (Sigma). The typical response was a reversible increase in blood flow. Experiments were terminated if a preparation lacked vasomotor tone or if systemic arterial blood pressure decreased below 70 mm Hg.

Effect of Cyclooxygenase Inhibitors on Angiotensin Response. After a steady-state baseline, angiotensin II (Sigma, human sequence) was added to the suffusates to concentrations of 1–150 nM for 5–10 minutes. After a 10–30-minute washout, a cyclooxygenase inhibitor was added to the suffusates (30 μM). To minimize nonspecific artifacts caused by a cyclooxygenase inhibitor, structurally dissimilar compounds (meclofenamate or indomethacin) were used on alternating days. Previous studies have shown the efficacy of this dose in the intestine and skeletal muscle of the rat. After at least 10 minutes had passed to allow equilibration of the cyclooxygenase inhibitor with the tissue, angiotensin was added again to both suffusates.

Effect of Topical ANF on Angiotensin Plus Cyclooxygenase Inhibitor Response. The tissue was suffused continuously with solutions containing a cyclooxygenase inhibitor in Ringer's (30 μM meclofenamate or indomethacin) alone or a cyclooxygenase inhibitor plus ANF (30 nM). After a stable baseline, angiotensin II was added to both suffusates to final concentrations of 1–350 nM in ascending sequence for 5–10 minutes at each dose. After the angiotensin was washed out of the suffusates for 10–30 minutes and the baseline was restored, the protocol was repeated.

Effect of Topical ANF on Norepinephrine or Arginine Vasopressin Response. The tissue was continuously suffused with solutions containing either Ringer's alone or Ringer's plus ANF (30 nM) until the baseline was stable. Thereafter, either norepinephrine hydrochloride (Sigma) or arginine vasopressin (Sigma) was added to the suffusates in ascending sequence. The norepinephrine concentrations ranged from 0.01–2.5 μM. The vasopressin concentrations ranged from 0.5–1.0 nM. The tissue was exposed to...
each agonist concentration for 5–10 minutes. After a 10–30-minute washout and the restoration of the original baseline, the protocol was repeated.

In some animals, this protocol was repeated with a cyclooxygenase inhibitor in the suffusates.

**Effect of Intravenous ANF on Arginine Vasopressin, Norepinephrine, or Angiotensin Response**

The tissue was continuously suffused with a Ringer's saline (vehicle) or ANF was infused into the suffusates until a new steady state was maintained. After washout, the protocol was repeated.

In some animals, this protocol was repeated with a cyclooxygenase inhibitor in the suffusates.

**Statistical Analysis**

All values were expressed as means ± SEM. Most comparisons were paired, and ANF treatments were randomized with vehicle. In most cases, the same number of observations were made with constrictor plus ANF applied first and with constrictor plus ANF applied second. Differences were determined with paired t tests. Significance was assessed at the 95% confidence interval.

**Results**

The normalized arteriolar diameter and calculated blood flow data presented in Figures 1–4 were expressed relative to steady-state control baselines. The raw values from which these data were derived are presented in Tables 1 and 2.

**Effect of ANF on Baseline Hemodynamic Variables**

**Intestine.** The effect of topical or intravenous ANF on steady-state systemic arterial blood pressure, intestinal arteriolar diameter, and calculated blood flow is shown in Table 1. Topical ANF (30 nM) had no significant effect on any measured variable in the presence (n = 17 animals) or absence (n = 9 animals) of cyclooxygenase inhibitors. Intravenous ANF (0.1 nmol/min/100 g) caused a significant decrease in systemic arterial pressure relative to the paired value with intravenous saline (n = 6 animals), but there was no significant effect on diameter or calculated blood flow in submucosal arterioles. The topical application of 329 ± 81 μM adenosine to the serosa caused an increase in calculated blood flow to 45 ± 5 nl/sec (239 ± 48% of control), which demonstrates that these intestinal arterioles were capable of dilating. Since systemic arterial pressure decreased but calculated blood flow remained constant during intravenous administration of ANF, the intestine autoregulated during ANF infusion. Within 1 hour after infusion of ANF, systemic arterial pressure returned to the pre-ANF value.

The topical application of either angiotensin (≤500 nM) or norepinephrine (≤5 μM) to the intestine caused no change in systemic arterial pressure regardless of whether the suffusates contained a Ringer's vehicle, a cyclooxygenase inhibitor. ANF, or a cyclooxygenase inhibitor plus ANF.

**Skeletal Muscle.** The effect of topical or intravenous ANF on baseline hemodynamic variables in the spinotrapezius muscle is shown in Table 2. The topical or intravenous administration of ANF caused no detectable change in calculated blood flow or arteriolar diameter. In contrast, the topical application of 31 ± 7 μM adenosine caused a calculated blood flow increase to 17 ± 4 nl/sec (396 ± 70% of control), which demonstrates that these skeletal muscle arterioles were capable of dilating.

The topical application of either angiotensin (<3 nM), arginine vasopressin (<3 nM), or norepinephrine (<1 μM) to the spinotrapezius muscle with or without cyclooxygenase inhibitors in the suffusate caused no change in systemic arterial blood pressure.

| Table 1. Baseline Hemodynamic Variables in Intestinal Microcirculation During Application of Various Exogenous Substances |
|---|---|---|---|
| **n** (Animals) | Treatment | Systemic arterial blood pressure (mm Hg) | Arteriolar diameter (μm) | Calculated blood flow (nl/sec) |
| 9 | Vehicle | 90 ± 4 | 61 ± 5 | 22 ± 4 |
| | Vehicle and tANF | 85 ± 2 | 61 ± 4* | 21 ± 3 |
| | Vehicle and tADO | 95 ± 4 | 72 ± 4* | 45 ± 5* |
| 17 | Cyclo mnhb | 91 ± 3 | 57 ± 4 | 26 ± 3 |
| | Cyclo mnhb and tANF | 88 ± 3 | 55 ± 4 | 24 ± 3 |
| 6 | Vehicle and saline infusion | 90 ± 1* | 56 ± 7 | 28 ± 6 |
| | Vehicle and ANF infusion | 81 ± 1* | 60 ± 7 | 27 ± 4 |

Blood flow calculated from arteriolar diameter and red blood cell velocity measurements. Vehicle, bicarbonate-buffered Ringer's solution suffusing serosa and mucosa. tANF, topical application of synthetic atrial natriuretic factor (30 nM) to serosa and mucosa, ANF or saline infused during suffusion of serosa and mucosa with vehicle, TADO, topical application of adenosine (300 μM) to serosa; cyclo mnhb, topical application of cyclooxygenase inhibitor (30 μM meclofenamate, n = 9, 30 μM indomethacin, n = 8) to serosa and mucosa. Each animal within a group was exposed to all treatments in random sequence.

*Arterial blood pressure was significantly decreased by ANF infusion (p < 0.05); both diameter and calculated blood flow were significantly increased by tADO (p < 0.05). Otherwise, there were no significant differences within a treatment group.
These results suggest that a cyclooxygenase product probably modulated the vasoconstriction caused by angiotensin. Which confirms earlier work (see text) containing a cyclooxygenase inhibitor (30 μM; meclofenamate, n = 5; indomethacin, n = 5) and 3 different angiotensin concentrations, once with the suffusates containing Ringer's alone and once with the suffusates containing Ringer's plus ANF (30 nM). Angiotensin (162 ± 23 nM) and a cyclooxygenase inhibitor caused significant dose-related decreases in calculated blood flow that averaged 19 ± 4%. With ANF in the suffusate, the blood flow decreases evoked by angiotensin averaged only 8 ± 5% and were not significant. The paired difference between treatments averaged 13 ± 4% and was significant.

In Figure 1 (right panel), the intestine of each of 5 animals was exposed twice to 500 nM angiotensin with no cyclooxygenase inhibitor in the suffusate; once during an infusion of saline and once during an infusion of ANF into the jugular vein. Angiotensin caused decreases in calculated blood flow that averaged 40 ± 2% during saline infusion, but the response was significantly reduced to 23 ± 5% during ANF infusion. The paired difference between treatments averaged 17 ± 6% and was significant.

Thus, both topical and intravenous ANF reduced the angiotensin response by about 50%.

Calculated blood flow increased >240% of control after topical 500 μM adenosine, which indicates the level of spontaneous vasomotor tone in the population of intestinal arterioles (resting diameter, 60–70 μm) (Figure 1).

Skeletal muscle. In 5 animals, a continuous suffusion of 2.2 ± 0.04 nM angiotensin produced no significant effect on calculated blood flow in steady-state conditions. The same dose of angiotensin produced significant decreases in calculated blood flow that averaged 29 ± 4% during suffusion with meclofenamate (n = 3) or indomethacin (n = 2). In these pilot experiments, angiotensin usually produced a transient vasodilation (probably a direct vascular effect) followed by a sustained vasoconstriction (probably an indirect vascular effect caused by prostaglandins). This secondary vasoconstriction was prevented by adding a cyclooxygenase inhibitor to the suffusate solutions.

To minimize the effects of vasoactive prostanoids...
Spontaneous muscle arteriolar diameter and calculated blood flow changes caused by topical application of 1–2 nM angiotensin during topical application of cyclooxygenase inhibitor were significantly attenuated by topical ANF (30 nM) in 7 animals (right panel). In a separate series of 5 animals, cyclooxygenase inhibitors potentiated the vasoconstriction caused by topical angiotensin, which confirms earlier work (see text). Continuous suffusion of 0.5 μM adenosine caused steady-state blood flow increases that averaged 2400% of control in these arterioles (20–30 μm), which indicates presence of spontaneous vasomotor tone. Note that in this tissue, topical angiotensin caused a greater vasoconstrictor response at a concentration less than 1/100 of that used in the intestine but that ANF reduced the effect of angiotensin to the same extent in both tissues.

In summary, systemic or local application of high concentrations of ANF did not cause vasodilation in skeletal muscle or intestinal arterioles. However, the vasoconstriction caused by topical angiotensin was reduced by approximately 50% in both skeletal muscle and intestine, despite a marked regional difference in the efficacy of angiotensin. In the intestine, >150 nM angiotensin produced blood flow decreases that averaged <30%, whereas <1.5 nM angiotensin produced blood flow decreases >30% in skeletal muscle.

Effect of ANF on Norepinephrine and Vasopressin-Induced Vasoconstriction

Intestine. The left panel of Figure 3 shows responses in 6 animals, each exposed twice to 3 different norepinephrine concentrations, once with the suffusates containing a cyclooxygenase inhibitor (30 μM; meclofenamate, n = 3; indomethacin, n = 3) and once with the suffusates containing a cyclooxygenase inhibitor plus ANF (30 nM). Norepinephrine (3.4 ± 0.5 μM) caused dose-related decreases in calculated blood flow that averaged 22 ± 7% with cyclooxygenase inhibitor alone and 27 ± 7% during cyclooxygenase inhibitor plus ANF. There was no significant effect of ANF treatment.

The middle panel of Figure 3 shows the effect of topical ANF on the response to norepinephrine in the absence of cyclooxygenase inhibitors. The intestine in each of 7 animals was exposed twice to norepinephrine: once with the suffusate containing the Ringer’s vehicle and once with the suffusates containing Ringer’s vehicle plus ANF (30 nM). The decrease in calculated blood flow during norepinephrine averaged 45 ± 5% without ANF and 37 ± 7% with ANF. There was no significant effect of ANF treatment.

The right panel of Figure 3 shows the effect of intravenous ANF on the norepinephrine response in the absence of cyclooxygenase inhibitors. The intestine of 5 animals was exposed twice to 3.5 ± 0.6 μM norepinephrine: once during a saline infusion and once during an ANF infusion. Topical norepinephrine caused decreases in calculated blood flow that averaged 50 ± 11% during saline infusion and 64 ± 7% during ANF infusion.
during ANF infusion. There was no significant effect of ANF treatment.

The presence of spontaneous vasomotor tone was verified by the topical application of 500 μM adenosine, which caused blood flow increases >250% of control in these arterioles (resting diameter, 50–60 μm).

**SKELETAL MUSCLE.** Pilot experiments were conducted in the spinotrapezius muscle to determine the effect of ANF on the norepinephrine response. In 4 animals, 100 ± 50 nM norepinephrine produced a vasoconstriction that averaged 28 ± 4% in the absence and 23 ± 6% in the presence of ANF. In an additional 3 animals in which the tissue has been treated with a cyclooxygenase inhibitor, 50 nM norepinephrine caused a vasoconstriction that averaged 36 ± 6% in the absence and 35 ± 7% in the presence of ANF. Although the spinotrapezius muscle microcirculation was more sensitive to norepinephrine than the intestinal microcirculation, ANF did not alter the norepinephrine response.

The left panel of Figure 4 shows the effect of ANF on vasoconstriction induced by arginine vasopressin. The spinotrapezius muscle in 5 animals was first treated with a cyclooxygenase inhibitor (meclofenamate, n = 2; indomethacin, n = 3) and then exposed twice to the same sulfamate concentration of vasopressin: once with the sulfamate containing only the Ringer’s vehicle and once with the sulfamate containing ANF. In the presence of cyclooxygenase inhibitor, the topical application of 0.9 ± 0.2 nM arginine vasopressin caused 68 ± 7% decreases in calculated blood flow in the absence and 70 ± 7% decreases in the presence of ANF.

The right panel shows results from a separate group of 5 animals in which the spinotrapezius muscle was treated with a cyclooxygenase inhibitor (meclofenamate, n = 3; indomethacin, n = 2) and then exposed twice to the same sulfamate concentration of vasopressin, once during a saline infusion and once during an ANF infusion. In the presence of cyclooxygenase inhibitor, the topical application of 0.8 ± 0.1 nM arginine vasopressin caused decreases in calculated blood flow that averaged 59 ± 4% during a saline infusion and 56 ± 4% during an ANF infusion.

The presence of spontaneous vasomotor tone in this population of skeletal muscle arterioles (resting diameter, 20–30 μm) was verified by the topical application of 50 μM adenosine, which caused blood flow increases that averaged >400% of control.

In summary, norepinephrine-induced vasoconstriction in skeletal muscle or intestinal arterioles or vasopressin-induced vasoconstriction in skeletal muscle arterioles was not significantly altered by topical or intravenous ANF.

**Discussion**

The major new findings from this study in the peripheral microcirculation are that 1) the vasoconstriction caused by topical angiotensin was attenuated by topical ANF or by systemically-infused ANF, 2) ANF had no effect on the vasoconstriction caused by topical norepinephrine or vasopressin, and 3) ANF had no direct vasodilator action. The implication is that ANF might functionally antagonize the vasoconstriction caused by angiotensin in the microcirculation but not that caused by norepinephrine or vasopressin. The mechanism of the antagonism probably does not involve a cyclooxygenase product.

**Comparison to Earlier Work**

Prostaglandins modulate the expression of vasopressin, norepinephrine, and angiotensin in many tissues, so it is not surprising that cyclooxygenase inhibitors potentiated responses to topically-applied vasoconstrictors in this study. Nevertheless, our data suggest no direct interaction between prostaglandins and the vascular effect of ANF because cyclooxygenase inhibitors did not alter the effect of ANF on angiotensin-induced vasoconstriction (Figure 1) or the lack of effect of ANF on norepinephrine-induced vasoconstriction (Figure 3). Thus, these present results support the hypothesis that the vascular actions of ANF are not dependent on prostaglandin release.

Many investigators have concluded that ANF has no direct vasoactivity outside the renal circulation, which is consistent with results in the intestinal (Table 1) or skeletal muscle (Table 2) vasculature. On the other hand, no extra fluid was administered to the animal during intravenous ANF to compensate for urinary losses, so plasma volume contraction might have induced reflex mechanisms that would have obscured a vasorelaxant effect of ANF.

Since previous studies have shown that ANF has an especially prominent effect on the vasoconstriction caused by angiotensin in isolated kidney and in isolated vascular smooth muscle, these present results (Figures 1 and 2) extend this property of ANF to
the intact microcirculation of skeletal muscle and intestine.

The data presented in Figures 3 and 4 contradict the general concept that ANF antagonizes the action of most vasoconstrictors, but an important feature of this experimental design could explain this contradiction. We studied small arterioles (resting diameter, 20–70 μm) with spontaneous vasomotor tone in intact tissues while other investigators have studied large arteries (resting diameter, >200 μm) in vitro with vascular tone induced by exogenous agonists. It is reasonable to expect some differences between responses observed in vivo in small arterioles and those observed in vitro in large conduit vessels.

Another aspect of this study concerns the method of applying substances directly to the tissue. Since there was no observable effect on systemic arterial blood pressure, it is unlikely that reflex compensatory mechanisms could have masked a direct action of topical ANF as in earlier whole-animal studies. On the other hand, the topically-applied vasoactive substances might have been inactivated partially by tissue peptidases, particularly in the intestine. For example, intestinal arterioles are any less sensitive than skeletal muscle arterioles to angiotensin cannot be concluded, even though >1.5 nM angiotensin produced >30% decreases in skeletal muscle blood flow while >150 nM angiotensin produced <30% decreases in intestinal arterioles.

A large fraction of the topically-applied ANF may have been inactivated by tissue peptidases before reaching its site of action at the vascular smooth muscle, particularly in the intestine. However, a subthreshold dose cannot explain the lack of effect of ANF on norepinephrine- and vasopressin-induced constriction for the following reasons: first, both the intestinal and skeletal muscle (approximate weights, 50 mg) were continuously suffused with solutions containing 100 ng ANF/ml (30 nM) and had been exposed to 40–60 μg ANF at the end of a typical experiment. For comparison, the total amount of atrial peptide in the whole rat averages only 10–20 μg, and normal rat plasma contains about 400 pg/immmunoreactive atriopeptin/ml during anesthesia and less than 100 pg/ml in unstressed conditions. Thus, the suffusate ANF concentration would have been at least 250 times higher than the maximum theoretical tissue concentration of ANF during anesthesia. Assuming that the intestine and skeletal muscle extracellular space equilibrated with the suffusate, more than 99% of the ANF within the tissue could have been inactivated by various mechanisms, and the tissue concentration still would exceed the maximum theoretical ANF concentration of 400 pg/ml. Second, if the concentration of ANF had been subthreshold, then there should have been no effect on the angiotensin response. Third, ANF administered by two different routes in two different tissues produced similar effects on basal vasomotor tone and hormone-induced vasoconstriction. Thus, it is unlikely that a subthreshold dose of ANF could explain these present results.

Interpretation of Results

Although high concentrations of ANF blunted angiotensin-induced vasoconstriction, ANF did not alter vascular tone in unstimulated arterioles, norepinephrine-stimulated arterioles, or vasopressin-stimulated arterioles, nor did it completely eliminate the vasoconstriction caused by angiotensin. This action of ANF is probably not an expression of angiotensin receptor blockade because ANF inhibits angiotensin-stimulated aldosterone production in isolated zona glomerulosa cells by a mechanism that is distal to the receptor. If ANF does not prevent angiotensin binding in vivo, then it might interfere with the cellular mechanism that causes vasoconstriction.

Since the responses evoked by norepinephrine and vasopressin were not attenuated by ANF, there might be more than one mechanism causing vasoconstriction in the microcirculation, only one of which is sensitive to ANF. There is evidence that the renal vasculature reacts by at least two distinct mechanisms. Cooper and Malik have concluded that renal vasoconstriction produced by activation of angiotensin receptors depends on extracellular calcium but not on calmodulin, while activation of norepinephrine receptors depends on both calmodulin and extracellular calcium. In addition, these authors concluded that angiotensin and norepinephrine stimulated renal prostaglandin synthesis by a process that was distinct from the vascular effect.
the perivascular space would have been different. In summary, in the skeletal muscle and intestinal microcirculation, exogenous ANF reduced the vasoconstriction caused by topical angiotensin by approximately 50% without altering basal vasomotor tone or the active response induced by norepinephrine or vasoressin. The mechanism for this differential effect of high concentrations of ANF is unknown, but it is probably not dependent on a cyclooxygenase product. Based on the assumption that ANF does not block angiotensin receptors, we speculate that angiotensin, vasoressin, and norepinephrine cause arterial vasoconstriction by at least two mechanisms in the microcirculation and that a fundamental difference between the mechanisms was unmasked by ANF. If physiologic concentrations of endogenous ANF produce a similar effect in unanesthetized animals, then ANF could be an important modulator of hormone-induced vasoconstriction in the peripheral circulation.

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