SUMMARY The atrial natriuretic factor (ANF) has pharmacological actions resulting in lower atrial and arterial pressures. Atrial distention stimulates ANF release, suggesting that ANF is an effector limb of a feedback loop for controlling cardiac filling pressure. To test this hypothesis it will be necessary to determine whether physiological atrial distention releases ANF in sufficient amounts to exert biological actions. Immunoblockade of endogenous ANF and attenuation of ANF release by atrial ablation inhibited volume-induced natriuresis in rats. Infusion of ANF in rats at doses mimicking those observed during experimental volume expansion produced a natriuresis sufficient to partly account for the volume-induced response. Infusion of ANF at doses expected to change plasma ANF levels minimally decreased arterial pressure in hypertensive rats over 7 days. In dogs, some studies suggest that increased plasma ANF levels following experimental changes in atrial pressure were not sufficient to exert acute cardiovascular or renal actions, whereas others support such a notion and indicate that ANF inhibited barostimulated renal renin release. This last action could alter arterial pressure in the long term by allowing sodium equilibrium at lower renal arterial pressure. Infusion of ANF in humans that produced plasma levels in the upper physiological range caused increased sodium excretion and decreased plasma renin activity. Although data are exiguous, justifying neither acceptance nor rejection of the hypothesis that ANF functions physiologically to regulate body fluid volume and arterial pressure, the current evidence slightly favors acceptance. (Hypertension 10 [Suppl I]: I-122-I-127, 1987)

KEY WORDS • plasma atrial natriuretic factor concentration • cardiovascular and renal actions • prolonged infusion
sure. Whether ANF release is modulated by neurotransmitters, circulating factors, or heart rate per se is less certain.

**Physiological Effects of ANF**

To evaluate the importance of ANF as a hormone it is necessary to determine if this peptide exerts biological actions at circulating levels encountered during physiological conditions or in pathophysiological situations. The present state of knowledge precludes categorical conclusions, however. First, there is little information about the effects of blockade of endogenous ANF. Second, as with other peptide hormones, some physiological actions may require several days to develop fully, and thus may only be apparent if ANF is administered for a prolonged time; information of this nature is sparse. Finally, although numerous reports have appeared concerning plasma concentration of immunoreactive ANF in humans and experimental animals, only a few studies documented the plasma levels of immunoreactive ANF after exogenous administration to be within the range of those measured during various physiological or pathophysiological states.

It is important for two reasons that such comparisons be done in each laboratory where these studies are conducted. One reason is that the loss of peptide by adsorption to the delivery system can markedly decrease the actual dose from that calculated. Because the amount of loss can vary among laboratories, this precludes extrapolation of the plasma concentration from the calculated dose. Another reason is the variation in the radioimmunoassay of ANF among laboratories. To obviate interference from plasma proteins, most workers now measure ANF immunoreactivity in plasma extracts rather than by direct assays. Using plasma extract, a majority of studies show mean values for basal plasma ANF of healthy volunteers in the range of 10 to 60 pg/ml. However, much variation still exists among laboratories, with individual values ranging as low as 2 to 12 pg/ml or as high as 27 to 152 pg/ml for basal levels of plasma ANF in healthy subjects. Differences in procedures for collecting and storing samples, extraction recoveries, specificities of antibodies, and purities of standards probably account for these disparities.

Thus, in this discussion, assessment as to whether or not a reported plasma ANF level after infusion of exogenous ANF falls within the physiological range is based only on comparison to the range of plasma values actually measured in the same laboratory where the infusion was carried out and using the same assay procedures as were used to measure the plasma levels after infusion. Also, unless otherwise specified, only values obtained from extracted plasma are discussed.

**Rats**

With regard to blockade of endogenous ANF, in two studies antibodies against ANF were administered intravenously to Inactin-anesthetized rats. Injection of an anti-ANF antiserum caused a marked decrease in urine volume output and urinary sodium excretion last-

ing 30 minutes, a significant increase in plasma renin activity, but no changes in arterial blood pressure or plasma aldosterone concentration. Although plasma ANF was not measured in this study, we have found that plasma immunoreactive ANF is similar in conscious (91 ± 6 pg/ml, n = 17) and Inactin-anesthetized (75 ± 22 pg/ml, n = 5) rats (R.W. Barbee, unpublished data, 1987). Thus, these findings suggest that in rats, plasma ANF circulating at a level not greater than that observed during resting, conscious conditions exerts a diuretic-natriuretic effect and inhibits plasma renin activity, but does not influence the control of arterial blood pressure or plasma aldosterone concentration.

In contrast to these findings, others found that intravenous injection of mouse ascitic fluid containing monoclonal antibodies against ANF into Inactin-anesthetized rats did not result in antinatriuresis. This suggests that endogenous ANF at resting (or lower) levels does not influence sodium excretion. There is currently no explanation for the different results in these two studies.

More consistent conclusions have been reported among laboratories with respect to the effects of endogenous ANF during rapid volume expansion in rats. Removal of the atrial appendages, which are major storage sites for proANF, blunted the volume-induced increase in plasma ANF and reduced the ability of anesthetized rats to excrete an acute intravenous volume load. This suggested that less ANF was available for release in the rats that had undergone atrial appendectomy, and that an attenuated increment in plasma ANF level accounted for the blunted natriuretic and diuretic responses. Also consistent with these studies is the observation that pretreatment of anesthetized rats with monoclonal antibodies directed against ANF resulted in nearly total blockade of the natriuretic and diuretic responses to experimental volume expansion. Moreover, in an infusion study, the natriuretic response to experimental volume expansion was compared with that after administration of ANF at a dose that mimicked the volume-expanded plasma ANF level. Compared with a control group, plasma ANF increased threefold in both the volume-expanded and ANF-infused rats, and urinary sodium excretion increased 11.8- and 4.6-fold in the two groups, respectively. These results suggest that approximately 38% of the increase in urinary sodium excretion observed during volume expansion could be accounted for by the natriuretic effect of circulating ANF. Thus, in rats evidence from several different lines of investigation supports the concept that the plasma level of ANF achieved during short-term experimental volume expansion is great enough to enhance urinary sodium excretion.

**Dogs**

Unlike the results in rats, experimental volume expansion in conscious dogs that had undergone atrial appendectomy caused diuresis and natriuresis that did not differ from those observed in the sham-operated
In healthy subjects, this plasma ANF level followed laboritories that plasma ANF concentration in dogs increased in response to elevated atrial pressure and deoxycorticosterone acetate (DOCA) treatment, resulted in significant effects on the cardiovascular and renal systems. For instance, increasing right or left atrial pressure by 3 to 5 mm Hg caused plasma ANF concentration to increase from 50 to 55 pg/ml to over 200 pg/ml. Administration of DOCA in dogs caused plasma ANF to increase from 42 to 99 pg/ml by Day 8 of treatment. During infusion of ANF at 10 ng \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \), plasma ANF increased to 134 pg/ml as compared with 55 pg/ml for the time control, while urinary sodium excretion doubled, mean arterial pressure decreased by 9 mm Hg, and heart rate did not change. These results suggest that physiologically feasible plasma levels of ANF could have an influence on renal sodium excretion and arterial blood pressure in dogs. Currently, there is no clear explanation for the discrepancy between studies. Thus, although agreement exists among laboratories that plasma ANF concentration in dogs increases in response to elevated atrial pressure and intravascular volume expansion, the results with respect to whether these circulating levels exert physiological effects are at odds.

**Humans**

Continuous infusion of ANF into healthy subjects at approximately 50 ng \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) for 30 to 60 minutes increased plasma ANF levels to the upper range observed in pathophysiological states. In healthy subjects on a high salt diet, this plasma ANF level follow-

**Prolonged Infusion**

Rat ANF (101–126) was infused by osmotic pumps into rats with various forms of hypertension at approximately 5 ng \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \). Systolic blood pressure, measured indirectly, decreased gradually over 7 days in spontaneously hypertensive rats (SHR) and in rats with one-kidney, one clip or two-kidney, one clip renal hypertension. Compared with noninfused rats, urinary sodium excretion was increased, not different, or decreased, perhaps depending on the type of hypertension. No changes in systolic blood pressure or natriuresis were observed in normotensive rats infused with the same dose of ANF.

In a similar study, human ANF (99–126) was infused at approximately 7 ng \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) into SHR and normotensive rats maintained on high or low sodium intake. Systolic blood pressure decreased within 24 hours after implanting the peptide-filled pump in all four groups and remained depressed throughout the 7-day infusion. Urinary sodium excretion gradually increased only in the SHR maintained on the high sodium diet, supporting previous findings that the de-
pressor effect of prolonged infused ANF was not initiated by sodium depletion. These findings, from two different laboratories, that prolonged infusion of ANF at doses not expected to increase plasma ANF concentration by a detectable amount, lowered arterial blood pressure in hypertensive and, in one study, normotensive rats are intriguing. These studies should be extended to include measurements of arterial blood pressure by direct methods, other hemodynamic variables, and plasma ANF concentration.

Similar novel results occurred in patients with severe congestive heart failure who received ANF infusion at 8 ng·min⁻¹·kg⁻¹ for 2 hours. Glomerular filtration rate and renal plasma flow increased markedly despite the low dose of ANF, and the fact that it was administered to patients with a disorder known to be associated with elevated levels of circulating ANF. Clearly, more work is needed to verify these early observations on the effects of low level infusions of ANF and to examine their potential importance.

In other studies, the relative potencies of rat ANF (103-126), ANF (103-125), and ANF (103-123) were compared for smooth muscle relaxing activity, acute hypotensive activity, and long-term hypotensive action (7 days) in SHR. Although differences in potencies were observed for the first two biological actions, the three peptides had equal potencies for the long-term blood pressure-lowering effect when infused at 0.4 nmol·h⁻¹·kg⁻¹ (approximately 15 ng·min⁻¹·kg⁻¹).

It is reasonable to assume, although it was not documented, that the circulating level of ANF achieved by this dose did not exceed the physiological range. These findings suggest that the mechanisms by which ANF alters the long-term control of arterial pressure may be different from those operating over the short term or in relaxing aortic smooth muscle.

Higher doses of ANF (40-100 ng·min⁻¹·kg⁻¹) infused for 3 to 7 days were hypotensive but not natriuretic in the following rat models of hypertension: two-kidney, one clip; angiotensin II-infused; and norepinephrine-infused. Although it is unclear whether these doses mimicked physiological, pathological, or pharmacological blood levels of ANF, the following information can be gleaned from these studies. It is unlikely that the antihypertensive effect of ANF was initiated by urinary loss of water and sodium, although an altered relationship between renal perfusion pressure and urinary sodium excretion obviously occurred. A 3-day infusion of ANF completely reversed the hypertension caused by norepinephrine infusion, yet only slightly lowered arterial pressure in angiotensin II-infused rats. However, this does not indicate that ANF is more effective at reversing the effects of the sympathetic nervous system than those of the renin-angiotensin system. It may be quite effective at inhibiting renin release and thus may have a profound influence on this system under more natural conditions. Also, it is noteworthy that even though on a molar basis the dose of angiotensin II was 18 times greater than that of ANF, the latter had a slight antihypertensive effect. Finally, except for one study, no depressor or natriuretic effects were noted when ANF was infused in normotensive rats, suggesting that in rats the homeostatic mechanisms for long-term blood pressure control must be initially activated or altered from basal activity for ANF to have a sustained hypotensive effect.

In contrast, when ANF was administered at 50 ng·min⁻¹·kg⁻¹ into normotensive dogs, producing an 11-fold increase in plasma ANF concentration, mean arterial pressure decreased significantly over the 5-day infusion. No long-term effects were observed on glomerular filtration rate, urinary sodium excretion, plasma renin activity, or plasma aldosterone concentration. The lack of increase in plasma renin activity despite the decrease in arterial pressure and presumably renal perfusion pressure suggested a sustained inhibitory effect of ANF on barostimulated renin release. This notion is supported by the observation that long-term infusion of ANF into rats with two-kidney, one clip hypertension decreased plasma renin activity to normal levels.

A powerful effect of ANF to inhibit barostimulated renin secretion was also demonstrated in short-term studies in dogs. Infusion at only 5 ng·min⁻¹·kg⁻¹, which increased plasma ANF concentration 70%, abolished the effectiveness of decreased renal perfusion pressure to increase plasma renin activity. These studies demonstrate that physiological plasma levels of ANF have the potential to modulate the activity of an important pressure–fluid volume homeostatic control system.

Thus prolonged infusion of ANF into hypertensive rats and normotensive dogs at doses ranging from low physiological to high pathological or pharmacological resulted in sustained decreases in arterial blood pressure without consistent changes in urinary sodium excretion. This suggests that ANF infusion altered the long-term renal pressure-natriuresis mechanism such that sodium equilibrium was maintained at a lower renal perfusion pressure. ANF could effect this new equilibrium by acting directly in the kidneys or by modulating other systems that impinge on cardiovascular and renal functions, such as the renin-angiotensin-aldosterone system and the sympathetic nervous system. This has important therapeutic implications if ANF analogues are to be used for treating patients with hypertension or heart failure. Although it is not established that ANF has this capacity under physiological conditions, evidence suggests that exogenously administered ANF has an antihypertensive effect at fairly low plasma concentrations. This observation requires verification by direct measurement of arterial pressure, plasma ANF concentration, and other hemodynamic variables.

**Perspective**

The available data justify neither acceptance nor rejection of the hypothesis that ANF functions under physiological conditions to regulate urinary sodium excretion and arterial blood pressure. Although some
of the data are conflicting, the greatest obstacle in assessing the possible physiological importance of ANF is the paucity of crucial information. After synthetic ANF became available, a surge of in vivo studies were conducted using pharmacological doses. These provided important information regarding possible therapeutic effects of ANF and verified the biological actions observed earlier with crude atrial extract. Indeed, more studies of this nature are warranted so that mechanisms of action can be determined.

A second wave of studies designed to examine the actions of ANF at physiological levels is just under way. Critical interpretations of these data should include some of the following considerations. First, ANF administered at physiological levels might have only subtle, nondetectable effects in the short term, but if administered over a period of days or weeks the effects could be amplified by its concurrent actions on other regulating systems, such as the renin-angiotensin-aldosterone and autonomic nervous systems.

Second, the importance of ANF as a hormone may differ among species. For instance, a reflex mechanism linking atrial stretch and renal function appears to be prominent in dogs but less important in nonhuman primates. Perhaps the humoral component of this atrial-stretch-natriuresis response is more important than the neural element in some species.

Third, administration of ANF at physiological doses may cause important cardiovascular and body fluid volume alterations that could go unnoticed if certain variables are not measured. For instance, ANF infusion in humans led to a decrease in stroke volume without causing a change in arterial blood pressure, presumably partly because of reflex vasoconstriction. At pharmacological doses, ANF decreased blood volume and central venous pressure in anephric rats, presumably because of fluid migration into the interstitium and perhaps because of increased resistance to venous return. If only arterial blood pressure is measured, possible cardiac output and venous return effects of ANF may not be observed, and if only urinary sodium excretion is measured, possible blood volume changes may not be observed.

Finally, the importance of the renal pressure-natriuresis mechanism in maintaining sodium and water balance should be taken into account. According to this concept, arterial pressure has a dominant long-term influence on sodium and water excretion that could override the natriuretic effects of ANF and other natriuretic factors. This mechanism could be the fundamental force causing sodium retention in congestive heart failure, despite high circulating levels of ANF. However, the presumed dominance of the renal pressure-natriuresis mechanism does not preclude an important role for ANF in sodium homeostasis and arterial blood pressure regulation. Just as angiotensin II displaces this relationship to higher pressures, ANF could shift it in the opposite direction. Further studies are needed to compare the potencies of these two counteractive peptides in altering the long-term relationship between renal perfusion pressure and natriuresis.

It is reasonable to expect that, given the widespread interest in this putative new hormone, the physiological studies essential for assessing its possible contribution to body fluid regulation and arterial blood pressure control will be forthcoming in the near future. Furthermore, studies of this nature will surely be spurred if and when an ANF antagonist becomes available. As of this writing, a few studies suggest that ANF has cardiovascular, renal, and hormonal actions at physiologically feasible blood levels in rats, dogs, and humans, although the evidence in dogs is controversial.

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