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Diverse Biological Actions of Atrial Natriuretic Peptide

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I. INTRODUCTION

Atrial natriuretic peptide (ANP) is a recently discovered hormone secreted primarily by atrial myocytes in response to local wall stretch (i.e., increased intravascular volume). The combined actions of ANP on vasculature, kidneys, and adrenals serve both acutely and chronically to reduce systemic blood pressure as well as intravascular volume (27, 29). The reduction in blood pressure is the consequence of reduced peripheral vascular resistance (in part mediated by direct relaxation of vascular smooth muscle), diminished cardiac output, and decreased intravascular volume. In the kidney, ANP acts on specific receptors in renal microvasculature and tubule epithelium to induce hyperfiltration, inhibition of Na^+ transport, and suppression of renin release, all of which are effects responsible for natriuresis, diuresis, as well as diminished arterial blood pressure. Also ANP acts to lower blood pressure and intravascular volume by inhibiting aldosterone biosynthesis, both indirectly by inhibiting renin secretion from the renal juxtaglomerular apparatus and directly by a receptor-mediated action on adrenal glomerulosa cells. Finally, ANP facilitates transudation of plasma water to interstitium, providing yet another means for reducing intravascular volume and blood pressure. The aim of this review is to summarize current understanding of the structure of ANP; its synthesis, secretion, and removal from the circulation; its cellular and target organ actions; and, finally, its role in various pathophysiological states.

II. STRUCTURE OF ATRIAL NATRIURETIC PEPTIDE

The discovery by DeBold and co-workers (69–71) of the potent diuretic and natriuretic properties of atrial extracts led to the prompt identification of the molecular structure of circulating ANP and its precursors in atrial tissue. The sequence of preproANP, deduced by cloning and characterizing cDNAs from mRNA (302, 362), is shown in Figure 1. The human sequence consists of 151 amino acids (239, 302) and shares strong homol-

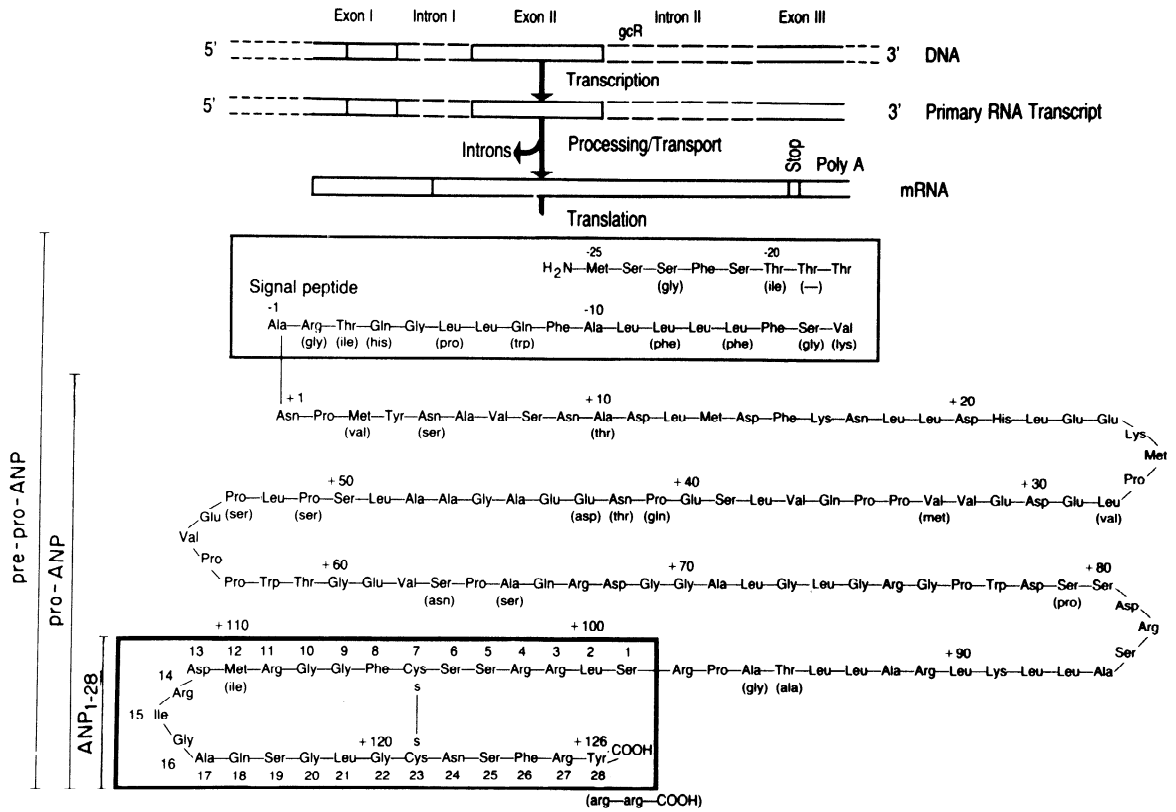

FIG. 1. Transcription and translation of ANP gene. See text for details.

ogy with peptides from rat (42, 196), dog (238), and rabbit (238). The amino-terminal methionine, which typically initiates protein sequences in eucaryotes, is followed by a sequence rich in hydrophobic residues, not unlike "leader" sequences for other secretory peptides. Cleavage of this "signal" peptide yields proANP-(1—126), the principal storage form of the peptide (42). Bioactive peptides are derived from the carboxy-terminus, with the predominant circulating form being ANP-(99—126) or the more commonly denoted ANP-(1—28)[1] (Fig. 1). In humans, ANP-(1—28) is identical to that in rats except for the substitution of methionine for isoleucine at position 12 (156a, 239). Throughout the remainder of this review, the terms ANP-(1—28) and ANP are used interchangeably, as are the terms proANP-(1—126) and proANP.

Active ANP analogues share a common central ring structure formed by a disulfide bridge between cysteine residues at positions 7 and 23 (Fig. 1). Disruption of this ring structure by hydrolytic cleavage of the Cys-Phe bond at positions 7-8 or Leu through Cys bonds at positions 21-23 invariably leads to loss of bioactivity (215). Although these cleavages occur to a limited extent as part of the normal metabolic degradation of ANP (288, 363), the bulk of the peptide in plasma is "cleared" by receptor-mediated endocytosis (see sect. VI*A*). Studies of structure-function relationships also reveal the importance of the three carboxy-terminal residues Phe-Arg-Tyr (216). Although deletion of the terminal tyrosine fails to alter activity, deletion of all three residues markedly reduces natriuretic and vasorelaxant potency. Extensions or deletions of the amino-terminus tend to exert a less critical effect on bioactivity (215, 216); indeed even the storage form, proANP, has been shown to have ANP-like activity (156).

III. SYNTHESIS OF ATRIAL NATRIURETIC PEPTIDE

Electron-dense granules in atrial myocytes (Fig. 2) were recognized by Kisch (161) and Jamieson and Palade (153), with the latter workers emphasizing their predominant ultrastructural localization at the nuclear pole near the Golgi apparatus. Jamieson and Palade (153) and subsequently DeBold et al. (73) likened them to the secretory granules seen in other endocrine tissues. The number and density of the granules were shown by DeBold (70) to change with alterations in salt

[1] As discussed in detail in the next section, early attempts to determine the molecular structure of circulating ANP led to the identification of a number of similar peptides of varying length with the same basic structure. These other peptides are now recognized to be nonphysiological artifacts of overly harsh separation procedures. Nevertheless, the names of these species appear in many early papers under the designations auriculin A, auriculin B, and atriopeptins I, II, and III. Cardionatrin and hANP are previously used terms that correspond to ANP-(1—28).

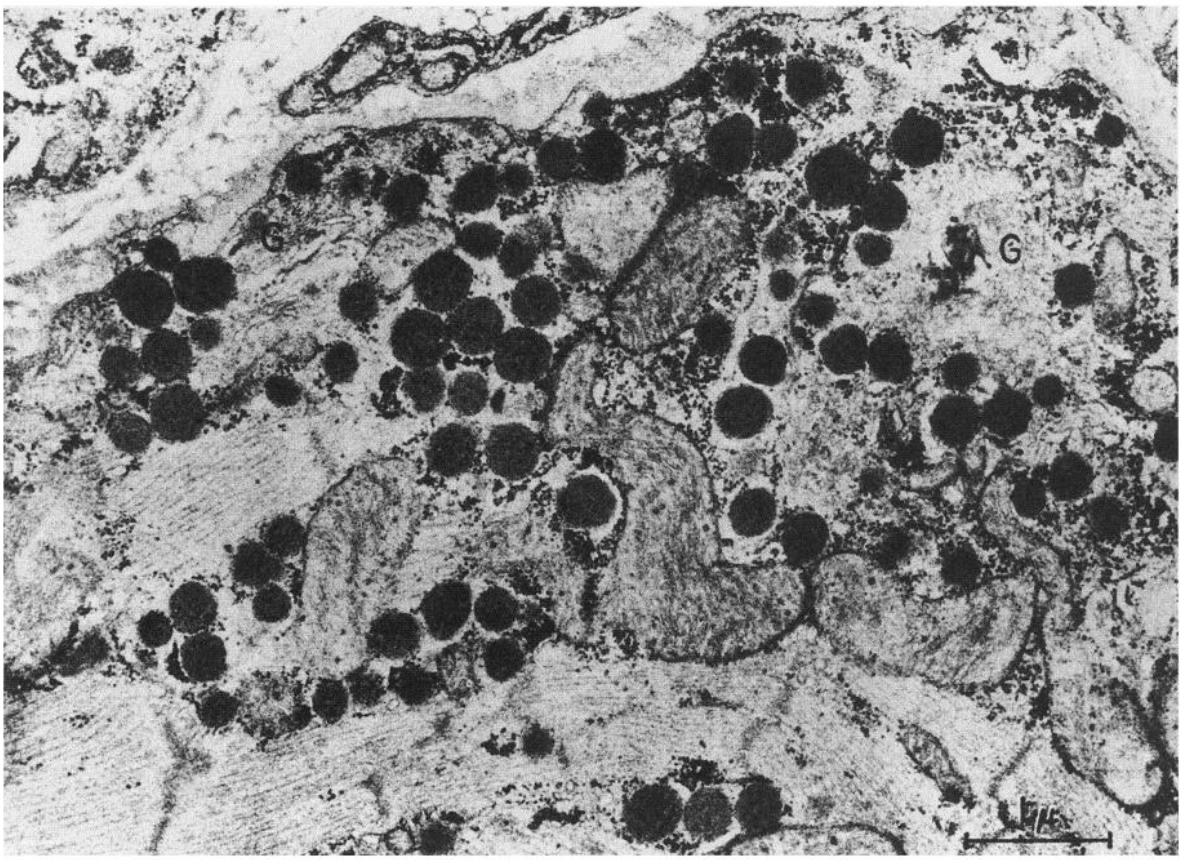

FIG. 2. Characteristic electron-dense granules in an atrial myocyte. G, golgi apparatus. [From Jamieson and Palade (153).]

and water intake, implying a functional role in the control of extracellular volume. DeBold et al. (71) further demonstrated that granule-enriched extracts of rat atrial tissue were capable of inducing a prompt, short-lived but profound natriuresis and diuresis in recipient rats and confirmed the active component to be a peptide that they designated atrial natriuretic factor. Specific antibodies directed against either the amino- or carboxy-terminus of preproANP revealed immunoreactive material to be concentrated in these secretory granules (211, 333). With the use of a full-length cDNA as a probe, preproANP mRNA was detected as a single abundant species in rat atria, constituting 0.5–3% of total atrial mRNA in the basal state (301).

In addition to atrial myocytes, ANP has been shown to be synthesized and stored in a variety of tissues, albeit at levels far lower than those found in atria, and they are therefore unlikely to contribute substantially to plasma levels, at least under physiological conditions. Bloch et al. (43) reported the formation but not storage of ANP in fetal ventricles and suggested that, in this tissue, specific differences in hormone processing reflect a different functional role for ventricular compared with atrial ANP. Atrial natriuretic peptide content and mRNA levels are lower in fetal atria than in ventricles, and these distributions reverse in early postnatal life (353). In adult animals and humans, ventricles "under stress," as in left ventricular hypertrophy or congestive heart failure, express specific ANP mRNA or immunoreactive ANP, suggesting recruitment of these cells to secrete ANP in pathophysiological states (43, 82, 210a, 336a). Atrial natriuretic peptide gene expression has also been observed in aortic arch (104), lung (105), anterior pituitary (105), hypothalamus (105), brain (284), adrenals (241), and kidney (280). In brain, ANP-like immunoreactivity has been detected in the paraventricular nuclei (which synthesize vasopressin and influence anterior pituitary and autonomic nervous system function) and the anteroventral region of the third ventricle (the "AV3V" region believed important in blood pressure regulation), suggesting that ANP functions as a neuropeptide and may suppress the release of AVP (281, 319). Indeed, ANP binding density in the paraventricular nucleus is increased in vasopressin-deficient Brattleboro rats and in rats after hypophysectomy. Of interest in this regard, Sutoh et al. (323) recently identified a novel 26-amino acid peptide in porcine brain with potent natriuretic, diuretic, and spasmolytic actions similar to ANP-(1—28). This peptide, designated brain natriuretic peptide (BNP), shows considerable sequence homology with ANP-(1—28) but also shows important differences indicative of encoding by a separate gene. Brain natriuretic peptide competes effectively with ANP-(1—28) for binding to ANP receptors in renal glo-

meruli and papillae, thereby accounting for similar renal actions (121).

The genes encoding for preproANP have been sequenced in humans, rats, and mice and reveal high degrees of homology (18, 230, 300). The human gene, located on the short arm of chromosome 1 (1pter), consists of three exons (peptide coding regions) interrupted by two introns (intervening sequences) (Fig. 1). The first exon encodes the leader sequence and the first 16 residues of proANP, whereas the third exon encodes only the carboxy-terminal tyrosine. Thus the second exon encodes the intervening peptide sequence containing the functionally active portion of the molecule. A potential glucocorticoid receptor binding site has been found on the second intron, raising the possibility of regulation of ANP gene expression by glucocorticoids (see Refs. 98, 106, 301; see next section).

Initial reports revealed that peptides of varying length derived from the carboxy-terminus of proANP had natriuretic activity (22, 193, 229, 299a, 336). Most are now believed to be nonphysiological cleavage products obtained by overly harsh proteolytic digestion during extraction procedures. Flynn et al. (92) identified ANP-(1—28) (the terminal 28 amino acids of proANP) in atria, a finding soon confirmed by others (156a, 173). Schwartz and co-workers (229) provided the definitive sequence of the 28-amino acid circulating form of human ANP-(1—28). Sugawara et al. (325) demonstrated the presence of ANP-(1—28) in plasma from humans, whereas atrial tissue yielded the proANP-(1—126) isoform and a 56-amino acid dimer of ANP-(1—28). Coronary sinus plasma from humans exhibits a threefold greater preponderance of ANP-(1—28) over shorter forms of the peptide (173). Rat atrial myocytes in culture also release ANP-(1—28) (124, 317) but store the prohormone (348). Proteases in atrial tissue and serum are capable of cleaving proANP to ANP-(1—28). A protease in rat atrial homogenates [termed atrioactivase (150)] and in bovine atrial tissue cleaves the Arg-98 to Ser-99 bond of proANP yielding ANP-(1—28) (35, 149). Others, however, suggest that cultured atrial myocytes can secrete proANP-(1—126) (112). Bloch et al. (44) found that a specific protease in serum (but not in plasma) cleaves proANP to an inactive 14-kDa fragment and the active 3-kDa peptide ANP-(1—28). Because this enzyme activity is not present in plasma, however, its physiological importance is in doubt. Inagami and Imada (150) believe this protease is thrombin. Trippodo et al. (340) also demonstrated the conversion of proANP-(1—126) to ANP-(1—28) by incubation in medium containing platelets. Thus processing of proANP to ANP-(1—28) certainly occurs immediately before and possibly even soon after secretion from the myocyte, resulting in ANP-(1—28) as the predominant form entering coronary sinus blood (35, 42, 44, 112, 149, 340, 348).

IV. REGULATION OF ATRIAL NATRIURETIC PEPTIDE SECRETION

Mechanical and humoral stimuli are capable of inducing ANP secretion. The notion of a natriuretic factor released in response to cardiac distention was considered by Henry et al. (137) and others (24) based on the finding that inflation of a balloon in the left atrium of dogs induced a marked increase in urine flow, whereas prevention of atrial stretch in volume-expanded animals abolished this renal response (113). Isolated rat heart-lung preparations (Langendorff preparations) release bioactive or immunoreactive ANP in response to atrial stretch induced by volume expansion (76). Pacing frequency (heart rate) per se has no effect on ANP secretion in isolated, perfused, beating rat atria (72). Similarly, in anesthetized dogs, increased atrial pressure rather than rate is the principal stimulus for elevations in plasma ANP levels (349). Many other studies have confirmed that atrial stretch augments ANP secretion in vivo. Ledsome et al. (179) observed increased plasma ANP levels in dogs subjected to mitral valve obstruction. The release of ANP also occurs in response to acute as well as chronic volume loading in rats (25, 174, 331) and in humans (277). Atrial distention and raised plasma ANP levels also attend central hypervolemia induced by head-down tilt (145) or head-out water immersion (86), the latter being associated with brisk natriuresis and diuresis. As is discussed in section XI, plasma ANP levels are also elevated in clinical states associated with increased atrial pressures, including rapid tachyarrhythmias, congestive heart failure, and various disorders associated with expansion of extracellular fluid volume (i.e., acute and chronic renal failure). The mechanism(s) by which increases in wall tension lead to rapid secretion of ANP remains unclear: DeBold and DeBold (73a) have recently demonstrated that stretch-induced release of ANP is independent of Ca^{2+} and that removal of Ca^{2+} from superfusate increases the rate of ANP release.

Several lines of evidence indicate that glucocorticoids may stimulate ANP secretion. Garcia et al. (98) demonstrated that adrenalectomized rats do not respond to increased atrial pressure with increased atrial and plasma ANP levels unless pretreated with mineralocorticoids and glucocorticoids. Furthermore, the glucocorticoid dexamethasone alone can increase ANP gene transcription (106) in keeping with evidence of a glucocorticoid binding site on the ANP gene and with the known regulatory effect of glucocorticoids on transcription of mRNAs encoding other hormones. Thus glucocorticoids permit the atria to respond to changes in volume status and may themselves induce ANP secretion.

Several other factors also influence ANP secretion. Acetylcholine, epinephrine, and vasopressin all cause release of a natriuretic substance from rat atrial tissue in vitro, as detected by bioassay (317, 361). The ANP secretory response to isoproterenol has recently been shown to be dependent on both superfusate Ca^{2+} and release of Ca^{2+} from intracellular stores. The effect of isoproterenol was mimicked in this preparation by dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP), implicating this pathway (295). Likewise, ANP secretion is enhanced from isolated rat atria in response to increases in osmotic pressure and Na^+ con-

centration or hypoxia (19, 88, 185a). In vivo, intravenous administration of vasopressin, angiotensin II (ANG II), or phenylephrine raises plasma ANP levels in rats, possibly due to their systemic vascular effects, since the rise in plasma ANP correlates closely with elevations in mean arterial blood pressure (197). Hoffman and Keiser (145a) have recently stressed the primacy of increased right atrial pressure as the stimulus for ANP release, having compared ANP levels after cardiac pacing in normal and hypophysectomized rats. Endothelin, a newly discovered potent vasoconstrictor peptide elaborated from endothelial cells in response to increased shear stress and a variety of chemical agonists (i.e., thrombin, epinephrine, phorbol esters) (for review see Ref. 46), has been shown to augment ANP release from isolated cardiac myocytes (96), from isolated contracting right atria (359a), and from isolated heart preparations (320). Plasma levels of ANP also rise after endothelin administration intravenously (320). These findings suggest that ANP and endothelin may be important biological antagonists.

At least two mechanisms may be involved in regulation of ANP secretion, namely rapid conversion of proANP-(1—126) to ANP-(1—28) and/or release of stored ANP-(1—28) and enhanced synthesis of mRNA encoding for preproANP, leading to increased levels of proANP-(1—126) and ANP-(1—28). The former mechanism may be involved in the initial, rapid response to such stimuli as increased atrial tension and changes in tonicity, whereas changes in synthesis of preproANP mRNA probably participate in more chronic stimulation of ANP secretion. Although levels of preproANP mRNA in atria comprise between 0.5 and 3% of total mRNA expressed in these cells in the basal state (224, 300, 301), the concentration of message for preproANP rises sharply with changes in salt and water intake (330). Nakayama et al. (224) showed a marked decrease in mRNA levels in rats after 2 days of water deprivation, whereas Ballermann et al. (26) reported a greater than twofold increase in plasma ANP levels coupled with increased preproANP mRNA expression in the atria of rats given deoxycorticosterone acetate (DOCA) for 12 h. In rats with congestive heart failure induced by myocardial infarction, Mendez et al. (210) have also shown an increase in specific atrial preproANP mRNA, which paralleled atrial hypertrophy. Similarly, recruitment of preproANP mRNA synthesis occurs in other cell types, such as ventricle, aortic arch, and lung. Thus several stimuli of ANP secretion are capable of enhancing transcription of preproANP mRNA.

V. CLEARANCE OF CIRCULATING ATRIAL NATRIURETIC PEPTIDE

The half-life of intravenously injected ANP is 2–4 min in animals and humans (191, 334, 364). After incubation with tissue homogenates, Tang et al. (334) found the rank order of degradative potency to be kidney > liver > lung > plasma > heart. Nevertheless, in one study the bulk of ANP activity was found to be recoverable after a single passage through lungs and kidneys (356) so that the primary sites of extraction and degradation in vivo have not been established with certainty. The brush border of the proximal tubule is very rich in degradative enzymes and plays a major role in degrading other peptides. Two groups have recently reported the cleavage of the Cys-7 to Phe-8 bond of ANP by a metalloendopeptidase variously termed enkephalinase or neutral endopeptidase EC 24.11. This cleavage, which disrupts the ring structure of ANP (240, 321), is inhibited in vitro and in vivo by phosphoramidon or thiorphan, inhibitors of endopeptidase EC 24.11 (172, 318), whereas captopril, an inhibitor of angiotensin-converting enzyme, is without effect. Ura et al. (343) have shown also that infusion of phosphoramidon causes an increase in urinary Na^+ excretion rate ($U_{Na}\dot{V}$), an effect attributed to inhibition of breakdown of either kinins or ANP. Lafferty et al. (172) found that phosphoramidon infusion in rats with reduced renal mass leads to parallel increases in plasma ANP levels, urinary guanosine 3′,5′-cyclic monophosphate (cGMP) (the second messenger of ANP), and $U_{Na}\dot{V}$. Seymour et al. (303a), using another similar neutral endopeptidase inhibitor, showed an augmentation of both the magnitude and duration of the hypotensive, natriuretic, and urinary cGMP responses to ANP in spontaneously hypertensive rats (SHR). Recently, two synthetic orally active endopeptidase inhibitors (so-called atriopeptidase inhibitors UK69578 and UK79300) were shown in mice to potentiate the natriuresis, diuresis, and increment in plasma ANP levels induced by volume loading (282). The inhibitor UK69578 was also administered acutely to six patients with mild congestive heart failure and in this setting again augmented plasma ANP levels and Na^+ excretion accompanied by significant reductions in mean right atrial and pulmonary artery wedge pressures, possibly reflecting venodilation (234). These studies suggest a potentially important therapeutic role for selective inhibitors of EC 24.11. In addition to degradation by ectoenzymes, ANP is also removed from the circulation by binding to the so-called "clearance" receptor (discussed in detail in sect. VI*A*). This plasma membrane protein is expressed in large abundance on vascular endothelial cells (5, 184, 195). It has a long extracellular ANP binding domain and a very short intracellular domain, thus resembling other clearance receptors, including those for low-density lipoproteins and mannose 6-phosphate. Biologically inactive ANP analogues that bind specifically to ANP clearance receptors increase circulating ANP levels (195).

Thus, since the demonstration of the biological activity of atrial peptides in 1981, the tools of modern biochemistry and molecular biology have helped to elucidate ANP gene structure and transcription products. It is now clear that the predominant secreted form is the 28-amino acid peptide ANP-(1—28). The critical importance of the intact ring structure and carboxy-terminal residues have also been demonstrated. The regulation of peptide synthesis and secretion by increased intravascular volume and other factors has been clearly shown. The importance of the kidney not only as a target organ

but also as a major site of degradation has been defined. Under normal circumstances, ANP is rapidly removed from the plasma, but plasma half-life may be prolonged by selective blockade of degradative enzymes or clearance receptors.

VI. CELLULAR MECHANISMS OF ATRIAL NATRIURETIC PEPTIDE ACTION

As with other peptide agonists, ANP must first bind to stereospecific cell surface receptors in order for physiological responses to be evoked in target cells. This hormone-receptor interaction in turn activates a plasma membrane-associated guanylate cyclase to convert MgGTP to cGMP. Newly generated cGMP stimulates cGMP-dependent protein kinases, with the latter responsible for phosphorylation of a large number of intracellular proteins, biochemical events essential to ultimate expression of physiological actions induced by ANP. In addition, ANP also inhibits adenylate cyclase activity in a number of tissues, raising the possibility that some physiological effects of ANP are brought about by interference with agonist-stimulated cAMP generation. Finally, ANP, in part via its action to raise cGMP, may decrease levels of cytosolic calcium.

A. Atrial Natriuretic Peptide Receptors

Specific ANP binding sites have been revealed by autoradiographic techniques in all ANP target tissues studied, including most notably adrenal, kidney, and vasculature but also central nervous system, pigmented epithelium and ciliary process of the eye, hepatocytes, gallbladder, colonic smooth muscle, and lung parenchyma (38, 39, 198, 202, 207, 264, 265). In the kidney, ANP binding sites are concentrated in large renal vessels, glomeruli, and renal medulla (28, 30, 61, 169, 207; Fig. 3). In the adrenal, ANP binding is limited primarily to the zona glomerulosa (198, 207; Fig. 3). Binding sites are also expressed on a number of cell types, including adrenal glomerulosa cells, renal inner medullary collecting duct (IMCD) cells, renal glomerular mesangial and endothelial cells, arterial smooth muscle and endothelial cells, and the pig kidney epithelial cell line LLC-PK_1 (75, 120, 142, 143, 184, 202, 207, 226, 250, 292, 294).

Although radioligand equilibrium binding techniques initially characterized ANP receptors as conforming to a single class, more recent affinity cross-linking experiments demonstrate the presence of several distinct cell surface ANP binding sites in most cells and tissues (31, 168, 184, 202, 203, 206, 216, 249, 293, 307). Unfortunately, complete agreement regarding the nature, function, and, in some instances, the existence of these different receptors is still lacking among workers in the field. Most agree, however, on the existence of at least three receptors: ANP-receptor 1 (ANP-R1), ANP-R2, and ANP-R3.

The receptor ANP-R2 (also termed ANP clearance receptor or C-receptor) is a plasma membrane-associated protein that binds ANP with high affinity and has an apparent molecular mass of 120–130 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions (64–70 kDa in the presence of reducing agents, indicating disulfide-linked subunits) (184, 195, 293, 328, 329). This ANP binding site has been purified to homogeneity (293, 328, 329), its cDNA cloned (265), and the gene product expressed in *Xenopus* oocytes (97). The purified protein is devoid of guanylate cyclase activity, and the binding site in intact cells is not coupled to guanylate cyclase (293, 328, 329, 341). Indeed, evidence that binding of ANP to this protein elicits a specific cellular response is almost entirely lacking. Whereas other ANP receptors display a high degree of selectivity for ANP-(1—28), ANP-R2 also binds ANP fragments and internally ring-deleted ANP analogues with high affinity (5, 195, 289). These findings together with the abundance of ANP-R2 on vascular endothelial cells suggest that ANP-R2 serves to clear ANP from the circulation (5, 184, 195, 289).

A second cell membrane-associated protein that binds ANP specifically and with high affinity also has an apparent molecular mass of ~130 kDa; unlike ANP-R2, however, reducing agents do not alter the ap-

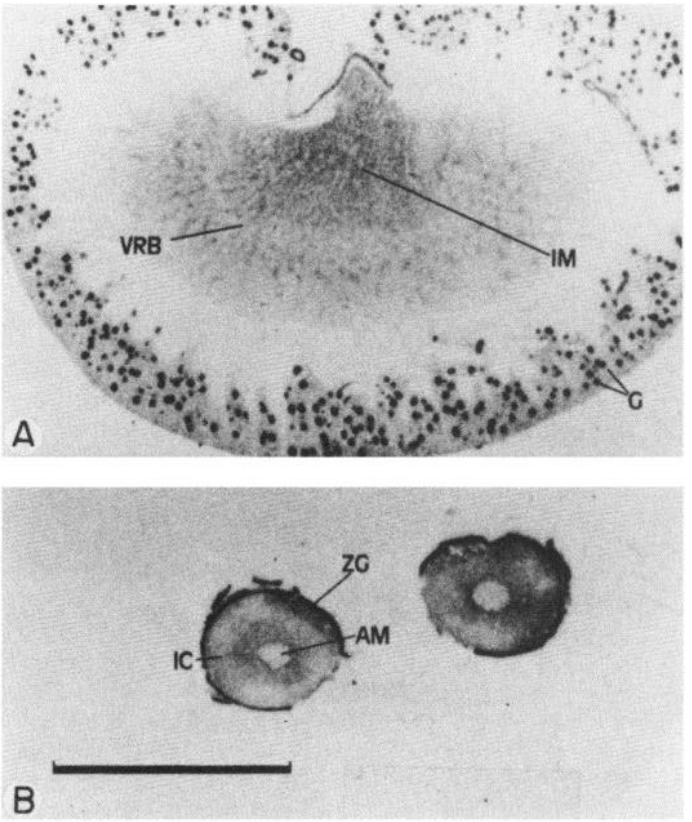

FIG. 3. Autoradiographic localization of ANP binding in rat kidney (*A*) and rat adrenal (*B*). AM, adrenal medulla; G, glomeruli; IC, inner cortex; IM, inner medulla; VRB, vasa recta bundles; ZG, zona glomerulosa. [From Mendelsohn et al. (207).]

parent molecular mass of this protein (31, 184, 328, 329), designated ANP-R1. Atrial natriuretic peptide-receptor 1 exhibits selectivity for ANP-(1—28) over ANP-(5—25) (184). The interaction of ANP with ANP-R1 activates particulate guanylate cyclase (185, 328, 329). Atrial natriuretic peptide-receptor 1 has been purified to apparent homogeneity (170). The binding activity of ANP and guanylate cyclase activity copurified over several chromatographic steps, although on solubilization responsiveness of the cyclase to ANP was lost. The complete nucleotide sequence of the ANP-R1 gene was recently elucidated, confirming the ANP binding site and guanylate cyclase activity on the same transmembrane protein (64a). Atrial natriuretic peptide-receptor 1 possesses a single transmembrane domain and shows ~30% sequence homology with ANP-R2 in the extracellular ANP binding domain. The cytoplasmic tail of ANP-R1 is longer than that of ANP-R2 and shares appreciable sequence homology with soluble guanylate cyclase and protein kinases (64a). Transfection of Cos-7 cells with mutant ANP-R1 clones in which the protein kinase-like domain was deleted resulted in enhanced basal expression of guanylate cyclase activity and loss of ANP responsiveness compared with cells transfected with the wild-type ANP-R1 gene. Furthermore, whereas ATP augmented ANP-stimulated cGMP generation threefold in cells transfected with the wild-type gene, ATP had little effect on guanylate cyclase activity in cells transfected with the kinase-deleted mutant gene. Thus the kinaselike domain was found to be necessary for regulation of guanylate cyclase activity by ANP and by ATP. Chinkers and Garbers (64) therefore suggest that the kinaselike domain of the ANP-R1 receptor is necessary to repress guanylate cyclase activity in the basal state and that a potential mechanism whereby ANP activates guanylate cyclase is to induce a conformational change that alters interactions between the cyclase and the kinase domains resulting in guanylate cyclase activation.

The nucleotide sequence of a cDNA encoding a third ANP receptor (ANP-R3) distinct from ANP-R1 and ANP-R2 was also delineated by two groups after screening cDNA libraries of human placenta and rat brain. This receptor appears structurally similar to ANP-R1 with >70% homology in the region coding for the intracellular tail (kinaselike and guanylate cyclase domains). Furthermore, a highly conserved region in the extracellular portion of ANP-R3, ANP-R1, and ANP-R2 may represent the ANP binding domain of the three receptors. Although it was suggested that ANP-R3 may be relatively more selective for BNP over ANP when compared with ANP-R1, further evidence is required to establish relative potency orders for ANP peptides in activating ANP receptor subtypes (62a, 299).

A 180-kDa membrane protein with guanylate cyclase activity was also purified to homogeneity from rat adrenocortical carcinoma cells (251). This isoenzyme binds ANP with a 1:1 stoichiometry, strongly suggesting coexpression of ANP binding sites and guanylate cyclase activity. The fact that the 180-kDa ANP binding site may represent a distinct ANP receptor is suggested by the finding shown in Figure 4, which is based on affinity cross-linking studies in glomeruli and which depicts specific ANP binding to bands at 130 and 180 kDa, whereas similar studies of renal inner medulla reveal only a single band at 130 kDa (122). The 180-kDa receptor in glomeruli was labeled on Western blots with antiserum raised against the 180-kDa guanylate cyclase obtained from rat adrenocortical carcinoma cells, and the antibody inhibited ANP-stimulated guanylate cyclase activity in glomerular membranes (31). Sequencing of the gene that encodes this 180-kDa ANP binding site will be required to establish whether it represents a distinct ANP receptor or currently known ANP receptors associated with other membrane-bound protein(s).

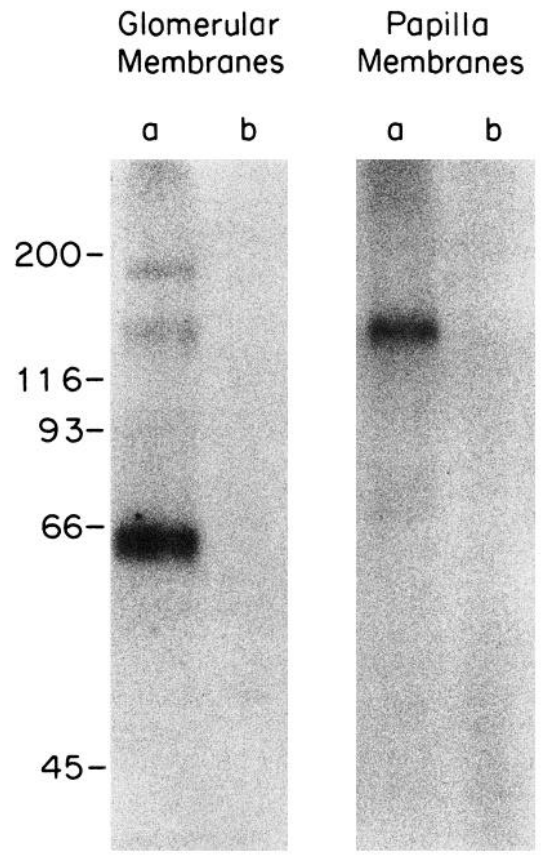

FIG. 4. Affinity cross-linking of radiolabeled ANP-(1—28) to membranes prepared from glomeruli and papilla of rat. Binding of radioligand was performed in absence (*lanes a*) or presence (*lanes b*) of excess (10^{-6} M) unlabeled ANP.

The existence of a 140-kDa ANP binding site consisting of two 70-kDa subunits has also been suggested (151, 250). Although this binding site is not directly coupled to guanylate cyclase, antibodies directed against it inhibit ANP-stimulated cGMP accumulation (151). Whether this 140-kDa site is distinguishable from ANP-R2 remains to be determined.

Equilibrium dissociation constants (K_d) for ANP receptors (ANP-R) range from 50 to 500 pmol/l (for re-

view see Ref. 202), which is similar to levels of ANP in plasma. Interaction of ANP analogues with ANP-R requires the presence of an intact internal disulfide bridge and conservation of residues within the ring formed by the disulfide bridge (75, 143, 288, 290). Furthermore, except for the R2 receptor, an intact carboxy-terminal Phe-Arg is essential for binding, and deletion of residues at the amino-terminus of ANP reduces the affinity of analogues for receptors (120, 288). In contrast, ANP-R2 binds ANP analogues devoid of the carboxy-terminal Phe-Arg-Tyr, those with extensive deletions at the amino-terminus or within the ring, and even some devoid of an intact disulfide bridge (203, 289, 290).

A number of reports have concluded that ANP receptor density in tissues varies inversely with the circulating ANP concentration (25, 26, 31, 99, 108, 109, 140, 297, 306). However, this apparent regulation of ANP receptors appears to be explained, in large part, by occupancy of ANP-R2 rather than alterations in the expression of plasma membrane ANP receptor protein (203).

B. Guanosine 3',5'-Cyclic Monophosphate: Major Second Messenger for Atrial Natriuretic Peptide Action

The spasmolytic effect of ANP on vascular smooth muscle is but one of the many actions of this peptide that correlates closely with local elevations in cGMP concentration. Atrial natriuretic peptide stimulates cGMP accumulation by activation of plasma membrane-associated guanylate cyclase (also referred to as "particulate" guanylate cyclase) (125, 219, 220, 358). Bioavailable analogues of cGMP mimic the physiological actions of ANP, including smooth muscle relaxation and inhibition of renal tubule Na^+ transport, confirming cGMP's role as a second messenger for ANP action (188, 219, 237, 368). Atrial natriuretic peptide stimulates particulate guanylate cyclase in a concentration-dependent manner in responsive tissues, including rabbit IMCD cells where ANP, acting via the ANP-R1 receptor, stimulates maximum enzyme activity (V_{max}) without altering the Michaelis constant (K_m) of the enzyme (122, 292, 338). Soluble guanylate cyclase activity is not affected by ANP, nor does desensitization of soluble guanylate cyclase by nitrovasodilators affect ANP-sensitive particulate guanylate cyclase activity (220). Thus ANP raises intracellular levels of cGMP exclusively by increasing the V_{max} of particulate guanylate cyclase.

The mechanism of activation of particulate guanylate cyclase by ANP appears to vary with receptor subtype. In the case of ANP-R1, cyclase activity is represented by a specific molecular domain of the receptor peptide, suggesting that binding of ANP to the receptor portion of the molecule induces conformational changes in an adjacent catalytic domain, thereby increasing the rate of cGMP formation. As discussed in the previous section, the catalytic activity is repressed by the presence of a kinaselike domain interposed between the binding site and the catalytic unit (64a). For the 180-kDa guanylate cyclase-linked ANP binding site, solubilization of guanylate cyclase activates the enzyme and results in loss of ANP sensitivity (31, 251). Thus the ANP-sensitive particulate guanylate cyclase associated with this protein appears to be under tonic inhibitory control by a protein or lipid component of the plasma membrane, and ANP binding to this component may reverse this inhibition. The ANP-stimulated accumulation of cGMP is also regulated by other intracellular messenger systems. Activation of protein kinase C inhibits ANP-stimulated, but not basal, particulate guanylate cyclase activity (225, 311). In addition, in vascular smooth muscle, calcium-mobilizing hormones appear to stimulate cGMP phosphodiesterase activity, suggesting an additional point of control of cellular cGMP responsiveness (311).

In vascular smooth muscle, increments in intracellular cGMP levels result in the activation of cGMP-dependent protein kinases and phosphorylation of a number of intracellular proteins (185, 219, 268). In addition, cGMP induces dephosphorylation of myosin light chains in vascular smooth muscle cells, a precondition for smooth muscle relaxation (219, 268). In other cell types, phosphorylation by cGMP-dependent protein kinases appears to mediate the actions of ANP (90, 235) but by mechanisms that are as yet poorly understood. In IMCD cells and in other epithelia, increments in intracellular cGMP induced by ANP lead to inhibition of an amiloride-sensitive cation Na^+ channel (188, 366, 368). The direct application of cGMP to inside-out patches of IMCD cell membranes also inhibits Na^+ channel activity (188). Whether membrane-associated cGMP-dependent protein kinase accounts for this effect or whether cGMP interacts directly with the channel remains to be determined.

C. Atrial Natriuretic Peptide Actions on Adenylate Cyclase

Atrial natriuretic peptide inhibits basal and hormone-stimulated adenylate cyclase activity in some tissues, including aorta, mesenteric and renal artery, homogenates of anterior and posterior pituitary, particulate fractions of renal glomeruli, and adrenal glomerulosa membranes (7–10, 12, 271). No inhibitory effect on adenylate cyclase was demonstrated, however, in collecting duct, proximal tubule, adrenal medulla, testis, spleen, or skeletal muscle (115, 133, 231, 232). In addition, ANP also inhibits basal, hormone-stimulated (glucagon, norepinephrine, isoproterenol), and forskolin-stimulated adenylate cyclase activity, which are effects dependent on addition of GTP (12, 271). The latter finding implies involvement of an inhibitory guanine nucleotide regulatory protein (G_i). Not surprisingly, therefore, pertussis toxin, a bacterial toxin that ADP ribosylates and thereby inactivates G_i, abolishes the action of ANP on adenylate cyclase (7, 11, 12, 271). Although ANP appears to be inhibitory to adenylate cyclase in broken cell preparations in the hands of some, but not all (133), investigators, ANP-induced reductions

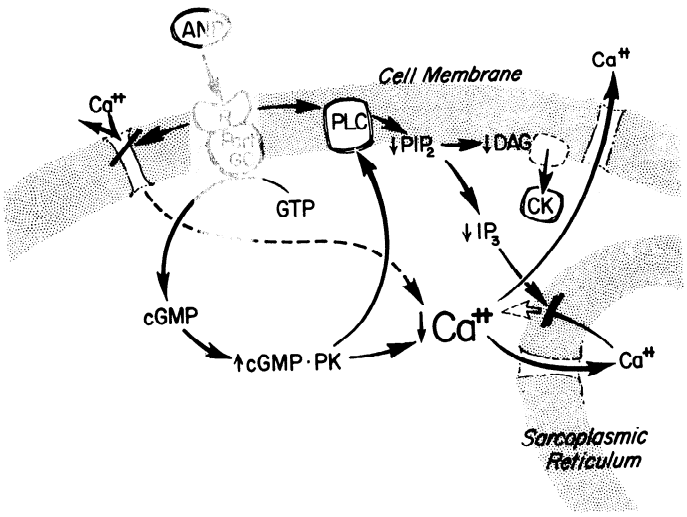

FIG. 5. Role of ANP in modulation of intracellular calcium. Potential mechanisms by which ANP promotes a decrease in intracellular Ca^{2+} are outlined. These include inhibition of Ca^{2+} release from sarcoplasmic reticulum, inhibition of Ca^{2+} influx, and enhancement of active Ca^{2+} extrusion. cGMP·PK, cGMP-dependent protein kinase; CK, protein kinase C; DAG, diacylglycerol; IP_3, inositol trisphosphate; Part GC, particulate guanylate cyclase; PIP_2, phosphatidylinositol bisphosphate; PLC, phospholipase C; R, ANP receptor. [From Lewicki (186). In: *Contemporary Issues in Nephrology. Atrial Natriuretic Peptide*, Churchill Livingstone, New York, 1989.]

in cell-associated cAMP levels have not been demonstrated (227, 342), casting some doubt on the functional significance of this effect in intact cells and in vivo.

D. Effects of Atrial Natriuretic Peptide and Guanosine 3',5'-Cyclic Monophosphate on Intracellular Calcium Mobilization and Phosphoinositide Turnover

Because ANP opposes the action of a number of agonists that stimulate smooth muscle cell contraction and because smooth muscle cell contraction involves a rise in cytosolic calcium (Ca_i^{2+}), cGMP is believed to interfere with this intracellular ionic process. As demonstrated for several agonists capable of stimulating smooth muscle cell contraction, it is generally believed that receptor occupancy leads to activation of phospholipase C, which liberates inositol trisphosphate (IP_3) and diacylglycerol (DAG) from membrane-associated inositol phospholipids (36, 37, 201). In turn, IP_3 stimulates release of Ca^{2+} from intracellular stores, i.e., the initial agonist-stimulated rise in smooth muscle Ca_i^{2+} is independent of extracellular Ca^{2+}. Maintenance of smooth muscle cell contraction is thought to involve activation of protein kinase C by DAG and uptake of Ca^{2+} into the cells through receptor-gated plasma membrane Ca^{2+} channels (201). These events are summarized diagrammatically in Figure 5.

Effects of ANP and cGMP on agonist-stimulated phosphatidylinositol turnover have been sought (185, 267). Cyclic GMP interferes with thrombin-induced phosphatidylinositol turnover in platelets (327) and significantly blunts inositol monophosphate accumulation in vascular smooth muscle cells (267); however, convincing evidence for an effect of ANP or cGMP on IP_3 generation is lacking. Because ANP is more effective in opposing the contractile effects of receptor agonists such as ANG II and norepinephrine than that elicited by depolarization with K^+, it has been suggested that ANP exerts its effects on vascular smooth muscle cells by inhibiting Ca_i^{2+} release. Support for this possibility was obtained in rabbit aortic strips where, in the absence of extracellular Ca^{2+}, norepinephrine-, histamine-, and caffeine-induced contractions were inhibited by ANP in a concentration-dependent fashion (205). Also ANP inhibited norepinephrine-induced $^{45}Ca^{2+}$ efflux in the same preparation. The increment in Ca_i^{2+} induced by agonists is also inhibited by ANP in vascular smooth muscle cells (129, 214), rat mesangial cells (15, 130), and adrenal glomerulosa cells (63). The ANG II-induced calcium transients were sensitive to ANP in some (101, 327) but not other studies (59). Whether technical and methodological differences among studies account for this discrepancy is unclear. Of interest, in permeabilized cells cGMP was without effect on IP_3-mediated Ca^{2+} release from intracellular stores (205, 335). Whether ANP inhibits Ca_i^{2+} release through mechanisms that do not involve cGMP remains a possibility, since some ANP analogues that fail to stimulate cGMP generation in vascular smooth muscle also inhibit contraction (185).

The mechanism of ANP-induced inhibition of calcium mobilization could involve regulation at the level of Ca^{2+} release from intracellular stores, reuptake of Ca^{2+} into these stores, or Ca^{2+} influx or efflux across plasma membranes or membranes bounding intracellular organelles (Fig. 6). In frog cardiac myocytes, ANP inhibits isoproterenol-induced Ca^{2+} influx through L-type Ca^{2+} channels (111, 129). Because isoproterenol-induced Ca^{2+} influx is mediated in these cells by cAMP, the interaction between the second messengers of isoproterenol and ANP were probed. Cyclic AMP and cGMP were found to have opposing effects on cardiac myocyte L-type channel activity, with cAMP opening the channels, whereas cGMP inhibited this effect (129), perhaps by cGMP-induced stimulation of cAMP phosphodiesterase. The fact that cGMP may be involved in the removal of Ca^{2+} from the cell is suggested by the finding that Ca_i^{2+} stores, normally depleted with repeated agonist stimulation, are depleted more rapidly in the presence of agents that elevate cytosolic cGMP (163). In keeping with an effect of ANP on Ca^{2+} exit, ANP reduces basal Ca_i^{2+} concentrations in rat and aortic smooth muscle cells (17) and rat glomerular mesangial cells (15, 130). Furthermore, peak and postpeak plateau Ca_i^{2+} levels in response to ANG II are reduced by ANP in vascular smooth muscle cells and by ANG II and vasopressin in mesangial cells (101, 327). The intracellular Ca^{2+} response to K^+-induced hyperpolarization is also blunted in vascular smooth muscle cells (270). Similarly, ANP blunts ANG II-induced increments in Ca_i^{2+}. Be-

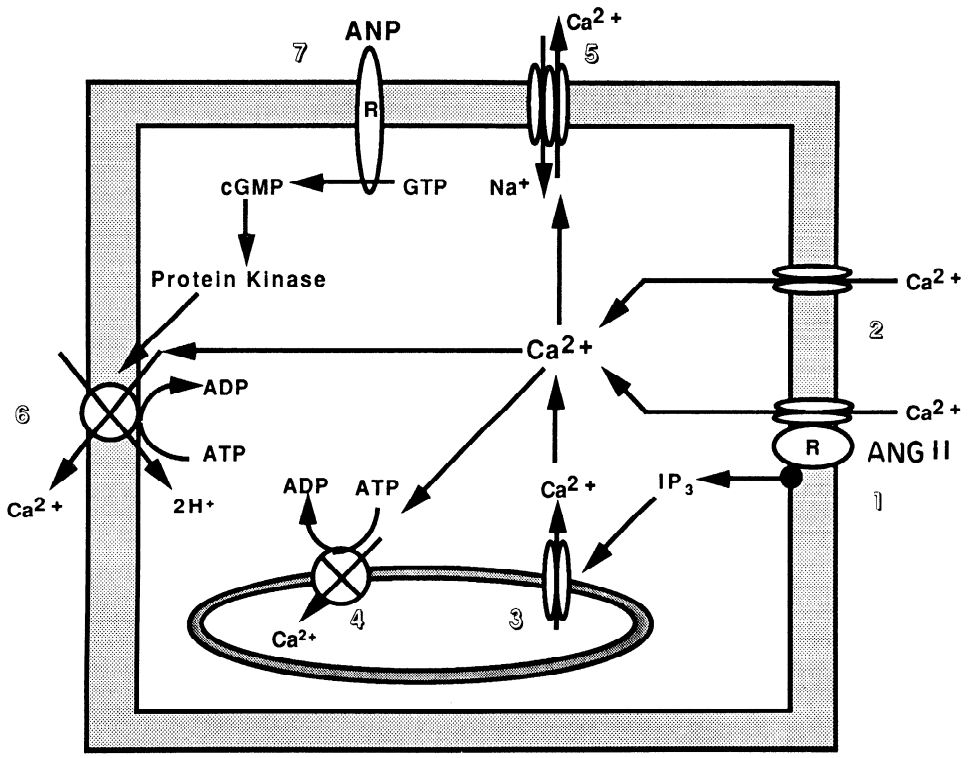

FIG. 6. Mechanisms of transcellular and intracellular transport of calcium. *1*, receptor-gated Ca^{2+} channel; *2*, voltage-sensitive Ca^{2+} channel; *3*, IP_3-gated calciosome Ca^{2+} channel; *4*, calciosome Ca^{2+}-ATPase; *5*, Na^+-Ca^{2+} exchanger; *6*, Ca^{2+}-H^+-ATPase; and *7*, ANP receptor-guanylate cyclase. ANG II, angiotensin II; IP_3, inositol trisphosphate. See text for details.

cause agonist-induced increments in Ca_i^{2+} result, at least in part, from activation of phospholipase C with consequent IP_3-induced liberation of Ca^{2+} from intracellular stores, whereas K^+-induced increments in Ca_i^{2+} are dependent on influx from extracellular stores, the findings suggest that ANP reduces Ca_i^{2+} by mechanisms other than inhibition of phosphatidylinositol turnover.

As with ANP, cGMP lowers basal as well as KCl- and hormone-stimulated Ca_i^{2+} in vascular smooth muscle (163). Also cGMP enhances sequestration of $^{45}Ca^{2+}$ into subcellular storage pools in vascular smooth muscle cells (270) but has no effect on IP_3-induced $^{45}Ca^{2+}$ release from these stores. The fact that ANP and cGMP augment Ca^{2+} extrusion via the plasma membrane Ca^{2+}-ATPase but not the Na^+-Ca^{2+} exchanger is suggested by the findings that ANG II-stimulated $^{45}Ca^{2+}$ efflux was augmented and Ca_i^{2+} reduced by sodium nitroprusside, ANP, and 8-bromo-cGMP (8-BrcGMP) in a Na^+-independent fashion (97a). Cyclic GMP also activates Ca^{2+}-ATPase in crude membranes prepared from vascular smooth muscle cells (270) and in plasma membrane-enriched fractions containing calmodulin-sensitive Ca^{2+}-ATPase (260, 324). The fact that this effect of cGMP on plasma membrane Ca^{2+}-ATPase is mediated by cGMP-dependent protein kinase can be concluded from the finding that antibodies to cGMP-dependent protein kinase abolish the stimulatory effect of cGMP on plasma membrane Ca^{2+}-ATPase (260). It is of interest that the intermediary effect of cGMP-dependent protein kinase was found in a purified membrane preparation, emphasizing that cGMP-dependent protein kinase is a membrane-associated protein (324).

The available evidence thus favors the view that ANP, through stimulation of guanylate cyclase and cGMP-dependent protein kinase, activates smooth muscle plasma membrane Ca^{2+}-ATPase activity, thus augmenting removal of Ca^{2+} from the cell, although activation of Ca^{2+}-ATPase activity by ANP per se has not been shown. Whether, in addition, there is an effect of ANP on Ca_i^{2+} release from storage pools remains to be established. Because dephosphorylation of myosin light-chain kinase occurs in response to activation of soluble guanylate cyclase (90) and because myosin light-chain kinase is calmodulin dependent, ANP may reduce myosin light-chain activity via cGMP-induced reductions in Ca_i^{2+}. An additional effect of cGMP on Ca^{2+} homeostasis in cardiac myocytes results from the inhibition of cAMP-induced Ca^{2+} influx, perhaps mediated via activation of cAMP phosphodiesterase.

VII. ACTIONS OF ATRIAL NATRIURETIC PEPTIDE ON CARDIOVASCULAR SYSTEM

Acute infusion of pharmacological doses of ANP into normal and hypertensive subjects leads to rapid and sustained reductions in mean arterial pressure. Chronic infusions of ANP at levels not to exceed those achieved by endogenous peptide release also lower blood pressure (89, 103, 118). Mechanisms by which ANP reduce blood pressure include diminished cardiac output, reductions in peripheral vascular resistance, and decreased intravascular volume. The relative importance of each of these mechanisms varies with the basal condition of the experimental subject insofar as other factors that regulate blood pressure, such as autonomic

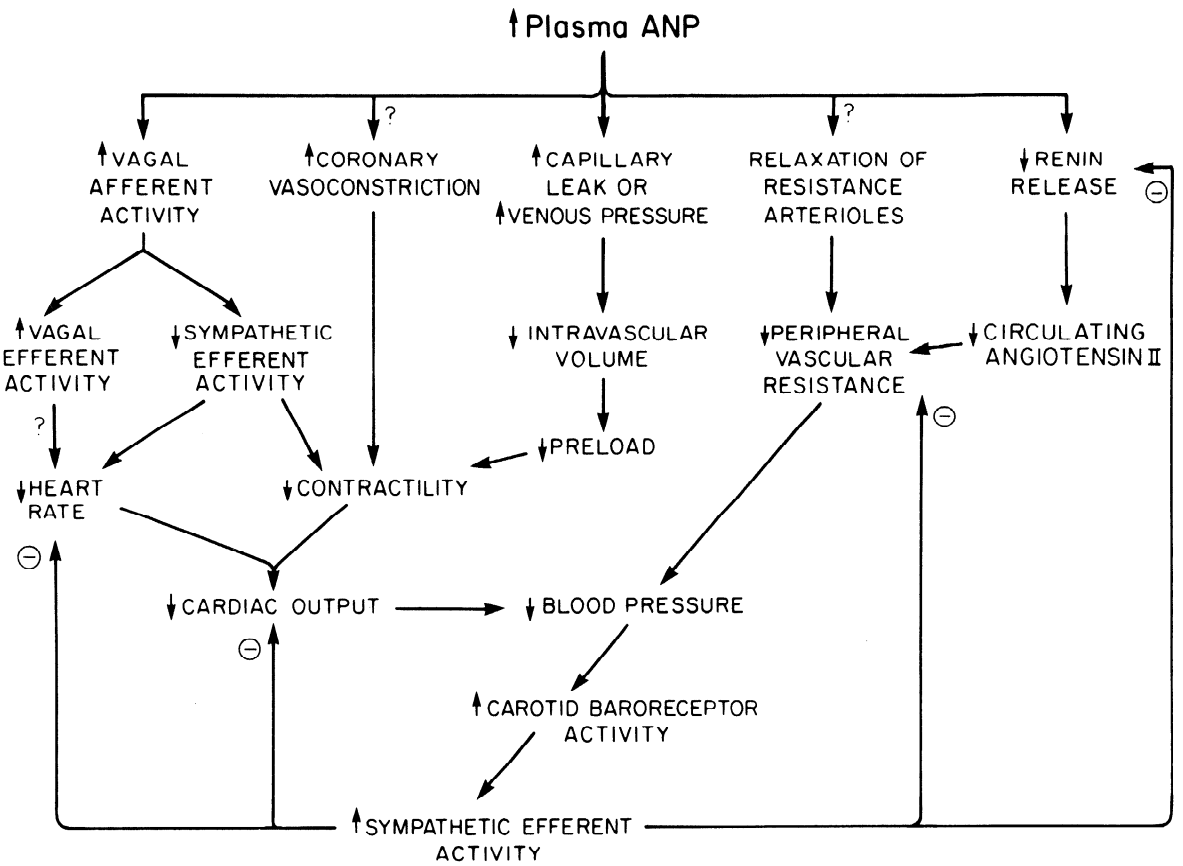

FIG. 7. Schema depicting various actions of ANP to influence blood pressure. See text for details.

tone, volume status, and levels of circulating angiotensin and norepinephrine, also exert important influences on the overall cardiovascular response to ANP. A scheme depicting the known actions of ANP on blood pressure is given in Figure 7; the components of this model are considered in detail next.

A. Effects of Atrial Natriuretic Peptide on Cardiac Output

In conscious and anesthetized rats, dogs, and sheep, ANP infusion at doses of ~100 pmol·kg^{-1}·min^{-1} decreases stroke volume and cardiac output; heart rate often decreases or fails to rise despite marked falls in blood pressure (3, 4, 47, 162, 175, 250a, 286, 287). In these models, the initial ANP-induced declines in cardiac output are due at least in part to effects of the peptide on both parasympathetic and sympathetic nervous systems (Fig. 7). In longer term studies (5 days) of low-dose ANP infusion in sheep, Parkes et al. (250a) have shown a return to normal cardiac output and a reduction in the calculated total peripheral resistance, thereby maintaining the hypotensive effect. Unmyelinated vagal afferent fibers innervate the myocardium, originating both in chemo- and mechanoreceptors (200). Chemoreceptor activation by autacoids, such as prostaglandins or kinins, and mechanoreceptor activation by cardiac distention serve to increase vagal afferent traffic. The resulting heightened vagal efferent traffic and diminished sympathetic discharge tend to slow heart rate and diminish contractility, leading not only to reduced cardiac output but to other systemic effects, such as reduced peripheral vascular resistance and reduced renal Na^+ reabsorption (200). In anesthetized rats, Ackermann et al. (3) provided evidence that atrial extracts reduce cardiac output by stimulating vagal afferents. As shown in Figure 8, rats injected with atrial extract sufficient to induce a 20-fold increase in $U_{Na}\dot{V}$ exhibited marked falls in blood pressure, heart rate, stroke volume, and cardiac output, whereas prior vagotomy was associated with a lesser hypotensive effect of the extract, no decrease in heart rate, and an augmentation in stroke volume. Furthermore, denervation of the carotid sinus in vagotomized animals also prevented the increase in stroke volume induced by atrial extracts. These results have recently been confirmed using purified ANP where vagotomy again markedly attenuated the hypotensive response (337). The role of vagal efferents in the cardiovascular response to ANP remains unclear (Fig. 7). In conscious and anesthetized rats, atropine in doses sufficient to prevent changes in heart rate after administration of acetylcholine failed to alter the hypotensive and negative inotropic responses to ANP (3, 4, 287, 337), although in one study atropine did prevent ANP-induced bradycardia (4).

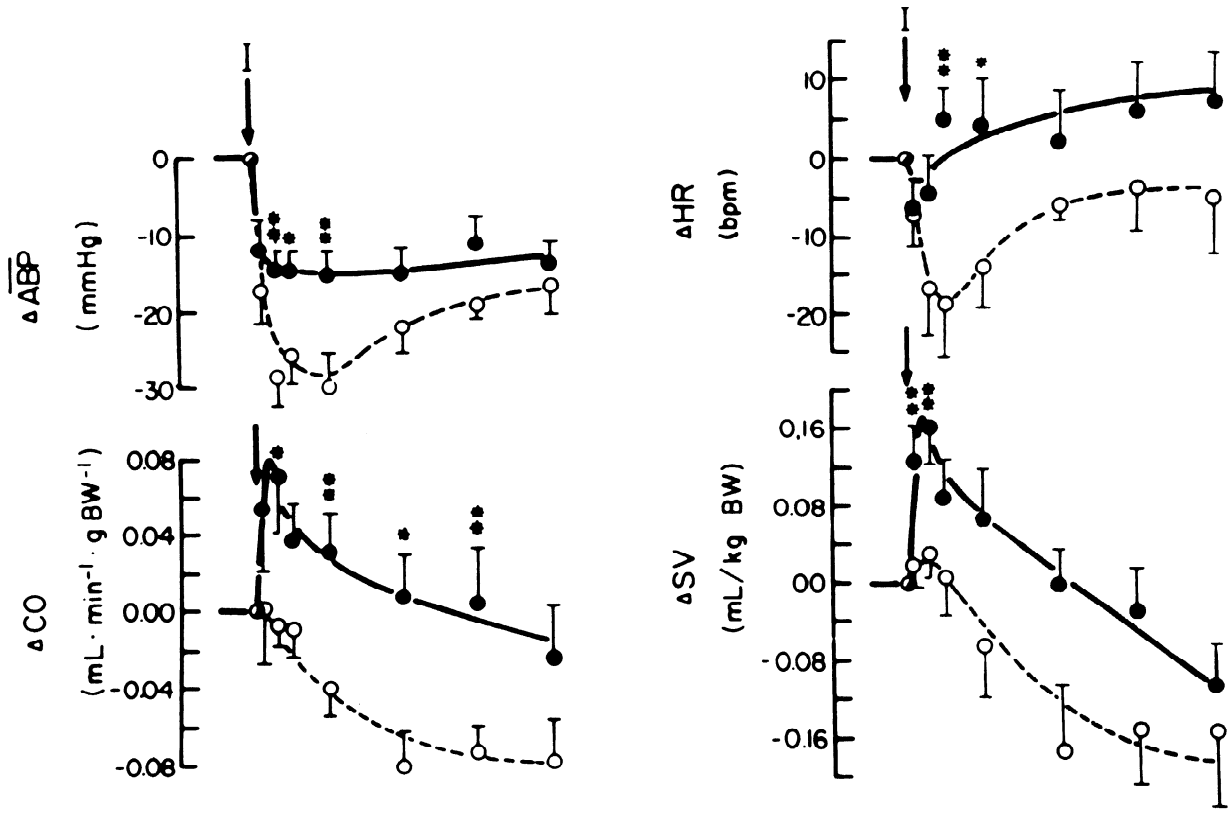

FIG. 8. Cardiovascular effects of intravenously injected atrial extract in vagotomized (open circles) rats. ΔABP, change in mean arterial blood pressure; ΔCO, change in cardiac output; ΔHR, change in heart rate; I, injection; ΔSV, change in stroke volume. Note that ANP lowered heart rate, stroke volume, cardiac output, and blood pressure to a greater degree in sham-operated vs. vagotomized animals. * $P < 0.05$; ** $P < 0.025$. [From Ackermann et al. (3).]

In animals given ANP intravenously, the fall in blood pressure tends to stimulate sympathetic nerve activity, whereas the stimulation of cardiac vagal afferents tends to oppose this effect. Thus the underlying autonomic state of the animal determines whether in response to ANP the activity of the sympathetic nervous system is stimulated, inhibited, or unaltered. These relationships are emphasized in a recent study in which renal sympathetic nerve activity in response to ANP was measured directly (337). Administration of two active ANP analogues to intact animals markedly reduced mean arterial pressure but failed to augment heart rate or renal sympathetic nerve activity. In contrast, similar reduction in blood pressure induced with nitroprusside markedly stimulated heart rate and renal sympathetic activity. Thus, unlike nitroprusside, ANP causes hypotension and suppresses the stimulatory effect of baroreceptor activation on sympathetic nerve activity. Moreover, when ANP is administered to vagotomized animals, sympathetic nerve activity increases and the hypotensive effect of ANP is attenuated. These results indicate that in the intact animal, ANP both inhibits sympathetic activity by activating vagal afferents and stimulates sympathetic activity at baroreceptors by reducing blood pressure. Vagotomy unmasks the stimulatory effect of ANP-induced hypotension on sympathetic nerve activity. As noted, Ackermann et al. (3) reported similar results with atrial extracts, including stimulation of cardiac output in vagotomized animals.

However, ANP does not reduce cardiac output solely by diminishing sympathetic efferent activity. Destruction of sympathetic efferents by spinal cord transection or inhibition with propranolol failed to alter the response of stroke volume to atrial extract; blood pressure fell and stroke volume failed to rise (2). In conscious sheep, ganglionic blockade with trimethophan camsylate, a drug known to reduce sympathetic efferent activity, not only failed to prevent the decrease in cardiac output in response to ANP but may have potentiated it (47). These results suggest that factors other than withdrawal of sympathetic tone must account in part for the fall in cardiac output caused by ANP.

The fall in cardiac output caused by ANP can be attributed in part to decreases in preload measured as a reduction in central venous or right atrial pressure (47, 65, 146, 255, 304; Fig. 7). The decrease in right atrial filling with ANP, eventually leading to decreased stroke volume and cardiac output, is secondary to dilation of capacitance veins, increased resistance to venous return, and/or to transudation of plasma from intravascular to extravascular sites. In intact dogs and in animals under sympathetic blockade (hexamethonium-nadolol

together with constant infusion of norepinephrine), ANP failed to alter effective venous compliance but did lower central venous pressure (304, 339). These results suggest that the reduction in venous pressure results from decreases in intravascular volume. Because decreases in venous pressure in response to ANP often precede large urinary volume losses and have even been observed in anephric subjects (6, 91, 339), any reduction in intravascular volume must be due in part to a redistribution of fluid from intra- to extravascular compartments. Indeed, since the original description by DeBold et al. (71), several investigators have noted that administration of ANP leads to increases in hematocrit and plasma protein concentrations, indicating loss of a red blood cell and protein-free transudate from the vascular space (6, 91, 339, 354, 355). This net efflux of plasma volume could be due to increased capillary permeability or changes in the balance between hydraulic (favoring filtration) and oncotic (favoring absorption) forces acting across capillary walls. Because ANP tends to raise plasma protein concentrations, the resulting increases in plasma oncotic pressure would tend to favor net absorption of fluid into the capillaries. Increased transudation must therefore be due to increases in capillary permeability and/or increases in transcapillary hydraulic pressure gradients. Evidence for increased capillary permeability is conflicting (85a, 148, 325a, 343a). Huxley et al. (148) have shown in capillaries of the frog mesentery that ANP in pharmacological doses (10^{-5} M) causes a fourfold increase in capillary permeability. In vivo attempts to demonstrate this permeability change have relied on assessment of circulating volumes in anephric animals. After infusion of ANP, Valentin et al. (343a) found a 9% increase in hematocrit and a 4% increment in albumin concentration in anephric rats, suggesting fluid transudation, whereas the experiments of Sugimoto et al. (325a), using ^{51}Cr-labeled red blood cells in functionally anephric rats suggested a 1.5-fold increase in capillary filtration coefficient. However, Eliades et al. (85a) measured skin and muscle small vessel pressures and lymph flow in the dog forelimb perfused under constant pressure and failed to demonstrate any change in pressures or lymph flows. In support of the raised hydraulic pressure hypothesis, ANP has been shown to increase blood pressure in at least one specialized capillary bed, the renal glomerulus, leading to increased glomerular filtration rate (80), a process analogous to fluid transudation. Thus ANP appears to decrease preload primarily by reducing intravascular volume and not by dilating capacitance veins.

B. Direct Effects of Atrial Natriuretic Peptide on Cardiac Function

With the use of isolated perfused hearts from guinea pigs, rats, and dogs, ANP has been shown to sharply reduce coronary blood flow and cardiac output (47, 350). However, in the guinea pig Langendorff preparation, when perfusion flow was maintained constant despite the constrictive effects of ANP (using a constant flow technique), ANP no longer reduced contractility, suggesting that the peptide acted to decrease contractility by decreasing coronary perfusion (350). In these isolated heart preparations, actions of autonomic nerves are prevented and effects of preload are directly controlled. In apparent disagreement with these results, Ackermann (2) reported no effect of atrial extracts on contractility of isolated rat hearts. Others have reported that ANP did not alter rate or contractility of isolated rat atria or papillary muscle (255); in addition, ANP did not alter ventricular performance curves in open-chest rats. In studies of coronary artery segments in vitro, ANP exerted either no effect or served to relax vessel rings precontracted with K^+ (1). Vasoconstriction was not observed, nor were effects of ANP on resting coronary vasculature examined. Measurements of the effect of ANP in vivo have generally revealed increases or no change in regional blood flow (139, 247, 255, 358), although one study demonstrated reductions (176). Thus it remains unclear whether ANP reduces cardiac output by increasing resistance of coronary arteries to blood flow, but the greater number of workers suggest that ANP acts primarily as a vasodilator.

C. Effects of Atrial Natriuretic Peptide on Peripheral Vascular Resistance

The effects of ANP on vascular resistance have been studied in vitro and in vivo. In vitro studies of isolated medium-sized arteries, the vessels that mediate peripheral vascular resistance, have yielded conflicting results. Atrial natriuretic peptide relaxes precontracted aortic rings, rabbit facial vein, and portal vein (359). In contrast, a relaxant effect of ANP in mesenteric, femoral, coronary, or cerebral arteries preconstricted with high K^+ solutions was not observed, nor was a relaxant response to ANP seen in untreated or precontracted cerebral or mesenteric arteries (1, 247). Thus it has been difficult to demonstrate ANP-induced vasodilation in the vessels that regulate peripheral resistance.

The effects of ANP on regional hemodynamics have also been examined. Using microspheres after bolus injection of ANP into conscious rats, Garcia et al. (102) found marked increases in blood flow to the lungs, heart, spleen, mesentery, kidneys, and testes without significant increments to brain, skin, or muscle. Other investigators, however, using similar techniques, found little or no increment in regional blood flow to any vascular bed after ANP or atrial extract in Wistar-Kyoto (WKY) rats and SHR (95, 175, 176, 254). Indeed, in conscious SHR, ANP increased vascular resistance in renal, mesenteric, and hindquarters vascular beds (175, 176). In studies using conscious and anesthetized dogs, ANP likewise failed to alter coronary, mesenteric, or iliac blood flow while reducing renal vascular resistance (139, 304). These seemingly conflicting results are probably related to differences in resting vascular and autonomic tone of the various experimental animal models employed.

In anesthetized rats, large doses (as judged by natriuretic effect) of ANP led to decrements in peripheral vascular resistance (3). In conscious rats and dogs, on the other hand, ANP infusion served to increase peripheral vascular tone; hypotension occurred because of the inhibitory actions of ANP on cardiac output (4, 162, 175, 286). In studies in rats and dogs under sympathetic blockade, ANP infusion led to decreases in peripheral vascular resistance (95, 287, 304), again suggesting that the response of vascular resistance to ANP depends on the base-line level of autonomic activity in the animal. The hypotensive effect of lowered cardiac output and reduced intravascular volume stimulates sympathetic activity that, in many cases, results in increased peripheral vascular resistance. When this sympathetic output is blocked, ANP causes vasodilation. Even in cases where resistance rises in response to ANP, it could be argued that the vascular tone would have risen further were it not for the vasodilatory actions of ANP.

VIII. RENAL ACTIONS OF ATRIAL NATRIURETIC PEPTIDE

Atrial natriuretic peptide infusion induces marked natriuresis and diuresis accompanied by similarly marked increases in phosphate, calcium, magnesium, chloride, and cGMP excretion (13, 49, 50, 54, 158, 360, 370), especially when supraphysiological levels of the peptide are reached in plasma. On the other hand, at plasma levels that can be achieved by endogenous secretion in vivo, renal responses to ANP may be less marked because of the activity of other neurohumoral and mechanical systems that regulate salt and water excretion. This variability in responsiveness to ANP has led some to question whether ANP plays a physiological role in volume regulation (41, 114, 119). However, brisk natriuresis and diuresis occur in response to ANP infusions in doses designed to mimic plasma ANP levels achieved endogenously (13, 32, 212, 252, 314, 370), if care is taken to avoid volume depletion. In this regard, the recent study of Mizelle et al. (217) is particularly noteworthy. In conscious, chronically instrumented dogs with indwelling catheters in both renal arteries, ANP was infused at physiological levels into one kidney and the vehicle into the other kidney, and the split bladder technique was utilized to monitor solute excretion separately from ANP-infused and contralateral vehicle-infused kidneys. The kidney receiving ANP exhibited a clear increase in salt excretion that persisted for the duration of the infusion, whereas Na^+ excretion declined from base-line values in the contralateral kidney, thereby helping to maintain overall Na^+ balance in this animal model. Although the kidney receiving ANP was also subjected to the same volume-retentive stimuli that lowered salt excretion in the contralateral kidney, a persistent natriuresis and diuresis was achieved, reflecting the potent overriding action of ANP.

Evidence in favor of a physiological role for ANP in the regulation of salt excretion also comes from studies utilizing inhibitors of ANP degradation as well as specific anti-ANP antisera. Protease inhibitors, such as phosphoramidon, or truncated ANP analogues that bind exclusively to ANP-R2 receptors (see sect. VI*A*), have led to modest increases in circulating ANP levels accompanied by increased salt and water excretion (172, 234, 282). In rat models of congestive heart failure, diabetes mellitus and subtotal nephrectomy, and DOCA salt hypertension, administration of specific anti-ANP antibodies reduced urine volume and Na^+ excretion, indicating that endogenous circulating levels of ANP had served to augment Na^+ and water excretion in these settings (23, 152a, 228, 244, 245). Similar antinatriuretic effects of anti-ANP antibody were seen in normal rats challenged with acute volume expansion (144).

The excretory effects of ANP were initially attributed largely to changes in renal hemodynamics and in particular the associated increase in glomerular filtration rate (GFR) (66, 147). More recently, effects of ANP on Na^+ transport by the renal tubules have assumed a prominent role in descriptions of the renal actions of ANP (183, 315, 316). Figure 9 summarizes the various renal actions of ANP. These include increases in GFR and filtration fraction, possible effects to inhibit net Na^+ reabsorption in the proximal tubule, suppression of renin secretion in the macula densa, and inhibition of net Na^+ reabsorption and vasopressin-mediated water reabsorption in the cortical and inner medullary portions of the collecting duct. Each of these loci of action is considered in detail below.

A. Effects on Renal Hemodynamics

Atrial natriuretic peptide infusion in animals and humans exerts variable effects on renal blood flow (45, 50, 54, 139, 164, 259, 303, 313, 331). In several studies showing augmented renal blood flow, the increase was transient, lasting <1 min (50, 139). As discussed in section VII*A*, this variability of response is not surprising, since changes in sympathetic nervous tone and the circulating levels of vasoconstrictors can modify overall vascular responsiveness to ANP. Thus, in isolated perfused rat kidneys, atrial extract increased renal vascular resistance above base-line values; however, after preconstriction of the preparation with ANG II, norepinephrine, or vasopressin, atrial extract exerted a vasodilatory effect (54). In conscious as well as anesthetized rats, ANP tends to reduce renal blood flow; prior denervation of the kidney attenuates this effect (141). Because total renal blood flow may remain unchanged or decline in animals undergoing a brisk natriuresis in response to ANP, it is evident that changes in renal blood flow per se are not the major factors responsible for enhanced renal solute excretion (54, 259, 313). Redistribution of renal blood flow from cortex to medulla has been observed in some studies (45, 331).

Many studies have demonstrated that ANP increases GFR and filtration fraction (54, 66, 147, 303, 365). Atrial natriuretic peptide dilates preglomerular

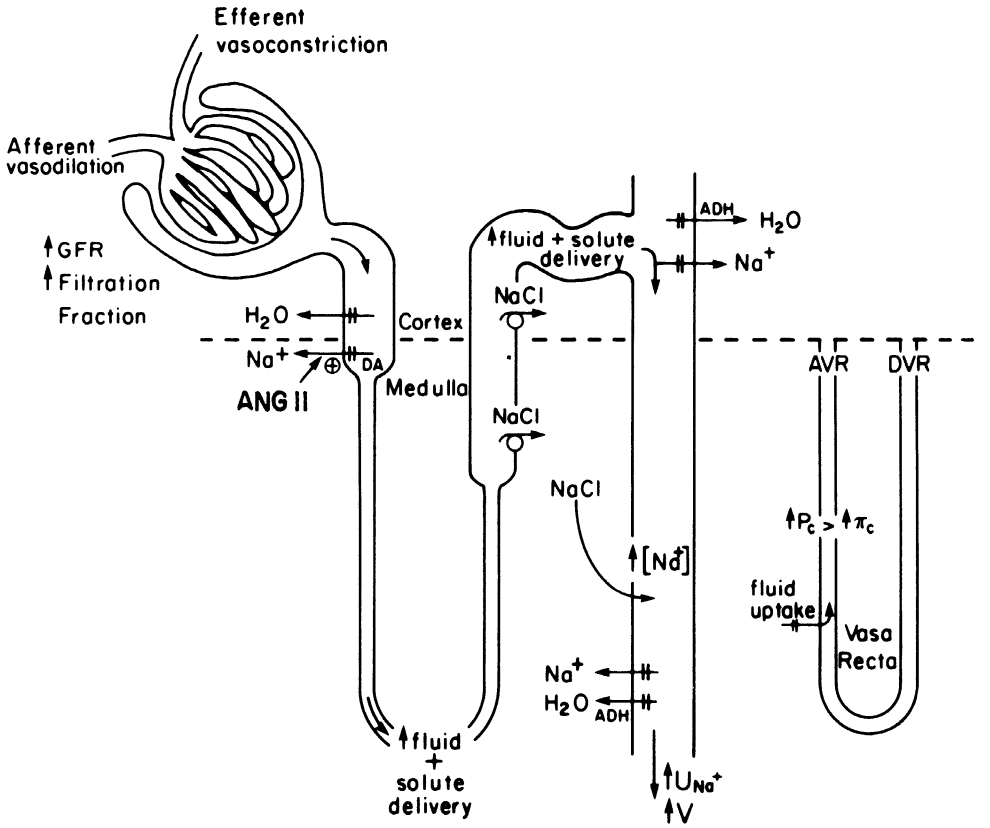

FIG. 9. Renal actions of ANP. ADH, antidiuretic hormone; ANG II, angiotensin II; AVR, ascending vasa recta; DA, dopamine; DVR, descending vasa recta; GFR, glomerular filtration rate; π_c, colloid osmotic pressure; P_c vasa recta capillary hydraulic pressure, U_{Na}, urinary Na^+; V, volume. See text for details.

(afferent) arterioles and constricts postglomerular (efferent) arterioles, leading to increased hydraulic pressure within glomerular capillaries (80) but offsetting effects on glomerular blood flow. In addition, ANP may act to relax glomerular mesangial cells, an effect believed likely to increase filtration surface area (17, 40, 81, 308). Using quantitative video microscopy, Marin-Grez et al. (199) examined the diameter of renal microvasculature in hydronephrotic kidneys of anesthetized rats before and during administration of ANP and observed dose-dependent dilatation of arcuate, interlobular, and proximal afferent vessels and constriction of efferent arterioles. These results were recently confirmed in the rat with the use of an in vitro blood-perfused kidney preparation (346). The effect of ANP to dilate arcuate arteries was confirmed in measurements of tension of arteriolar rings in vitro (1). Atrial natriuretic peptide relaxed arcuate arterioles precontracted with K^+, norepinephrine, or 5-hydroxytryptamine but had little influence on mesenteric, femoral, cerebral, or coronary vasculature. In contrast to these results, Edwards and Weidley (84) were unable to demonstrate any response of microdissected rabbit afferent and efferent arterioles to ANP either at base line or after preconstriction with norepinephrine or ANG II. A recent preliminary report has shown that efferent arteriolar vasoconstriction is a dominant effect and occurs at lower concentrations of ANP than cause afferent relaxation (364a). Since Marin-Grez et al. (199) found no dilation of the segment of afferent arteriole immediately adjacent to the glomerulus, it is possible that Edwards and Weidley (84) obtained negative results because they examined this portion of the afferent arteriole primarily.

Afferent arteriolar vasodilation and efferent vasoconstriction serve to augment glomerular capillary hydraulic pressure. Direct measurement of the determinants of glomerular filtration in vivo have confirmed these responses (80). After administration of ANP, hydraulic pressure was elevated in glomerular capillaries, efferent arterioles, and Bowman's space (proximal tubule). Calculated resistances in afferent and efferent arterioles were in accord with the video image analysis described (199) in that afferent arteriolar resistance fell while efferent arteriolar resistance rose. In agreement with these results, Fried et al. (94), examining isolated perfused dog glomeruli, reported significant increases in glomerular hydraulic pressure and efferent resistance.

An additional determinant of GFR and filtration fraction is the glomerular capillary ultrafiltration coefficient, K_f; K_f increased significantly after addition of ANP to isolated perfused dog glomeruli (94). In addition, glomeruli freshly isolated from rats and precontracted with ANG II were shown to dilate when exposed to ANP, suggesting ANP-induced relaxation of mesangial cells (40). Data also in support of a direct effect of ANP on K_f were obtained in studies of cultured glomerular mesangial cells (17, 308). It is believed that these cells regulate the surface area for filtration and K_f in vivo by contracting and relaxing and thereby closing or opening regions of the capillary tuft for perfusion and filtration. Thus ANG II, which lowers K_f in vivo, induces

contraction of glomerular mesangial cells in culture (81). These cells possess specific, high-affinity ANP receptors and respond to ANP with striking elevations in cGMP accumulation (30). In addition, ANP relaxes cultured mesangial cells precontracted with ANG II (17, 308). These results imply that ANP also relaxes glomerular mesangial cells in vivo, resulting in expansion of capillary surface area available for filtration, and an increase in K_f. Changes in K_f in response to ANP in vivo may be more readily demonstrable in animals with high mesangial cell tone (i.e., high renin states or after administration of ANG II or norepinephrine).

Some investigators have proposed that the increase in GFR alone can account for the natriuresis and diuresis induced by ANP (66, 147), whereas others have argued that ANP also directly alters tubule Na^+ and water reabsorption (49, 78, 128, 181, 222, 232, 233, 315, 316, 344, 345, 367). In many studies, ANP has stimulated natriuresis and diuresis without producing detectable alterations of GFR (49, 222, 315, 316). In several of these studies, infusion of ANP at doses low enough to simulate the variations encountered in endogenous ANP levels results in natriuresis and diuresis without detectable changes in GFR; at higher doses, the increase in GFR is more marked (50, 222, 248, 253, 303, 370). These studies suggest that the natriuresis at these lower doses occurs independent of changes in GFR. However, because the filtered load of Na^+ and water is very large compared with the amount excreted in the urine, even during brisk natriuresis, it has been argued that undetectable changes in GFR could, in themselves, contribute to the natriuresis observed with ANP (192).

Nevertheless, several observations indicate that changes in GFR alone do not account for the bulk of the natriuresis and diuresis observed in response to ANP infusion. First, in toadfish, a species that lacks glomeruli, ANP induces a striking natriuresis (181). This result emphasizes the antiquity of responsiveness to ANP in phylogeny and demonstrates that natriuresis and diuresis can occur by mechanisms unrelated to changes in glomerular function. In addition, increases in Na^+ and water delivery to the distal nephron after ANP administration are usually insufficient to account fully for the observed natriuresis and diuresis (49, 315, 316). Finally, there is now considerable evidence for direct effects of ANP on collecting duct Na^+ and water transport (78, 128, 232, 233, 315, 316, 366, 367). Because the renal tubule consists of several highly specialized segments arranged in series, actions of ANP at several sites contribute to the overall natriuresis and diuresis.

B. Renal Epithelial Actions of Atrial Natriuretic Peptide

Given the striking increase in sodium, chloride, calcium, phosphate, and magnesium excretion evoked by atrial extracts in early studies, a direct effect of ANP on proximal tubule ion transport was proposed (45, 126, 158, 303). Clearance studies have shown increased lithium and phosphate excretion in response to ANP, and these results have been taken by some (126, 127) but not others (61, 147, 192) to indicate that the hormone inhibits proximal tubule solute transport. Micropuncture studies have yielded conflicting results. In the studies of Cogan and associates (66, 147), infusion of ANP led to significant increases in GFR and a 30% increment in solute delivery out of the proximal tubule; this increment in solute delivery was abolished when, during ANP infusion, aortic constriction was performed to return the GFR to base-line values. These results were interpreted to mean that the increase in solute delivery out of the proximal tubule was caused by an excessive filtered load and not by actions of ANP on proximal tubule cells. Van de Stolpe et al. (344), however, have recently demonstrated that ANP can increase fractional delivery of Na^+ out of the proximal tubule in animals in which GFR does not rise in response to ANP. These results suggest that ANP reduces proximal Na^+ reabsorption independent of changes in GFR. In agreement with these results, Harris et al. (128) provided evidence that ANP can inhibit proximal fluid reabsorption. In these studies, volume reabsorption in the superficial proximal tubule was measured by the shrinking droplet technique. Perfusion of the adjacent peritubular capillary network with ANG II (1.1×10^{-12} M) increased the rate of volume reabsorption; addition of ANP antagonized the angiotensin-induced increment in volume reabsorption in a concentration-dependent manner. At 1.8×10^{-8} M ANP, the stimulatory effect of ANG II on proximal tubule volume reabsorption was abolished, whereas exposure of the tubule to ANP alone, in the absence of ANG II, had no measurable effect on the rate of volume reabsorption. These results suggest that the effects of ANP to reduce proximal fluid reabsorption are critically dependent on the basal transport rate. Studies of isolated proximal tubules perfused in vitro support this conclusion. Under basal conditions, ANP does not alter volume reabsorption, whereas angiotensin-stimulated volume reabsorption is reduced by ANP (34, 107). In contrast, a recent in vivo study using free-flow micropuncture techniques failed to demonstrate antagonism of heightened angiotensin-induced proximal tubule solute reabsorption by ANP (189). Evidence that ANP may alter transport properties of the proximal tubule has also come from studies of sodium and phosphate transport in renal cortical brush-border vesicles derived primarily from the proximal tubule. Vesicles isolated from rats pretreated with ANP exhibited diminished sodium-phosphate cotransport compared with vesicles isolated from control rats (127). Of interest, however, it has not been possible to demonstrate ANP receptors in microdissected proximal tubule segments (53, 60), and although autoradiography of renal slices consistently reveals strong labeling of glomeruli and blood vessels, proximal tubule labeling is not seen (169, 207, 221). Similarly, although glomeruli and IMCD cells exhibit striking increases in cGMP after exposure to ANP, proximal tubule cells were unresponsive in three

studies and only minimally responsive in another study (60, 231, 323, 338).

A possible means for reconciling the in vivo data demonstrating an action of ANP on proximal tubule ion transport and the lack of proximal ANP receptors is that ANP acts via an intermediary pathway to influence transport in this segment. In this regard, evidence has recently been obtained by Ortola et al. (246) to suggest that dopamine may mediate the actions of ANP in this segment in vivo (246). Phosphate excretion, a reciprocal index of proximal solute reabsorption, was found to increase together with Na^+ in rats given a low-dose ANP infusion intravenously, and these increments were subsequently abolished by concomitant infusion of a highly specific and potent dopamine DA_1 receptor antagonist. Likewise in rats with high endogenous plasma ANP levels induced by subtotal nephrectomy, DA_1 receptor antagonism significantly blunted basal phosphate as well as Na^+ excretion. Katoh et al. (157) have furthered these findings with the demonstration that the ANP-induced increments in urinary volume, Na^+ concentration, and the fractional excretion of sodium are diminished in the presence of haloperidol and the more specific DA_1 receptor blocker Sch 23390 and also in the presence of carbidopa. However, when dopamine was readministered in the continuing presence of carbidopa the ANP effect was restored to normal (157). Whether other signal transduction systems also contribute to the action of ANP on proximal tubule ion and water transport remain to be determined.

There is currently little evidence that ANP-induced inhibition of salt or water transport in the thin limb of Henle, the thick ascending limb, or distal convoluted tubule contributes to the natriuretic or diuretic effects of this hormone. Microperfusion studies reveal no effects of ANP in thin descending limb or cortical and medullary thick ascending limb in vitro (167) or in thick asending limb in vivo (258). Moreover, these segments are devoid of receptors for ANP (53, 132, 169, 221), and cGMP accumulation in response to ANP is either absent or far lower than that observed in glomeruli or IMCD cells (60, 231, 338). Of interest, ANP has been shown to inhibit Na^+-K^+-$2Cl^-$ cotransport in the intestine of the winter flounder, an absorptive epithelium with transport properties analogous to those in the renal thick ascending limb segment in mammals (236). Micropuncture studies in rats have revealed modest increments in solute delivery out of the late distal convoluted tubule in response to atrial extracts or ANP, but these have generally been too small to account for the observed natriuresis and diuresis (49, 315), especially in view of the known ability of the medullary collecting duct to increase solute reabsorption in response to increased delivered load (154).

Considerable evidence indicates that ANP effects natriuresis and diuresis in part by inhibiting net Na^+ and water reabsorption in the collecting duct. In early microcatheterization studies, in which fluid was sampled by passing thin catheters retrograde for varying distances up individual collecting ducts, infusion of atrial extracts did not alter delivery of Na^+ and water to the loop of Henle but led to increases in Na^+ and water delivery from outer medullary collecting duct (315). Between the outer medullary collecting duct and the final urine there was an enormous increment in fractional Na^+ excretion (315). The increased delivery to the outer medullary collecting duct was consistent with inhibition of net salt and water reabsorption in the cortical collecting duct. Furthermore, because both micropuncture and microcatheterization studies had shown that the IMCD is capable of reabsorbing large increments in outer medullary collecting duct solute load (154), it seemed clear that atrial extract interfered with this normal load-dependent increase in salt reabsorption in this segment. More recent microcatheterization studies using synthetic ANP have confirmed these earlier results, and micropuncture studies of the late distal convoluted tubule and papillary tip have also demonstrated that ANP infusion augments NaCl delivery to the base of the accessible papilla without altering delivery out of the late distal convoluted tubule (48, 345). In addition, reabsorption of NaCl between the puncture site and the tip of the papilla was significantly decreased in ANP-treated animals compared with the controls. Because the length accessible to micropuncture is only a small fraction of the overall length of the collecting duct segment (154), the increment in delivery to the papillary base may reflect decreased reabsorption along inaccessible portions of the cortical and medullary collecting ducts. It is also possible, of course, that this increased delivery of Na^+ represents an unduly large contribution of deep nephrons to the collecting duct fluid sampled in these studies.

Although the in vivo studies cited above demonstrate that ANP inhibits net Na^+ and water reabsorption in the collecting duct, they do not assess whether the hormone acts directly on the epithelial cells or alters the transepithelial driving forces. Some evidence indeed suggests a role for ANP in altering transepithelial driving forces in the renal medulla; unfortunately, no information is presently available pertaining to the cortical collecting duct. Papillary micropuncture studies demonstrated that ANP increases hydraulic pressures in loops of Henle, collecting ducts, and vasa recta (208). Because pressures in the vasa recta increased more markedly than pressures within the lumina of adjacent papillary collecting ducts, ANP created unfavorable hydraulic gradients for net Na^+ reabsorption in the IMCD. The sensitivity of ANP-induced natriuresis to transepithelial gradients is further emphasized by observing the effects of altering peritubular hydraulic and oncotic pressures and thus favoring net reabsorption of Na^+. In the presence of hyperoncotic albumin, ANP-induced natriuresis was markedly attenuated (208). Administration of ANG II in high concentrations increased peritubular capillary hydraulic pressure, favoring net Na^+ excretion, and this peptide markedly potentiated the natriuretic effect of ANP (208). Taken together, these results indicate that ANP can increase the hydraulic gradient from vasa recta to papillary collecting duct,

favoring reduced net volume and Na^+ reabsorption in IMCD. Similar Starling effects also help to explain decreased fractional reabsorption of Na^+ and water in proximal tubule segments.

Another proposed action of ANP to alter transepithelial driving forces in the IMCD involves washout of medullary solute gradients (48, 67, 147, 193, 345). Atrial natriuretic peptide increases vasa recta blood flow, leading to dissipation of medullary solute gradients (67, 345). With the loss of medullary solute, water abstraction from the descending limb of Henle is thought to be reduced, leading to decreased luminal concentration of salt delivered to the thick ascending limb and reduced NaCl transport in this segment. Reduced thick ascending limb NaCl transport would tend to contribute to the natriuretic actions of ANP. On the other hand, medullary washout would also lead to diminished backleak of solute from interstitium to lumen along the IMCD, thus tending to reduce natriuresis. Measurement of medullary solute content during administration of ANP indicates that urea is indeed lost from the medullary interstitium into final urine (67). Measurements of medullary and vasa recta blood flow indicate that ANP increases these parameters, within seconds in one study (208, 209), but only after a significant diuresis and natriuresis has ensued in another study (160). Thus it is presently unclear whether medullary washout is a consequence or cause of the natriuresis and diuresis induced by ANP; nevertheless, increased medullary blood flow serves to reduce medullary solute content, thus acting to sustain the natriuretic response.

In the cortical collecting tubule perfused in vitro, ANP applied to the basolateral membrane inhibits the hydraulic conductivity response to vasopressin but not to forskolin (which stimulates the catalytic subunit of adenylate cyclase) or exogenous cAMP (78). These results suggest that ANP prevents the vasopressin-mediated increase in cAMP generation and thereby inhibits the hydrosmotic response. Presumably, this inhibition of water transport contributes to the diuresis induced by ANP. Of interest, it has not been possible to demonstrate that ANP alters basal or vasopressin-stimulated cAMP accumulation in microdissected cortical collecting ducts of rats or rabbits (342) or primary cultures of rabbit cortical collecting duct cells (227). Thus whether ANP inhibits the hydrosmotic response to vasopressin by reducing adenylate cyclase activity or a step beyond formation of cAMP remains unclear. Atrial natriuretic peptide also reduces net Na^+ reabsorption in perfused cortical collecting ducts isolated from rats pretreated with mineralocorticoids (232). Inhibition of cortical collecting duct Na^+ reabsorption may account for the increased delivery of Na^+ to the medullary collecting duct observed in microcatheterization studies (315, 316). Although ANP receptors are not demonstrable in rat cortical collecting tubules (53, 169), ANP has been shown to increase cGMP levels slightly in freshly prepared cortical collecting tubules (60, 231, 338). In addition, in primary cultures of cortical collecting tubule cells, ANP markedly stimulates cGMP accumulation and at concentrations of the peptide that inhibit Na^+ and water transport (227). Addition of cGMP analogues to the basolateral solution reduced Na^+ reabsorption (232). Taken together, these results suggest that the inhibitory effect of ANP on Na^+ transport in cortical collecting tubule is mediated by cGMP.

The IMCD is highly branched and difficult to perfuse in vitro, hence the early studies of ANP action in this segment were performed on freshly prepared suspensions of rabbit IMCD cells (367). In this preparation, ANP inhibits transport-dependent oxygen consumption without altering this parameter in cells derived from the outer medullary collecting duct or thick ascending limb. Measureable responses to ANP occur at 10^{-11}–10^{-10} M, which are levels that are readily achieved in the plasma of animals under basal and volume-expanded conditions. Examination of the interaction of ANP with amiloride, ouabain, and amphotericin using oxygen-consumption measurements suggested that the peptide inhibited Na^+ entry into these cells (367). Atrial natriuretic peptide reduces isotopic Na^+ uptake in these suspensions by two-thirds at concentrations identical to those found effective in oxygen-consumption studies (366). In primary cultures of rat IMCD, application of ANP to the cell-attached patch preparation reduced the open time of the amiloride-sensitive cation (Na^+) channel, providing direct confirmation that ANP acts in this tissue by reducing Na^+ flux across conductive cation channels (187, 188). Inhibition of Na^+ channels by ANP has also been shown in LLC-PK_1 cells, a renal epithelial cell line that exhibits characteristics of proximal and distal tubule epithelia (57, 115). Thus, as shown in Figure 10, ANP inhibits a Na^+ or cation channel, probably located on the luminal surface of the IMCD cell (285).

Not only does ANP reduce reabsorptive flux of Na^+ in this segment, but it may also stimulate a secretory flux (316), possibly mediated by a basolateral Na^+-K^+-$2Cl^-$ cotransporter. Preliminary measurements of isotopic Na^+ transport in isolated perfused rat IMCD suggest that a basolateral furosemide-sensitive pathway may mediate net secretion of Na^+; this pathway appears to be stimulated by vasopressin (275). In cultured endothelial cells and in winter flounder intestine, a bumetamide-sensitive Na^+-K^+-$2Cl^-$ transporter is inhibited by ANP and by cGMP (234a, 236). However, in rabbit IMCD cells, furosemide has no effect on oxygen consumption, isotopic Na^+ uptake, or K^+ fluxes, suggesting that Na^+-K^+-$2Cl^-$ cotransport is not an important pathway for Na^+ transport in this segment in the rabbit (366, 367). In support of an action of ANP to stimulate Na^+ secretion, however, preliminary measurements of isotopic Na^+ fluxes in isolated perfused rat IMCD indicate that ANP has dual effects on Na^+ transport, inhibiting lumen-to-bath Na^+ flux while stimulating a furosemide-sensitive and vasopressin-stimulated bath-to-lumen Na^+ flux (275). These results suggest that in rat IMCD, ANP inhibits net Na^+ reabsorption by reducing entry of Na^+ into the cell from the lumen and stimulating a basolateral Na^+-K^+-$2Cl^-$ cotransporter. Recently, Sands et al. (283) failed to demonstrate an

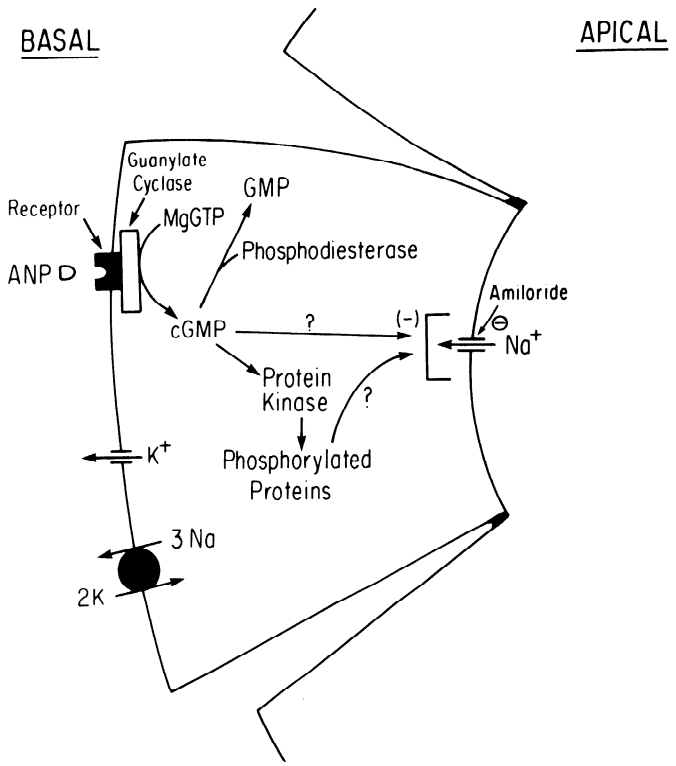

FIG. 10. Mechanisms of Na^+ reabsorption and ANP action in IMCD cell. See text for details.

alteration in passive Na^+ permeability in isolated perfused rat IMCD in response to ANP, which is in apparent contradiction to the hypothesis that ANP stimulates Na^+ secretion in this segment. Thus an effect of ANP to stimulate a secretory Na^+ flux in the IMCD remains unproven.

The signal transduction mechanisms mediating ANP inhibition of conductive Na^+ entry into IMCD cells have been thoroughly characterized and are also summarized in Figure 10. Freshly prepared suspensions of intact rabbit IMCD cells express a single class of cell surface ANP receptors with an apparent K_d of 10^{-10} M and a molecular mass of 120–130 kDa, with the use of affinity cross-linking techniques (120, 122). In vascular smooth muscle this class of receptors is known to be tightly linked to a membrane-associated or particulate guanylate cyclase. Indeed, the cDNA encoding for a single peptide possessing both guanylate cyclase and ANP binding activities has recently been cloned (64a) as discussed in section VI*A*; it is likely that the IMCD ANP receptor is either identical or homologous to this peptide. Several lines of evidence indicate that cGMP is the mediator of the effects of ANP on Na^+ transport in IMCD. Atrial natriuretic peptide increases intracellular cGMP with a concentration-response relationship and with a time course consistent with the known actions of this peptide on transport (231, 338, 368). Indeed, ANP stimulates particulate guanylate cyclase prepared from IMCD cells by tripling the V_{max} of the enzyme at levels of ANP that effect inhibition of Na^+ transport (122). Several studies have also shown that cGMP can reproduce the transport effects of ANP. In freshly prepared IMCD cells, increasing cGMP by exposure to nitroprusside (which stimulates soluble guanylate cyclase), by inhibition of phosphodiesterase with isobutylmethylxanthine, or by addition of the cGMP analogue, 8-BrcGMP, mimics the inhibitory effect of ANP on transport-dependent oxygen consumption and on uptake of Na^+ via the conductive channel (366, 368). In primary cultures of rat IMCD cells, patch-clamp studies have demonstrated that cGMP applied to the cell-attached membrane reduces the open time of the amiloride-sensitive cation channel (188). Taken together, these results fulfill Sutherland's criteria for demonstrating that a response to a peptide hormone is medated by synthesis of a cyclic nucleotide and establish cGMP as the mediator of ANP regulation of Na^+ transport in the IMCD. The patch-clamp data (188) suggest that cGMP binds directly to the cation channel but do not rule out the activity of a cGMP-dependent protein kinase on or adjacent to the plasma membrane.

As in the cortical collecting tubule, ANP antagonizes the hydrosmotic action of vasopressin in IMCD, contributing to the diuretic action of ANP. In isolated perfused rat IMCD, application of ANP to the basolateral, but not apical, surface inhibits vasopressin-stimulated water flow (232); however, ANP did not alter cAMP accumulation in this segment in response to vasopressin at concentrations of 10^{-11}–10^{-10} M (231, 232). In addition, ANP also inhibited the osmotic water permeability response to a cAMP analogue (232). Thus ANP inhibits vasopressin-stimulated water reabsorption, apparently at a site distal to the increase in cAMP. Because cGMP duplicated the action of ANP on vasopressin-stimulated water flow, it is likely that cGMP also mediates the diuretic effects of ANP in IMCD.

As summarized in Figure 9, ANP causes natriuresis and diuresis by concerted actions at several nephron segments. Here ANP increases GFR and filtration fraction by dilating afferent and constricting efferent arterioles, leading to increased hydraulic pressure in the glomerular capillaries, thereby increasing the driving force for ultrafiltration. Atrial natriuretic peptide disrupts glomerulus-proximal tubule balance such that increased delivery of filtrate to the proximal tubule is not reabsorbed proportionately, in part due to dopamine-mediated inhibitory effects of ANP on the proximal tubule epithelium. An increased load of Na^+ and water is passed through ANP-insensitive descending and ascending limbs of Henle and the distal convoluted tubule to the cortical and medullary collecting duct where ANP reduces Na^+ and vasopressin-dependent water reabsorption.

As discussed, ANP not only enhances delivery of tubule fluid and solutes to more distal nephron segments but also preserves salt transport in the medullary thick ascending limb of Henle (167, 258), with the latter promoting maintenance of a medullary interstitium more rich in sodium than is observed with administration of loop-active agents, such as furosemide (67). The

infusion of ANP also results in an elevation of vasa recta hydraulic pressure in excess of increments in oncotic pressure, thereby rendering local Starling forces less favorable for capillary fluid reabsorption not only in the papilla but in the entire postglomerular microcirculation surrounding juxtamedullary nephrons (209). Furthermore, the imbalance of hydraulic pressures between vascular and tubule elements could promote recycling of the relatively hypernatric papillary interstitial fluid favoring convective movement of sodium to adjacent collecting ducts. Although the medullary collecting duct under antidiuretic conditions has a high electrical resistance, the permeability of the IMCD during diuretic or volume-expanded conditions is unknown. Indeed, preliminary in vitro studies have suggested that ANP may actually facilitate secretion of sodium by the IMCD (275). This movement of sodium and water into the collecting duct lumen could occur through paracellular channels formed by permeable tight junctions known to be present in IMCD epithelia. The resulting recycling of sodium from relatively hypernatric papillary interstitium to collecting duct lumen together with direct effects of ANP on IMCD sodium transport all contribute to the large magnitude of hypernatric character of ANP-induced natriuresis and diuresis.

IX. EFFECTS OF ATRIAL NATRIURETIC PEPTIDE ON RENIN SECRETION

The infusion of ANP markedly lowers renin secretory rate and plasma renin concentration (50, 135, 136, 194, 243; Fig. 11). The mechanism of this inhibition of renin secretion is not fully identified and probably involves multiple pathways, direct and indirect. In primary cultures of juxtaglomerular cells, ANP inhibits basal renin release, an effect mediated by cGMP (171). Inhibitors of phosphodiesterase potentiate ANP action to increase cGMP accumulation, making it likely that ANP acts in these cells by stimulating guanylate cyclase (134). Studies in renal cortical slices obtained from rats and primates have recently confirmed the ability of ANP to inhibit renin release in vitro (135, 136). Thus it seems likely that ANP inhibits renin release at least in part by stimulating guanylate cyclase and increasing cytosolic levels of cGMP. Opgenorth et al. (243) reported an inhibitory effect of ANP on renin secretion in the kidney undergoing filtration while renin secretion from the control perfused but nonfiltering kidney was not altered, suggesting that the inhibition of renin secretion was a function of delivery of solute to the macula densa. In cardiac-denervated dogs, electrical atrial pacing caused a rise in ANP levels but no inhibition of renin secretion or augmentation of urinary volume, implying that the inhibition of renin secretion may also be dependent on hemodynamic factors (356a). As discussed in section VII*A*, ANP may cause inhibition of sympathetic stimulation to the kidney, thereby reducing the stimulus for renin secretion (337). In dogs with caval constriction, Scheurer et al. (294a) have recently shown that

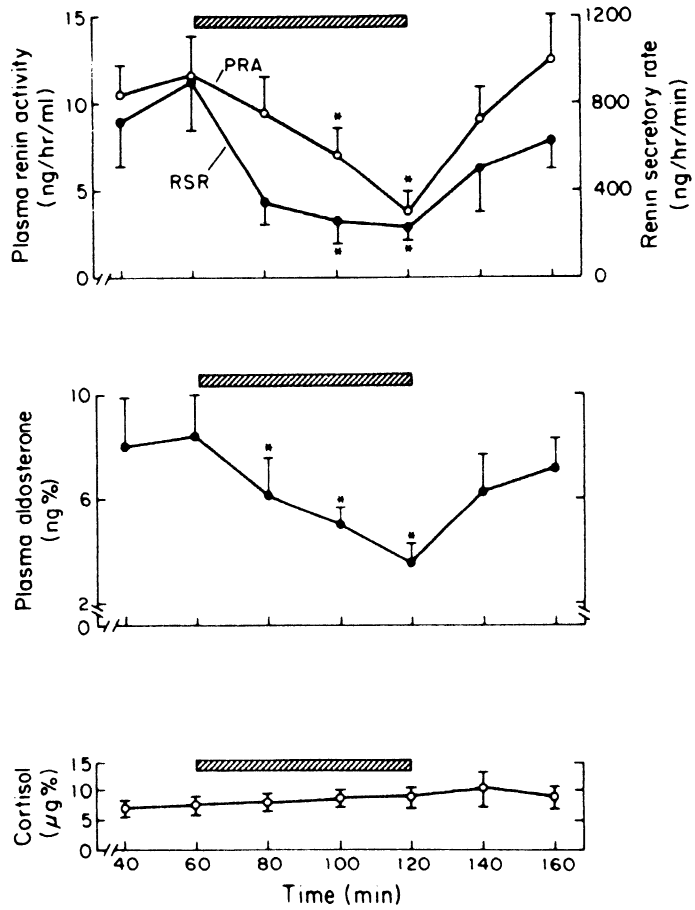

FIG. 11. Effects of synthetic ANP infusion on renin secretory rate (RSR), plasma renin activity (PRA), and plasma aldosterone and cortisol concentrations in normotensive anesthetized dogs. Hatched bars denote ANP infusion periods. [From Maack et al. (194).]

propranolol or ANP inhibited the rise in plasma renin activity, but these effects were additive, suggesting that ANP acted independent of the sympathetic pathway. Thus, although convincing primary evidence exists for a direct ANP-mediated inhibition of renin secretion, renal autoregulatory effects and the effects of inhibition of neural stimuli may also contribute.

X. ADRENAL AND OTHER ENDOCRINE ACTIONS OF ATRIAL NATRIURETIC PEPTIDE

The action of ANP to restore Na^+ and water balance in volume-expanded states involves direct stimulation of renal salt and water excretion as well as inhibition of aldosterone synthesis and release (Fig. 11). Atrial natriuretic peptide inhibits aldosterone secretion both by reducing renin secretion from the renal juxtaglomerular appartus and by direct effects on the aldosterone-secreting glomerulosa cells of the adrenal cortex. In the latter, ANP inhibits basal, ANG II-, K^+-, and ACTH-stimulated aldosterone release (20, 21, 63, 74, 117, 165, 166, 263, 295a) and reduces aldosterone secretion in vivo

(194, 213, 354, 355, 370). Inhibition of secretion occurs at 10^{-11} M ANP, a level achieved endogenously in plasma. Atrial natriuretic peptide stimulates guanylate cyclase activity and cGMP accumulation in adrenal glomerulosa cells (33). However, whereas half-maximal inhibition of aldosterone synthesis occurs at concentrations of ANP at 10^{-11} M, stimulation of guanylate cyclase is not observed below concentrations of 1 nM (204). In addition, increasing intracellular cGMP by stimulation of soluble guanylate cyclase or exposure to membrane permeable analogues of cGMP fail to influence aldosterone secretion (204). Thus, at this time, there is little evidence supporting the view that cGMP is the second messenger that mediates the action of ANP to inhibit aldosterone secretion from adrenal glomerulosa cells. The possibility that ANP alters Ca^{2+} influx has also been examined, with contradictory results. Atrial natriuretic peptide failed to affect ANG II- and K^+-stimulated $^{45}Ca^{2+}$ uptake, although inhibition of K^+-, ACTH-, and ANG II-stimulated $^{45}Ca^{2+}$ uptake by ANP in adrenal glomerulosa cells has been reported (14, 59, 63, 132, 326).

Inhibition of aldosterone biosynthesis by ANP is thought to take place early in steroidogenesis, at a step that involves the delivery of cholesterol to the inner mitochondrial membrane cytochrome *P*-450 enzyme complex (53, 62). A second site of inhibition, of quantitatively lesser importance, however, is at the conversion of corticosterone to aldosterone (56, 295a), the main site of secretogogue (ACTH, K^+, ANG II) stimulation. The various sites of ANP action in zona glomerulosa cells is summarized schematically in Figure 12 (190). Atrial natriuretic peptide exerts effects on other steroidogenic tissues, including testicular Leydig cells that express ANP receptors and produce testosterone by a cGMP-dependent mechanism (218).

Atrial natriuretic peptide has also been shown to act on other endocrine tissues. It has been shown to inhibit arginine vasopressin secretion in response to two potent stimuli, hemorrhage and prolonged dehydration (281), and ANP has been shown to inhibit the firing of vasopressin neurons in the paraventricular nuclei of anesthetized rats (319). Intracerebroventricular infusion of ANP also lowers plasma vasopressin levels in conscious sheep and markedly increases urine volume and free-water excretion in rats, effects attributed to central suppression of vasopressin secretion (180). Because vasopressin, at least in lesser doses, augments ANP secretion (197), a negative feedback system for endocrine antagonism of water homeostasis is suggested.

XI. ROLE OF ATRIAL NATRIURETIC PEPTIDE IN PATHOPHYSIOLOGICAL STATES

The role of ANP in disorders of volume regulation, such as congestive heart failure, hypertension, liver disease, nephrotic syndrome, and acute and chronic renal failure, has been explored in humans and in animal models. Although an exhaustive treatment of this subject is beyond the scope of this review, a brief section follows and includes references to timely and comprehensive reviews.

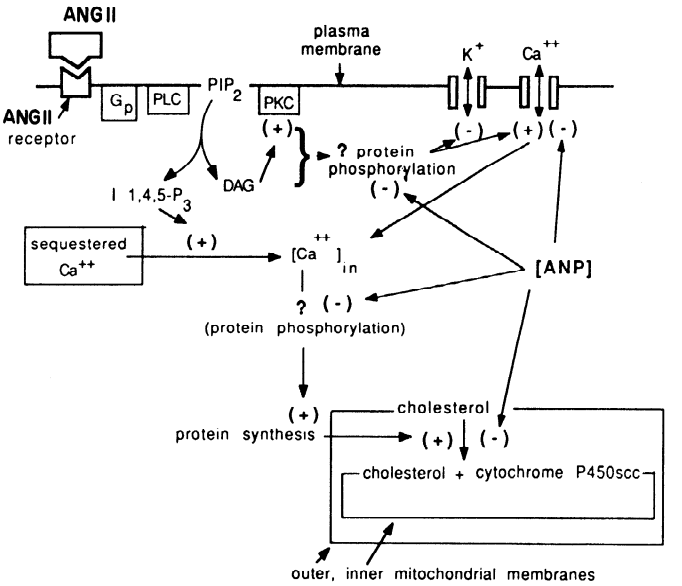

FIG. 12. Schema depicting various steps in ANP-mediated inhibition of angiotensin II receptor-coupled second messenger systems, with latter serving as an example of agonist stimulation of aldosterone biosynthesis. ANG II, angiotensin II; $[Ca^{++}]_{in}$, intracellular Ca^{2+} concentration; *P*-450scc, cytochrome cholesterol side-chain cleavage enzyme; DAG, diacylglycerol; G_p, guanine nucleotide binding protein; $I_{1,4,5}$-P_3, inositol trisphosphate; PIP_2, phosphatidylinositol bisphosphate; PKC, protein kinase C; PLC, phospholipase C; +, stimulation; −, inhibition; ?, mechanism unknown. [From Lotshaw and Mulrow (190). In: *Contemporary Issues in Nephrology. Atrial Natriuretic Peptide*, Churchill Livingston, New York, 1989.]

A. Heart Failure

Because ANP is secreted in response to atrial stretch, it is hardly surprising that plasma ANP levels are elevated in patients with congestive heart failure (CHF) (93). Normal individuals and patients with heart disease who do not suffer from CHF exhibit plasma ANP levels ranging from 10 to 50 pmol/l, whereas patients with CHF typically exhibit levels in excess of 100 pmol/l, with wide individual variation (51, 65, 77, 157a, 266, 274, 279, 296). The plasma level of ANP correlates closely with indices of the severity of the CHF, varying directly with right atrial and pulmonary capillary wedge pressures and inversely with cardiac index, stroke volume, blood pressure, and New York Heart Association functional class (51, 65, 77, 157a, 274, 279, 296). In animal models of CHF, high plasma levels also correlate inversely with atrial tissue concentrations, denoting prompt secretion and little tissue storage despite high ANP mRNA levels (210). As noted, ventricular ANP gene expression has also been observed in humans and in animal models of CHF (68, 79, 82, 110, 178, 182). Effective therapy for CHF leads to reductions in plasma

ANP levels usually in proportion to improvement in clinical status and cardiac performance (157a). As in normal subjects, the proximate signal that induces the failing heart to augment ANP levels appears to be increased atrial transmural pressure (i.e., atrial stretch) (77). Increased atrial stretch occurring in the course of supraventricular tachyarrhythmias also induces ANP secretion (273).

Although plasma ANP levels are often markedly elevated, patients with CHF typically exhibit evidence of volume overload, increased preload, and increased systemic vascular resistance, implying an acquired insensitivity to the effects of endogenous ANP (51, 65, 266, 274, 279). However, recent studies have demonstrated that despite reduced sensitivity to its actions, ANP helps modulate the renal, hemodynamic, and endocrine effects of CHF. Thus infusion of specific anti-ANP antibody to rats with CHF secondary to myocardial infarction acutely suppressed renal salt and water excretion (23), implying that fluid retention would have been even more marked were it not for the modest natriuretic and diuretic responses to chronic elevations in plasma ANP levels. Despite the high plasma levels of ANP and the relative insensitivity to its actions observed in patients with CHF, administration of exogenous ANP has occasionally led to favorable hemodynamic, renal, and hormonal responses, presumably by reducing preload, effecting redistribution of plasma volume to the extravascular space, and reducing afterload by direct vasodilation (52, 65, 274, 279).

B. Hypertension

The natriuretic and hypotensive effects of ANP suggest a key role of this peptide in chronic regulation of blood pressure. Moreover, ANP may act chronically to counterbalance the salt retaining and vasoconstrictive actions of ANG II and norepinephrine. At doses that cause only modest reductions in blood pressure in normal rats, ANP may profoundly reduce blood pressure in rats made hypertensive using the two-kidney, one-clip model, as well as in dogs and rats subjected to infusions of ANG II (83, 177, 280a, 347). In these states of angiotensin excess, ANP reduces blood pressure at least in part by antagonizing the vasoconstrictor actions of ANG II on resistance vessels.

The concentrations of ANP in atrial tissue are reported to be higher in Dahl salt-sensitive (i.e., hypertension prone) than in salt-resistant rats, allegedly due to impaired secretion, a pattern even evident in prehypertensive animals (242). In SHR, immunoreactive ANP levels in atria tend to be higher than in WKY controls even before overt hypertension, again implying a defect in ANP secretion (123). In Dahl salt-sensitive rats, renal papillary collecting tubule cells in culture generate less cGMP in response to ANP than do cells from salt-resistant rats (16). Intracerebroventricular infusion of ANP also preferentially suppresses the exaggerated salt appetite of SHR relative to WKY rats (152). Although ANP binding to surface receptors of vascular smooth muscle cells from SHR and WKY is similar, cGMP accumulation is blunted in SHR (223). These various findings have been taken to reflect innate differences in ANP actions in genetic rat models of hypertension.

Several studies have also shown that hypertension augments circulating levels of ANP. In hypertension mediated primarily by volume overload, such as the one-kidney, one-clip and DOCA salt models, the increases in atrial pressures induced by the attendant volume expansion are associated with elevated ANP levels in plasma (100). In DOCA salt rats, transcription rates of preproANP mRNA and plasma ANP rose sequentially, preceding a natriuresis that eventually reduced the volume overload experienced by these animals (26). These results are in keeping with evidence demonstrating that ANP is an important mechanism by which the body "escapes" from the salt-retaining effects of mineralocorticoid excess (26, 116). In clinical forms of hypertension, plasma ANP levels vary widely and have not yet proven to be of value in differentiating among the diagnostic causes of high blood pressure (110, 110a, 278). Ferrier et al. (89a) have suggested from preliminary data that an inability to respond with a normal increase in ANP secretion in the face of a salt load exists in children of hypertensive compared with normotensive parents (89a). On the other hand, ANP administration to hypertensive patients with the use of a variety of protocols has usually resulted in lowering of blood pressure (52, 155, 272a, 354). This response to ANP infusion may result in a more marked renal loss of salt and water and more marked inhibition of plasma renin activity and aldosterone than seen in normotensives (272). However, the use of ANP as an antihypertensive agent has been limited by the lack of an orally effective analogue, thereby precluding much needed long-term studies. The recent availability of intranasally administered ANP preparations or the use of inhibitors of ANP breakdown, may partly overcome this obstacle (52). In acute studies, ANP has been shown to have a narrow therapeutic index, with lower doses having little effect and slightly higher doses inducing intolerable hypotension (52, 155, 272a, 354). The use of low doses of ANP in combination with other agents also remains to be explored.

C. Liver Disease

Renal sodium and water retention, hallmark features of advanced cirrhosis, have motivated many studies to evaluate the possible role of ANP (for reviews see Refs. 93, 351). In general, plasma ANP levels are elevated, particularly in patients with ascites, thus supporting the "overflow" theory of ascites formation. As in CHF, raised plasma ANP levels in the setting of volume retention implies a state of relative refractoriness or "resetting of responsiveness" to ANP. In this regard, peritoneovenous shunting in six cirrhotic patients with high base-line plasma ANP levels led to further elevations of plasma ANP levels, prompt increased urinary

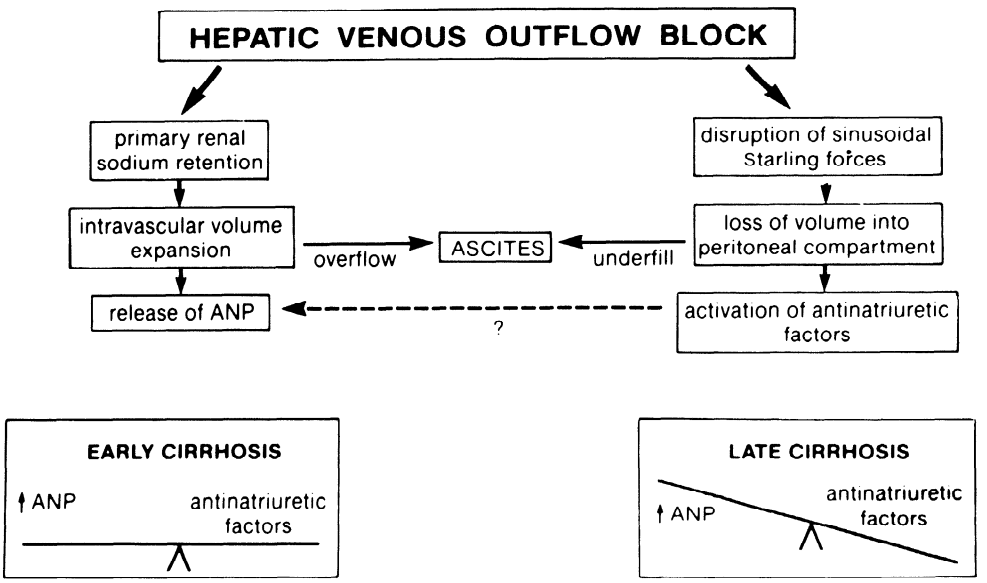

FIG. 13. Schema depicting role of ANP in renal sodium retention in cirrhosis. [From Warner et al. (351). In: *Progress in Atrial Peptide Research*, ©1989, Raven Press, New York.]

cGMP excretion, marked natriuresis and diuresis, and reciprocal declines in plasma aldosterone in five patients; in the sixth patient in whom there was mechanical failure of the shunt, no changes were measured in any of the parameters tested (55, 351). In another protocol designed to examine responsiveness to acute shifts in body fluids, 12 cirrhotic subjects were subjected to head-out water immersion for 3 h. This maneuver serves to translocate interstitial fluid from the lower extremities to the central intrathoracic vascular compartment (86, 87), thereby increasing ANP levels that are at least in part responsible for the natriuretic response (265a). In six subjects, natriuresis and enhanced urinary cGMP excretion paralleled further elevations in plasma ANP, whereas in the remainder of the subjects, despite equivalent increments in plasma ANP and urinary cGMP levels after immersion, renal excretory responsiveness was not apparent (309, 351). Moreover, none of the latter group suppressed plasma aldosterone levels <1.4 mM/l, a level considered necessary for ensuring a natriuretic response. When the base-line clinical features of "responders" and "nonresponders" were compared, the former proved to have less advanced disease than the latter. Figure 13 provides a tentative means for interpreting the data obtained by Skorecki and co-workers (309, 351). According to this view, hepatic venous outflow blockade, the major pathophysiological defect in cirrhosis, leads to primary renal sodium retention, intravascular volume expansion, and compensatory elevations in plasma ANP concentrations. At early stages of hepatic injury, this rise in ANP levels adequately counterbalances other antinatriuretic adaptations, albeit only partially so that volume expansion and ascites formation persist. With disease advancement, hepatic architectual remodeling impairs hepatic sinusoidal fluid exchange, leading to ascites formation. The resulting "underfilling" of the plasma compartment promotes antinatriuretic renal responses and also reduces the stimulus for ANP secretion so that plasma ANP levels no longer effectively offset antinatriuretic mechanisms. Clearly this hypothetical explanation requires further testing.

Short-term infusion of exogenous ANP into cirrhotic subjects with ascites and edema resulted in modest transient natriuresis and diuresis but unfortunately was often complicated by serious hypotension. The latter side effect seems to be less prominent when ANP is given by bolus administration (for review see Refs. 52, 93).

D. Renal Disease

The nephrotic syndrome is an intense Na^+-retentive state often sufficient to induce edema formation. Although total body content of Na^+ and water are often elevated in patients with nephrotic syndrome, plasma ANP levels are often reduced, suggesting diminished effective arterial and venous blood volumes (93, 257). After head-out water immersion in nephrotic subjects, however, plasma levels rise appropriately, but renal excretory responses remain blunted. In keeping with the possibility that renal responsiveness to ANP may be impaired in nephrotic subjects, natriuretic responses to infused ANP are blunted in rats with experimental nephrotic syndrome (138, 159, 256a, 357). Nevertheless, in some studies, ANP stimulated GFR to a similar extent in control and nephrotic kidneys, confirming a specific tubular insensitivity to the natriuretic effect of ANP (256a, 357). The cellular basis for this insensitivity (acquired receptor defect, impaired step in signal transduction, or effector response) remains to be studied. Perico et al. (256) has recently shown in the adriamycin model of nephrotic syndrome in rats that ANP receptor densities do not differ between the medullas of treated or untreated kidneys. A defect in renal responsiveness to infused ANP was not observed in one human study (369).

Circulating natriuretic factors have been implicated in the maintenance of body fluid volume homeostasis in progressive renal failure (310). The activity of these factors tends to vary with salt intake; reduction of salt intake in parallel with the reduction in GFR often eliminates the increased natriuretic activity (298). In models of reduced renal mass, plasma ANP levels correlate closely with salt intake and excretion, reaching higher values on high- versus low-salt diets and higher than those attained on equivalent diets in animals with normal renal function (312). Similarly, in patients undergoing regular triweekly hemodialysis for chronic renal failure, plasma ANP levels have been used as an index of volume status, being higher immediately before each dialysis and lower soon after treatment (85, 269). In addition, ANP infusion into animals with reduced renal mass leads to natriuresis and diuresis, indicating a considerable reserve of functional ability to augment GFR and Na^+ excretion. This effect has recently been studied in humans by Windus et al. (357a), who demonstrated in a cohort of patients with renal failure of mixed etiology the ability to augment Na^+ excretion by 65% in response to ANP infusion. Infusion of specific anti-ANP antibody into rats with reduced renal mass led to marked reductions in fractional and absolute excretion of Na^+, Ca^{2+}, and PO_4^{2-} under conditions in which GFR and renal plasma flow were unchanged (245). These findings suggest that in the presence of reduced renal mass a major regulatory effect of ANP is directed toward the augmented excretion of Na^+ and other solutes, presumably reflecting actions involving the proximal tubule.

Under certain circumstances, ANP has been shown to ameliorate renal damage in response to an ischemic insult. In studies of acute renal failure induced by renal artery clamping, ANP in pharmacological doses increased GFR and urinary volume when administered during or immediately after the ischemic episode (291, 305). A beneficial effect of ANP on cisplatin-induced nephrotoxicity in the rat has also been reported (58). The mechanisms of this protective effect of ANP are as yet unknown.

Extracellular fluid volume is known to increase during periods of complete bilateral ureteral obstruction and prompt excretion of this retained volume usually occurs after relief of obstruction. Given this expansion of extracellular volume it is hardly surprising that a role for atrial peptides has been suggested. In recent studies by Purkerson et al. (262), rats subjected to bilateral ureteral obstruction for 24 h demonstrated plasma ANP levels more than twice those seen in control rats or in rats with unilateral ureteral obstruction also of 24-h duration. Of note, heparin, which is known to bind atrial peptides and interfere with their biological actions (352), served to decrease the magnitude of the natriuresis and diuresis observed after relief of bilateral obstruction but exerted little measurable influence on salt or water excretion in rats after relief of unilateral obstruction. These findings implicate endogenous ANP in the genesis of the natriuresis and diuresis after relief of bilateral urinary tract obstruction.

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