Atriopeptins as Cardiac Hormones

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Numerous observations have implicated humoral factors in the homeostatic regulation of fluid and electrolyte balance. This review will focus on a recently discovered peptide that is synthesized and stored in cardiac atrial myocytes and exerts potent, selective, and transient biological effects. Characterization of the cardiac substance has shown that it is not related to endogenous Na₉,K-ATPase inhibitors, which may also act as natriuretic hormones. The atrial peptide is a potential hormonal mediator of changes in body fluid composition, extracellular volume, and systemic blood pressure. An earlier review describing the initial isolation and chemical and biological characterization of the atrial peptides has been published (see ref. 2).

Morphological and Physiological Evidence for Atrial Participation in Fluid and Electrolyte Balance

Early ultrastructural studies of mammalian atrial myocytes suggested that these contractile cells may also have a secretory function. Auricular myocytes, from humans and other species, possess spherical osmophilic granules formed in the Golgi apparatus. These granules are not present in mammalian ventricular muscle fibers; however, morphological examination of nonmammalian vertebrates has revealed the presence of granules in ventricular, as well as atrial, cardiocytes.

The suggestion that the atria are involved in water and electrolyte homeostasis was based on the observation that the granularity of rat atrial myocytes was decreased by sodium loading and that both sodium restriction and water deprivation caused increased granularity. The critical role for an atrial substance was demonstrated with the discovery that atrial, but not ventricular, extract exhibited a natriuretic-diuretic effect when injected into assay rats. This natriuretic-diuretic bioactivity of the crude atrial extract copurified with the atrial specific granules. Recent immunocytochemical studies have demonstrated that the biologically active peptides are localized in the atria and specifically within the atrial granules.

Anatomical and physiological studies demonstrate that the atria are sites of low pressure baroreceptors involved in the regulation of plasma volume, which is consistent with the proposal that the atrial myocytes are endocrine cells involved in the regulation of fluid and electrolyte balance. Stimulation of the atrial volume receptors by distention causes diuresis, bradycardia, hypotension, and decreased systemic vascular resistance. The clinical literature contains documentation of patients that exhibit polyuria associated with elevated atrial pressure occurring during paroxysmal atrial tachycardia or fibrillation. The simplicity of a cardiac volume-pressure detection system coupled to a potential endocrine hormone that regulates renal fluid and electrolyte transport and systemic vascular resistance is readily apparent. Thus, increased plasma volume has been proposed to stimulate the atrial receptors to release atrial peptide into the circulation, and subsequently activate target organs, such as kidneys, adrenal cortex, and vasculature, to reduce the plasma volume. The link between these effects of atrial distention and the bioactive atrial peptides remains to be established.

Peptide and Gene Structure Elucidation

Assay of Biological Activity

When injected into intact rats, extracts prepared from rat atria produce marked increases in urinary sodium excretion and in urine volume. This seminal observation by deBold and colleagues provided the
primary bioassay system for measurement of atrial peptide activity and served as the stimulus for efforts to isolate and identify the active species. Shortly thereafter, we recognized that atrial (but not ventricular) extracts produced a relaxation of isolated vascular (rabbit thoracic aorta) and intestinal smooth muscle (chick rectum) preparations. The use of an in vitro assay system thus afforded a much more rapid and quantitative bioassay, which markedly facilitated purification of these peptides. It was noted that (unlike the qualitative and relatively nonspecific in vivo rat diuresis-natriuresis assays) relaxation of aorta required the presence of the Phe-Arg carboxy-terminal moiety. All the on the other hand, chick rectum was unaffected by the high molecular weight atrial peptides, and partial proteolysis (i.e., by pretreatment with low concentrations of trypsin or kallikrein) was required for maximal intestinal spasmodic activity. The in vivo natriuresis assay would not discriminate between the high and low molecular weight fractions of atrial extracts of the atrial peptides.

Isolation and Purification of the Atrial Peptides

A variety of methods typically employed for small peptides have been used for extraction and purification. Extraction of atrial tissue has most often been performed with 1 M acetic acid (alone or supplemented with 20 mM HCl, 1 M HCl-1% NaCl, or peptidase inhibitors such as ethylenediaminetetraacetic acid, pepstatin, phenylmethylsulfonyl fluoride). The one exception has been the employment of phosphate buffered saline with pepstatin and phenylmethylsulfonyl fluoride. Purification has consisted of an initial brief heat step (100 °C), followed by adsorption to C-18 silica (ODS) columns, gel chromatography (Sephadex G-50, G-75, or Biogel P-10) in acetic acid, ion exchange (i.e., SP-Sephadex) and reverse phase high-performance liquid chromatography on C-8 or C-18 columns.

Structures of the Atrial Peptides

From the efforts of numerous laboratories a variety of pure atrial peptides have been obtained from rat and human tissue, their amino acid sequences established, and the structures of putative precursor peptides elucidated by complementary DNA (cDNA) sequencing. From rat atrial tissue a large variety of peptides have been obtained ranging from 21 to 126 amino acid residues in length. From human tissue only three distinct peptides, consisting of 28, 56, and 126 residues respectively, have been detected. The β (56 amino acid) human peptide has the remarkable structure of a dimer of 850 to 900 bp in poly(A) + RNA from atria, while no message was detected in ventricle or liver. Message obtained by cleavage at Ala (residue 24 in the rat or 25 in the human putative signal sequence). Interestingly, the N-terminal sequences of these 126 residue peptides closely resemble the 30 residue sequence reported for cardiogliatrin, an M750 peptide isolated from porcine atrial tissue.

It is tempting to suggest that the multiplicity of atrial peptides isolated from the rat compared with the few detected in human tissue may indicate differences in cellular processing in these two systems. Alternatively, the truncated peptides obtained may have been generated by proteolysis during the extraction procedure. Resolution of this matter must await elucidation of the steps involved in biosynthesis and processing and of the structure of secreted forms of the atrial peptides.

Gene Studies of the Atrial Peptide

Following purification and sequencing of the atrial peptides, it has been possible to isolate cDNA and genomic copies of the gene encoding the precursor polypeptide. The cDNA cloning of the rat and human genes has been reported in a number of nearly simultaneous papers. The genes were isolated from cDNA libraries prepared from atrial poly(A) + message RNA (mRNA), which were screened by colony hybridization using synthetic oligonucleotide mixtures deduced from the peptide sequence. For both the rat and the human peptides, the least degenerate 14 nucleotide probe mixtures corresponded to the sequences Ser-Cys-Phe-Gly-Gly and Met-Asp-Arg-Gly respectively. Positive candidates from the initial screening were probed with a second oligonucleotide mixture corresponding to a different region of the hormone, and clones positive in both screens were sequenced. In both cases, full-length (750-850 base pairs) cDNA copies of the mRNA were obtained. The cDNAs for the precursor (APG) molecules of 152 amino acids in rat and 151 amino acids in human (see Figure 1) are very similar: peptide and nucleotide homology is 85% between the two species. Each pre-atriopeptigen (pre-APG) is characterized by a hydrophobic leader peptide at the N-terminus with an attendant leader peptidase cleavage site. The active hormone is located at the extreme C-terminal end. In rat, the nucleotide sequence predicts the sequence Tyr-Arg-Arg before a stop codon, while the human sequence predicts that the C-terminal Tyr is followed by a termination codon, apparently the result of a single nucleotide change in the first Arg codon. The peptide sequences between the leader and the biologically active hormone exhibit striking conservation, suggesting the possibility of other activities distinct from the atriopeptins expressed at the C-terminus. However, though APG and various high molecular weight molecules have been isolated, no functional role for the N-terminal fragment has yet been described.

The cDNA clones for APG have been useful as probes for examining the level of gene expression. Northern analysis revealed a single, abundant message of 850 to 900 bp in poly(A) + RNA from atria, while no message was detected in ventricle or liver. Message...
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10 20

Human Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phc Leu Leu Leu Ala Phe Gin Leu Leu Gly Gin Thr Arg Ala Asn Pro Met
Mouse Gly — Ile Leu Gly Phe Val Trp Pro His Ile Gin Thr Val
Rat Gly — Ile Lys Gly Phe Val Trp Pro His Ile Gin Thr Val

30 40 50

Human Tyr Asn Ala Val Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu Glu Lys Met Pro Leu Glu Asp Glu
Mouse Ser Thr Val Thr
Rat Ser Thr Val

60 70 80

Human Val Val Pro Pro Gin Val Leu Ser Asp Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val Pro Pro Trp Thr
Mouse Met Ala Glu Gin Thr Ser
Rat Met Ala Glu Gin Thr Asp Ser Ser

90 100 110

Human Gly Glu Val Ser Pro Ala Gin Arg Asp Gly Gly Ala Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
Mouse Asn Pro Leu Ser Ser Arg Ser
Rat Asn Ser Pro

120 130

Human Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly
Mouse Ala Gly
Rat Ala Gly

140 150

Human Ala Glu Ser Gly Leu Gly Cys Asn Ser Phe Tyr
Mouse Arg Arg
Rat Arg Arg

FIGURE 1. A comparison of the complete amino acid sequences of rat, human, and mouse pre-atriopeptigen. The complete sequences are those predicted from cDNA nucleotide sequencing, human 25 and rat. Residues 1-25 compose the putative signal sequence. Residues 26-151 (atriopeptigen) represent the ordered sequence of isolated peptides. The rat peptides: residues 128-148 (atriopeptin IAP), 128-150 (AP I), 128-151 (AP II), 124-151 (cardionatrin) 30-31; 127-151 (Arg-AP III) 32-33; 119-151 34; 104-126 rat atrial natriuretic peptide (ANP) 5; 79-151 (atriopeptigen) 26-151. The human peptides: residues 124-151 (human ANP) 5; 26-151. Levels were also examined in water-deprived rats and showed a decrease in atriopeptigen expression that correlated with the length of deprivation. 42

Using cDNA probes, Southern analysis revealed a single band in restriction digests of genomic DNA, which suggests that pre-APG is encoded in a single gene. The pre-APG sequence is localized on three exons (Figure 2). Exon 1 carries the sequence corresponding to pre-APG 1-41 (1-40 in mouse). Exon 2 carries the sequence 42-150 (41-149 in mouse), and the C-terminal Tyr (Tyr-Arg-Arg) sequence is on exon 3, which is separated by a large intervening sequence. While the genomic structures are typical of eukaryotic genes, it is quite unique for a single amino acid and termination codon to be spliced from a separate exon. Whether this structure is related to the sequence requirements for splicing of the RNA or is a method of retaining a residue essential for biological activity remains to be determined. It has been shown that Des-Tyr analogues of atriopeptin are fully active in vitro and in vivo bioassays.

The next step in genetics experiments will be to elucidate the control mechanism responsible for the specific expression observed in the atrium and to explore expression in other tissues, particularly in brain. Using genetic probes, it will also be interesting to determine whether this structure is involved in regulating expression levels in the atrium.
to investigate the possible role in disease states of defects in the APG gene. The cloned genes will also be useful in preparing larger quantities of APG for studying its biological processes.

**Nomenclature**

A number of low molecular weight peptides having natriuretic-diuretic and vasodilatory activities have been isolated from atrial extracts and their structure established (as indicated above). Each group of investigators has assigned its own name to the peptide they isolated. It is now clear that all of the active peptides must arise from a single high molecular weight precursor. To allow comparison of the various peptides described in the literature, we have selected a common nomenclature pattern for this review. We have focused on the 24 amino acid peptide with the sequence Ser-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr. We have named this material atriopeptin III (AP III) because of its source and its varied biological activity (i.e., it is not just a natriuretic hormone). The legend for Figure 1 indicates the structure and the sequence of the various low molecular weight atrial peptides that have been described. For convenience, and to facilitate comparisons, the 28 amino acid peptide reported by Flynn, deBold, and deBold as cardionatin will be referred to as Ser-Leu-Arg-AP III, and the 25 amino acid peptide named auriculin will be referred to as Arg-AP III.

**Renal Effects of Atrial Natriuretic Factors**

**Solute and Water Excretion**

Numerous studies have corroborated the initial observation that atrial extracts possess natriuretic and diuretic activity. Sodium excretion was observed to rise sixfold to 90-fold, and urinary volume increased 12- to 30-fold; this variability was related to baseline excretion values and concentrations of extract administered. Chloride and osmolar excretions were found to parallel that of sodium. Calcium excretion increased sixfold to 10-fold. The duration of action was transient: increases in urinary volume and electrolyte excretion occurred within 5 minutes after injection and returned to baseline by 20 minutes. The atrial extracts were effective in awake and anesthetized animals, in water-deprived and salt-deprived animals, and in those with 1.5 to 10% volume expansion.

Numerous low molecular weight peptides purified from atrial extract produce a quantitatively similar natriuresis and diuresis. Their potencies are variable; however, those peptides containing the C-terminal amino acids Phe-Arg-Tyr convey the greatest potency (discussed further in the section Atriopeptin Structure-Activity Studies). A high molecular weight precursor also exhibits natriuretic properties.

Prolonged infusion, a technique that reduces the artifacts produced by rapid changes in urine flow, into intact dogs or rats has provided data during steady state influences of atrial peptides. Infusion of Arg-Arg-AP III into the renal artery of dogs increased urinary volume and sodium, potassium, lithium, and phosphate excretion. Intrarenal infusion of AP III increased electrolyte excretion and volume in a dose-responsive manner. The effects of Arg-AP III administered intravenously were similar. Atrial extract infused intravenously into rats also produced a diuresis and a natriuresis.

**Effects of Atriopeptins on Regulators of Sodium and Water Excretion**

**Glomerular Filtration Rate**

Changes in glomerular filtration rate (GFR) are known to affect Na+ excretion. Assuming that plasma Na+ concentration remains stable, an elevation of GFR increases the amount of Na+ filtered and thus delivered to the tubule. Unless conditions promoting avid tubular Na+ reabsorption prevail, increased Na+ is excreted.

In rats administered bolus atrial extract, GFR was not observed to change. During constant infusion of atrial extract, however, GFR measured at stable urine flow rate increased. Similarly, constant infusion of synthetic peptides into intact dogs or rats has produced a sustained increase in GFR, which is consistent with the finding observed when partially purified extract is infused into isolated perfused kidneys.

Microperfusion studies revealed no increase in single nephron GFR (SNGFR) when low doses (=3 atria equivalents) were infused, but at larger doses (6 atria equivalents) both SNGFR and total kidney GFR rose. Increases in GFR with subsequent increased delivery of Na+ to the distal tubule usually are associated with a subsequent decrease in GFR, the so-called tubuloglomerular feedback. In microperfusion experiments, the increased distal Na+ delivery during atrial extract infusion was not associated with a reflex decrease in SNGFR. Thus, it would appear that atrial extract inhibits tubuloglomerular feedback, which allows an increased filtered load of Na+ to persist. Therefore, the increase in GFR, at least with high doses, and the inhibition of tubuloglomerular feedback appear to contribute to the natriuresis seen with atrial peptides.

**Renal Blood Flow, Filtration Fraction, and Renal Vascular Resistance**

Atrial peptides are selective renal vasodilators when tested at low dose. However, the data on the effects of both crude extract and synthetic peptides on renal blood flow are conflicting. Bolus administration of prolonged infusion of atrial extract produced either no change or an increase in renal blood flow. Infusion of Arg-Arg-AP III into dogs caused a transient increase or no change in RBF. In contrast, AP III infused into anesthetized dogs produced a dose-dependent increase in renal blood flow. The latter study employed doses up to sevenfold larger than the former, and renal blood flow during control periods was comparatively low, which suggests an intense
renal vasconstriction probably resulting from the anesthesia and the acute surgical trauma, both of which would induce catecholamine and renin release. Others found that in the presence of renal vasconstriction produced by angiotensin II, atrial extract reduced perfusion pressure and vascular resistance in the isolated perfused kidney. 66, 67

In all studies in which it was measured, filtration fraction rose after atrial peptide administration irrespective of whether renal blood flow changed. 39, 57, 62-4, 67 An increase in filtration fraction produces an increase in peritubular capillary oncotic force, a factor promoting maintenance of glomerular tubular balance by increasing the reabsorption of the increased filtered Na⁺ load. 69 Therefore, although the increases in GFR produced by atrial peptides likely contribute to the observed natriuresis and diuresis, the coincident increases in filtration fraction may partially offset the effects of the increased GFR.

**Medullary and Papillary Blood Flow**

Renal vasodilation has been proposed to cause an increase in urinary sodium excretion by dissipating the medullary hypertonicity (‘medullary washout’). 70 Studies of drug-induced vasodilation have shown that, in addition to medullary washout, the natriuresis depends on an increase in distal delivery of sodium, 71 an increase in papillary plasma flow, 72 or an increase in interstitial hydrostatic pressure. 73

Analysis of renal blood flow distribution and papillary plasma flow in the rat before and after atrial extract showed that total renal blood flow rose after extract and that a greater percentage was distributed to the inner cortex. 46 The associated increase in urinary sodium excretion correlated significantly with the calculated increases in inner cortical blood flow. Similarly, papillary plasma flows were increased after atrial extract injection. In contrast, studies in dog 62 found that total renal blood flow fell throughout infusion of Arg-Arg-AP III, after a transient rise, and no increase in interstitial hydrostatic pressure was detected. Most recently, in experiments in trained, conscious dogs renal blood flow redistribution was measured with microspheres during administration of atriopeptins I and III. 74 Atriopeptin I, the 21 amino acid peptide lacking the Phe-Arg C-terminal residues, produced no changes, in either total renal blood flow or distribution to four cortical layers; however, AP III increased total renal blood flow briefly and prompted a significant redistribution of cortical blood flow toward the superficial (zone 1 96% increased) and midcortical regions (zone 2, 199%; zone 3, 139%). The blood flow in zone 4, the juxtamedullary region, did not change significantly. These data would suggest that blood flow is shunted away from the salt-retaining nephrons.

**Atriopeptin Inhibition of Tubular Reabsorption**

Atrial peptides may exert part of their renal effect by altering the proximal tubular reabsorption of solute and water. The urinary excretion of lithium, a marker of proximal sodium reabsorption, rose twofold to fivefold during Arg-Arg-AP III infusion. 62 Similarly, atriopeptin infused into thyroparathyroidectomized rats produced a sixfold increase in phosphaturia, as well as a doubling in the fractional excretion of bicarbonate. The renal cortical brush border membrane vesicles from these rats exhibited a reduced Na⁺ gradient-dependent uptake of phosphorus and Na⁺H⁺ exchange in the presence of atriopeptin. 75 Administration of Ser-Leu-Arg-Arg-AP III to dogs with severe sodium retention did not increase urinary Na⁺, but a significant kaliuresis was observed, 76 which is consistent with an increased delivery of Na⁺ from the proximal tubule to distal sites where Na⁺ is exchanged for K⁺. On the other hand, micropuncture studies have refuted the idea of a direct inhibition of proximal reabsorption, since neither atrial extract 47, 52 nor Arg-AP III 63 reduced proximal fluid and Na⁺ reabsorption of superficial nephrons of rats. However, this finding does not negate the possible inhibition of reabsorption in juxta-medullary nephrons not accessible by micropuncture.

**Renal Mechanisms of Atrial Peptide-Induced Natriuresis**

The GFR appears to be the major mechanism initiating natriuresis. In many studies GFR rose 50 to 100%, 61-63, 66, 67 and the filtered load of Na⁺ increased proportionally. A recent micropuncture study in which Arg-AP III was infused indicated that the fractional reabsorption of Na⁺ was appropriate for the degree of filtered load of Na⁺ produced by the peptide-induced elevations in GFR. 63

The mechanisms for the increased GFR are unknown. Changes in renal blood flow are unlikely to be causally related, since they rise either transiently or not at all. 50, 51, 62, 63 while GFR remains comparatively elevated. Furthermore, the natriuresis associated with increased renal blood flow during drug-induced vasodilation occurs only when interstitial hydrostatic pressure rises, a finding not observed with atrial peptides. 62 Atrial peptides may produce an increase in GFR by increasing glomerular capillary permeability or by causing preferential afferent arteriolar dilation, thereby increasing hydraulic pressure in the glomerular capillary.

It is unlikely that increased GFR is the sole mechanism causing the atrial peptide–induced natriuresis. Some studies have detected no change in GFR while sodium excretion increased. Further, GFR tends to fall throughout atrial peptide infusion and falls before natriuresis abates. It has been suggested that the observed increases in medullary or papillary blood flow might influence the natriuresis. 33, 46, 52 The association of increased GFR and increased medullary or papillary blood flow may produce a greater natriuresis than either mechanism alone. 71

Atrial peptides appear to have direct tubular actions as well. 62 The increase in lithium and phosphate excretion and the alteration in Na⁺-dependent phosphate transport caused by atriopeptin suggests a proximal tubular effect. It would seem likely, however, that under most euolemic or volume-expanded experimental conditions proximal rejection of Na⁺ plays a
lesser quantitative role in atrial peptide–induced natriuresis, while increases in GFR influence natriuresis more profoundly. Suggestions have been made that atrial peptides reduce Na⁺ reabsorption in distal segments, such as the medullary collecting duct. In the water-deprived animal, in which any increase in distal delivery of Na⁺ would be expected to be avidly absorbed in the distal nephron, natriuresis and diuresis are observed. Finally, increased cyclic GMP levels were observed in glomeruli, ascending limb, and collecting duct fractions of dog kidney when they were incubated with atriopeptin, which suggests that these regions are specific target areas for atrial peptide recognition and response.

Atriopeptin Inhibition of Aldosterone and Renin Secretion

Crude atrial extracts as well as synthetic atriopeptins were found to inhibit basal, adrenocorticotropic hormone–stimulated, and angiotensin II–stimulated aldosterone production from isolated rat adrenal zona glomerulosa cells. The status of sodium balance in the rat from which cortical cells are prepared can determine whether basal aldosterone production will be affected by atriopeptin. Sodium-depleted rats, but not control rats, yield cortical cells with an elevated aldosterone production that is inhibited by atriopeptins.

The action of atriopeptins on adrenocorticotropic hormone–stimulated aldosterone production does not appear to be at the level of receptor blockade. Atriopeptin inhibited the steroidogenic actions of both potassium and dibutyryl-cAMP on bovine adrenal zona glomerulosa cells. Both of these agents exert their steroidogenic actions at steps distal to hormonal receptors. Atriopeptins inhibited both basal and angiotensin II–stimulated pregnenolone accumulation (i.e., the early pathway in steroidogenesis). The production of aldosterone from exogenous progesterone and exogenous 25-hydroxycholesterol (i.e., the late pathway) was either inhibited or unchanged by the addition of atriopeptin.

Corticosterone production in zona fasciculata cells was unaffected by atriopeptin. The effect of changes in aldosterone levels on renal sodium handling is not observed for 30 to 60 minutes, yet the natriuresis induced by atrial peptides begins within 5 minutes of injection or infusion. Thus, it seems unlikely that plasma aldosterone is involved in the acute natriuresis stimulated by atrial peptides. It is tenable, however, that the diminution in aldosterone levels in chronic volume expansion might be, at least in part, mediated by atrial peptides.

Atrial peptides may exert a more pronounced effect on renin and aldosterone secretion in conditions of elevated baseline levels. Indeed, Arg-AP III decreased plasma aldosterone in the 2-kidney, 1 clip hypertensive rat (i.e., high renin) sensitive to saralasin. In a dog model of severe sodium retention with ascites exhibiting high plasma renin activity and aldosterone levels, which was induced by inferior vena cava constriction, infusion of Ser-Leu-Arg-Arg-AP III produced parallel suppression of renin and aldosterone concentrations. Similarly, the elevated renin levels of the 2-kidney, 1 clip rat were diminished by long-term (7-day) infusion of Arg-Arg-AP III.

Effects of Atriopeptin on Blood Pressure

Injection of crude atrial extracts into rats produced a profound natriuresis and diuresis and lowered arterial blood pressure. In contrast, others have reported little or no change in blood pressure following injection of atrial extract in spite of large natriuretic and diuretic responses. Such experiments are complicated by contamination of the crude atrial extract with other biologically active compounds that could have pressor or depressor activities. The discovery that atrial extracts possessed spasmolytic activity in vitro raised the possibility that the hypotensive actions of these extracts could be due to a direct effect on the vasculature in addition to the known effect on fluid volume.

The ability of atrial peptides to produce a vasodepression is enhanced when the animal is anesthetized or has undergone sinoatrial denervation. The presence or absence of an intact baroreceptor reflex may account for some of the discrepancy between laboratories. The discovery that atrial extract in spite of large natriuretic and diuretic responses. The ability of atrial peptides to produce a vasodepression is enhanced when the animal is anesthetized or has undergone sinoatrial denervation. The presence or absence of an intact baroreceptor reflex may account for some of the discrepancy between laboratories. Specific binding to rabbit kidney cortex revealed two types of atriopeptin binding sites with apparent affinities of 0.05 nM and 470 nM. Binding to both the aorta and kidney membranes was reversed both the hypertensive and elevated plasma renin levels in two-kidney, one clip rat. Whether or not the antihypertensive effects of the atriopeptin were due to its spasmolytic effects on vascular smooth muscle or partially due to a suppression of renin levels is not clear.

Atriopeptin Receptors

A single class of atriopeptin–specific binding sites have been reported on rabbit aorta membranes. These binding sites have a density of 96 fmol/mg of protein and an affinity of approximately 0.1 nM using Leu-Ala-Gly-Pro-Arg-AP III as the iodinated ligand. This affinity corresponds well with the inhibitory concentration, 50% (IC₅₀), value for relaxation of this tissue. Specific binding to rabbit kidney cortex revealed two binding sites with apparent affinities of 0.05 nM and 490 nM. Binding to both the aorta and kidney membranes was not displaced by biologically inactive atriopeptin analogues or by several unrelated hormones including angiotensin II, insulin, somatostatin, substance P, thyrotropin-releasing hormone, and vasopressin. Binding sites for the human 28 amino acid peptides have been described on cultured vascular smooth muscle cells. The affinity of this site for the human peptides was 1 to 2 nM.

High affinity atriopeptin binding sites have been described for membranes from adrenal zona glomerulosa cells. The affinity of specific atriopeptin binding sites on rat zona glomerulosa cells corresponds well
with the IC$_{50}$ values for atriopeptin inhibition of aldosterone production. The integrity of the atriopeptin disulfide bond is an absolute requirement for both receptor binding and inhibition of steroidogenesis. Atriopeptins appear to be specific for binding to these sites since labeled atriopeptin cannot be displaced by angiotensin II, adrenocorticotropic hormone, vasoactive intestinal peptide, somatostatin, Leu-enkephalin, dynorphin, N-terminal pro-opiomelanocortin. These sites have affinities on the order of 140 pM for cultured bovine zona glomerulosa cells and 52 pM for rabbit kidney cortex. Specific receptors in the central nervous system have been localized using autoradiographic techniques (discussed in the section on brain immunocytochemical localization).

**Atriopeptin Stimulation of Guanylate Cyclase**

The spasmylytic effects of atriopeptins on vascular smooth muscle have been correlated with elevations in cyclic GMP (cGMP). This relationship is analogous to that for the spasmylytic effects of the nitroso-vasodilators. Synthetic atrial peptide has been found to elevate cGMP levels in kidney slices, isolated renal tubule cells, urine of intact animals, and cultured vascular smooth muscle cells. Atriopeptin-induced elevations in plasma cGMP probably are not the result of atriopeptin actions on the kidney since renal arterial cGMP levels exceed renal venous cGMP levels during atriopeptin infusion. Circulating cGMP does not appear to mediate the natriuretic or diuretic response to intravenous infusion to atriopeptin.

The particulate guanylate cyclase activity (effective concentration, 50% [EC$_{50}$] = 10 nM) in homogenates from rat kidney, aorta, testes, intestine, lung, and liver has been shown to be stimulated by Arg-Arg-AP III. The ability of atriopeptin to both relax isolated thoracic aorta segments and elevate cGMP levels has been shown to be independent of the presence of an intact endothelium, which excludes the endothelial-dependent relaxing factor from mediating the atriopeptin-induced cGMP changes. The elevation of cGMP levels following incubation of atriopeptin with tissues is suggestive of specific receptor recognition and some resultant biological effect. Exposure of canine kidney fractions to Arg-Arg-AP III caused no change in cGMP levels in the proximal tubule, a twofold increase in the thick loop of Henle, a threefold increase in the collecting duct, and a 50-fold increase in the glomeruli. These sites of atrial peptide recognition suggest that natriuresis may result from glomerular influences on GFR and alteration of tubular sodium transport.

Although atriopeptin had no stimulatory effect on adenylate cyclase from kidney membranes, interactions of atriopeptin with adenylate cyclase from a variety of tissue have been observed. Atriopeptin inhibited particulate adenylate cyclase from homogenates of rat aorta, mesenteric arteries, and renal arteries with an apparent inhibitor constant ($K_i$) of 0.1 to 1 nM. Both receptor-dependent and receptor-independent activations of adenylate cyclase were inhibited by atriopeptin.

**Atriopeptin Release**

Little direct data are available on the physiological stimuli for atriopeptin secretion. Low molecular weight, but not high molecular weight, atriopeptins have been shown to be released from isolated, perfused rabbit hearts. In rat heart-lung preparations, atrial distension from increased perfusion pressure caused the release of a substance that increased urine flow and fractional sodium and fractional potassium excretion when injected in intact rats. Isolated rat atria have been reported to release a natriuretic factor in response to acetycholine, epinephrine, and arginine vasopressin. Atriopeptin activity was assayed for by the natriuretic response to infusion of conditioned Krebs buffer into intact rats. Unfortunately, adequate controls (i.e., incubation of atrial tissue alone) were not included in these experiments. The release of natriuretic activity was unaffected by ouabain, isoproteranol, 40 mM potassium, and deamino-8-D-arginine vasopressin. Further validation will be necessary to demonstrate secretory activities of these agents in a physiological setting.

**Atriopeptin Structure-Activity Studies**

In our laboratories as well as in others, experiments using synthetic atriopeptin analogues have contributed to the elucidation of trends in structure-function relationships. Conclusions have been based on in vitro vascular smooth muscle relaxation assays and, in rat and dog, on stimulation of natriuresis and diuresis and on renal vasodilation. The spasmolytic effects of atriopeptin on vascular smooth muscle have been correlated with elevations in cGMP levels in kidney slices, isolated renal tubule cells, urine of intact animals, and cultured vascular smooth muscle cells. Atriopeptin-induced elevations in plasma cGMP probably are not the result of atriopeptin actions on the kidney since renal arterial cGMP levels exceed renal venous cGMP levels during atriopeptin infusion. Circulating cGMP does not appear to mediate the natriuretic or diuretic response to intravenous infusion to atriopeptin.

A graphic comparison of the influence of structural modifications on the biological activity of related atrial peptides is shown in Figure 3. These peptides have been isolated from rat atrial extracts, and as previously indicated, they represent truncated versions of a single high molecular weight precursor (atriopeptigen). Striking differences in biological processes, especially in the intact dog, occur depending on the site of atriopeptigen cleavage. For the sake of comparison, the 24 amino acid peptide AP III has been selected as the reference compound. Use of the intestinal (chick rectum) smooth muscle assay did not discriminate potency differences amongst the peptides; however, rabbit thoracic aorta strips proved reliable predictors of the relative potency of the atrial peptides. The N-terminal extension of AP III moderately enhanced the in vitro vasorelaxant activity and markedly facilitated the in vivo natriuretic response to intrarenal arterial injections of the peptide (see Figure 3). The most pronounced natriuretic influence in the dog appears to derive from the N-terminal addition of an Arg residue. The C-terminal loss of the Tyr residue does not alter the vasodilator or natriuretic activity since AP III...
result in a marked reduction (98%) in vasorelaxant and natriuretic-diuretic potency (Figure 3).

The primary structural determinant for vasodilation is an Arg residue having a specific spatial orientation relative to the cyclic portion of the peptide. In diuretic-natriuretic experiments in the rat and dog, this appears to also be responsible for renal vasodilatation activity with its contribution to the overall, composite diuretic-natriuretic effect.

It has been noted that the cysteine disulfide forming the cyclic structure Cys 3 to Cys 19 is essential for in vitro and in vivo activity. The synthetic core peptide atriopeptin (residues 3–19) while retaining significant activity in intestinal smooth muscle is essentially inactive on aorta and in vivo in a natriuretic-diuretic experiment in dog or rat. These observations are congruent with the hypothesis that the core peptide serves a delivery role for the C-terminal fragment that determines fundamental response. Perhaps the cyclic peptide can form an amphiphilic species similar to other peptide hormones of this size. The N-terminus, which is probably in close proximity to the active C-terminal fragment, serves a modulating function determined by only several residues. This hypothesis serves as a working model for further studies in structure-function relationships.

Radioimmunoassay

Initial quantitative studies of the atrial peptides used either whole animal (natriuresis-diuresis) or isolated smooth muscle (contractile) bioassays. The level of sensitivity of these assays is at best in the low nanogram range for the smooth muscle assay and in the low-microgram range for the in vivo studies. Precise quantitation is difficult using such biological systems. In spite of this drawback, these assays proved satisfactory for qualitative and semiquantitative measurement and purification of atrial tissue extracts. However, detection of peptide levels in plasma or other possible tissue sites where the amounts of material are well below nanogram levels required the development of an assay with much greater sensitivity. When synthetic atriopeptins became available for immunization and preparation of radiolabeled tracer, efforts for the development of a specific radioimmunoassay capable of detecting picogram levels of the peptide were undertaken.

The methods used to generate polyclonal antisera have been similar. In general, a low molecular weight form of the peptide (for example, AP III) was coupled to a carrier molecule (such as thyroglobulin) and emulsified in complete Freund’s adjuvant. Nakao et al. immunized with a conjugated 12 amino acid C-terminal atrial peptide fragment. Rabbits received 100 to 200 μg of the conjugated material intradermally for the primary immunization, followed by monthly boosts with similar amounts of the conjugate. Antisera were detected 6 to 12 weeks after the primary injection. In addition, we have produced antisera using a high molecular weight (the 93 amino acid cyanogen bromide fragment of the precursor, atriopeptigen). In rabbits, thyroglobulin or keyhole limpet hemocyanin coupled atriopeptin produces antisera that are effective at final concentrations of 1:1000 to 1:5000. We have also generated antisera in guinea pigs against thyroglobulin coupled peptide, which can be used at dilutions of 1:5000 to 1:150,000. The sensitivities of these antisera are between 3 and 60 pg. To date, validation of the immunoassay has involved demonstrating parallel displacement curves for authentic standard and tissue atrial peptide samples. In addition, biologically inactive or unrelated peptides do not displace labeled atriopeptin from the antisera. The definitive validation of the assay, which remains to be demonstrated, is the
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quantitative recovery of biologically active material (e.g., from cardiac or plasma samples) and sequence analysis of the immunoreactive material.

Standard immunoassay methods have been employed. The tracer used is a low molecular weight peptide (24–33 amino acids in length) iodinated by the chloramine-T method. After iodination, the labeled peptide is purified either on an octadecasilyl mini-column or by high-performance liquid chromatography. Specific activities vary between 120 and 500 to 900 μCi/μg. Separation of antibody-bound and free atrial peptide is done with either dextran-coated charcoal or secondary antibody (goat anti-rabbit immunoglobulin). Intraassay and interassay variabilities are low (<5–10%) with either method. As far as we know, no group has reported using a high molecular weight (atriopeptigen) tracer molecule, which means there is no way to test for antisera specific for this species.

Initial efforts at quantitation by immunoassay have focused on measurement of cardiac atriopeptin levels. Atrial peptide levels in right atrium are significantly greater than those in the left atrium, with combined levels of 10 to 20 μg per rat. A high salt diet (1% NaCl as drinking water) increases peptide levels in both atria almost twofold. Variability in reported atrial peptide levels should be expected, since atrial extracts are composed of a mixture of high and low molecular weight peptides and the polyclonal antisera used by the different groups may have differing cross-reactivities with respect to the high molecular weight peptide.

Demonstration of atrial peptides in the plasma is critical in order to classify a substance as a hormone. With the greater sensitivity that the radioimmunoassay provides, atrial peptides have now been detected in the plasma. Different procedures have been used to prepare the plasma samples for radioimmunoassay. Blood, collected in the presence of kaikirein inhibitors and trypsin inhibitors and ethylenediaminetetraacetic acid to prevent proteolysis, was passed over a Sep-Pak cartridge, eluted with methanol, evaporated, lyophilized, and reconstituted in buffer. Normal rat plasma collected as described contains 150 fmol/ml immunoreactive atriopeptin. If the circulating form is mainly a low molecular weight species, this corresponds to about 400 pg/ml. A high salt diet (1% NaCl in the water) increases circulating levels slightly more than twofold. Alternatively, blood, collected in the presence of ethylenediaminetetraacetic acid and protease inhibitors (pepsatin and phenylmethylsulfonyl fluoride), was purified either on immunoaffinity columns (Sepharose-4B antiatropeptin) or by Vycor glass. The peptide was eluted with acetic acid and 60% acetonitrile, lyophilized and reconstituted before assaying. This method yielded 1 ng/ml of immunoreactive atriopeptin. It remains to be determined which form(s) of the peptide is present in the circulation. Gel filtration chromatography of processed plasma samples yields immunoreactive material that comigrates with the low molecular weight atrial peptides.

The determination of which of the numerous low molecular weight fragments isolated from atrial tissue is responsible for immunoreactivity must be resolved.

Atriopeptin Immunocytochemical Localization in Heart and Brain

The availability of antisera to the atriopeptins has allowed immunohistochemical localization of these substances in the heart and brain of the rat. In the heart, antisera to partially purified atrial extracts, small peptide fragments, AP III, and a cyanogen bromide fragment of precursor peptide-atriopeptigen have revealed granular material within the myocytes of the left and right atria (Figure 4B). The staining is most dense in the perinuclear region of the atrial myocytes, as expected from the distribution of secretory granules within the myocytes. Electron microscopic studies have confirmed that the immunoreactive material is located within these secretory granules. No immunoreactive material has been seen in the ventricle. Atriopeptin immunoreactive neurons have been observed in the brain of the rat with the use of antisera to AP III and the cyanogen bromide fragment of atriopeptigen. Neurons are stained mainly in two areas, the hypothalamus and the pontine tegmentum. In the hypothalamus, there is a dense cluster of small, bipolar neurons in the anteroventral periventricular nucleus, adjacent to the anterior tip of the third ventricle (Figure 4A). Additional immunoreactive neurons and fibers are located nearby in the adjacent medial preoptic area, in the preoptic part of the bed nucleus of the stria terminalis, and in the ventral pallidum. Caudally, the neurons extend along the wall of the third ventricle. At the level of the paraventricular nucleus of the hypothalamus, immunoreactive neurons and fibers are found within the parvocellular divisions of this nucleus and, in the perifornical region, the periventricular and arcuate nucleus. A separate collection of large, multipolar cell bodies is found in the lateral hypothalamic area at this level (Figure 4C); these large cells extend back as far as the mammillary region. Other small clusters of neurons and fibers have been observed in the amygdala, the habenular nucleus, and medial and lateral mammillary nuclei. Rat hypothalamic homogenates have been reported to exhibit detectable immunoreactive levels of atrial peptides, which were decreased in animals on a high salt diet.

In the pons, atriopeptin neurons are found primarily within the laterodorsal tegmental nucleus and the pedunculopontine nucleus. A dense collection of fibers is present in the interpeduncular nucleus. The function of atriopeptin-containing neurons in the brain is largely unknown; however, it seems likely that the anteroventral periventricular nucleus will be of particular interest. The organum vasculosum of the lamina terminale, a neural structure with an attenuated blood-brain barrier, lies just anterior to anteroventral periventricular nucleus, which suggests that substances in the blood may have access to the atriopeptin...
neurons of the anteroventral periventricular nucleus. This region of the brain has extensive connections with structures that are important in cardiovascular regulatory functions, including the paraventricular nucleus of the hypothalamus (D.G. Standaert et al., unpublished observation, 1985) and the parabrachial nucleus. Animals with lesions that include the anteroventral paraventricular nucleus show profound alterations in the regulation of fluid balance. They do not drink sufficient quantities of fluid and thus become hypovolemic and hypernatremic. A recent autoradiographic study has demonstrated atriopeptin binding sites in the brain, specifically in the subfornical organ, area postrema, nucleus tractus solitarii, and the olfactory bulb.

All of these findings suggest that atriopeptin may serve as a central neurotransmitter or neuromodulator as well as a conventional peripheral hormone in the regulation of cardiovascular function. A similar sort of central and peripheral dual regulatory function has been proposed for cholecystokinin in the regulation of feeding, for corticotropin releasing factor in cardiovascular regulation, and for angiotensin II in the control of blood pressure.

Pathophysiological States Involving Atrial Peptides

Abnormalities in endocrine systems have been shown to result in pathophysiological conditions related to a defect in synthesis, release, or recognition of the hormone. Abnormal changes in atrial peptide levels may be associated with conditions of fluid imbalance, altered vascular tone, or cardiac degeneration. Cardiac extracts from spontaneously hypertensive rats produce less natriuresis (in normal assay rats) compared with normotensive Wistar-Kyoto controls. Further, infusion of Arg-Arg-AP III caused a more pronounced fall in blood pressure in the spontaneously hypertensive than in the Wistar-Kyoto rats. Concomitant changes in urine flow rate and sodium and chloride excretion were greater in the Wistar-Kyoto than in the spontaneously hypertensive rats, and cGMP excretion was also significantly less in the spontaneously hypertensive rats.

The effects of prolonged (7-day) delivery of Arg-Arg-AP III at 1 µg/hr has been studied in the two-kidney, one clip hypertensive rat. Beginning on the
third day the blood pressure of the chronically infused rats was significantly lower than those two-kidney, one clip animals who received no atrial peptide. Two possible explanations were proposed: 1) either atrial peptide productions is reduced in spontaneously hypertensive rats or 2) long-term atrial peptide infusion causes depletion of cardiac stores. The experiments presented do not discriminate between these possibilities since the assay was indirect and the data lack proof of complete dose dependency.

In the Dahl-salt sensitive rat, which develops hypertension when administered a high salt diet, significantly elevated cardiac levels of atrial peptide were observed in cardiac extracts when compared with the control Dahl resistant strain of rat. The atrial peptide levels were independent of the amount of salt in the diet. In addition, a short-term (5-day) high salt diet increases cardiac levels in the salt-resistant rat comparable to those in the salt-sensitive rat (on long-term high or low salt diet). Large doses of extract were used in these experiments to assess natriuretic activity in normal rats, and again, dose response curves were not obtained. When injected with atrial extract, the salt-sensitive rats proved to be hyporesponsive (excreting less sodium) compared with the response of salt-resistant controls. Neither of these studies was performed in prehypertensive animals to determine whether altered atrial peptide levels occurred before the onset of hypertension.

Syrian hamsters with hereditary cardiomyopathy have reduced cardiac atriopeptin levels (measured with the natriuresis assay) relative to those of controls. Of course, measurement of a static level of active atrial peptide extracts does not indicate turnover rates or amounts released into the circulation. In fact, one interpretation of diminished atrial levels is that the depletion resulted from chronic release.

Outlook

More than 100 publications involving atrial peptides have appeared since the announcement of the structure a little more than a year ago. A published review in a rapidly changing field may quickly become dated. At this moment, however, a number of powerful tools (e.g., antisera, gene clones, radioligands, analogues) are available for the quick elucidation of several important issues. We expect that the participation of atriopeptin in physiological and pathological processes will be rapidly defined; this will necessitate the establishment of the postranslational peptide processing, the release mechanisms, and the identification of the circulating form of the atrial peptide. Studies of the anatomy and physiology of atriopeptin-containing neuron systems in the brain will help to establish the function of these systems and may uncover interactions between circulating atriopeptins and atriopeptin-containing neurons in the brain.

Since biological activity is retained in a peptide as small as 21 amino acids, analogues can readily be synthesized. Thus, a search for more potent, longer acting, and more selective agonists and antagonists is a logical area of research focus. A continued evaluation of receptor sites and other biological effects is also anticipated. Physiological stimuli, including plasma volume, pressure, electrolyte and fluid levels, and neuronal and hormonal influences will be evaluated for their ability to manipulate atrial peptide synthesis storage and release. Serum assays will aid in characterizing the kinetics of atriopeptin release in diseases involved in fluid, electrolyte, and blood pressure homeostasis. Candidate diseases include hypertension, renal diseases, and congestive heart failure. Pharmacological strategies will be tested in an attempt to modify gene expression, processing, and storage and to facilitate peptide release.

Perhaps the most striking realization is the imminent prospect of clinical trials for atrial peptides, barring unforeseen toxicity problems. The application of modern technology has markedly condensed the interval between observations made with crude rat heart extract and initiation of patient testing of this novel peptide for possible therapeutic benefit.

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