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COMMENTARY

RENAL AND CARDIOVASCULAR EFFECTS OF ATRIAL NATRIURETIC FACTOR

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The discovery of atrial natriuretic factor (ANF) by Adolpho de Bold and co-workers in 1981 [1] opened a new and exciting chapter in the physiology and pharmacology of the renal and cardiovascular systems The progress in the field in the past 4 years has been nothing short of remarkable, particularly in the characterization of the chemical structure and the functional properties of ANF [2–4]. In the present short article, we will consider the effects of ANF on the renal and systemic vasculature as well as on cardiovascular dynamics, without attempting to be all inclusive. For a general overview of the chemical and functional properties of ANF, the reader is directed to three recent reviews on the subject [2–4] and to a recent Symposium on Atrial Natriuretic Factor (Annual Meeting of FASEB, Anaheim, CA, April 1985) (*Fedn Proc.* **45** (7), 1986).

The first issue to be addressed is that of the nomenclature of ANF, which became a source of confusion even among investigators in the field. Almost simultaneously several groups of investigators, including our own, purified structurally related atrial natriuretic peptides of different lengths and provided particular names (cardionatrins, atriopeptins, auriculins), followed by letters or numbers (e g. auriculin A and B, atriopeptin I, II and III) [2–12]. The numeration of the amino acids in the ANF molecule also became arbitrary, number 1 being the first N-terminal amino acid of the ANF precursor or the first N-terminal amino acid of a particular sequence chosen as the basic structure of ANF [2–12]. Recently, it has been determined that the 28 amino-acid cardionatrin I, first characterized by de Bold's group [5], is the main circulating form of ANF [13] Furthermore, it is possible, and even likely, that the other peptides may be simply the result of hydrolytic artifacts during the purification procedure [3]. Consequently, in the present article, cardionatrin I will be considered the basic ANF peptide and will be identified as (1-28)ANF. The other peptides will be identified accordingly, i e (1-28)hANF = αhANP, (4-27)ANF = auriculin A, (5-25)ANF = atriopeptin I, etc. (see Fig. 1).

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Based on the results of our early studies, we have proposed that, from a point of view of its vascular actions, ANF may be described as a potent antagonist of vasoconstrictors with a weak but significant direct or indirect agonist (vasoconstrictive) action of its own [14]. As will be described below, the antagonist action is not due to a competition between ANF and vasoconstrictors at a receptor level but is probably due to alterations in intracellular calcium homeostasis with cGMP acting as a putative secondary messenger. The agonist (vasoconstrictive) action of ANF is particularly important in the kidney where it is at least partly responsible for the increase in glomerular filtration rate (GFR) and sodium excretion which are the major physiological effects of ANF on this organ [3, 14]. The characterization of ANF as a functional partial agonist will be used in the present article as the main conceptual framework to understand the complex and sometimes apparently contradictory effects of ANF on the renal vasculature, on isolated vascular smooth muscle preparations, and on cardiovascular dynamics

EFFECTS OF ANF ON THE RENAL VASCULATURE AND ON RENAL FUNCTION

The concept that ANF behaves as a functional partial agonist originated from our initial observations on the effects of crude atrial extract on the renal vascular resistance (RVR), hemodynamics, and excretory functions in a functioning isolated perfused rat kidney preparation [14]. These observations were then confirmed using synthetic atrial peptides in the isolated preparation [6, 14, 15] as well as in intact anesthetized or conscious dogs [3, 16] Figure 2 illustrates the effects of ANF on the renal vascular resistance in vasodilated or vasoconstricted isolated rat kidneys. When either crude atrial extract or synthetic (4-27)ANF (auriculin A) is added to a perfusate of a functioning isolated rat kidney preparation, perfused in the absence of other vasoactive substances, there is a slow, prolonged and relatively small increase in RVR (upper panel) In contrast, when the isolated kidney is precontracted with angiotensin II or other hormonal (norepinephrine, vasopressin) or non-hormonal (ouabain, tetracaine) vasoconstrictor agents [6, 14, 16], there is a prompt and sustained decrease in RVR towards control levels (lower panel)

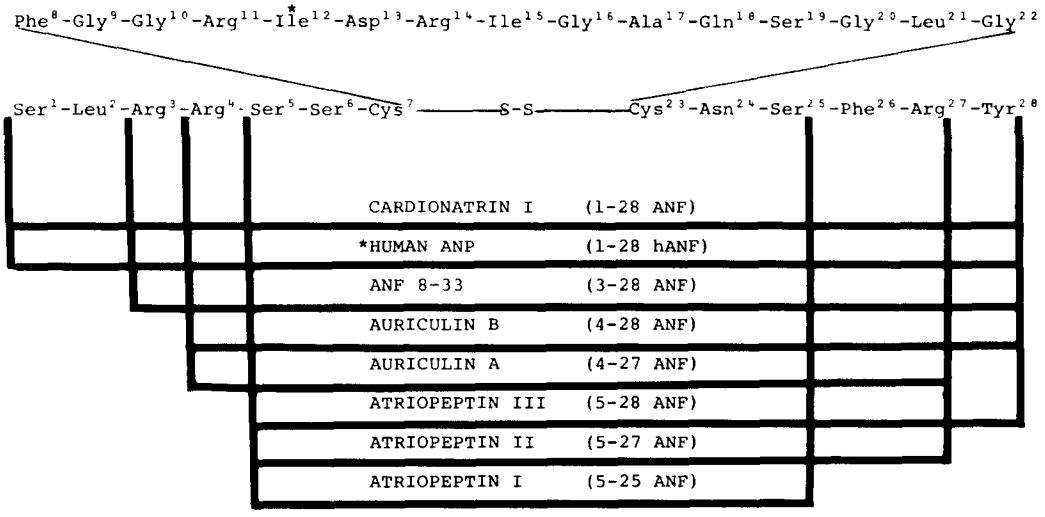

Fig 1 Amino acid sequence of atrial natriuretic peptides isolated, characterized, and synthetized by several groups of investigators [2–12] See text for nomenclature

The agonist (vasoconstrictive) effect of ANF is accompanied by a hemodynamic profile which encompasses a major increase in filtration fraction and GFR, indicating that ANF has an efferent arteriolar vasoconstrictive effect [3, 6, 14]. Recently, direct evidence was obtained in isolated perfused glomeruli of the dog [17] and in intact anesthetized Munich–Wistar rats [18] demonstrating that, indeed, ANF is a preferential efferent arteriolar vasoconstrictor This effect leads to an increase in glomerular capillary hydrostatic pressure [17, 18] which, in turn, is responsible, at least in part, for the increase in GFR Ultimately, the increase in GFR, together with a wash-out of the inner medulla, accounts wholly or in great part for the ANF-induced natriuresis [3, 14, 16, 19, 20] The agonist effect of ANF in the isolated perfused rat kidney is abolished at low levels of extracellular [Ca^{2+}] (0 2 mM) and is blocked by verapamil [14]. Under these conditions, the increase in GFR and the ANF-induced natriuresis are also abolished [14] Despite initial claims to the contrary [7], there is no indication of a separation of vasoactive and natriuretic effects of ANF with any of the atrial peptides tested to date [3] The antagonist (vasorelaxant) action of ANF is partly independent of extracellular [Ca^{2+}] since ANF is able to block a tetracaine-induced contraction in the virtual absence of calcium and in the presence of 1 mM ethyleneglycolbis(amino-ethylether)tetra-acetate (EGTA) [15]. Thus, the natriuretic effect of ANF depends mostly on its agonist rather than on its antagonist action (see below)

From the foregoing, it is clear that ANF does not behave as a powerful renal vasodilator as it has sometimes been assumed [21] This contention is strengthened by the fact that classical renal vaso-

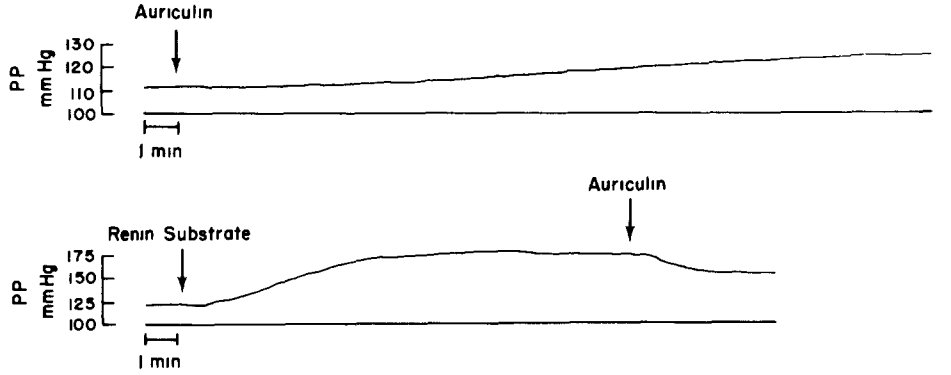

Fig 2 Comparative effects of synthetic (4-27)ANF (auriculin A) on the renal vascular resistance of isolated rat kidneys perfused in the absence or presence of vasoconstrictors (4-27)ANF was added to the perfusate at a final concentration of 5 nM *Upper panel* In isolated rat kidneys perfused in the absence of vasoconstrictors, (4-27)ANF slowly increases the perfusion pressure (PP) (and the renal vascular resistance) to a new plateau level which is significantly above control levels *Lower panel* In isolated kidneys preconstricted by the addition of renin substrate to the perfusate (generation of angiotensin II), (4-27)ANF causes a prompt large decrease in PP and in renal vascular resistance See text and Refs, 3, 6 and 14 for details Modified from Maack *et al* [3]

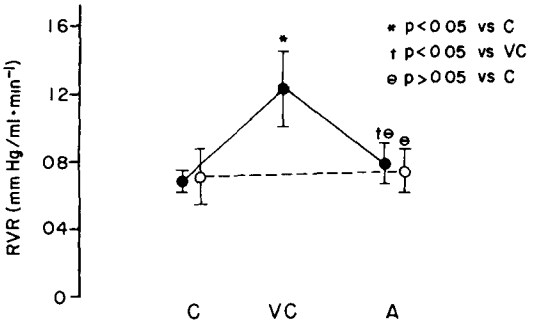

Fig 3 Steady-state effects of auriculin (A) on the renal vascular resistance (RVR) of normal (C) and vasoconstricted (VC) kidneys of the dog The open circles and dashed line show that (4-27)ANF does not alter the steady-state RVR in dogs with an initial normal renal vascular tone. The closed circles and solid line show that, when the initial renal vascular tone is raised (C to VC), (4-27)ANF returns the RVR to normal basal levels (VC to A) These data indicate that administered ANF tends to normalize renal vascular resistance and renal blood flow in intact dogs (see text) (4-27)ANF was infused i v at a dose of 0 1 $\mu g \cdot min^{-1}$/kg body weight RVR was determined by the ratio of the mean renal perfusion pressure and the mean renal blood flow as determined by electromagnetic flowprobe Based on data from Sosa *et al* [20]

dilators such as acetylcholine and bradykinin, contrary to ANF, are able to decrease the RVR of isolated rat kidneys perfused in the absence of vasoconstrictors. It should be noted that *in vitro* or *in situ* pharmacological preparations of perfused rat kidney, contrary to the physiological preparation used in our study, have a very high initial renal vascular tone It is, therefore, not surprising that in such preparations ANF acts fundamentally as a vasorelaxant [7, 21].

In addition to differences regarding the calcium-dependence of the agonist and antagonist effects of ANF, the former differentiates from the latter by its much slower onset and rise to a plateau level (see Fig. 1) [3, 6, 14, 19]. It is possible that the efferent arteriolar vasoconstrictive effect of ANF may be due to a secondary release of a vasoconstrictor rather than to a direct effect of the peptide [3, 6, 14, 19]. This would explain the reason why the agonist effect of ANF cannot be observed in isolated vascular strip preparations (see below) Our attempts to verify whether prostaglandins or leukotrienes are involved in such action of ANF have so far proven negative (M J. F. Camargo and T Maack, unpublished observations)

Membrane preparations obtained from homogenates of kidney cortex contain specific high affinity binding sites for ANF with a K_d of about 50 pM [22] The K_d of binding of (1-28)ANF to a whole isolated perfused rat kidney is of the same magnitude, the bulk of the binding sites being present in the renal cortex but with a significant number of lower affinity binding sites in the papilla [23] Recent autoradiographic evidence clearly indicates that the renal binding sites are localized in vascular structures, particularly in glomeruli and in vasa recta [24]. Isolated glomeruli also show high capacity ANF-specific binding sites with approximately the same K_d as in whole kidney tissue [25]. The localization of specific receptors for ANF in the kidney and the elucidation of the kinetics of receptor–ANF interaction in particular structures of the kidney may be decisive pieces of information to fully understand the physiological role of ANF and the mechanisms of the renal effects of ANF In this respect, the results obtained to date strongly favor the view that the natriuretic effect of ANF is due to its renal hemodynamic and vascular actions, particularly to the increase in GFR and the wash-out of the inner medulla [3, 6, 14, 16, 19, 20]. ANF leads to large increases of cGMP in isolated glomerular preparations [26], but it remains to be determined whether this increase represents a primary or secondary effect of ANF. In any event, the initial studies referred to above strengthened the notion that ANF is primarily a renal vasoactive substance

The results obtained in isolated perfused rat kidneys permitted the prediction that the net effect of ANF on the renal vasculature of intact animals would depend on the initial renal vascular tone. In intact anesthetized or conscious dogs with a normal initial renal vascular tone, constant infusion of ANF leads initially to a very transient decrease in RVR which lasts only 1–3 min [3, 16, 19, 27] For the remainder of the infusion of ANF (30 min), RVR returns to, or even above, control levels [3, 16, 19, 27]. However, filtration fraction, glomerular filtration rate, and sodium excretion remain elevated during the entire period of the ANF infusion [3, 16, 27]. In contrast, when the initial renal vascular tone of the intact dog kidney is raised, e.g by manipulating the renal pedicle, ANF leads to a sustained decrease in the RVR towards control basal levels [19, 20]. Consequently, as illustrated in Fig 3, administered ANF tends to normalize the renal vascular resistance and, in this manner, the renal blood flow in intact animals

Since ANF may increase filtration fraction and GFR in face of an unchanged RVR [3, 16, 19, 20], it must be assumed that, under this condition, the efferent vasoconstrictive effect is balanced by a corresponding afferent arteriolar vasodilation [18, 27, 28]. In our view, however, the latter phenomenon may be simply due to the normal autoregulatory response of the kidney to the ANF-induced decrease in arterial blood pressure (see below). Whatever the case, it is noteworthy that in steady-state conditions ANF does not decrease the total RVR to below basal levels (Fig. 2) [19, 20]. This supports the notion that ANF does not behave as a classical renal vasodilator but as a strong antagonist of renal vasoconstriction. In addition, the results obtained to date clearly indicate that the natriuretic effect of ANF is unrelated to changes in total RVR or renal blood flow (RBF) [3, 16, 27, 28] Indeed, the ANF-induced increase in GFR and sodium excretion is of the same magnitude in kidneys with an initial normal or decreased renal blood flow [19, 20].

The mechanism of the natriuretic effect of ANF has been the subject of considerable controversy but the results obtained to date strongly favor the postulate that the ANF-induced increase in sodium excretion is due wholly or in great part to its renal

hemodynamic effects, particularly to the combination of the increase in GFR and the decrease in inner-medullary hypertonicity [3, 14, 16, 19, 20] The reason for the latter effect remains to be elucidated but it may be due to increases in vasa-recta blood flow, luminal flow rate, passive permeability of papillary collecting ducts or to a combination of these events Despite intensive efforts by several groups of investigators, there is no direct evidence to date supporting the notion that ANF has a direct tubular Na^+-transport inhibitory action (for review see Ref 3). It cannot be ruled out, however, that ANF may increase the passive permeability of papillary collecting ducts to sodium, a phenomenon which, if present, may contribute to its natriuretic effect [29] In any event, the primary role of the increase in GFR and the decrease in inner-medullary hypertonicity in the ANF-induced natriuresis has been demonstrated conclusively in recent "early clamp" experiments in the dog [20]. In these experiments the ANF-induced increase in sodium excretion was fully abolished when perfusion pressure of the kidney was decreased in such a manner as to block the increase in GFR and the decrease in inner-medullary hypertonicity [20].

One of the main inferences to be derived from the results described above is that administered ANF, by behaving as a functional partial agonist, modulates renal vascular tone in such a manner as to keep renal vascular resistance and renal blood flow within normal limits [3, 16, 19, 20] This is a remarkable property which, to our knowledge, is not shared by any other endogenous vasoactive substance Whether homeostatic regulation of renal blood flow is also one of the main physiological roles of ANF remains to be determined

EFFECTS OF ANF ON ISOLATED BLOOD VESSELS

The original observation of Deth *et al* [30] that crude atrial extract relaxes isolated rings of the rabbit aorta pre-contracted with catecholamines was confirmed and extended in several laboratories, including our own [2–4, 31–33]

ANF antagonizes both receptor- (e g angiotensin II, norepinephrine, histamine) and non-receptor- (e.g potassium depolarization) induced contractions in rabbit aortic rings [2–4, 6, 30–33] Contrary, however, to what has been shown in the isolated kidney ANF does not have a detectable agonist (vasoconstrictor) action in isolated strips or rings of the rabbit aorta [31, 32] As pointed out above, this lack of agonist effect may be due to the absence of the release of a secondary vasoconstrictor in isolated blood vessel preparations.

Although ANF counteracts the effects of all vasoconstrictors tested to date, we have found that it is a particularly powerful antagonist of angiotensin II-induced contractions in isolated rings of the rabbit aorta [32]. Figure 4 (top panel) shows that ANF, in addition to shifting the angiotensin II dose–response curve to the right, also markedly blunts the maximal angiotensin II-induced contraction The bottom panel of Fig. 4 shows that, whereas ANF also shifts the dose–response curve of norepinephrine contraction to the right, it does not decrease the maximal

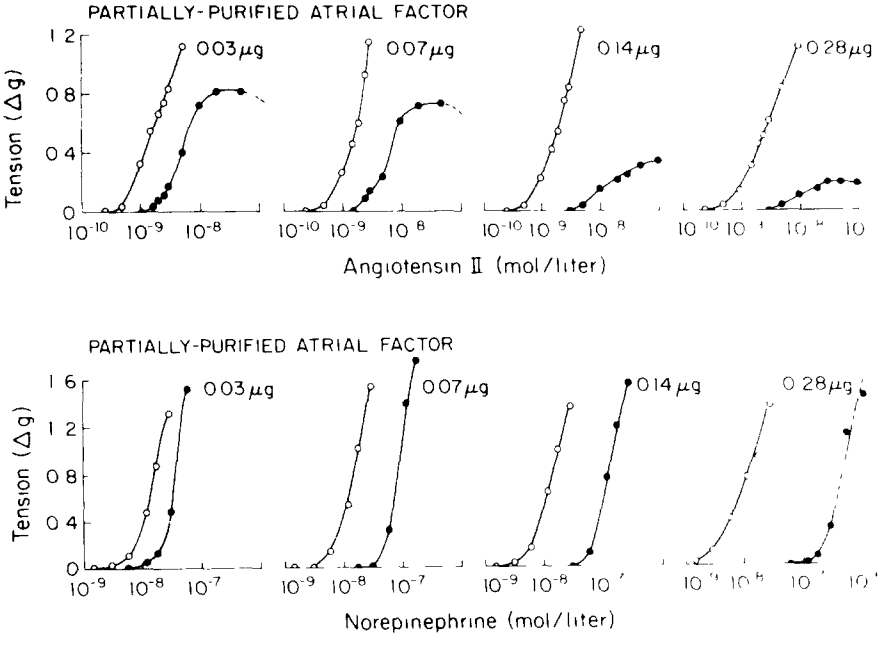

Fig 4 Antagonist effect of partially purified atrial natriuretic factor (ANF) on norepinephrine- and angiotensin II-induced contraction of isolated rings of the rabbit aorta *Upper panel* ANF shifts the dose–response curve of angiotensin to the right and decreases the maximal angiotensin-II-induced contraction *Lower panel* ANF shifts the dose–response curve of norepinephrine to the right without changing the maximal contraction These data indicate that ANF is a particularly strong antagonist of angiotensin II For details, see text and Ref 32 Modified from Kleinert *et al* [32]

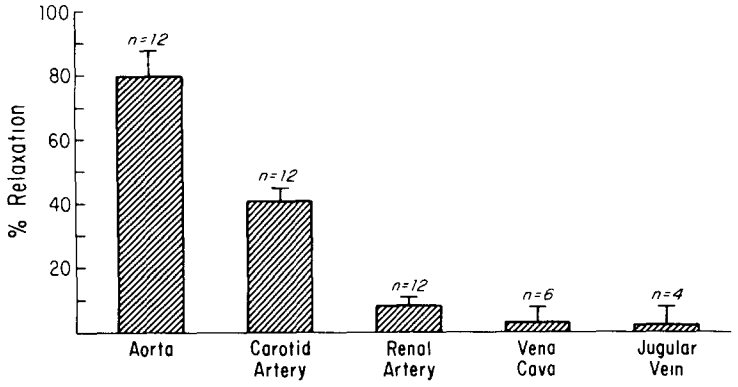

Fig 5 Vasorelaxant effect of synthetic (4-27)ANF (auriculin A) on various rabbit isolated blood vessels pre-contracted with histamine Optimal resting tension was adjusted for each blood vessel Histamine was added to the bathing solution at a final concentration of 6 μM and (4-27)ANF at a final concentration of 24 nM. The aorta is the most responsive artery, followed by the carotid and renal arteries The vena cava and jugular veins are practically unresponsive to the effect of (4-27)ANF Based on data from Kleinert *et al* [37]

contractile response as compared to controls. Furthermore, high doses of norepinephrine, but not of angiotensin II, are able to overcome fully the maximal inhibitory effect of ANF on the contractile response of the rabbit aorta [32]. This finding led to the postulate that ANF may be a particularly powerful antagonist of angiotensin II-induced contractions in intact mammals [32]. As will be described in the section on effects of ANF on the cardiovascular system (see below), comparisons between the blood-pressure lowering effect of ANF in renin-dependent and -independent models of hypertension tend to support this hypothesis [34].

The sensitivity of isolated arteries and veins to the antagonistic action of ANF is very variable [33, 35–38]. Figure 5 shows the results of our studies on the responsiveness of histamine-precontracted arteries and veins of the rabbit to the vasorelaxant effect of (4-27)ANF. Of the arteries studied, the aorta was the most sensitive followed by the carotid and main renal artery. The vena cava and the jugular vein were essentially unresponsive to the vasorelaxant action of (4-27)ANF [37]. Winquist *et al.* [33] reported that the facial vein of the rabbit is exquisitely sensitive to ANF, but this vein has a thick vascular wall and high basal tone. In this respect, the facial vein of the rabbit resembles an artery more than a vein. These authors also reported [36] that the coronary, iliac and femoral arteries are poorly responsive to ANF, whereas the renal artery has a higher responsiveness than that observed in our study Differences in preparation, the agonist used to induce contraction, and the nature and dose of particular atrial natriuretic peptides may account for the apparent discrepancies among the results of different laboratories. In general, it is more difficult to study physiological or pharmacological responses of isolated preparations of veins than of arteries because veins are mostly composed of connective tissue and depend on an intact sympathetic nervous system for their tone Thus, it remains worthwhile to consider that, despite the lack of responsiveness of isolated veins, ANF may produce venodilation *in vivo* (see below).

The reasons for the differences in the response of isolated vessels to the antagonist effect of ANF are not entirely clear. It is to be expected that responsive vessels have a high density of specific ANF receptors and this seems, indeed, to be the case for the aorta and the facial vein of the rabbit [38]. However, some veins (e.g the renal and jugular veins) which have a high density of specific binding sites for ANF, are very insensitive to its effects, at least in *in vitro* conditions [38]. Further studies are needed to clarify the sensitivity of vascular beds to the antagonist action of ANF. In particular, it is also necessary to study the effects of ANF on smaller resistance and capacitance vessels

Extensive structure–activity relationship studies indicate the relative importance of the C- and N-terminal portions of the ANF molecule for the vasorelaxant action of ANF in isolated strips or rings of the rabbit aorta. For the following discussion the reader is directed to Fig. 1 The disulfide bridge between Cys^{7} and Cys^{23} is essential for the vasorelaxant as well as for all other known biological activities of ANF [3, 4, 6, 7, 12] The C-terminal Tyr^{28} is apparently of little importance, whereas deletion of the C-terminal Phe^{26}-Arg^{27} markedly reduces but does not abolish the vasoactive or the natriuretic effects of ANF [2–4, 12] More difficult to interpret is the role of the N-terminal portion of the ANF molecule. A more extended N-terminal portion tends to increase the apparent relative biological activity of ANF in the rabbit aorta [4, 35] Part of the differences may be due, however, to alterations in biological half-life rather than to intrinsic biological activity In any event, from the data gathered to the present, one may summarize the order of vasorelaxant activity of ANF as (1-28)ANF = (2-28)ANF = (3-28)ANF > (4-28)ANF = (4-27)-ANF > (5-28)ANF = (5-27)ANF $\gg$ (5-25)ANF Recently it has been found that (1-28)ANF is the main circulating form of ANF and, apparently, it

also has the most potent biological activity of all peptides tested to data [4, 13] Since most of the functional characterization of ANF has been done with (3-28)ANF, (4-27)ANF and (5-27)ANF, it is fortunate that the differences in the actions of the atrial peptides considered above seem to be more quantitative than qualitative in nature [2–4].

The ANF relaxation of agonist-induced contractions in isolated aorta has been found to be independent of the presence of endothelium and not mediated by prostaglandins [39, 40]. ANF leads to major increases in cGMP and activates cGMP-dependent protein kinase in intact isolated vessels and in vascular smooth muscle cells in culture [39, 41–43] Since the antagonist effect is apparently independent of the presence of intact endothelium, it is somewhat surprising that ANF binds to cultured endothelial cells derived from bovine aorta with an approximately 10-fold higher affinity than to cultured smooth muscle cells and also generates an approximately 10-fold higher increase in cGMP [43] The functional meaning of these major phenomena in cultured endothelial cells remains to be elucidated. The increase in cGMP is due to an activation of guanylate cyclase rather than to an inhibition of phosphodiesterase [39, 42, 43]. The increase in cGMP in aortic smooth muscle seems to be independent of extracellular $[Ca^{2+}]$ [39], in accordance with the lack of calcium-dependence of the relaxant effect of ANF in the renal vasculature (see above and Ref 15). Thus, the vasorelaxant effect of ANF has certain similarities with that of nitrate drugs. Contrary to these drugs, however, ANF mainly increases particulate (membrane bound) rather than soluble guanylate cyclase [39, 42, 43]. Furthermore, contrary to ANF, sodium nitroprusside does not increase cGMP in cultured smooth muscle or endothelial cells derived from bovine aorta [43]

Specific high affinity binding sites for ANF were demonstrated in isolated vessels and in vascular smooth muscle cells in culture [2, 22, 41, 43] The K_d of binding is similar to that found in renal tissue, namely 10^{-9}–10^{-10} M. The K_d of binding corresponds closely with the IC_{50} of the ANF-induced relaxation of pre-contracted vessels, suggesting that the specific binding is closely correlated with the biological action of the peptide [22] Unfortunately, the correlation between the kinetics of specific binding of ANF and the dose–response curve of the generation of cGMP is not as consistent. Thus, in smooth muscle cells in culture, the dose–response of the increase in cGMP seemed bimodal with very small increases being detected at ANF concentrations up to ten times the K_d of binding [41] Large increases in cGMP were obtained only with ANF concentrations of 10^{-7}–10^{-6} M, a value at least two orders of magnitude larger than the K_d of ANF binding in the preparation [41, 43]. As pointed out by Schenk *et al.* [43], this discrepancy may be due to the non-equilibration conditions used in the measurement of ANF-induced generation of cGMP in the cultured cells

From the foregoing it is clear that much more work is needed to clarify the relationship between specific binding of ANF to smooth muscle, cGMP generation and the antagonist action of ANF Nevertheless, the hypothesis that cGMP is the secondary messenger of the antagonist (vasorelaxant) effect of ANF is an attractive one Recent work of Popescu *et al* [44] has shown that cGMP activates Ca^{2+}-ATPase in sarcolemmal sheets prepared from coronary arteries of pigs Such an effect would result in the extrusion of Ca^{2+} from the cells and, therefore, to a decrease in cytosolic Ca^{2+} activity L M Popescu has suggested (personal communication) that this could be the fundamental cellular mechanism of the antagonist effect of ANF Investigations along these lines could lead to exciting developments in the understanding of the mechanisms of smooth muscle contractility as well as in the knowledge of the effects of ANF on this fundamental process

EFFECTS OF ANF ON THE CARDIOVASCULAR SYSTEM

ANF consistently decreases blood pressure of mammals [1–3, 16, 34, 45–51] The decrease in blood pressure in normotensive experimental animals and humans, albeit highly significant, is of small magnitude (10–20 mm Hg) [1–3, 16, 34, 45–51] In experimental models of hypertension, infusion of ANF leads to very large decreases in blood pressure towards normal levels [34, 46, 47, 49, 50] The decrease in blood pressure is not due to the diuretic effect of ANF since it occurs before any significant urinary losses take place [16, 51] Furthermore, strict replacement of urinary losses do not reverse the blood pressure lowering effect of ANF [16, 51] Pioneering studies by Ackerman [45], using crude atrial extract, indicated that the fall in blood pressure may be due to a decrease in cardiac output rather than to a decrease in total peripheral resistance Recent studies with synthetic atrial natriuretic peptides in conscious or anesthetized animals confirmed and extended the initial observations of this author [50–52]

Figure 6 illustrates the results of our studies on the effects of (4-27)ANF on some cardiovascular parameters in conscious dogs [51] ANF reversibly decreased blood pressure by about 20 mm Hg. This decrease was not accompanied by reflex tachycardia, probably because of a vagomimetic action of ANF [45, 52–54] Rather, a small but significant decrease in heart rate was detected in the second (E_2) but not in the first (E_1) experimental period Cardiac output decreased markedly, accompanied by a decrease in stroke volume [51], whereas calculated total peripheral resistance remained unchanged Maximal $+dP/dt$ was reduced during the infusion of ANF, but correction of this index for reductions in afterload rendered the values unchanged or even slightly improved in relationship to control values [51] We were also unable to detect a change in central venous pressure in these experiments Similar results were obtained in anesthetized dogs, except that heart rate did not decrease significantly in any of the experimental periods [51] These results clearly demonstrate that, in normotensive intact conscious or anesthetized dogs, ANF does not behave as a vasodilator and that its blood pressure lowering effect is mainly due to a decrease in cardiac output.

The reason for the ANF-induced decrease in cardiac output remains obscure. It is unlikely that the decrease in cardiac output is due to a direct negative

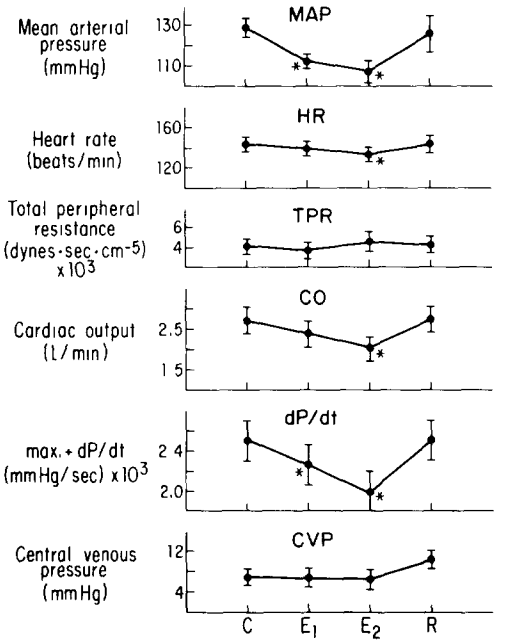

Fig 6 Effect of (4-27)ANF (auriculin A) on the cardiovascular system in conscious dogs (4-27)ANF was given i v as a bolus (3 μg min^{-1}/kg body wt) followed by a constant infusion (0 3 μg min^{-1}/kg body wt) C = control period, E_1, E_2 = sequential 10- and 30-min experimental periods during the infusion of ANF, R = recovery, 1 hr after suspension of the ANF infusion Results are means ± S E , N = 4, an asterisk (*) indicates $P < 0.05$ vs C The decrease in blood pressure is due to a decrease in cardiac output rather than to a decrease in total peripheral resistance See text and Ref 51 for description and discussion From Kleinert *et al* [51]

inotropic effect of ANF in heart muscle since maximal +dP/dt corrected for reductions in afterload was unchanged [51]. In addition, ANF has no effect on isolated guinea pig atrial or papillary muscle (H. Kleinert and R Levi, unpublished results) or in isolated Purkinje fibers of the dog (M. Pecker, T Maack and C. O. Lee, unpublished results) Recently, it has been reported that (5-27)ANF causes a marked coronary vasoconstriction in an isolated Langendorff preparation of guinea-pig heart [55]. If such a phenomenon occurs in the intact animal, it could lead to mechanical insufficiency of the myocardium and contribute to the reduction in cardiac output This effect, however, should be reflected, at least in part, by reductions in contractility which do not appear to occur in normal dogs. Although we could not detect a decrease in central venous pressure or pulmonary wedge pressure, it is possible that these measurements were not sensitive enough and that the decrease in cardiac output is due to a decrease in venous filling pressure [51] In this regard, Lappe *et al* [50] reported that infusion of (5-27)ANF into normotensive or spontaneously hypertensive (SHR) rats leads to significant decreases in blood pressure, cardiac output, central venous pressure and left atrial pressure. Such an effect could be due to either venodilation or a decrease in blood volume by, for example, a shift of fluid from the intravascular to the interstitial compartment Certainly further work is needed to clarify the mechanism(s) by which ANF decreases cardiac output Whatever the case, the finding that ANF has such an unexpected effect requires a great deal of caution in the clinical evaluation of the effects of ANF in humans, particularly in individuals who already have a low cardiac output

In intact normotensive animals, ANF may cause a very transient vasodilation but this effect is followed immediately by a return of the peripheral vascular resistance to control, or even above control, levels [50, 51]. This phenomenon is reminiscent of the effects of ANF on the renal vascular resistance presented in the first section of this article As pointed out, this may suggest that ANF has a dual vasodilatory–vasoconstrictive effect and that the latter may be due to the secondary release of a vasoconstrictor substance(s) or, in intact animals, to a reflex-mediated vasoconstriction Whatever the case, the results obtained to date clearly demonstrate that, in steady-state conditions, the ANF-induced decrease in blood pressure in normotensive animals is not due to a net peripheral vasodilation

ANF markedly reduces the blood pressure in all experimental models of hypertension tested to date [34, 46, 47, 49, 50]. Relatively low doses of synthetic (4-27)ANF reduce the blood pressure of the renin-dependent two-kidney, one-clip (2K-1C) Goldblatt hypertensive rats to a much greater extent than of the renin-independent one-kidney, one-clip (1K-1C) hypertensive rat, despite similar initial levels of high blood pressure [34] When the 1K-1C rat is made renin-dependent by administering a low-salt diet, the blood pressure lowering effect of (4-27)ANF is equivalent to that obtained in the 2K-1C rat [34] These results are consistent with the hypothesis derived from our studies in isolated rings of the rabbit aorta (see above) that ANF may be a particularly strong antagonist of angiotensin-induced contraction. In general, however, it can be expected that, at appropriate doses, ANF will reduce the peripheral resistance in all cases of hypertension characterized by an increase in vascular tone

Careful dose–response relationship studies are necessary to further characterize the magnitude and nature of the blood pressure lowering effect of ANF in hypertensive animals. The results obtained to date are, however, consistent with our general hypothesis that ANF behaves as a functional partial agonist and a strong antagonist of vasoconstrictors The physiological role of ANF in the homeostatic control of blood pressure remains to be elucidated. Nevertheless, as a whole, the advances in knowledge on the cardiovascular effects of ANF raise the hope that this endogenous substance may become an important pharmacological tool in the investigation of the mechanisms involved in the control of blood pressure

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