ROLE OF ATRIAL PEPTIDES IN BODY FLUID HOMEOSTASIS

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SUMMARY. Extracts of mammalian atria, but not ventricles, induce marked diuresis, natriuresis, and reduction in blood pressure when infused systemically in rats and dogs. These extracts also inhibit aldosterone biosynthesis and renal renin release. Natriuretic peptides, 21 amino acids and longer, have been isolated from atria of rodents and man, and share a nearly homologous amino acid sequence at the carboxyterminus. Natriuretic activity resides in a 17-amino acid ring formed by a disulfide bridge, and the C-terminal Phe-Arg appears necessary for full biological potency. The deoxyribonucleic acid-encoding atrial natriuretic peptides have been cloned and the gene structure elucidated. Reduction of the diuretic and natriuretic responses to an acute volume load by right atrial appendectomy first suggested a role for atrial peptides in the physiological response to plasma volume expansion. Subsequently, release of peptides with natriuretic and spasmolytic properties from isolated heart preparations in response to right atrial distension was demonstrated by bioassay and radioimmunoassay. The presence of these peptides in normal rat and human plasma in concentrations of 20–100 pM, and the findings of increased levels in response to acute and chronic plasma volume expansion, rapid atrial tachyarrhythmias, systemic hypertension, congestive heart failure, and renal insufficiency imply that they play an important role in body fluid homeostasis. The mechanisms by which atrial peptides increase renal salt and water excretion are as yet unclear. Renal vascular effects have been consistently demonstrated, and limited evidence for direct actions on tubule ion transport has also been reported recently. In vitro, these peptides cause precontracted vascular and nonvascular smooth muscle to relax, mediated by a direct action on smooth muscle cells. Specific receptors for these peptides have been characterized in crude membranes prepared from whole kidney homogenates and adrenal glomerulosa cells, in intact glomeruli and cultured glomerular mesangial cells, and in intact bovine aortic smooth muscle and endothelial cells. Natriuretic peptides stimulate cyclic guanosine monophosphate accumulation in target tissues, and augment particulate guanylate cyclase activity in membrane fractions, suggesting that cyclic guanosine monophosphate is the second messenger mediating their cellular action. (Circ Res 58: 619–630, 1986)
now known to be a polypeptide and has been characterized in terms of the amino acid sequence of the prohormone and the biologically active molecule(s), as well as the structure and nucleotide sequence of the gene that encodes the biosynthetic precursor (Seidman et al., 1985). Various names have been applied to these atrial peptide(s). We will refer to these atrial natriuretic and vasoactive peptides by the abbreviation ANP, and subscripts will be used to indicate specific sequences, with ANP$_{1-28}$ (numbered from amino- to carboxyterminus) serving as the reference moiety (Fig. 1). The biosynthetic precursor translated directly from messenger ribonucleic acid (mRNA) is referred to as pre-pro-ANP, and the pro-hormone storage form is termed pro-ANP.

In view of the rapid pace of progress in this field, this review by necessity cites many preliminary observations that thus far have been published in abstract form only.

**Molecular Structure**

The sequence of pre-pro-ANP, deduced from complementary deoxyribonucleic acid (DNA) copies of ANP-specific mRNA, contains 151 and 152 amino acid residues in humans and rodents, respectively (Nakayama et al., 1984; Kanagawa et al., 1984c; Maki et al., 1984; Yamanaka et al., 1984; Currie et al., 1984a; Oikawa et al., 1984). As shown in Figure 1, cleavage of a signal peptide results in pro-ANP, 126 amino acids in length, which is thought to represent a storage form of ANP (Nakayama et al., 1984; Kanagawa et al., 1984c; Bloch et al., 1985). Several smaller, biologically active peptides, all of which are derived from the carboxyterminus of pro-ANP, have been isolated from rat atria (Thibault et al., 1983, 1984a; Kanagawa and Matsuo, 1984; Kanagawa et al., 1984a; Misono et al., 1984a, 1984b; Atlas et al., 1984). Purification of the natriuretic peptide found in human atria revealed a 28 amino acid sequence (Flynn et al., 1983; Kanagawa and Matsuo, 1984a). A similar sequence, which differs from human ANP$_{1-28}$ by a single substitution of isoleucine for methionine at position 12, has been isolated from rat atria (Kanagawa et al., 1984a). Biological activity of ANP depends on a 17 amino acid ring structure formed by a disulfide bridge between two cysteine residues (Misono et al., 1984a), and on the presence at the carboxyterminus of Phe-Arg (Currie et al., 1984a; Sugiyama et al., 1984). Aminoterminal residues beyond the disulfide bridge are not required for biological action, but elongation at the aminoterminal to very large sequences, such as pro-ANP itself, is associated with loss of activity (Thibault et al., 1984b). Experimental evidence suggests that ANP$_{1-28}$ is the circulating form in both rats and humans (Sugawara et al., 1985; Schwartz et al., 1985). Other sequences iso-

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**Figure 1.** The biosynthetic pathway, and the sequences of the human pre-pro-ANP gene, pre-ANP, and ANP$_{1-28}$ ANP$_{1-28}$ is numbered from amino- to carboxyterminus. Specific amino acids in the pre-ANP and ANP sequences are given by single letter code [alanine = A, arginine = R, asparagine = N, aspartic acid = D, cysteine = C, glutamine = Q, glutamic acid = E, glycine = G, histidine = H, isoleucine = I, leucine = L, lysine = K, methionine = M, phenylalanine = F, proline = P, serine = S, threonine = T, tryptophan = W, tyrosine = Y, valine = V (Lehninger, 1982, p. 96)].
lated from atria, which differ from ANP<sub>5-28</sub> only by the length of the aminoterminal extension, probably reflect varying degrees of proteolysis occurring during extraction of the peptide(s) from atria.

**Control of ANP Secretion**

A substance that induces natriuresis and diuresis in bioassay rats is released in vitro from rat heart-lung preparations in response to high atrial perfusion pressure (Dietz, 1984). Also, rabbit hearts perfused in vitro release a substance into the coronary venous effluent that mimics the smooth muscle relaxant effects of ANP (Currie et al., 1984b). In the latter study, the substance in the effluent was further analyzed by chromatographic techniques, and was found to resemble ANP<sub>5-28</sub>. No evidence for release of larger molecular weight pro-ANP was found. ANP release from isolated rat hearts in vitro in response to elevated mean right atrial pressure has been confirmed by radioimmunoassay (Lang et al., 1985). Thus, ANP release appears to be stimulated by atrial distension or stretch. Arginine vasopressin (AVP), phenylephrine, and angiotensin II infusion in vivo also produce a marked, though transient, increase in plasma ANP levels (Manning et al., 1985). A specific antagonist of pressor AVP receptors abolished the release of ANP in response to AVP; the nonpressor analog of vasopressin (dDAVP), known to interact with a different receptor, did not stimulate ANP release (Manning et al., 1985). Rat atria in vitro also release ANP-like bioactivity in response to AVP but not dDAVP, and in response to epinephrine (Sonnenberg and Veress, 1984), suggesting that these humoral agents may stimulate atrial myocytes directly to secrete ANP. By contrast, dibutyryl cAMP has recently been shown to inhibit ANP release in vitro (Schiebinger, 1985). Cultured rat myocardial cells actively synthesize and secrete only low molecular weight ANP, while low molecular weight ANP is not released, suggesting that rat myocardial cells (at least in culture) do not possess the enzymes required for cleavage of pro-ANP prior to secretion (Bloch et al., 1985). Conversely, since only low molecular weight ANP is found in human plasma (Gutkowska et al., 1985a; Sugawara, 1985; Yamaji et al., 1985a), it is likely that human ANP is cleaved prior to secretion, as shown in Figure 1, although this issue awaits further clarification.

The presence of ANP in rat and human plasma has been confirmed by radioimmunoassay, with chromatographic analysis consistently showing single peaks in human plasma (Gutkowska et al., 1985a; Sugawara, 1985; Yamaji et al., 1985a), and one major peak with smaller amounts of large molecular weight ANP in rat plasma (Gutkowska et al., 1984; Lang et al., 1985). Circulating ANP concentrations in normal humans of 7.5 pm (23 pg/ml) (Yamaji et al., 1985b) and 21 pm (63 pg/ml) (Gutkowska et al., 1985a) have been reported. Circulating ANP levels vary directly with dietary salt intake in humans (Gutkowska et al., 1985b; Shenker et al., 1985); similarly, acute central volume expansion, as well as supine posture, is associated with a rise in circulating ANP levels (Yamaji et al., 1985b; Hollister, et al., 1985). Atrial pacing (Gutkowska et al., 1985) and paroxysmal atrial tachyarrhythmias (Yamaji et al., 1985a) also cause plasma ANP levels to rise. Furthermore, elevations in plasma ANP concentrations have been demonstrated in patients with chronic congestive heart failure (Shenker et al., 1985) and systemic hypertension (Arendt et al., 1985). Whether high plasma ANP levels in these two studies resulted from chronic elevations in atrial pressures, or were mediated by other mechanisms, remains to be determined.

In normal rats, plasma ANP concentrations of 20-550 pm have been reported (Tanaka et al., 1984; Gutkowska et al., 1984; Lang et al., 1985). Acute expansion of extracellular fluid volume (Lang et al., 1985) or dietary salt excess (Tanaka et al., 1984) also leads to raised plasma ANP levels, similar to the findings in humans. In addition, 12 hours after initiation of deoxycorticosterone administration, immunoreactive ANP concentrations in rat plasma rose 3-fold (Ballermann et al., 1986), suggesting that ANP is secreted in response to mineralocorticoid-induced volume expansion. In addition, this plasma secretory response was accompanied by a pronounced rise in specific atrial pre-pro-ANP mRNA activity, suggesting that ANP participates in the phenomenon of mineralocorticoid escape. Plasma ANP levels also rise dramatically in rats in response to acute as well as chronic ablation of renal mass (Smith et al., 1986). Reduction in dietary sodium intake in proportion to the reduction in renal mass obviates this plasma ANP response, suggesting that ANP plays an important role in the adaptation to higher sodium excretion per nephron necessitated by advancing renal disease. Thus, the diuretic and natriuretic responses to atrial distension by stretch (Henry et al., 1956), the release of ANP from the heart in response to atrial pacing (Gutkowska et al., 1985) or tachyarrhythmia (Yamaji et al., 1985a), and the increase in circulating levels of immunoreactive ANP with acute (Yamaji et al., 1985b) and chronic (Ballermann et al., 1986; Smith et al., 1986) plasma volume expansion all suggest that ANP is an important humoral component of body fluid homeostasis.

**Intracellular Storage**

The finding of characteristic granules in mammalian atrial myocytes was one of the first clues to the existence of an atrial secretory product (Kisch, 1956; Jamieson and Palade, 1964). Specific antibodies directed against an aminoterminal fragment of pro-ANP have been used to localize pro-ANP to these granules (Tang et al., 1984). Whether biologically active ANP is present in granules is as yet unclear, as specific antibodies directed against ANP also interact with the larger prohormone. The fact that extracts of atria contain large molecular weight natriuretic peptides which require activation by pro-
teolytic enzymes (Currie et al., 1983) supports the view that ANP is stored, at least in part, as a relatively inactive prohormone. Indeed, a 16.4 kD peptide corresponding to pro-ANP has been shown to be the predominant intracellular storage form in cultured rat atrial myocytes (Bloch et al., 1985). Atrial myocyte granule content has been noted to vary with salt and water intake (DeBold, 1979; Cantin et al., 1982). Apart from the atria, pro-ANP like material has also been demonstrated at several locations within the central nervous system (Needleman et al., 1985; Saper et al., 1985), suggesting that pro-ANP biosynthesis and storage take place at these sites as well.

**ANP Receptors**

In the rat, accumulation of radiolabeled ANP in renal glomeruli, in medullary and papillary vasa recta, and in several locations within the central nervous system by autoradiographic techniques suggests the presence of specific ANP receptors at these sites (Bianchi et al., 1985; Needleman et al., 1985). Radioligand-binding techniques have revealed the presence of specific ANP receptors in several tissues, including crude membrane fractions prepared from bovine and rat adrenal glomerulosa (DeLean et al., 1984a; Schiffrin et al., 1985a), rat mesenteric artery and rabbit aorta, and rat and rabbit kidney, and cultured renal epithelial (LLC-PK₁) cells (Napier et al., 1984). Cultured rat atrio smooth muscle cells (Hirata et al., 1984, 1985), bovine aortic smooth muscle and endothelial cells (Leitman et al., 1985), human platelets (Schiffrin et al., 1985b), and rat glomerular mesangial cells (Ballermann et al., 1985) also possess ANP receptors. ANP receptors characterized in isolated rat renal glomeruli are thought to be located primarily on mesangial cell surfaces (Ballermann et al., 1985). ANP binding sites described thus far have a high affinity for ANP as reflected by equilibrium dissociation constants ranging in the various preparations from 0.05 to 2 nM, and are saturable at concentrations of 5–10 nM (Napier et al., 1984; Ballermann et al., 1985; Hirata et al., 1985). Furthermore, the binding sites display a high degree of specificity for ANP in that many unrelated peptides, including angiotensin II, vasopressin, somatostatin, parathyroid hormone, and glucagon, fail to compete for binding to the receptor (DeLean et al., 1984a; Napier et al., 1984; Hirata et al., 1984; Ballermann et al., 1985). The structural requirements for ANP binding to specific receptors are similar to the requirements for biological activity, namely, the presence of the disulfide bridge and the carboxyterminal Phe-Arg residues (DeLean et al., 1984; Ballermann et al., 1985; Hirata et al., 1985). Clear-cut evidence that ANP receptors are located on the cell surface exists only for glomerular mesangial cells, where ANP binds with high affinity at 4°C, conditions under which internalization is highly unlikely, and where ANP binding is more than 90% reversible from intact cells exposed to an acidic medium (Ballermann et al., 1985). A 140 kD glycoprotein from rat kidney membranes, specifically labeled with ANP by photoaffinity techniques, was identified by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and is thought to represent the ANP receptor (Yip et al., 1985). Regulation of ANP receptors in response to changes in ambient ANP concentrations has been reported in isolated rat glomeruli and in cultured aortic smooth muscle cells. Glomerular ANP receptor density was 4-fold higher in rats fed a low-salt diet, which had suppressed plasma ANP levels, compared to rats given a high-salt diet in which circulating levels of ANP were also high (Ballermann et al., 1985; Ballermann and Brenner, 1986). Exposure of cultured smooth muscle cells to ANP for 24 hours also led to a marked decline in receptor density (Hirata et al., 1985). Thus, receptors in target tissues display a high affinity and specificity for ANP, which would allow ANP binding at concentrations similar to those found in plasma. As is the case for most hormone-receptor systems, ANP receptors in target tissues are regulated reciprocally with alterations in ambient ANP concentrations in plasma. These characteristics of ANP receptors strongly support a role for ANP as a circulating hormone.

**ANP-induced Changes in Cyclic Nucleotides**

The accumulation of cGMP in response to ANP has been observed in a number of tissues, leading some investigators to suggest that this cyclic nucleotide is the intracellular mediator of ANP action (Waldman et al., 1984). A marked rise in urinary cGMP excretion was observed when atrial extracts were infused into rats, with a large proportion of the urinary cGMP due to renal production (Hamet et al., 1984). Plasma cGMP levels also rise with atrial extract infusion (García et al., 1985a, 1985b). Within the kidney, ANP-induced cGMP accumulation in various isolated nephron segments correlates well with the baseline activity of guanylate cyclase (Tremblay et al., 1985). The most significant accumulation of cGMP in response to ANP occurred in glomeruli, and a modest response was seen in thick ascending limbs and in collecting ducts, whereas the proximal tubule was devoid of activity (Tremblay et al., 1985). ANP activates membrane-bound guanylate cyclase and is without effect on the soluble enzyme in fractions prepared from rat kidney, aorta, lung, liver, intestine, and testes. Half-maximal stimulation of guanylate cyclase occurs at concentrations of approximately 10 nM (Waldman et al., 1984). ANP is the first endogenous hormone known to stimulate membrane-bound guanylate cyclase activity. ANP-induced cGMP accumulation in vascular smooth muscle preparations is not dependent on an intact endothelial cell layer (Winquist et al., 1984), and is associated temporally with smooth muscle relaxation. In addition, the spasmodic activity of
ANP in smooth muscle preparations can be diminished by metylene blue, an inhibitor of guanylate cyclase (Ohlstein and Berkowitz, 1985). In cultured vascular smooth muscle cells and in isolated rat renal glomeruli, there is a strong correlation between ANP receptor binding and cGMP accumulation, indicating that only those ANP analogs that compete for binding to the ANP receptor are capable of stimulating cGMP accumulation (Ballermann et al., 1985; Hirata et al., 1984a).

Thus, experimental evidence suggests that ANP, by interaction with specific cell-surface receptors, activates guanylate cyclase, and that cGMP thus generated acts as the second messenger which mediates smooth muscle relaxation and perhaps other, as yet unidentified intracellular events. There are, however, observations which temper the above conclusion. Binding studies have demonstrated that half-maximal occupancy of ANP receptors occurs at ANP concentrations below 1 nM. In addition, ANP concentrations that elicit half-maximal smooth muscle relaxation are in the range of 0.1–1.0 nM, and aldosterone synthesis is inhibited at concentrations in the low nanomolar range. In most hormone-receptor systems, occupancy of only a small percentage of receptors is required to elicit the maximal physiological response. However, threshold ANP concentrations required to elicit a cGMP response have been in the range of 10 nM or above, concentrations at which ANP receptors are expected to be fully occupied, indicating that guanylate cyclase activation may only be a secondary phenomenon triggered by ANP receptor occupancy. If, on the other hand, cGMP is the second messenger that mediates ANP action, increments in cGMP too low to measure would have to account for the entire physiological response.

ANP has also been reported to inhibit adenylate cyclase activity. In aorta, mesenteric artery and renal artery particulate fractions ANP3-25 lowered basal and hormone-stimulated adenylate cyclase activity in a dose-dependent fashion, with half-maximal inhibition at ANP concentrations of 0.1–1 nM (Anand-Srivastava et al., 1984). Forskolin and sodium fluoride-stimulated adenylate cyclase activity was also reduced, indicating that inhibition of adenylate cyclase activity in this system must occur at the level of the guanine nucleotide regulatory unit or the catalytic unit of adenylate cyclase (Anand-Srivastava et al., 1984). A similar inhibitory action of ANP on adenylate cyclase was also observed in homogenates of anterior and posterior pituitary (Anand-Srivastava et al., 1985). Others have failed to demonstrate effects of ANP on adenylate cyclase activity in kidney homogenates (Waldman et al., 1984), on urinary cAMP excretion (Hamet et al., 1984), or on cAMP accumulation in rabbit aortic strips (Winquist et al., 1984; Ohlstein et al., 1985), suggesting that this may not be a universal phenomenon. Finally, a recent report suggests that dibutyryl cAMP inhibits ANP secretion by rat atria in vitro (Schiebinger, 1985).

Vascular Actions of ANP

ANP is a potent inhibitor of smooth muscle contraction. When human or rat atrial extracts were applied in vitro to rabbit aortic or chick rectum strips, which were precontracted with norepinephrine or carbachol, respectively, contractile tone was lost (Currie et al., 1983). A similar spasmolytic action has been reported for defined, synthetic sequences of ANP. In rabbit aortic strips, sequences lacking carboxyterminal Phe-Arg residues were several orders of magnitude less potent than ANP sequences possessing this dipeptide moiety (Currie et al., 1984a; Oshima et al., 1984). Although early reports suggested that this structural requirement is not necessary for ANP action on chick rectum or for natriuretic activity (Currie et al., 1984a; Oshima et al., 1984; Geller et al., 1984a, 1984b), it now appears that full biological activity, regardless of the assay involved, requires an intact carboxyterminus (Sugiyama et al., 1984; Thibault et al., 1984b; Wakitani et al., 1985a, 1985b).

There is a significant variability in sensitivity of different vascular beds to ANP. Renal artery and isolated aortic strips in vitro respond readily to the spasmolytic effects of ANP (Currie et al., 1983, 1984a; Garcia et al., 1984a; Ishihara et al., 1985), whereas mesenteric, coronary, femoral, vertebral, and carotid arteries are relatively insensitive (Garcia et al., 1984a; Ishihara et al., 1985). Effects of ANP infusion in vivo are not as clearcut, however. ANP infusion in conscious rats led to increments in renal, splanchnic, coronary, testicular, lung and spleen blood flows, without change in cardiac output, as determined by the microsphere method (Garcia et al., 1985). In conscious dogs in which regional blood flows were assessed by implanted flow probes, renal vascular resistance declined significantly, whereas mesenteric, coronary, and iliac blood flows were unaffected (Hintze et al., 1985). In another study, ANP infusion resulted in a significant decrease in mean arterial pressure in conscious rats due to reduction in cardiac output, with a consequent rise in renal, femoral, and mesenteric vascular resistances (Lappe et al., 1985). Similarly, partially purified atrial extracts given to conscious, spontaneously hypertensive rats (SHR) or their Wistar-Kyoto nonhypertensive controls resulted in a decline of cardiac output, a fall in mean arterial pressure, and elevations of regional vascular resistances (Pegram et al., 1985). The fall in mean arterial pressure associated with ANP infusion in conscious rats appears therefore to be the result of a reduction in cardiac output rather than a fall in peripheral vascular resistance. Reduction in venous filling pressures of both right and left atria suggests that this fall in cardiac output is mediated, at least in part, by a decrease in venous return (Lappe et al., 1985), although a direct nega-
tive inotropic effect of ANP on ventricular myocardium cannot be ruled out.

Thus, conclusions regarding the physiological actions of ANP in regulating total and regional vascular resistances are difficult to draw at this time. Circulating concentrations of ANP achieved by infusion are probably much greater than those produced by endogenous ANP release, making it difficult to determine whether inhibition of vasoconstriction occurs under physiological conditions. Interpretation of infusion studies is further complicated by the fact that ANP antagonizes the effects of several vasoconstrictors such as norepinephrine, angiotensin II, and vasopressin. The degree of activation of specific vascular ANP antagonists.

regional hemodynamics awaits the development of •

clear definition of the fact that ANP antagonizes the effects of several infusion studies is further complicated by the distribution of specific ANP receptors on different adrenal cell types. Plasma aldosterone levels decline upon infusion of ANP into anesthetized dogs (Maack et al., 1984) and conscious rats (Chartier et al., 1984b), without change in plasma cortisol levels. Since ANP also inhibits renin release (see below), two mechanisms may be involved in the suppression of plasma aldosterone: a direct effect of ANP on adrenal glomerulosa cells and reduced stimulation of adrenal aldosterone release due to lower circulating angiotensin II levels. Whereas chronic activation of ANP release may modify renal sodium reabsorption by suppressing adrenal aldosterone release, it is unlikely that suppression of aldosterone plays a role in the acute natriuretic response to ANP infusion.

Inhibition of Renin Release

Plasma renin activity and renal renin secretion are significantly reduced by ANP infusion in the dog (Maack et al., 1984; Burnett et al., 1984). There are several mechanisms that might be responsible for this effect of ANP on renin release. First, ANP results in preglobular vasodilation causing glomerular capillary hydraulic pressure to rise (Ichikawa et al., 1985). A similar rise in hydraulic pressure within the afferent arteriole could enhance juxtaglomerular cell stretch and thus inhibit renin release. Second, enhanced delivery of NaCl to the macula densa region of the ascending limb of Henle is also associated with a reduction in renin release. Thus, enhanced sodium delivery to this tubule segment, known to be a result of ANP infusion (Huang et al., 1985), may mediate the reduction in renin release, although recent evidence against this possibility has also been presented (Villarreal et al., 1985). Finally, it is possible that ANP acts directly on juxtaglomerular cells to inhibit renin release. Preliminary evidence indicates that renin secretion remains constant in the nonfiltering kidney during ANP infusion, suggesting that macula densa perfusion is required for the inhibitory action of ANP on renin release (Oppenorth et al., 1985). A direct effect of ANP on renin release from renal cortical slices has also been reported, however (Hennich et al., 1985). The effect of ANP on renin release in vivo is dependent on the baseline activity of the renin-angiotensin system. In conscious rats, plasma renin activity was reduced only in the presence of chronic early step in aldosterone biosynthesis. The inhibitory action of ANP on aldosterone release is dependent on an intact intramolecular disulfide bridge (Chartier et al., 1984a), similar to the structural requirements for smooth muscle relaxation. Specific, high-affinity receptors for ANP have been demonstrated in adrenal glomerulosa membranes, with binding again critically dependent on an intact disulfide bridge (DeLean et al., 1984a; Schiffrin et al., 1985; Chartier et al., 1984a). ANP has been found not to influence corticosterone biosynthesis (DeLean et al., 1984b), which may reflect the distribution of specific ANP receptors on different adrenal cell types. Plasma aldosterone levels decline upon infusion of ANP into anesthetized dogs (Maack et al., 1984) and conscious rats (Chartier et al., 1984b), without change in plasma cortisol levels. Since ANP also inhibits renin release (see below), two mechanisms may be involved in the suppression of plasma aldosterone: a direct effect of ANP on adrenal glomerulosa cells and reduced stimulation of adrenal aldosterone release due to lower circulating angiotensin II levels. Whereas chronic activation of ANP release may modify renal sodium reabsorption by suppressing adrenal aldosterone release, it is unlikely that suppression of aldosterone plays a role in the acute natriuretic response to ANP infusion.
activation of the renin-angiotensin system, as in two kidney-one clip Goldblatt hypertension, whereas, in normal rats and in rats with volume-dependent hypertension (one kidney-one clip Goldblatt hypertension with a normal salt intake), ANP failed to alter plasma renin activity (Volpe et al., 1985). Similarly, in conscious dogs with inferior vena cava ligation, a state of chronic hyperreninemia, ANP markedly suppressed plasma renin activity, whereas no response was seen in normal dogs (Freeman et al., 1985). Thus, under conditions of a stimulated renin-angiotensin axis, such as anesthesia, renin-dependent hypertension, extracellular fluid volume depletion, and inferior vena cava constriction, ANP infusion is associated with inhibition of renin release.

Renal Actions of ANP

Whether given by infusion or by bolus injection, ANP induces a dramatic increase in renal salt and water excretion. The intrarenal mechanisms whereby ANP induces this natriuresis and diuresis are as yet incompletely understood. An immediate, but transient, increase in the urine flow rate, associated with enhanced sodium, chloride, potassium, calcium, magnesium, and phosphorus excretion, follows bolus administration of atrial extracts (DeBold et al., 1981; Sonnenberg et al., 1982; Briggs et al., 1982; Keeler and Azzarolo, 1983; Hammond et al., 1985a). The massive solute diuresis led to the suspicion that ANP inhibits tubule ion transport at a nephron segment(s) beyond the medullary thick ascending limb (Sonnenberg et al., 1982; Briggs et al., 1982; Keeler and Azzarolo, 1983). However, direct inhibition of epithelial ion transport by ANP has not been demonstrated convincingly until quite recently. Atrial extracts failed to inhibit renal Na+,K+-ATPase activity (Pollock, Mullins and Banks, 1983) and were without effect on sodium, chloride, or volume transport in various isolated epithelia (Throckmorton and Gilmore, 1983; Pammani et al., 1984), including isolated rabbit renal proximal tubules perfused in vitro (Baum and Toto, 1986). By contrast, inhibition of sodium transport in LLC-PK₁ cells (a cell line derived from pig kidney) and in suspensions of isolated rabbit papillary collecting ducts has recently been reported (Cantiello and Ausiello, 1986; Zeidel et al., 1986). Possible inhibitory effects of ANP on adenylate cyclase activity in isolated tubule segments have also been examined, with negative results in one preliminary study (Umemura et al., 1985), and the finding that ANP inhibits ADH-stimulated adenylate cyclase activity in rabbit cortical collecting duct (Werness et al., 1986) in another. The latter observation is in keeping with the finding in isolated perfused cortical collecting tubules that ANP₅₋₁₈ inhibits ADH- but not forskolin-stimulated transepithelial water flow (Dillingham and Anderson, 1986). Direct inhibition of proximal tubule phosphate transport is suggested in clearance experiments in which ANP infusion produced a brisk phosphaturic response in the absence of changes in glomerular filtration rate (GFR) (Hammond et al., 1985a). The finding that sodium-phosphate cotransport is reduced in proximal tubule brush border membrane vesicles prepared from rats previously infused with ANP would support this possibility (Hammond et al., 1985b). However, conclusions regarding a direct action of ANP on the proximal tubule should be considered tentative at present, since this nephron segment is devoid of ANP receptors (Cantin and Genest, 1985), and since a direct effect of ANP on brush border vesicles or on proximal tubules could not be demonstrated when the peptide was added only in vitro (Hammond et al., 1985b; Baum, 1985).

Since ANP is a powerful inhibitor of renal vasoconstriction, it is not surprising that ANP-induced alterations in renal hemodynamics are commonly observed. Increases in GFR following bolus administration of large doses of atrial extracts (Briggs et al., 1982; Keeler and Azzarolo, 1983) or infusion of atrial extracts (Beasley and Malvin, 1985) or synthetic ANP (Maack et al., 1984; Burnett et al., 1984; Huang et al., 1985) are usually, though not invariably, observed. Bolus administration of ANP₁₋₂₈ in humans also increases GFR transiently (Kuribayashi et al., 1985). Alterations in renal blood flow (RBF) in response to ANP are less consistent. Total RBF, measured by the microsphere method, was increased after bolus administration of atrial extracts or infusion of synthetic ANP in rats (Borenstein et al., 1983; Garcia et al., 1985b), or infusion of synthetic ANP₅₋₂₈ or ANP₅₋₂₇ in dogs (Wakitani et al., 1985a). Other studies have reported no change in total RBF with ANP infusion in dogs and rats (Burnett et al., 1984; Huang et al., 1985), although a selective increase in blood flow to the inner cortex has been described (Salazar et al., 1985). The latter zonal increase in inner cortical flow was associated with increasing urinary excretion of PGE₂ and PGI₂. The response of isolated, perfused rat kidneys to atrial extract infusion was found to depend on the degree of baseline vascular tone. Under vasodilated conditions, atrial extracts acted as vasoconstrictors, with significant increases in renal vascular resistance accompanied by an increase in GFR, and thus marked elevation of the filtration fraction. In contrast, when baseline renal vascular resistance was maintained at high levels by angiotensin II, vasopressin, or norepinephrine, isolated perfused kidneys responded to atrial extract infusion with overt vasodilatation (Camargo et al., 1984). The most consistent finding in studies addressing renal hemodynamic responses to atrial extracts or to synthetic ANP is an increase in the filtration fraction. Studies of the determinants of glomerular ultrafiltration using micropuncture techniques have demonstrated that ANP infusion leads to an increase in single nephron GFR and filtration fraction, brought about
by a rise in glomerular capillary hydraulic pressure (Ichikawa et al., 1985). These changes were due to reduction in afferent arteriolar resistance accompanied by small increments in efferent arteriolar tone. Efferent arteriolar resistance was also observed to rise in isolated perfused glomeruli when ANP was added to the perfusate (Fried et al., 1985).

It has been proposed that increments in GFR can account fully for the natriuresis evoked by ANP (Huang et al., 1985). However, solute and fluid delivery to the last accessible portion of the distal tubule were noted to increase only 2- to 3-fold with ANP or atrial extract administration (Briggs et al., 1982; Huang et al., 1985), far less than the 10- to 50-fold increases in final urinary solute excretion that commonly occur. It is therefore reasonable to conclude that ANP-induced natriuresis does not result solely from increments in GFR, or from a decrease in proximal tubule sodium reabsorption, but must be due in large part to ANP actions at other renal sites. In support of this possibility, Sonnenberg et al. (1982) reported that atrial extract administration failed to increase sodium delivery to the end-proximal or to the distal convoluted tubule, whereas sodium delivery to the outer medullary collecting duct was increased 3-fold, and to the papillary collecting duct nearly 20-fold. Thus, the bulk of the increase in sodium excretion that followed ANP administration was attributed to reduced net collecting duct sodium reabsorption. As microcatheterization techniques were used in this study, increased addition of sodium into papillary collecting ducts from deep nephrons could also have accounted for the findings. In the study by Briggs et al. (1982), although whole kidney sodium excretion increased 10-fold, these workers also found no effect of atrial extracts on proximal tubule function, and only a minimal decrease in loop of Henle sodium transport. In addition, their observation that urinary potassium excretion increased only slightly with atrial extract administration led these workers to conclude that a site distal to the potassium secretory segment, namely, the terminal collecting duct, must be involved. The finding that synthetic ANP stimulates cGMP accumulation in cultured papillary collecting duct cells in vitro (Appel and Dunn, 1985) adds further support for an action of ANP on terminal nephron segments.

The uniform finding of urinary sodium concentrations above those of plasma following ANP infusion might provide a possible clue for further resolution of the issue of localization of ANP action within the kidney. A likely source for this high urinary sodium concentration (often in excess of 200 mEq/liter) is the hypernatric renal papillary interstitium. Evidence for net addition of sodium along the papillary collecting duct has been presented by Sonnenberg et al. (1982), and by Rocha et al. (1985), and preliminary observations reveal that vasa recta Starling forces become less favorable for papillary interstitial fluid uptake following systemic ANP infusion in the rat (Dunn et al., 1986). Recycling of the resulting accumulated hypernatric papillary interstitial fluid to papillary collecting ducts may then account for enhanced urinary sodium concentration and absolute sodium excretion rate. The finding that ANP enhances bath-to-lumen NaCl permeability in isolated rat papillary collecting ducts perfused in vitro adds further support for this possibility (Rocha et al., 1985), as does the finding that ANP inhibits Na* entry-dependent oxygen consumption in fresh suspensions of rabbit inner medullary collecting duct cells studied in vitro (Zeidel et al., 1986).

In support of the hypothesis that alterations in peritubular capillary Starling forces play an important role in contributing to the natriuretic response to ANP, Mendez et al. (1986) observed that angiotensin II infusion enhanced ANP-induced renal sodium excretion, whereas hyperoncotic albumin infusion strongly suppressed the natriuretic and diuretic responses to even maximal doses of ANP administered intravenously.

Two studies of the potential role of ANP in the chronic regulation of sodium excretion have recently been performed in the authors' laboratory. Ballermann et al. (1986) examined the potential role of ANP in mediating the natriuretic response regularly seen after chronic mineralocorticoid administration (the so-called "escape" phenomenon). In their study, the initial sodium retention induced by deoxycorticosterone acetate (DOC) was soon followed by natriuresis and a return to normal external sodium balance, the latter in all likelihood secondary to the initial expansion of extracellular fluid volume. This transient retention of salt and water stimulated enhanced synthesis of atrial pre-pro ANP mRNA and also triggered increased secretion of ANP, leading to impressive elevation in plasma immunoreactive ANP concentrations.

Smith and coworkers (1986) recently explored the role of endogenous ANP in the adaptation to higher sodium excretion per nephron that regularly occurs in response to reduction in nephron number. Rats were subjected to 5/6 nephrectomy or sham operation and were fed low, normal, or high levels of sodium. Urinary sodium excretion increased with increasing dietary sodium in both groups, and sodium excretion per nephron was increased in 5/6 nephrectomized rats as compared to sham-operated rats on the higher salt intakes. As shown in Figure 2, plasma immunoreactive ANP levels were unaffected by dietary sodium manipulations in sham-operated rats, but rose progressively in 5/6 nephrectomized rats with increasing sodium intake. Despite extensive nephron reduction, however, plasma ANP levels failed to rise in uremic rats on low sodium diets, and in this group sodium excretion per nephron also failed to rise. These findings afford strong evidence that ANP plays an important role in promoting the adaptive increase in sodium excretion per nephron in chronic renal failure. Furthermore, restriction of dietary sodium in the setting of reduced...
GFR obviates the stimulation of ANP secretion as well as the adaptive increase in sodium excretion rate per nephron.

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