Structure and Function of Atrial Natriuretic Peptides

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The literature on natriuretic peptides is critically appraised, with emphasis on chemical rather than physiological aspects.

Borst and Borst-de Geus (1) first suggested that the kidney's control of sodium excretion is paramount in the maintenance of blood pressure. Since then, knowledge on the nature of human hypertension has accumulated in favor of their suggestion (2). The evidence is strong that miniscule deficiencies in renal sodium excretion can, over the course of years, lead to cardiovascular pathology (2).

Each day an adult human filters approximately 25,000 mmol of sodium into the renal tubules because of the high glomerular filtration rates that are required to clear the body of those substances that can enter the urine only by filtration. All but 200 mmol of this sodium is reabsorbed. The regulation of sodium filtration and reabsorption has been investigated intensively. It is known to include physical factors such as filtered sodium load as well as humoral agents such as the adrenal hormone, aldosterone. There are additional mechanisms, probably of a humoral nature (3). Their identity, though it has been vigorously pursued, has not been established, but two particular substances, named "natriuretic hormone" and "atrial natriuretic factor" (ANF), have attracted much research attention. The first has a low molecular mass (500–1000 Da), most likely originates from the hypothalamus (4, 5), increases its concentration in plasma after extracellular volume expansion (6), and acts via inhibition of Na⁺/K⁺-transporting ATPase (EC 3.6.1.37) activity (7). The natriuresis produced by this factor is small in magnitude and slow in onset (8). In contrast, the natriuretic action of ANF is potent and rapid (9). ANF was first derived from extracts of atrial tissue and was localized to electron-dense spherical granules (10), which, in the mammal, are found in the atria but not in other parts of the heart (11, 12). Earlier experiments had shown that the number of these atrial-specific granules was inversely related to extracellular fluid volume (13, 14).

Soon after the first demonstration of the natriuretic potency of atrial extracts, Deth et al. (15) reported that the extracts also possessed smooth-muscle relaxant properties, and others (16) showed that it depressed cardiac activity in the whole animal.

The discovery of a factor in the low-pressure chambers of the heart that responds to changes in body fluid volume and that has natriuretic, vasodilator, and cardio-inhibitory effects, has kindled widespread research activity. Although the physiology and pathophysiology of ANF are still subject to a great deal of speculation, the growth of knowledge about its structure has been startling.

The purpose of the present brief review is to provide a critical appraisal of the rapidly expanding literature on atrial natriuretic peptides. It will not include historical aspects of atrial granules or of body-fluid volume regulation, these being described elsewhere (17). I will emphasize chemical rather than physiological aspects, because the latter have been well reviewed by Maack et al. (18).

Purification of Atrial Extracts

The crucial initial discovery that the atria of rats contain a potent natriuretic factor was made by using a crude tissue extract in isotonic saline (9). Only three years later, totally pure synthetic atrial natriuretic peptides became commercially available. This progress was astounding when one considers the heterogeneity of ground-up atrial tissue and the fact that approximately 10,000 rat atria were required to obtain enough of the substance for amino acid sequencing.

Histological evidence suggested that proteinaceous materials form a large proportion of the atrial-specific granules (19). de Bold (20) used acetic acid extraction and gel filtration on Sephadex G-75 in his initial attempts to purify the active principle. This yielded several peaks with natriuretic activity (20), although most of the activity was accounted for in peaks corresponding to molecular masses between 100 and 6000 Da. Other investigators used variations of de Bold's original procedure, and a wide range of apparent molecular masses was reported for atrial factors with natriuretic activity. There was a similar disparity among different laboratories in their initial reports of the amino acid composition of the factor (21-23). Collectively, the morphological investigations of de Bold et al. (19) and the finding that the natriuretic activity of atrial extracts was abolished by trypsin but not by heat or by concanavalin A (20, 24) suggested that the active molecule is a lipid- and carbohydrate-free polypeptide containing tryptophan as well as sulfur-containing amino acids. In light of current knowledge it is apparent that the variations in early results were due in part to artifacts of different purification procedures. Differences in molecular mass or amino acid composition might have resulted from differences in the pH of the supernatant fluid that was applied to the filtration column (24), differences in the acetic acid concentrations used during extraction or column elution (25), differences in the temperatures at which purification was carried out (26), differences in the purification step at which the temperature of the supernate was raised (26), or differences in the extraction media (some used phosphate-buffered saline, others used acetic acid). Only Trippodo et al. (24) attempted to
explain the differences among the findings of various laboratories. Among their explanations was the prophetic suggestion that the range of molecular masses that they (24) and others (20) had found was ascribable to the presence in the atrial-specific granules of a larger precursor molecule that is processed into natriuretic products of smaller molecular mass. Current, more complete information supports this early proposal (27–29).

Amino Acid Sequencing

In contrast to the range of amino acid compositions and contents of ANF published by different investigators, the amino acid sequences independently derived by several laboratories differed little with respect to the core of the molecule (27, 30–33). It is a 17-amino-acid ring formed by a disulfide bond between two cysteines (Figure 1). This grouping is now known to constitute the carboxy terminus of a 152-residue pre-peptide whose sequence was first described for the rat by Yamanaka et al. (34) and, simultaneously, by Oikawa et al. (35) for humans (Figure 2).

The pre-peptide is divided into three regions: a peptide that connects the signal peptide and the C-terminal ANF portion, which begins with Leu94 in both species. The disulfide bond shown in Figure 1 links Cys105 to Cys121 in Figure 2. This bond is essential for the biological activities of ANF (33).

The sequences of both the connecting peptide and of ANF are closely similar in the two species (Figure 2), and reportedly display considerable homology with mouse pre-proANF (36). Such similarity has been taken to suggest that these substances are important to the survival of these species (36).

The connecting peptide (residues 1 to 93, Figure 2) might well give rise to various smaller peptides with biological activities. For example, it has been asserted that a polypeptide consisting of residues 1 to 30, purified from pig atria, relaxes vascular smooth muscle but has no effect on renal volume excretion (37). As yet, this report is unconfirmed and the investigators provided no information that would allow a conclusion regarding the purity of the substances and no experimental results regarding renal actions of the substance. However, they did add the name "cardiodilatin" to the plethora of names already associated with atrial peptides.

![Fig. 1. Amino acid sequence of atrial natriuretic peptide isolated from rat atria by Misono et al. (33)](image)

Cys105 and Cys121 are linked by a disulfide bond. The human form of this peptide has the same composition except that the Ile at position 110 is replaced by Met

![Fig. 2. Complete amino acid sequences for human and rat pre-proANF: the two sequences are identical except in regions identified by reversed-out lettering](image)

Three portions of the pre-peptide are indicated: signal, connecting, and atrial natriuretic factor (ANF). The disulfide bond that is essential for biological activities links Cys105 to Cys121 (identified by white squared).

Names of Atrial Peptides

The naming of bioactive atrial peptides by different laboratories has been bewildering. Figure 3 summarizes the currently used names of bioactive compounds, their amino acid sequences, the relationship of each sequence to the pre-peptide shown in Figure 2, and a reference to the first

![Fig. 3. Summary of names and structures associated with (A) preproatrial natriuretic peptides in human, rat, and pig and (B) rat atrial natriuretic peptides](image)

The numbered references identify the first user of the name. &-hANP, a dimeric form of a-hANP, has 56 amino acids and a molecular mass of 8000 Da (double those for the usual, biologically active form, &hANP)
user of the respective name. Remember that little is known about the biological synthesis and processing of atrial peptides and that all compounds listed in Figure 3 are products of different approaches to in vitro extraction and purification of atrial tissue homogenates.

In Vivo Processing of Atrial Peptides

In vitro purification of atrial tissue typically requires 10 to 15 steps, which vary greatly among different laboratories. Therefore, no correlations with in vivo synthesis of atrial peptides are possible. In fact, it is not known whether more than one atrial peptide is produced in vivo. Present evidence on the matter is conflicting. Flynn et al. (39) have reported that the most-abundant forms circulating in rat plasma are the 126-amino-acid cardionatin IV (Figure 3A) and the 28-amino-acid cardionatin I. Cantin and Genest (17), citing a personal communication, stated that their group has identified a 26-amino-acid form as the peptide that is released into the medium of pure atrial cardiocyte cultures. Other investigators (39) have implied that the 24-amino-acid form, atriopeptin III, is released by volume expansion and that plasma enzymes then degrade this form. The degraded form eluted between the positions of atriopeptin II and atriopeptin III. Because these two peptides differ by only one amino acid (Figure 3B), a substance that elutes between them is not likely to be free of impurities. It must be concluded, therefore, that the naturally occurring forms of atrial peptides have not yet been identified. The most common site at which peptide prohormones are enzymatically cleaved by exopeptidases into a biologically active portion and an inactive remainder is between pairs of the basic amino acids lysine and arginine; arginine usually falls on the carboxyl side of the pair (40). This would make Lys-Arg or Arg-Arg the most probable cleavage site. Only the latter occurs in atrial propeptidase (at positions 101–102, Figure 2), making the 26-amino-acid peptide Arg101–Tyr126 (Figure 3B) the preferred candidate for naturally occurring atrial peptide—if the formation of such a peptide takes place via the exopeptidases that seem to be involved in the formation of many mammalian proteins (40).

Biological Activities of Atrial Peptides

The perceived excitement of atrial peptide research has led a number of investigators to try these substances in their experimental models and to report a variety of apparent biological actions. At the moment it is not possible to synthesize a coherent biological picture from many of these reports. There is, however, universal agreement that atrial peptides have large doses relax constricted smooth muscle and act on the kidneys to promote a brisk natriuresis. Early experiments in isolated rabbit aortas showed that atrial extract or synthetic ANP counteracts the vasoconstrictor action of norepinephrine, angiotensin II, serotonin, histamine, and methoxamine (41–43). More recently it was demonstrated that the vasorelaxant action of atrial peptides differs from that of acetylcholine, ATP, and bradykinin, in that these all require the presence of endothelium to be active (44) but ANP does not (45). It is currently believed that the effect of atrial natriuretic peptides on vascular smooth muscle involves cell-surface interactions that result in an increase in intracellular cGMP and activation of particulate guanylate cyclase (EC 4.6.1.2) near the cell membrane (46). This proposed mode of action differs from that of other cGMP-dependent vasodilators such as the nitrovasodiators and the endothelium-dependent dilators. All of them are associated with activation of soluble guanylate cyclase (47) than particulate guanylate cyclase.

Correlation between Structure and Biological Activity

Both the natriuretic effects (9) and relaxant effects on constricted aortic strips (15) or constricted chick-rectum strips (48) have been used as the basis for bioassays of atrial peptides (48,49), because these activities have been found to be inseparable in most peptides (22). The only exception is the 30-amino-acid substance named cardionatin by Forsemann et al. (37).

Ring Structure

Several investigators have shown that the ring structure imposed by the disulfide bridge between Cys105 and Cys121 (Figure 1) is essential for receptor binding (50) and for subsequent biological activities (33,49–51). In addition, the presence of the hydrophobic residue at position 110 within the ring (Met in h-ANP; Ile in r-ANP, Figure 1) has been shown to be required if receptor binding is to occur with full affinity, and it may be important for full biological activity of the peptide (50).

N-Terminal Amino Acids

Natriuretic activity. The natriuretic potencies of rat ANP, cardionatin I, and rat ANF 1–33 (Figure 3B) do not differ significantly (31). Extension of the N-terminal, yielding the peptide Ala-Leu-rat ANF 1–33, appears to reduce natriuretic potency slightly (31). These results were confirmed by Thibault et al. (52) and extended to the peptides Gly96-Tyr126 and Glu54-Tyr126. The latter two N-terminal-extension peptides showed slightly decreased natriuretic potencies, but all the other peptides from Cys105-Tyr126 to Arg110-Tyr126 showed equipotent natriuretic activity (52). Therefore, in rats, full natriuretic activity does not depend critically on any amino acids preceding Cys105. Dogs, however, respond differently. In them, N-terminal extensions to atriopeptin II or atriopeptin III (Figure 3B) reportedly increase natriuretic potency at all doses tested (53).

Smooth muscle activity. (i) Vascular smooth muscle: Binding of atrial peptides to rat vascular smooth-muscle receptors occurs with full affinity, and the cGMP response of rat vascular smooth-muscle cells occurs with undiminished magnitude, even when all amino acids preceding Cys105 have been removed (50). Garcia et al. have published identical dose–response curves for the relaxation of renal artery strips by Arg101-Tyr126 or by Gly95-Tyr126 (54). Katau et al. (55) reported similar conclusions when they tested the renal vasodilator potency of various N-terminal extended atriopeptins in anesthetized dogs. Therefore, N-terminal extensions to Cys105 do not appear to exert major effects on the vascular potency of atrial peptides.

(ii) Intestinal smooth muscle: Intestinal smooth-muscle responses appear to be more sensitive to N-terminal manipulations than are vascular smooth-muscle responses. Cys105-Tyr126 gives a significantly larger response than peptides with one, two, three, or four N-terminal residues added to Cys105 (52). However, it is not evident from the results available whether N-terminal length and intestinal smooth-muscle relaxation potency are directly correlated.
C-Terminal Amino Acids

One report (55) has suggested that removal of any C-terminal amino acid reduces the vasorelaxant potency of atrial peptides. However, at lower concentrations, removal of the C-terminal Tyr126 has little effect on any known biological activity of atrial peptides (50, 52, 53, 56). Removal of additional C-terminal amino acids affects natriuretic and vascular smooth-muscle relaxation potency before it affects intestinal smooth-muscle relaxation potency. Thus, atriopeptin I, which differs from atriopeptin II by the deletion of the C-terminal dipeptide Phe124-Arg125, shows unaltered relaxant potency for intestinal smooth muscle (49, 55), but markedly decreased natriuretic and vasorelaxant potencies (52, 56, 57). Removal of two more C-terminal residues from atriopeptin I further reduces natriuretic activity (52) and also diminishes potency in vitro relaxation of intestinal smooth muscle (52).

In summary, the present state of knowledge of ANF structure–activity relationships is that in rats the minimum length that gives full natriuretic activity is Cys105-Arg125, the minimum length that gives full vascular relaxant activity also is Cys105-Arg125, and the minimum length that gives full intestinal relaxant activity is Cys105-Cys121. The peptide Cys105-Asn122 apparently has not been tested yet. In dogs, more potent natriuretic activity is achieved with N-terminal additions to Cys105 (53).

Radioimmunoassay of Atrial Peptides

After the demonstration by Gutkowska et al. (58) that antibodies can be raised against atrial peptides, several laboratories have used either commercially available or locally produced antibodies to measure the concentration of immunoreactive atrial peptides, to localize immunoreactive sites in various tissues, and to localize putative ANF receptors in several tissues. The findings to date have been reviewed by Cantin and Genest (17). Some of them allow tantalizing speculations about the physiological role of atrial peptides. I will address these later. Others appear to contradict previously well-understood physiological mechanisms and, thereby, raise the question as to whether the doses of ANF that have been used in experiments are physiological doses, as well as questions of validity and specificity of available radioimmunoassays for ANF. Until more specific antibodies become available, reports based on the use of polyclonal antibodies should be interpreted with caution.

Physiological Role of Atrial Peptides

The guiding belief of investigators in this field is that ANF plays a major role in the homeostasis of body sodium and body fluids. This belief derives from observations that the number of electron-dense granules in atrial myocytes changes with chronic treatments designed to alter extracellular fluid volume (13, 14). The belief was strengthened firstly by the demonstration that the granules are the locus of a substance with potent natriuretic and diuretic action (19, 59) and secondly by the demonstration, after acute blood volume expansion, of increases in the concentration in plasma of an immunoreactive atrial peptide that is eluted from an HPLC column at the same volume as are highly purified granule contents (39). The further demonstration that ANF not only promotes natriuresis but also inhibits aldosterone secretion in vitro (60) and in vivo (61), enhances vasopressin release in vitro (62), relaxes blood vessels (15), and depresses cardiac output (16) suggests the tantalizing possibility that the atria can, with appropriate stimulation, release a peptide that interacts in an internally reinforcing manner with many components of the regulatory systems of body fluid volume and pressure. It remains to be demonstrated that any of these putative actions are of homeostatic significance in the whole, conscious animal.

Controversies and Future Directions

Although there is universal agreement regarding the natriuretic effect of atrial peptides, there is disagreement among investigators regarding the mechanism by which natriuresis is promoted. Some hold that the peptides have a direct tubular effect; others, that their natriuretic action occurs only as a secondary effect following renal hemodynamic changes. This controversy was described in a recent review (19).

A second area of disagreement concerns the effects of atrial peptides on arterial blood pressure. Most findings after acute systemic injection of purified atrial extract or synthetic atrial peptide into anesthetized rats or dogs indicate a decrease in mean arterial blood pressure (9, 16, 63–66), but one indicates no change (67). All those who have investigated the problem agree that synthetic peptides decrease mean arterial blood pressure in conscious hypertensive rats (68–71), whereas in conscious normotensive rats or dogs they may (68, 69, 72) or may not (63, 71) produce hypotension.

A comparison of the various reports suggests several possible explanations for the observations. The simplest is that the magnitude of arterial pressure reduction depends directly on the administered peptide dose (71). With reference to this possibility it is regrettable that those who reported no change in blood pressure after injection of atrial extract (56, 63, 67) or synthetic peptide (71) did not report whether their injections were sufficient to cause natriuresis.

An alternative explanation is suggested by the data of Volpe et al. (71). It is that the magnitude of the hypotensive effect of atrial peptides depends in part directly on the concentration of angiotensin II in the circulation. Maack et al. (18) have pointed out that this explanation is consistent with findings in isolated rabbit aorta constricted with various agents, including angiotensin II, and with findings in different hypertensive models characterized by low or high activity of renin (EC 3.4.23.15) in plasma.

An area of considerable controversy is the mechanisms by which atrial peptides are processed and released in the body. Several investigators have suggested that increased atrial stretch is a physiological signal for the release of ANF, based on evidence from bioassay studies on the perfuse of an isolated heart–lung preparation (73) as well as from measurements of immunoreactive material in the atria of rats with chronic pulmonary hypertension (74) or in plasma of dogs, either after left atrial balloon inflation (75) or after aortic cuff inflation (76). The time-course of release of immunoreactive material is rapid and independent of cardiac afferent or efferent innervation (75). If these results can be confirmed, they suggest that increased atrial stretch, independent of cardiac sympathetic or parasympathetic nerves, causes the release of immunoreactive atrial natriuretic peptides into the circulation.

It is tempting to believe that the missing link in the mechanisms by which the body regulates fluid and salt
content has been found. However, before this view can be accepted, two major problems must be resolved.

Firstly, the finding of nerve-independent, left atrial stretch-mediated increase in immunoreactive atrial natriuretic activity in plasma must be reconciled with the observation that cardiac denervation completely eliminates the natriuresis of left atrial stretch in conscious dogs (77) and greatly decreases the natriuresis of isometric volume expansion in the anesthetized dog (78). The results obtained to date suggest that the amount of ANF released in response to the well-known natriuretic stimulus of left atrial stretch may not be large enough to explain the natriuresis.

The second major problem to be resolved is the discrepancies among reports concerning mechanisms by which natriuretic peptides are released from the atria. Isoproterenol, a beta-adrenergic agonist, has been reported by one group to cause degranulation of atria in vivo (79) and by another group to have no effect on in vitro release of atrial natriuretic factor (80). In a similarly contradictory fashion, one group found that stimulation of cardiac autonomic alpha receptors causes in vitro release of ANF (81) and another group found that stimulation of cardiac sympathetic nerves has no effect on the in vivo release of immunoreactive atrial peptide (82).

Currently, all mechanisms of action of atrial peptides are being debated. Thus, natriuretic action is believed by some to result from direct tubular effects and by others to result secondarily from renal hemodynamic changes (19). The mechanism of vascular action might be via particulate guanylate cyclase or via soluble guanylate cyclase (17). The mechanism of cardiac output depression has been ascribed to inhibition of cardiac contractility (16) or to depression of cardiac preload (72).

While investigations into the mechanisms by which atrial peptides act are interesting, they cannot demonstrate that, functionally speaking, atrial peptides are more than an interesting observation. Unless it can be shown that these substances are physiological moderators of arterial blood pressure or of extracellular fluid volume, it cannot be shown that atrial peptides are mechanisms of homeostatic significance. However, this does not diminish their potential pharmacological significance.

References
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