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# THE BIOCHEMICAL PHARMACOLOGY OF ATRIAL PEPTIDES

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## ATRIOPEPTIN: OVERVIEW—VINTAGE 1988

### *Cardiac Biosynthesis*

A schematic overview is presented as a framework for the more comprehensive review of the atrial peptide system that follows (Figure 1). An expanded discussion of the specific details and references is detailed in the subsequent section. Briefly, granules located in the atria (but not the ventricles) contain the 126-amino acid prohormone termed atrial natriuretic factor (ANF), atrial natriuretic peptide (ANP), cardionatrin or atriopeptin (AP). cDNA probes have been used to characterize the amino acid sequence of a 152-amino acid rat atrial preprohormone. Non-atrial sites of AP synthesis have also been discovered in the central nervous system and the cardiac ventricle, which only expresses AP biosynthesis during fetal and neonatal development and during cardiac hypertrophy.

Recently, Flynn and colleagues announced (at the Third International Congress on Biologically Active Atrial Peptides, New York, June 25–26, 1988) the isolation and sequencing from rat atrial extracts of a unique atriopeptin-like peptide termed iso-rANP (rat atrial natriuretic peptide) (Fig-



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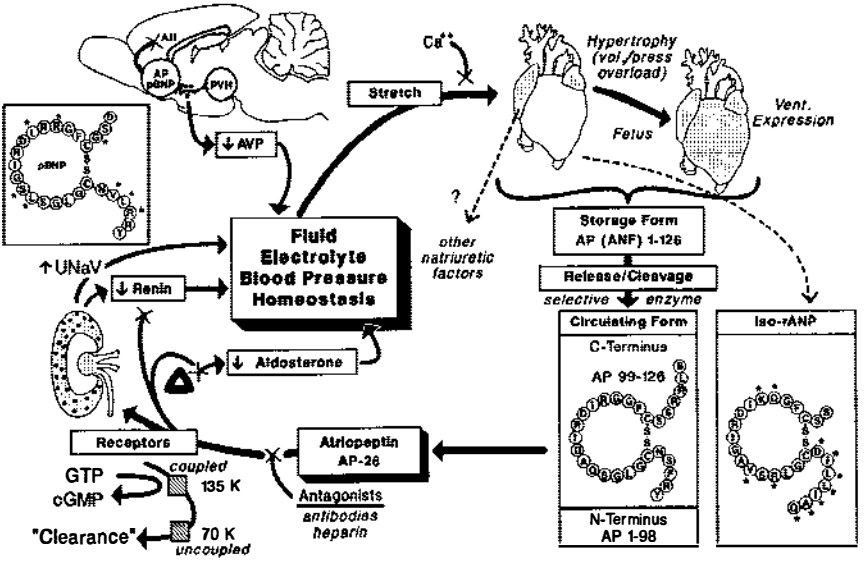


*Figure 1* The atrial peptide system

ure 1). This new peptide, together with the newly identified porcine brain natriuretic peptide (described below), suggests that there naturally are a family of closely related structural analogs of AP that appear to derive from separate, unique genes. Complementary or antagonistic activities or regulatory control of these related peptides could profoundly influence biological function and will surely become the focus of intensive investigation.

## *Release*

Release of the peptide into the circulation is stimulated by manipulations known to increase right atrial pressure, such as acute volume expansion, water immersion, and vasoconstrictor substances. Cultured cardiomyocytes only release prohormone, but clearly the myocardium contains a specific cleavage enzyme that selectively generates the C-terminal 28-amino acid peptide. The release process in cultured myocytes appears to be negatively regulated by intracellular calcium. Release from the intact heart (in vivo or in vitro) is accompanied by immediate selective proteolytic cleavage since the plasma circulation forms are the C-terminal peptide AP 99–126 (i.e. AP-28) and the N-terminal fragment (AP 1–98). In humans a 56-amino acid antiparallel dimer of AP–28 (termed $\beta$-ANP) has been demonstrated to be released especially in cardiac disease states. This dipeptide has a longer plasma half-life and longer duration of action than does AP-28. The $\beta$-ANP appears to be rapidly converted to AP–28 in plasma.

Acute volume expansion simultaneously causes AP release, natriuresis,

and diuresis. AP alone is not the sole mediator of this response since acute volume expansion of conscious animals could induce natriuresis without AP release. In addition, higher levels of exogenous AP are required to cause a natriuresis-diuresis in a variety of experimental animals than the levels of endogenous AP generated by acute volume expansion. Such results suggest the possibility of another endogenous natriuretic substance.

## *Receptors*

Once released, the circulating AP appears to interact with at least two receptors, i.e. approximately 60–, 70-kd and 130–, 180--kd peptides. The higher molecular weight receptor proteins appear to be directly coupled to particulate guanylate cyclase, and they exhibit strict structural- and stereo-specificity for AP agonist stimulation. On the other hand, the 70-kd peptide is not coupled to cyclic GMP synthesis and is very nonselective, binding either native, truncated, or linear fragments of AP. Analogs that bind to this low molecular weight receptor do not stimulate isolated blood vessel strips or the isolated perfused kidney, but chronic infusion in vivo causes a delayed natriuresis-diuresis and an accumulation of endogenous AP-28. These data suggest that the uncoupled 70-kd receptor may be a clearance site that serves as a "sink" for the binding and therefore inactivation of circulating AP-28.

The major biological effect of AP-28 results in changes in fluid and electrolyte metabolism and blood pressure homeostasis. AP-28 acts on renal receptors in the glomerulus, the medullary collecting ducts, and perhaps the juxta-glomerular cells causing a natriuresis-diuresis, and suppression of renin release. AP also acts on adrenal zona glomerulosa cells to suppress aldosterone synthesis and release.

## *Suppression of Biological Activity*

Unfortunately, there is no specific pharmacological antagonist of AP, thereby complicating efforts at definitive elucidation of the physiological and pathological role of AP. Three approaches for the manipulation of AP release and response have been developed including surgical atrial-appendectomy, antibodies (monoclonal or polyclonal) for passive or active (i.e. autoimmune animals) immunization, and exogenous heparin (which appears to tenaciously bind AP-28). Application of these approaches has been successfully applied blocking the natriuresis-diuresis produced by exogenous or endogenous AP induced by acute volume expansion. Other pharmacological strategies to manipulate the duration of action of AP under study include the use of either analogs that saturate the "clearance receptor" or agents that inhibit enkephalinase, E-24.21 activity responsible for the enzymatic destruction of AP-28.

### *Brain—AP System*

We know that AP in brain arises by de novo synthesis since Northern blot analysis has demonstrated the presence of AP mRNA in the hypothalamus. AP modulates vasopressin and LH release and inhibits the central nervous system actions of exogenous angiotensin. The actual levels of AP in the brain are extremely low, a condition that has hampered establishment of the molecular form and structure of AP stored or released in the brain. Recent efforts by Matsuo and colleagues to characterize porcine brain AP have led to the exciting discovery of a novel 26-amino acid peptide that is remarkably homologous with AP. Termed pBNP (porcine brain natriuretic peptide), there are seven amino acid replacements and the insertion of one arg residue compared with AP 103–126. This peptide appears to be equipotent with AP as a natriuretic-diuretic and vasodilator. Most likely other biological tests will uncover other actions of BNP.

Numerous earlier reviews have comprehensively discussed the initial isolation and characterization of the atrial peptides (1–6). This review highlights recent discoveries that provide a more thorough understanding of the mechanisms of AP regulation and function. Insights into novel receptors, novel atrial peptides, and the possibility of non-AP natriuretics predict a wealth of possibilities for new pharmacologic discoveries.

## GENE EXPRESSION

### *Biosynthesis and Storage*

The major site of AP gene expression is the atria of the heart. It has been possible, with the development of AP-specific antisera and of AP-mRNA specific oligonucleotide probes, to identify many non-atrial tissues as additional sites of AP synthesis, and storage. Convincing evidence for local synthesis of AP in non-atrial tissues depends on either the detection of AP-specific mRNA (which would indicate transcription of the AP gene) or the extraction and identification of the high molecular weight prohormone AP 1-126, which could only come from the tissue itself since the intact prohormone does not circulate (7). The extraction of AP-28 or one of the low molecular weight AP metabolites from a non-atrial tissue could indicate local synthesis and processing; however, it could also represent tissue accumulation of circulating AP-28 or AP metabolite through internalization and/or binding to AP receptors.

AP synthesis has been documented in the ventricles of the heart (8–10), brain (11, 12), peripheral ganglia (13–16), pituitary (10), vascular tissue (17, 18), and the lungs (10) at levels much lower than in atria. In all tissues studied, a 950–1000 nucleotide transcript hybridizes to AP cDNA. The transcription start site, as determined by either primer extension of S1

analysis, is the same as in atria. Atrial AP mRNA has an additional transcription start site which is 80bp upstream from the primary start site and lies 30bp downstream from an A+T rich sequence that may function as a weak promoter (10).

AP mRNA is expressed at very high levels in atria, where it constitutes 1–3% of the total mRNA. The ventricles of the heart appear to be next most abundant source, in which the abundance of AP mRNA is about 1% of that in atria. In situ hybridization in ventricular slices shows uniform distribution of AP mRNA throughout the ventricles except in the atrial proximal region where grain density is higher (9). Based on the abundance of AP mRNA, the immunoreactive AP (APir) in ventricles is lower than expected (about 400-fold less than that in atria). Since there is no translational difference of AP mRNA between atria and ventricles (E. Tolunay, unpublished observations), AP may be released constitutively from ventricles and the ventricular AP, due to the large tissue mass, might contribute substantially to circulating AP. This is conceivable since neonatal ventricular cardiomyocytes secrete AP constitutively in tissue culture (19).

In brain, pituitary, lung, aortic arch, and the abdominal aorta, the amount of AP mRNA is even lower than that in ventricles, i.e. several hundred-fold less than that in atria. In the brain AP mRNA is differentially distributed: It is most abundant in hypothalamus (about 200-fold less than that in atria), followed by cortex and septum, and found in moderate amounts in hippocampus, midbrain, spinal cord, olfactory bulb, pons and the medulla (12). APir cell bodies and fiber tracts have been detected histochemically in regions of the midbrain known to be involved in cardiovascular regulation (20).

Peripheral neurons have been found to be additional sites of AP storage. Rat adrenal chromaffin cells have been identified by immunohistochemical staining (16, 21) to contain AP in epinephrine-containing and, to a lesser extent, norepinephrine-containing cells. Sequence analysis of purified AP immunoreactivity from adrenal medullary extracts has identified both AP-28, and the prohormone, AP 1–126 (22).

APir has been detected in cell bodies of both the cholinergic and adrenergic ganglions in rats. AP-positive cells were first identified by immunohistological staining in peripheral ganglion in the rat superior cervical ganglion (13). Subsequently, AP has been detected in extracts of both the ganglia nodosa (14) and the superior cervical ganglion (15). APir has also been described in sparsely distributed, small, intensely fluorescent (SIF) cell bodies of the coeliac-superior mesenteric ganglion which also stained for dopamine $\beta$-hydroxylase, an enzyme marker for catecholamine synthesis (16).

APir has been localized to the adventitia of the aortic arch, the site of the vascular baroreceptors (17), and AP mRNA expression has been identified in

both the aortic arch and in distal segments of the aorta. APir and AP-mRNA have also been detected along the extra- and intra-pulmonary veins (18) in atrial-like striated muscle cells that are interspersed among the vascular smooth muscle cells. The importance of vascular or ganglionic AP is unknown, but since exogenous AP is known to inhibit the release of norepinephrine from adrenergic nerve terminals in vitro (23), vascular and/or ganglionic AP may play a role in the regulation of sympathetic transmission in vivo.

The concentration of AP mRNA in lungs is very low, several hundred-fold less than that in atria (10). AP prohormone has been identified in lung extracts, whereas low molecular weight AP is secreted by the isolated perfused lung (24). Lung tissue also contains low molecular weight AP that is thought to accumulate by extraction of AP-28 from the plasma (25). In light of the detection of AP mRNA by in situ hybridization in extra and intrapulmonary veins, the AP mRNA and AP prohormone detected in the lungs may be largely from these veins (18). Pulmonary AP could have local relaxant effects on either pulmonary vascular, or bronchial smooth muscle.

The expression of AP in tissues apart from the CNS and the atria lends credence to the speculation that AP also functions as an autocoid. AP may locally affect fluid and electrolyte metabolism, autonomic outflow, and/or endocrine secretions, apart or in concert with systemic AP.

## *Gene Structure and Organization*

Gene structure and organization of AP from various species have been reviewed comprehensively (26). The nucleotide sequence for preproAP has been determined from various species including the genomic sequence from human (27–29), rat (30–31), and bovine (32) and the cDNA sequence from human (33), rat (34), dog (35), and rabbit (35). The coding sequences for AP are divided between three exons. A short first intron (about 100 bp) and a long second intron (from 391 bp in rat to 1093 bp in human) separate the exons (27–30, 32). The position of the introns in the gene is exactly the same for all species studied. The first intron is located between amino acids 16 and 17, the second between amino acids 125 and 126 (27–30, 32). The combined information from genomic and cDNA sequences predicts two different C-terminal sequences for the peptide. Human and dog peptides end with *Tyr*, while rat, mouse, rabbit and bovine with *Tyr-Arg-Arg* (32, 35). However, in all species the isolated peptide terminates with *Tyr* suggesting that the two arginines are cleaved off during posttranslational processing. *Tyr* or *Tyr-Arg-Arg* are the only coding sequences present in the third exon. The significance of this is unknown.

The amino acid and DNA sequences of AP are highly conserved between species. Twenty-seven of twenty-eight amino acids of the circulating peptide

are identical in all the species studied. The single amino acid difference is at position 12, which is *Met* for human, dog, porcine, and bovine; and *Ile* for rat, mouse, and rabbit. The overall homology for the six species discussed is 73% for the amino acid sequences +1 to +126 (proAP) (32, 35). The high homology observed between the 5'-flanking regions suggests the presence of common regulatory sequences.

## *Regulatory Sequences*

The AP gene contains many of the regulatory sequences present in a typical eukaryotic gene. In the 5'-flanking region a typical TATA box is present 24–30 bp upstream from the transcription start site. A potential CAAT box upstream from the TATA box has been noted only in the human gene (28). In the 3'-flanking region an AATAAA polyadenylation signal is present about 250 bp downstream from the termination codon. The rat gene contains two such sequences. A sequence identical to the glucocorticoid receptor recognition core sequence (TGTYCT) is found in the second intron of the human AP gene but not in bovine, rat, or mouse genes (28, 29). In rats the expression of the AP gene is stimulated by glucocorticoids (dexamethasone) in atria and ventricles both in vivo and in tissue culture (36, 37). The glucocorticoid-responsive element has been mapped within the 2.4 kb sequence upstream of the transcription initiation site (38). However, sequence analysis within the 2.4 kb region detected only partial homology to the concensus glucocorticoid receptor recognition sequence, and no apparent clustering of potential receptor recognition sites was observed. This may explain the relatively low induction of AP mRNA in rats by dexamethasone as compared to that of mouse mammary tumor virus RNA or the human metallothionein gene (39, 40).

Recently, the 5'-flanking region of the rat AP gene has been sequenced up to −3637 bp (38). Various portions of this promoter region have been fused with the coding sequences of a prokaryotic reporter gene, chloramphenicol acetyltransferase (CAT), and the hybrid AP-CAT genes have been introduced into cultured cardiomyocytes from one-day-old or fetal rats. The CAT activity observed in cellular extracts of these cells was used to measure the effectiveness of these sequences in directing the tissue-specific expression as well as the developmental regulation of AP gene. The 3.4-kb region upstream from the transcription initiation site of the rat gene was found to contain all the necessary *cis*-acting elements for high-level tissue-specific expression, the developmental and hormonal regulation of the rat AP gene (38). A 0.64-kb fragment containing the TATA box and the transcription initiation site was not sufficient to support AP expression in vivo. In contrast, the analogous *cis*-acting elements in the human gene, with the exception of the glucocorticoid responsive element (located in the second intron), appear to be contained

within the 500-bp sequence upstream from the transcription initiation site (41). Using a short segment of human AP gene from -500 to +77 bp as the promoter for SV40 T antigen, Field has demonstrated tissue-specific and developmentally regulated expression of T antigen in transgenic mice (41).

### *Developmental Regulation*

Expression of AP is developmentally regulated in atria and ventricles of the heart (42, 43). The developing heart ventricle contains substantial AP mRNA that rapidly declines after birth. In contrast, atrial AP mRNA is initially low and increases through development. In the fetus and neonate, as in the adult, virtually all of the ventricular AP mRNA is expressed in the left ventricle. However, some species difference is observed in the decline of the ventricular AP mRNA through development. In one-day-old mice, AP mRNA level in the left ventricle is the same as in the adults, whereas in rats two weeks after birth AP mRNA in the left ventricle is still higher than that in the adult left ventricle. Tissue AP content parallels the mRNA during development in all compartments of the heart and the change in plasma APir parallels the ventricular APir; it is initially high and declines through development (43). During perinatal and postnatal development, the entire heart appears to have an endocrine function. Fetal heart responds to vasoconstrictor substances such as 1-desamino-arg8-vasopressin, or ductal closure following indomethacin treatment by releasing AP (43).

It is not clear which factors regulate the development tissue-specific expression of AP. One possibility, as suggested by Zeller et al (42), is that certain cells are committed for AP expression early during development. For example, the bulbus cordis which develops into the right ventricle has very low AP expression in day-9 embryonic heart, whereas the primitive ventricle that later develops into the left ventricle is replete with AP mRNA. Alternatively, the changes in atrial and ventricular AP expression following birth may reflect the changes in intracardiac pressures and vascular resistances (44).

## RELEASE

Initial studies demonstrated that elevations in atrial pressure correlated well with AP secretion, and it was thought that mechanisms that produced a rise in atrial pressure, such as intravascular volume expansion, would activate pressure sensitive secretory mechanisms. Raising right atrial pressure by either 1 or 5 mmHg in the isolated perfused rat heart resulted in a 1.5- to 3-fold increase in AP secretion into the cardiac effluent (45). Studies in the intact animal have further corroborated the relationship between atrial pressure and AP secretion. In the anesthetized rat, intravenous infusion of saline (45) or head-out water immersion (46), maneuvers which increase intravascular

volume, resulted in a five-fold increase in right atrial pressure and a similar increase in plasma AP levels. The administration of phenylephrine, vasopressin or angiotensin II, in hypertensive doses to anesthetized rats, produced an immediate rise in plasma AP levels (46). In these studies, the release of AP closely paralleled the rise in right atrial pressure and were dissociated from elevations in left atrial and mean systemic pressures. These data suggest that the right atrium, at least in the rat, is the major source of AP secretion in response to central hypervolemia. Studies in the dog suggest that the left atrium also secretes AP in response to elevated atrial pressures (47). In the anesthetized dog, acute mitral obstruction produced by inflation of a balloon in the left atria was immediately followed by a rise in plasma AP levels (48).

Atrial stretch receptors have been located in both the left and right atrium in several species and have been shown to traverse the vagus nerve (49). These receptors have been implicated in the modulation of heart rate (Bainbridge reflex) (50) and in the control of fluid and electrolyte balance (51). Neither bilateral vagotomy nor beta adrenergic receptor blockade with atenolol was able to attenuate AP release in the water-immersed rat (46) or the acutely mitral-obstructed dog (48) or even a cardiac denervated dog (47).

It is unclear whether atrial stretch and/or pressure is the primary physiological stimulus for AP secretion. Thus, the effect of resting tension (i.e. stretch) on AP secretion was examined in the isolated perfused rat left atria (52). An increase in resting tension of three to five-fold increased AP secretion approximately 30%, while a decrease in resting atrial tension by 50% resulted in a 30% decrease in AP secretion. Furthermore, pretreatment of the isolated atria with propranolol, phentolamine, and atropine did not affect the secretory pattern of AP in response to changing atrial tension. In vitro stretch of single cell suspensions of atrial myocytes, a preparation devoid of neural and humoral influences, has been induced by exposing cells to hypotonic media (53), a manipulation that acutely increases cell volume and stretches the plasma membrane. Atrial myocyte suspensions released AP as an inverse function of solution osmolarity, i.e. directly proportional to cellular stretch. Furthermore, in chronically instrumented conscious dogs, it has been demonstrated that plasma AP secretion best correlates with atrial wall stress rather than atrial pressure measurements (54). To further clarify whether atrial pressure or atrial stretch is the true stimulus of AP secretion, studies were performed in the open-chest dog subjected to cardiac tamponade and rapid intravascular volume expansion (55). Cardiac tamponade produced elevations in atrial pressure without measurable increase in plasma AP levels either in the presence or absence of acute volume expansion. The above studies collectively demonstrate that physical properties of atrial tissue such as compliance, stress, transmural pressures, and stretch are the physiological signals for AP release and appear to be independent of neural mechanisms.

The role of the central and/or peripheral nervous system in regulating AP secretion has been widely studied. In the isolated perfused rat heart, norepinephrine and epinephrine (but not isoproterenol) stimulated AP secretion (56, 57). The physiological significance of these observations is questionable since AP secretion in the acutely mitral obstructed dog model is not affected by bilateral cervical vagotomy or beta adrenergic receptor blockade (48), and secretion of AP by head-out water immersion in the rat was also not affected by bilateral cervical vagotomy (46). On the other hand, in the pithed rat or the hypophysectomized rat, AP secretion was markedly inhibited in response to acute volume loading (58, 59), but this suppressed effect could be due to the marked hypotension in the animals.

The precise cellular mechanism(s) coupling mechanical changes in the atrial muscle to hormonal release remains unresolved. By analogy to other secretory systems, it has been postulated that a rise in intracellular calcium and/or the activation of the phosphatidylinositol system is the stimulus for AP release. This intracellular signal pathway consists of two branches, inositol triphosphate which initiates release of calcium from intracellular stores, and diacylglycerol which activates protein kinase C. This hypothesis is supported by the demonstration that AP release from the isolated rat heart is moderately enhanced by the calcium ionophore A23187 (60) or the calcium channel agonist BAY k8644 (61). Furthermore, exposure of the isolated heart to BAY k8644 and a phorbol ester results in a synergistic stimulation of AP secretion when compared to either agent alone, therefore mimicking the presumed effects of the two branches of the phosphatidylinositol system. Hypoxia or pacing of the isolated perfused heart or atria has been shown to stimulate AP release (62, 63), and it would be expected that the mechanical alterations induced in the isolated perfused heart by A23187 or BAY k8644 would result in a tachycardia and tissue hypoxia.

Osmotic stretch or KCl depolarization of isolated atrial myocytes have been demonstrated to be a potent stimulus of AP secretion (53). When stretch or depolarized atrial myocytes were incubated in medium either lacking calcium or containing BAPTA AM, an intracellular calcium chelator, AP secretion was not inhibited but potentiated. Likewise, increased intracellular calcium levels by exposure to ionomycin-inhibited basal AP secretion and completely blocked release in response to osmotic stretch. Furthermore, in the conscious dog, AP secretion best correlates with atrial wall stress measured during passive diastolic atrial filling, a time when intracellular calcium concentrations will be at a nadir (54). These data taken together suggest that calcium is not the intracellular stimulus for AP release and may even negatively modulate AP release in a manner similar to that observed for renin (64).

AP is stored in the atrial granule as the mature 126-amino acid prohormone (65), but it is the C-terminal 28-amino acid fragment that is secreted from the

heart and that is physiologically active (66). Many hormones are secreted as the processed, physiologically active species; cultured atrial myocytes, however, secrete only the AP prohormone (67). To date, the site of AP prohormone processing has eluded detection. Several extracardiac sites of pro-AP proteolytic activity have been identified. Rat serum can generate AP-28 from pro-AP, an activity shared by thrombin (67). Because this activity did not reside in rat plasma, and the isolated perfused rat heart secretes only AP-28 (68), the absence of blood products demonstrates that this serum enzyme is of little physiological significance. It is currently believed that pro-AP proteolytic processing resides within the heart since the isolated perfused heart releases only AP-28 (68) and since exogenously applied pro-AP is converted to AP-28 by the isolated perfused heart (7). Furthermore, an enzyme from the particulate fraction of rat atrial homogenates that processes proAP has recently been purified (69). This enzyme was described as a serine protease because it was specifically inhibited by leupeptin, aprotinin, benzamidine, and disopropyl fluorophosphate. ProAP undergoes proteolytic activation in the heart before its secretion, but the cellular and subcellular site of the myocardial proteolytic activity has yet to be determined.



## METABOLISM

AP has a relatively short half-life in the circulation, ranging from 31 sec for AP-24 in rats (70) to 3.2 min for α-hANP in man (71). The kidney has the greatest capacity to degrade the peptide (72). Incubation of AP-28 with renal brush border membranes (73) or crude cortical membranes (74) results in a single cleavage of AP-28 at the $Cys^7$-$Phe^8$ bond which greatly inactivates the peptide. Incubation of AP-24, the amino-terminal truncated metabolite of AP-28, with renal brush border membranes resulted in the cleavage of the $Cys^7$-$Phe^8$ bond as well but, unlike AP-28, the peptide was also susceptible to hydrolysis at the $Ser^{103}$-$Ser^{104}$ bond and the $Ser^{123}$-$Phe^{124}$ bond (73). The enzyme responsible for this cleavage is most likely the Zn-metallo-endopeptidase, 3.4.24.11 (enkephalinase, E-24.11) (75), an ectoenzyme that cleaves following hydrophobic amino acids and has a molecular weight of 94 kd.

The 98-amino acid amino terminal fragment (AP 1–98) of the AP prohormone is also secreted by the heart in stoichiometric amounts with AP-28 (76) and accumulates in the plasma (7). Plasma levels of N-terminal fragment immunoreactivity (NTFir) are generally higher than those for AP in spite of the identical secretion rates because of the longer half-life for AP 1–98 (4.8 min) (70). Virtually all of the plasma AP 1–98 is cleared by the kidney which filters it and presumably degrades it on the brush border of the proximal tubule (7).

No biological activity has been described for the AP 1–98, although three consecutive synthetic fragments of AP 1–98, (AP 1–30, AP 31–67, and AP 79–98), have been synthesized and studied because of their postulated formation in plasma. All three are reported to relax porcine aortas (77), stimulate the production of cyclic guanosine 3', 5', monophosphate (cGMP) by aortic tissue and by renal particulate guanylate cyclase in vitro (78), and to lower blood pressure in vivo (79). The three peptides produced identical dose-response curves for the stimulation of guanylate cyclase even though they have no sequence homology between them.

Radioimmunoassays using antibodies raised to AP 1–30 and AP 31–67 reacted with human plasma and detected markedly enhanced immunoreactivity in the plasma of patients with chronic renal failure and elevated APir. Unfortunately, neither of the immunoreactive species detected in the plasma were characterized with regard to size by gel filtration or chromatographic mobility on either ion exchange or hydrophobic resins. Two separate studies using antibodies directed against AP 48–67 (79) and against AP 11–37 (80) failed to detect significant amounts of these low molecular weight peptides in rat plasma, but both detected the full length AP 1–98. Until the AP 1–30 and AP 31–67 immunoreactivity in human plasma is characterized, it is reasonable to expect that these two antisera are detecting AP 1–98. These findings and the inability of the AP 1–98, the species that is secreted by the heart, to have cardiovascular effects either in vivo or in vitro raise serious doubts as to the physiological relevance of the fragments AP 1–30, AP 31–67, and AP 79–98.

## RECEPTORS

Specific AP receptors have been demonstrated in rabbit aorta (81), kidney glomeruli, and medullary collecting ducts (82), adrenal cortical zona glomerulosa cells (83, 84), smooth muscle cells (85) and endothelial cells (86). Structure activity studies with N-terminal or C-terminal truncated analogs of AP-28 established that the C-terminal *Phe-Arg-Tyr* residues were critical for relaxation of rabbit aorta strips or for induction of a natriuresis-diuresis in dogs (46). Peptide analogs lacking the C-terminal *Phe-Arg-Tyr* were 100–1000 fold less active than the parent compounds in stimulating the particulate guanylate cyclase but had only 5 fold lower affinity as ligands for specific receptors on cultured bovine vascular smooth muscle cells (85). These analogs that bind to the receptors without stimulating guanylate cyclase were not inhibitors of the biological effects of the parent peptides (85). The dissociation of binding affinity, stimulation of cyclic GMP production and biological action of AP analogs suggested the existence of multiple receptors or binding sites.

AP binding sites have been specifically and covalently labeled either with photoaffinity ligands or by using cross-linking reagents. Such studies, in

adrenal zona glomerulosa (83, 84), rabbit aorta (81), rat kidney cortex (87), cultured vascular smooth muscle (88) or endothelial cells (86) demonstrated the presence of two major proteins of apparent molecular mass of approximately 70 kd and 130 kd. The 70-kd site was found to be guanylate cyclase-free and exhibited high affinity for intact peptide or for analogs lacking the C-terminal *Phe-Arg-Tyr* (86, 89). The 130-kd site is coupled to guanylate cyclase and has a much higher affinity for intact AP (86). This higher molecular weight protein seems to represent a bifunctional macromolecule rather than a dimer with two separate activities. Solubilized rat lung was sequentially purified through multiple chromatographic steps, and both the AP receptor binding protein and the particulate guanylate cyclase were comparably enriched at each step (90). The purified 130-kd protein was isolated on a GTP affinity column that retained both activities and comigrated with the band generated by cross-linking $^{125}$I-ANF to rat lung. Cultured canine kidney tubular cells contained a distinct 130-kd protein that was not reduced by the sulfhydryl reagent which exhibited a preferential affinity for full length AP-28 (91). One other macromolecule receptor has been isolated from adrenocortical carcinoma cells (92) based on its particulate guanylate cyclase activity. The purified 180-kd protein also tenaciously binds AP. The relationship of this protein to the smaller 120-kd receptor coupled to the guanylate cyclase needs resolution. Rat thoracic aorta cells contained a 130-kd disulfide-linked AP binding protein that was reduced by dithiothreitol to a 70-kd band (91). The non-reducible 70 kd and the reducible 130 kd proteins showed strong affinities for full length or C-terminal truncated AP peptides. The purified cyclase-free 70-kd AP binding protein from bovine adrenal cells is not derived from the cyclase containing receptor as evidenced by the fact that truncated analogs prevented the photolabeling of the smaller receptor protein but not the 130-kd peptide, and two-dimensional peptide mapping demonstrated greater than 90% difference between the profiles of the two receptor subtypes (89).

The function of the guanylate cyclase-independent 70-kd AP binding protein is currently unknown. An intriguing possibility arises from studies that demonstrated that a ring-deleted analog (des [gln$^{18}$, ser$^{19}$, gly$^{20}$, leu$^{21}$, gly$^{22}$]ANF 4-23) of AP binds with high affinity in the isolated perfused rat kidney but was devoid of detectable renal or hemodynamic effects and did not inhibit the natriuretic actions of AP-28 (i.e. ANF 99–126) (91). Surprisingly, administration of the ring-deleted AP analog to intact rats caused a natriuresis and a hypotension. This biological response in vivo induced by an analog that is inactive in vitro was accompanied by an increase in plasma levels of endogenous AP-28. These data suggest that the cyclase-uncoupled receptor sites may represent storage and clearance binding sites that once saturated with the ring-deleted analog result in the accumulation of the endogenous AP-28.

## MAJOR BIOLOGICAL RESPONSES

### *Renal Actions*

The discovery of AP was based on its potent natriuretic action, yet, after years of intensive research, the underlying renal mechanism for AP-mediated increase in urinary sodium excretion remains unknown. The natriuretic response is influenced by factors other than the level of peptide administered. For instance, conscious euvolemic monkeys do not increase sodium excretion when infused with AP, but after modest volume expansion there is a marked increase in sodium excretion associated with the same level of AP infusion (93). Preventing the fall in arterial pressure normally associated with AP infusion by simultaneously administering angiotensin II or methoxamine greatly potentiates the AP-induced natriuresis (94, 95). Lowering arterial blood pressure to the kidney by constricting the aorta abolishes the natriuretic action (96), where a similar reduction in renal perfusion pressure did not block furosemide action (97).

Based on early studies in isolated perfused kidneys, increased glomerular filtration was thought to be essential to the atrial peptide-induced natriuretic response. Recently, numerous studies have revealed that an increase in glomerular filtration rate (GFR) is not requisite for a full natriuretic response to AP infusion (95, 96, 98–103). The mechanism for the increased GFR appears to be unique and involves simultaneous dilation of the afferent arteriole and constriction of the efferent arteriole (104, 105). Changes in glomerular surface area due to mesangial cell relaxation may also contribute to the observed alterations in GFR (106).

Increased renal blood flow is frequently associated with natriuresis, and AP is a relatively specific renal vasodilator (107). However, the renal vasodilation is short-lived during continued administration of AP (54, 98). Despite the lack of a sustained increase in total RBF, changes in distribution of blood flow toward the inner cortex and medulla following AP (108) have been observed. Doppler measurements do not support a causal relationship between papillary blood flow and AP-induced changes in sodium excretion (109, 110).

Lacking strong evidence to support a glomerular or hemodynamic explanation for the natriuresis, several investigators have focused their research on the direct inhibition of tubular reabsorption. Micropuncture studies of the proximal tubule and loop of Henle reveal no direct inhibitory effect on Na transport in these nephron segments in vivo (111, 112). In microdissected inner medullary collecting ducts, AP increased in cGMP levels (113). Inner medullary collecting duct cells exhibited decreased $O_2$ consumption when treated with AP or 8-bromo-cGMP, possibly secondary to inhibition of sodium transport (114, 115).

One difficulty that remains to be resolved regarding a far distal site of action for AP is the adequacy of sodium delivery to that nephron segment to

account for the magnitude of the natriuresis observed. Fractional Na excretion of 6–8% in anesthetized dogs has been produced by AP (98). It is generally believed that only about 4% of the filtered load of Na is delivered into the collecting tubule/duct system. Lacking any increase in distal delivery, either by increased GFR or decreased upstream reabsorption, it is difficult to understand the mechanism that might account for the magnitude of the natriuresis.

If AP is an important regulator of body fluid volume, its action must be ultimately reflected in a change in extracellular fluid volume. A chronic 7-day infusion of AP into cardiomyopathic hamsters with edema (116) did not cause a decrease in extracellular fluid volume. Furosemide, on the other hand, produced a large decrease in extracellular fluid volume in similarly treated animals. In another study in which AP was infused into spontaneously hypertensive rats for 5 days (117), arterial blood pressure was reduced but no changes were detectable in any of the extracellular fluid compartments. Similarly, it is difficult to reconcile the failure of the hormone to effect any of its putative actions in congestive heart failure (CHF). Atriopeptin levels are highest in CHF, yet the kidney retains most of the Na presented to it, and aldosterone, renin, and vasopressin levels are elevated (118). All of these should be suppressed by AP, yet none are. Receptors for AP may be down-regulated in CHF (119). Constriction of the thoracic inferior vena cava in dogs (i.e. an animal model of sodium retention) results in virtually total retention of Na by the kidneys and subsequent development of edema and ascites. During caval constriction, plasma AP levels are suppressed (120), which would be consistent with the maximal Na retention. Yet when the caval constriction is released and cardiac filling pressure rises, there is only a modest increase in plasma AP concentration, despite a large natriuresis (118).

## *Cardiovascular Effects*

AP has potent vasorelaxant activity, especially when tested in vitro in large conduit arteries (121). In smaller arteries (122), veins (123), and resistance vessels (124), AP has little or no relaxant effect. The only vascular bed that consistently demonstrates a reduction in resistance is the kidney, but this change is transient (125) and direct studies on renal resistance vessels (126) do not indicate a vasorelaxant effect in vitro. In vivo, however, afferent arterioles appear to dilate and efferent constrict (104). The mechanism of the vasorelaxation appears to involve activation of particulate guanylate cyclase (127). Unlike the nitrovasodilators that activate soluble guanylate cyclase and are inhibited by hemoglobin (128), AP action is not inhibited by hemoglobin and is potentiated by M&B 22,948, a cGMP-specific phosphodiesterase inhibitor. AP attenuates the rise in free intracellular Ca associated with norepinephrine or angiotensin II treatment of isolated rabbit aorta (129).

One of the more surprising aspects of AP action was the observation that the blood pressure lowering in conscious animals receiving a continuous intravenous infusion was not due to a decrease in peripheral resistance, but to a decrease in cardiac output (130, 131)). In fact, total peripheral resistance rose slightly. These data are consistent with the lack of vasorelaxant effect on resistance vessels. The mechanism of the decreased output is not negative inotropy or chronotropy (although heart rate frequently does not increase in association with AP-induced hypotension (131), but decreased venous return to the heart (132). Mean circulatory filling pressure, a measure of venous compliance, is not changed by AP administration, suggesting that venous pooling does not account for the decreased venous return (133). Venoconstriction may contribute to decreased venous return (134) by altering capillary absorption (132). This mechanism would result in partitioning of fluid into the interstitium due to a net increase in capillary pressure with resultant decrease in plasma volume. The observations of increased plasma protein concentration (132) and hematocrit (135) are consistent with this interpretation. The fact that protein concentration rises suggests that capillary permselectivity is not altered significantly.

AP has very little or no direct effect on cardiac function (e.g. dP/dt) even in animals with acute infarcts (136, 137). In both anesthetized (138, 139) and conscious dogs (140), AP induced a transient, balanced increase in coronary flow. AP induced modest reductions in vascular resistance in the coronary and renal beds and in the spleen, but not in blood flow to stomach, intestine, pancreas, liver or skeletal muscle (139).

An intriguing possibility for AP is a role in the development of hypertension and its potential usefulness as a therapeutic agent. The fact that AP lowers pressure, at least initially, by decreasing cardiac output does not argue strongly for its use to treat hypertension. A recent report shows, however, that after five days of continuous infusion into conscious sheep, the initial fall in cardiac output gives way to a decrease in total peripheral resistance (141). In various animal models of hypertension, including the spontaneously hypertensive rat (SHR), one and two kidney and DOC-salt hypertension, AP is an effective blood pressure lowering agent (99). Interestingly, AP is most effective in a volume expanded form of hypertension, DOC-salt, rather than vasoconstrictor types of hypertension (99). When infused continuously into SHR (142) or renal hypertensive rats (117), AP is an effective hypotensive agent for periods of up to 5–6 days. Whether AP can sustain a blood pressure reduction chronically remains to be determined.

## *Hormonal Interactions*

The most consistent finding regarding hormonal interaction of AP is its inhibitory action on aldosterone biosynthesis. First reported using atrial ex-

tracts on isolated zona glomerulosa cells (143), the fundamental observation of inhibition of basal and stimulated aldosterone biosynthesis has been repeatedly confirmed in vitro and in vivo using synthetic peptide (144). In vitro, AP blocks all of the known secretogogues for aldosterone biosynthesis, including angiotensin, ACTH, and potassium. However, the mechanism through which this inhibition is effected is not clear. Activation of guanylate cyclase does not account for the inhibitory action of AP on adrenal zona glomerulosa cells (144). Likewise, the inhibition is not accounted for by alteration in Ca fluxes, phosphoinositide metabolism, inhibition of protein synthesis or Na-K ATPase activity or cellular K depletion (145).

AP inhibits renin release not only to decreased renal perfusion pressure (146), but to isoproterenol and prostaglandin (147) as well, suggesting a fundamental action of AP to regulate renin secretion. The mechanism by which AP inhibits renin release is not clear, but the macula densa is not required, as evidenced by studies in the nonfiltering kidney (148). At the cellular level, cGMP appears important in the renin inhibitory mechanism. M&B 22,948, a cGMP-specific phosphodiesterase inhibitor, greatly potentiates the AP inhibitory action (149).

Despite the consensus that AP is involved in volume, and not osmolar regulation, there are important interactions between AP and vasopressin. The initial observation relating AP to vasopressin suggested a stimulatory action of AP on vasopressin release from the isolated posterior pituitary (150). Subsequently, in vivo studies have revealed an inhibitory action of AP on vasopressin release whether AP is administered intravenously (151), directly into the brain ventricles (152), or by direct microinjection of vasopressin containing cells in the hypothalamus (153) (see below for details). In addition to this interaction in the hypothalamus, AP has been purported to inhibit the vasopressin-induced increase in hydraulic conductvity in the renal tubule (154).

## ATRIOPEPTIN IN THE CENTRAL NERVOUS SYSTEM

### *Biosynthesis and Storage Forms*

APir is present in rat brain extracts at relatively low levels compared to the heart (about 5 ng in a rat brain, as opposed to 20 $\mu$g in a single atrium (155). In the rat and pig brain APir is found in both high molecular weight (presumably precursor) and low molecular weight (processed peptide) forms (155–158). The mRNA for AP precursor from the rat hypothalamus, cerebral cortex, and brainstem are identical to a cDNA for atrial AP precursor (11, 12).

Matsuo & colleagues (159) isolated a distinct species from pig brain extracts, termed porcine brain natriuretic peptide (pBNP). The synthetic

peptide was as potent as AP in rat diuretic-natriuretic and hypotensive bioassay. pBNP is closely related to AP; 17 of its 26 amino acids are identical (see Figure 1). Like AP, it contains an internal 17-amino acid ring that is formed by a cystine disulfide linkage. However, the amino acid substitutions are scattered throughout the pBNP sequence, and several of these are not convertible by single nucleotide substitutions. The brain pBNP system must be examined from a neuroanatomical and neurophysiological perspective, and compared with the AP system to evaluate its unique biological significance.

During dehydration, AP levels decrease in certain hypothalamic structures concerned with fluid and electrolyte homeostasis (e.g. the medial preoptic region and the supraoptic nucleus), but not in others (the paraventricular nucleus and median eminence) (151). Hypothalamic AP levels can also be depressed by high salt intake (155), but they rise during the development of hypertension in spontaneously hypertensive rats (160).

Although the levels of AP in the brain are 10,000 times less than in the heart (155, 161), the peptide is localized to a relatively small population of neurons in which it is present in sufficient quantity to allow immunohistochemical mapping. Several groups of investigators have reported the distribution of peptide immunoreactivity, and the results are in good agreement (20, 162–165).

## *Central Cardiovascular System*

The largest collection of APir neurons in the rat brain is found in the anteroventral periventricular nucleus, along the anteroventral margin of the third ventricle, a region implicated in the regluation of blood volume, pressure, and electrolyte composition, the same functions that AP controls as a systemic hormone (166). Additional APir neurons can be found extending posteriorly along the third ventricle and in the paraventricular and arcuate nuclei. APir axons can be traced from the periventricular region into the median eminence, where they densely innervate the external lamina, which contains the hypophysial portal vessels. Lesions of the paraventricular nucleus deplete AP in the median eminence by about 90%. However, adrenalectomy does not change the AP content of the median eminence, whereas it does increase the AP levels in the periventricular system. These observations suggest that the periventricular AP system contains at least two separate components that participate in regulation of fluid and electrolyte homeostasis.

The paraventricular nucleus also contains one of the densest APir terminal fields in the rat brain. APir fibers innervate not only the neuroendocrine components of this nucleus, but also a small-celled autonomic portion (167). A distinct population of larger APir neurons are found in the lateral hypothalamus in the perifornical region. These neurons contribute to the descending hypothalamic projections to the spinal cord (168) and the nucleus of the solitary tract (169).

In the brainstem, APir cell bodies are found in parts of the parabrachial nucleus and the nucleus of the solitary tract involved in central cardiovascular regulation (170). Some of these APir neurons project to the hypothalamus (20, 167). Both contain APir terminal fields, and some of the APir neurons in these structures may be interneurons.

Another, somewhat larger population of APir cell bodies in the brainstem is found in Barrington's nucleus which innervates the parasympathetic preganglionic column in the sacral spinal cord (171) and is responsible for coordinating micturition reflexes (172). Barrington's nucleus is also the site of a major APir terminal field, probably originating in the anteroventral periventricular nucleus (173, Saper, unpublished observations).



## *Brain Receptor Distribution*

The greatest AP radioligand-binding activity is found in the circumventricular organs and choroid plexus (174–176). AP binding is also present in certain parts of the brain that receive substantial AP innervation, such as the paraventricular and supraoptic nuclei, median eminence, medial habenular nucleus, and nucleus of the solitary tract (174, 175). AP binds in structures such as the olfactory bulb and cerebellum (174, 175, 177), where it has not been possible to identify APir fibers (165). Quirion & colleagues (174, 177) report dense AP receptor populations in the cerebellum of the monkey and the guinea pig (species where AP immunoreactivity has not been mapped), but Saavedra (175) does not find AP receptors in the cerebellum in the rat.

AP-binding sites in the rat brain are clearly responsive to physiological manipulation of fluid homeostasis. Dehydration, caused either acutely by dehydration or chronically in Brattleboro rats with a genetic deficiency of vasopressin, shows elevated levels of AP binding in the subfornical organ (175, 178). This structure, like other circumventricular organs, lacks a blood brain barrier, and thus is exposed to circulating AP as well as other cardiovascular hormones, such as angiotensin (179). Spontaneously hypertensive rats and prehypertensive Dahl salt-sensitive rats, by contrast, have lower levels of AP binding in the subfornical organ, area postrema, and nucleus of the solitary tract (175, 176, 180, 181). These observations suggest that AP receptors in these structures are physiologically regulated during alterations in fluid homeostasis. However, the locations of these binding sites in or adjacent to circumventricular organs leave open the possibility that they may be monitoring circulating, rather than brain, AP.

## *Function*

REGULATION OF VASOPRESSIN SECRETION Systemically, AP and vasopressin have diametrically opposing effects in most tissues (154). In the brain, APir fibers innervate the vasopressin cell groups in the hypothalamus (12,

165). Intracerebroventricular (icv) administration of AP has a diuretic effect (182–185), which is due at least in part to inhibition of vasopressin secretion (151, 152, 186, 187).

Injections of AP into the third ventricle and incubation of hypothalamic-pituitary explants with AP demonstrate that the peptide works at a hypothalamic level to regulate vasopressin secretion (186). In contrast, incubation of pituitary-neural lobe explants with doses of AP in the physiological range (up to $10^{-7}$M) did not affect AP release. Recordings of the activity of individual vasopressin neurons during the application of AP show that dosages of less than 5 femtomoles alter the duration of the bursts of firing, as well as the interburst interval, without affecting the firing rate during the bursts (153). Larger doses completely suppress the firing of vasopressin neurons. AP also inhibits the firing of other hypothalamic neurons (188–190) such that it may influence the secretion of vasopressin through the agency of interneurons as well.



REGULATION OF DRINKING AND SALT APPETITE Injection of AP icv suppresses spontaneous or dehydration-induced drinking (191–194). Even the drinking that is caused by intravenous or icv administration of angiotensin II can be inhibited by icv injection of AP (191, 193, 194). Conversely, icv injection of AP antiserum can induce spontaneous drinking (195).

Salt intake can be suppressed in rats by icv injection of AP (182, 196). Injection of icv AP also causes vagal stimulation of gastric acid secretion (197), which may affect the digestion of salt and other solutes that play an important role in fluid and electrolyte homeostasis.

## ATRIOPEPTIN EXPRESSION IN THE CARDIAC VENTRICLE

Pharmacologic doses of dexamethasone to rats can result in exaggerated AP synthesis and release by the atria, and synthesis of AP mRNA in the ventricle, lung, and pituitary (198). Treatment with dexamethasone produced a two- to three-fold increase in left ventricular AP and AP mRNA that were not affected by prior adrenalectomy (198a).

Ventricular hypertrophy is characterized by an increase in tissue mass due primarily to exaggerated protein and RNA synthesis and can be induced by pressure or volume overload of the heart. Ventricular tissue AP and AP mRNA levels increase in several experimentally and pathologically induced models of ventricular hypertrophy. The site and extent of AP synthesis correlates well with the site and extent of hypertrophy. Pressure overload produced by banding the abdominal aorta in rats results in left ventricular hypertrophy and a two- to three-fold increase in AP and AP mRNA in the left but not right ventricle (36). Combined aortic banding and dexamethasone

treatment produced a five-fold increase in left ventricular AP and AP mRNA. These interventions had no effect on AP expression in the right ventricle. Immunocytochemical staining for AP demonstrated profuse granular staining throughout the left ventricular myocardium, but none in the right ventricle. Purification of left ventricular tissue AP revealed that the major form is the 126-amino acid prohormone.

Right ventricular pressure overload produced by hypoxic pulmonary hypertension leads to substantial induction of right ventricular AP and AP mRNA (199). Rats exposed to a 10% oxygen environment for three weeks developed a nine-fold increase in right ventricular AP and a profound 160-fold increase in AP mRNA. Accumulation of AP in the right ventricle followed the time course of development of ventricular hypertrophy, and tissue concentrations of AP rapidly fell to control levels following return to normoxic conditions. Hypoxia has also been shown to stimulate AP release both in vivo (200) and in vitro (62). This suggests that AP may modulate pulmonary blood flow in hypoxic pulmonary hypertension, and myocardial hypoxia may be an important mechanism in ventricular AP expression.

Several models of acute and chronic volume overload in the rat induce ventricular AP expression, as determined by relative changes in tissue AP mRNA concentrations (201). The most striking induction of ventricular AP expression occurred in the aortocaval fistula, with an 11-fold increase in AP mRNA, and was associated with the most pronounced degree of ventricular hypertrophy (202).

Ventricular AP expression has been described in spontaneous and salt induced systemic hypertension in the rat. Ventricular AP levels are five-fold higher in the 15-week old spontaneously hypertensive rat (SHR), than in the corresponding normotensive Wistar Kyoto rats (203). The cardiac ventricle in the Dahl salt sensitive (DSS) rat also contains substantial AP and AP mRNA following chronic salt induce hypertension compared to the corresponding Dahl salt resistant strain (204). The major portion of AP in the ventricle represents the prohormone form. In contrast, the AP and AP mRNA levels in the atria are not affected.

The participation of the ventricle in AP expression in animal models of spontaneous ventricular hypertrophy and cardiomyopathy has been described. A subpopulation of Wistar Kyoto rats that develop spontaneous biventricular hypertrophy have a marked accumulation of AP and AP mRNA in both ventricles, with a six-fold increase in AP mRNA in the left ventricle. Atrial AP mRNA and AP were not significantly different from controls (205). The cardiomyopathic hamster, a model of spontaneous cardiomyopathy and congestive heart failure, has been extensively studied (206–208). In this model, plasma AP concentrations are markedly increased and correlate with the degree of congestive failure. Ventricular AP (primarily as prohormone) and AP mRNA increase substantially and correlate with the severity of cardiac

insufficiency; in severe congestive failure, the ventricle contains 15 times more AP and AP mRNA than do control ventricles. In severe congestive failure, the prohormone is detectable in the plasma, suggesting a defect in post-translational processing.

The cardiac ventricle also demonstrates AP expression in humans with congestive heart failure. Patients with dilated cardiomyopathy have immunoreactive AP granules within right and left ventricular myocytes, predominantly within the subendocardium (209). AP and AP mRNA in normal human ventricular tissue are detectable by immunoassay and Northern blots (209, 210). In patients with dilated cardiomyopathy, the total AP mRNA and immunoreactive AP content in the ventricle is 40–80 times greater than controls, and approximately 30% of that present in the cardiomyopathic atria and 85% of that present in control atria.

The stimulus for AP expression in the ventricle is not known. Hypertrophic and neonatal ventricular tissue possess similar ultrastructural characteristics including the reappearance of secretory-like granules (211). In addition, adult hypertrophic ventricular myocardium reexpresses fetal forms of actin (212) and mysoin (213). This suggests that ventricular AP expression in hypertrophy may represent a regression of the ventricular myocyte to an earlier developmental form with reexpression of the fetal phenotype. Intracellular hypoxia produced by myocyte stretch may represent the underlying stimulus for AP expression in the ventricle. The cardiac ventricle may synthesize, store, and release AP to decrease preload and afterload and ultimately reduce the metabolic demands of the ventricular myocardium.

Although it appears that the cardiac ventricle is capable of storing only a limited amount of immunoreactive AP, the potential for sustained synthesis and release is substantial. The total mass of ventricular tissue capable of expressing AP is about ten times greater than the mass of the atria. The total mass of AP mRNA expressed in the hypertrophic ventricle may approach one third to one half that present in the atria (36). Ventricular AP may participate in the maintenance of fluid, electrolyte, and blood pressure homeostasis during chronic hemodynamic overload. The association between hypertrophy and ventricular AP expression suggests that AP may have a local effect modulating the development of ventricular hypertrophy.

## WHAT'S ON THE HORIZON?

Numerous unresolved issues need further investigation. Division of the sequence of events in the production of AP and its response would include: synthesis, storage, release, processing, metabolism, receptors, response, and intracellular mediation. In each area a prominent question remains.

The regulation of *biosynthesis* has not yet been addressed. While we now have evidence that the 5'-terminal of the AP mRNA contains the cardiac

targeting signal, little is known about the endogenous events or proteins initiating or suppressing AP biosynthesis. Surely this is an actively regulated process as evidenced by the virtually instantaneous suppression of AP biosynthesis of the rat ventricle at birth or its rapid activation during hypertrophy.

The primary recognized stimulus for AP *release* is atrial wall stretch. How does local stretch control granular exocytosis? Are ion channels involved? Are there intrinsic mediators of stretch? Emerging data suggest that stretch simultaneously releases AP and mobilizes intracellular calcium. However, in isolated atrial myocytes, calcium appears to be a negative modulator by an unresolved mechanism. While progress is being made in the isolation and purification of a cardiac *processing* enzyme that could selectively cleave AP-126 at pro-arg$^{99}$ to release AP-28, the cellular type or site involved has not yet been identified. Is the enzyme localized in the granule, or on the surface of the atrial myocyte, or possibly on the adjacent cardiac mesenchymal cell?

Once AP-28 is released into the circulation, it apparently binds to at least two *receptor* sites, only one of which is coupled to guanylate cyclase. While the only current suggestion for the uncoupled site is for clearance and inactivation, another plausible and attractive possibility is that the second site subserves as yet unidentified actions of AP. The enzymatic *metabolism* of AP has been characterized—but the effect of the simultaneous blockade of metabolism and of the "clearance" receptors on the duration and potency of exogenous and endogenous AP needs investigation. Of course, we all await the discovery of a selective AP receptor site antagonist and must currently rely on passive or active immunization or the less selective use of heparin to interfere with AP biological effects.

Finally, the identification of two more unique but closely related natriuretic peptides, i.e. *pBNP* and iso-rANP in the brain and heart, respectively, opens a vast array of possibilities. Teleologically, it seems illogical to utilize separate genes to produce peptides with the same biological activity. We predict distinctly novel distributions and activities will be discovered for the newly characterized peptides which will be especially influential in the study of the physiology and pharmacology of this growing family of peptides.

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