

Review

Mechanisms of atrial natriuretic peptide secretion from the atrium

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Abstract

Since the discovery of atrial natriuretic peptide (ANP) more than 20 years ago, numerous studies have focused on the mechanisms regulating ANP secretion. From a physiological standpoint, the most important factor governing ANP secretion is mechanical stretching of the atria, which normally occurs when extracellular fluid volume or blood volume is elevated. In addition, the ability of several vasoconstrictors to increase ANP secretion can be traced to their indirect effects on atrial stretch via increases in cardiac preload or afterload. Whether vasoconstrictors such as angiotensin II and vasopressin have a direct positive or negative effect on ANP secretion has not been determined with certainty. Two paracrine factors derived from endothelial cells play important roles in modulating ANP secretion. Endothelin, a potent vasoconstrictor, stimulates ANP secretion and augments stretch induced ANP secretion. The dramatic increase in ANP release produced by cardiac ischemia appears to be mediated in part by endothelin. Nitric oxide (NO), an important vasodilator, is also produced by endothelial cells and inhibits ANP secretion acting through cyclic GMP as an intracellular messenger. Several recent studies have helped to define the cellular mechanism contributing to regulation of ANP secretion including stretch-activated ion channels, prostaglandins, cytochrome P450, G proteins and cell calcium. A number of steps in the cellular transduction of the ANP signal remain to be resolved. The release of ANP in disease states such as myocardial infarction and heart failure appears to be related to both mechanical and cellular events.

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1. Introduction

Atrial natriuretic peptide (ANP) is a cardiac hormone involved in the physiological maintenance of blood volume and arterial blood pressure. ANP transgenic mice with increased plasma ANP levels show chronic hypotension [1], whereas, ANP gene knock-out mice exhibit fluid retention and hypertension [2–4]. Thus, introduction of the ANP gene to increase the endogenous plasma levels has been used to treat experimental forms of hypertension [5]. Other peptides in the natriuretic peptide family, brain natriuretic peptide (BNP) and c-type natriuretic peptide (CNP), have hypotensive actions similar to ANP although CNP lacks significant natriuretic activity [6]. Both ANP and BNP are

thought to exert many of their actions through the A type natriuretic peptide receptor (NPR_A) which generates cyclic GMP (cGMP) to produce vasodilatation and natriuresis. NPR_A knockout mice exhibit increases in plasma ANP levels, salt and water retention, cardiac hypertrophy and salt-sensitive hypertension [7]. NPR_A knockout mice die by approximately 6 months of age due to congestive heart failure or aortic dissection [8]. Increased plasma levels of ANP play an important homeostatic role in the early stages of congestive heart failure to maintain adequate renal sodium excretion and sodium balance. These studies clearly demonstrate that the cardiac hormonal system is essential in volume and pressure homeostasis.

More than 30 years ago, Henry et al. [9] demonstrated that the cardiac atria could sense the “fullness” of the cardiovascular system and produce appropriate reflex changes in renal salt and water excretion to maintain a

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constant blood volume. They employed balloon inflation to stretch the atrium in anesthetized dogs and showed that atrial stretch resulted in a diuresis and natriuresis. However, they attributed the responses exclusively to reflex changes such as an inhibition of antidiuretic hormone secretion.

In 1980, the author was fortunate to attend a Fall meeting of The American Physiological Society in Toronto where Dr. Harald Sonnenberg, Adolfo De Bold and colleagues presented a fascinating series of experiments showing that saline extracts of rat atria produced a dramatic hypotension, diuresis and natriuresis when injected into bioassay rats (Fig. 1). The natriuresis occurred without a perceptible change in glomerular filtration rate, suggesting that the atrium contains a substance that can inhibit sodium chloride transport at the level of the renal tubule. De Bold et al. [10] later published these results as a classic paper in the journal *Life Sciences*. Several laboratories [11,12] soon isolated the 28 amino acid peptide which was named atrial natriuretic factor (ANF), although the terminology has since evolved to atrial natriuretic peptide (ANP).

From a physiological perspective, a wealth of information concerning the function of this cardiac hormone has been determined by studying the mechanisms regulating its secretion.

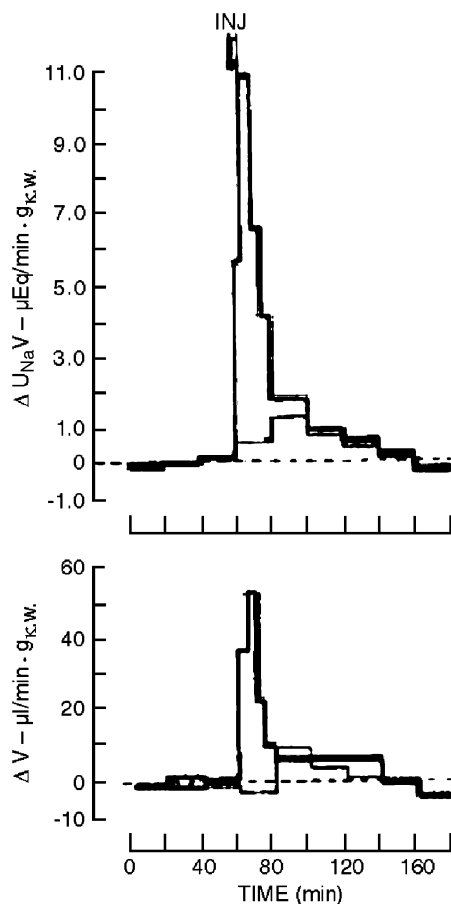


Fig. 1. Effect of rat atrial extracts on sodium excretion ($U_{Na}V$) and urine flow (V) in anesthetized bioassay rats, (from De Bold et al. [10], used with permission).

2. Atrial distension

Several early reports identified “specific atrial granules” in the heart. The granules were similar to those found in other organs that secrete peptide hormones [13,14]. The first hint that ANP secretion might be physiologically regulated was observed by De Bold [15] who showed that atrial granularity decreased significantly in rats on 2% NaCl plus mineralocorticoids while granularity increased in both water restricted or NaCl restricted rats. These results lead to the hypothesis that increases in blood volume might stimulate ANP secretion, thus, depleting it from the atrial granules. One obvious possibility was that increases in blood volume produced mechanical stretch of the atria, which was coupled to the secretory process. This seemed reasonable because the atria are extremely compliant structures that respond with marked distension to increases in volume status. To examine this hypothesis, the author’s laboratory developed a fluorocarbon perfused rat heart–lung circuit in which venous return and right atrial stretch were regulated to simulate an increase in blood volume [16]. Hearts were perfused at both low and high levels of venous return and the atria were observed to expand markedly at the higher pressure. Fluorocarbon perfusates from these two experiments were then infused into the abdominal aorta of anesthetized bioassay rats to assess natriuretic activity. As shown in the Fig. 2, the rats infused with the high central venous pressure perfusate (High CVP) showed a greater increase in urine flow than the Low CVP perfusate and a striking 14 fold greater increase in sodium excretion [16]. These experiments were the first to suggest that ANP is regulated by atrial distension and further suggested that cardiac innervation was not essential to natriuretic hormone secretion. This hypothesis was strengthened shortly thereafter by both in vitro and in vivo studies. Lang et al. [17], using a specific radioimmunoassay, showed that ANP is secreted by the isolated rat heart in response to changes in atrial pressure. They also demonstrated that the plasma concentration of ANP increases in response to volume expansion in anesthetized rats [17]. Experiments in chloralose-anesthetized dogs [18] demonstrated that mitral valve obstruction, which produces atrial distension, increased plasma ANP levels and this response was not attenuated by vagotomy. These experiments again supported the atrial stretch hypothesis and further showed that the release of ANP from the heart did not require cardiac reflexes. ANP was found to increase in humans with water immersion [19] and in clinical conditions such as heart failure [20] and renal failure [21]. The elevated ANP concentrations in each of these conditions correlate with the degree of atrial distension. In addition, contraction rate has been suggested to stimulate ANP secretion [22]. However, this effect could be attributed to the effect of frequency of contraction on extracellular translocation of ANP which has been shown by Cho et al. [23] to be an important determinate of ANP release from the heart. Cho et al. demonstrated that the

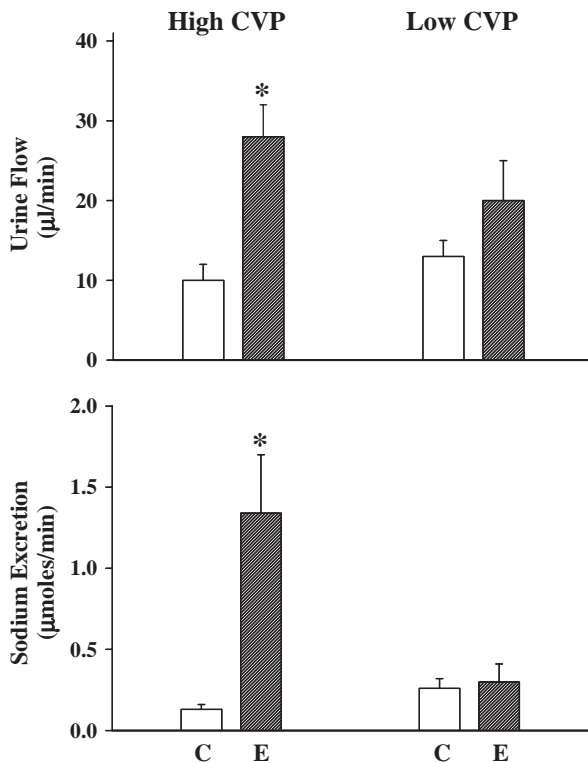


Fig. 2. Atrial stretch enhanced natriuretic activity in rat heart perfusates. “C”=control urine collection period and “E”=experimental period in bioassay rats, (redrawn from Dietz [16], used with permission).

effect of translocation on ANP release can be separated from myocytic ANP secretion [24].

Gene expression is also an important determinate of ANP secretion since the total amount of ANP stored will ultimately effect the quantity of the molecule released into the plasma. ANP secretion is substantially augmented in experimental animals with cardiac hypertrophy or heart failure where gene expression of the hormone is increased in both the atria and ventricles [25]. ANP gene expression can be up-regulated by glucocorticoids and mineralocorticoids which also enhance ANP secretion [26,27]. By contrast, adrenomedullin inhibits ANP gene expression in rat cardiomyocytes [28].

3. Cardiac ischemia: an important stimulus for ANP secretion

Hypoxia or ischemia is one of the most potent stimuli for ANP secretion [29–31] and it has been suggested that release of atrial natriuretic peptides could play an important role in the nocturia associated with sleep apnea [32]. The release of ANP in response to cardiac ischemia can be viewed as an important homeostatic mechanism since ANP can produce cardiac vasodilatation to increase blood flow and oxygen delivery to the heart as well as peripheral vasodilatation to reduce arterial pressure. The reduction in cardiac afterload will then result in a beneficial reduction in

cardiac oxygen demand. This response has been attributed, at least in part, to both alpha and beta-adrenergic stimulation of ANP release [30] but more recently endothelins have been implicated in this response. Our laboratory demonstrated that much of the increase in ANP secretion induced by ischemia could be attributed to the release of endothelins [33]. Fig. 3 shows that a period of anoxia in the isolated perfused rat atria results in a dramatic increase in ANP secretion, which is almost entirely blocked by the endothelin-1 receptor antagonist, BQ123. It should also be pointed out that during the recovery (normoxia) period, ANP secretion returned to base line as did atrial hemodynamics. Very recent studies attribute the nocturia seen with obstructive sleep apnea to increased secretion of ANP [34]. Plasma ANP levels in this sleep apnea study correlated directly with the degree of hypoxemia but also could be stimulated by hemodynamic mechanisms such as pulmonary hypertension, which elevates right heart pressures. Also, myocardial infarction leads to acute cardiac ischemia and a profound increase in the release of ANP in both animals [35] and humans [36].

4. Modulation of ANP secretion by paracrine factors

4.1. Endothelin

One of the most potent stimuli for ANP secretion is the endothelial cell derived peptide, endothelin-1. Lew and Baertschi [37,38] demonstrated this important relationship by co-culturing cardiac myocytes and endothelial cells, which resulted in enhanced ANP secretion. Fukuda et al.

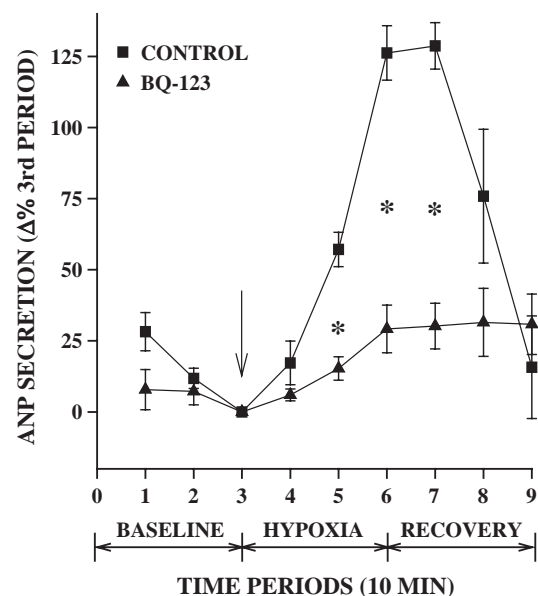


Fig. 3. Atrial natriuretic peptide (ANP) secretion from isolated perfused rat atria was stimulated by hypoxia. The endothelin-1 receptor blocker, BQ123, attenuated the ANP response to hypoxia (from Skvorak et al. [40], used with permission).

[39] showed that endothelin increases ANP secretion and up-regulates ANP messenger RNA in isolated rat cardiac myocytes. Skvorak et al., [40] demonstrated that endothelin augments the ANP secretory response to mechanical stretch in isolated perfused rat atria. Furthermore, they showed that a specific endothelin-1 receptor antagonist attenuates the ANP response to atrial stretch [40], thus demonstrating that endothelin plays an essential paracrine role in the stretch-activated ANP secretory process. Fig. 4 shows the synergistic effects of atrial stretch (high atrial pressure) and endothelin (50 nM) on ANP secretion from isolated rat atria [41]. High atrial pressure (8–10 mmHg) produced a marked increase in ANP secretion (30–60 min) compared to the low atrial pressure control period (30 min). Endothelin further augmented this response when added while atrial pressure was elevated (60–90 min).

4.2. Nitric oxide (NO)

Many years ago, experiments in animals showed that when acetylcholine was injected intravenously there was a pronounced vasodilatation and a significant drop in arterial blood pressure. This phenomenon was later attributed [42] to the release of an endothelial cell derived relaxing factor that has subsequently been identified as nitric oxide (NO). NO is a potent vasodilator which is produced from L-arginine and increases the production of cGMP in both cardiac muscle and vascular smooth muscle [43]. Stimulation of cGMP in the heart inhibits ANP secretion and decreases cardiac contractility and has been most recently shown as the mediator of the inhibitory action of C-type natriuretic peptide on ANP release [44]. In conscious rats, infusion of the NO synthase inhibitor, N^G-nitro-L-arginine

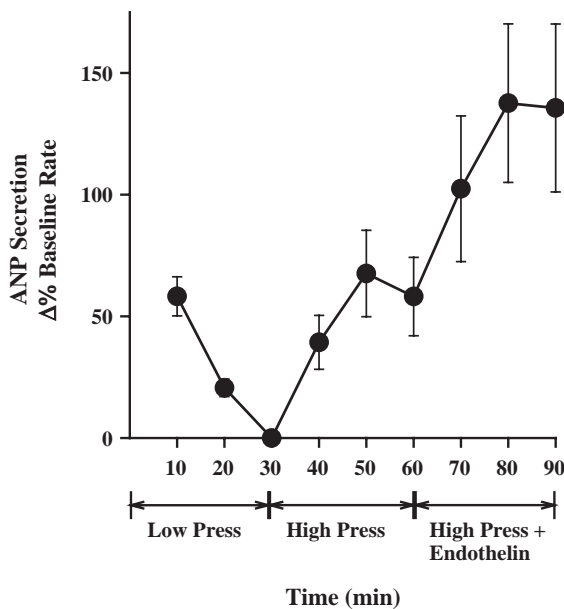


Fig. 4. Atrial natriuretic peptide (ANP) secretion from rat atria in response to atrial stretch (High Press) and 50 nM endothelin-1, (redrawn from Pollack et al. [41], used with permission).

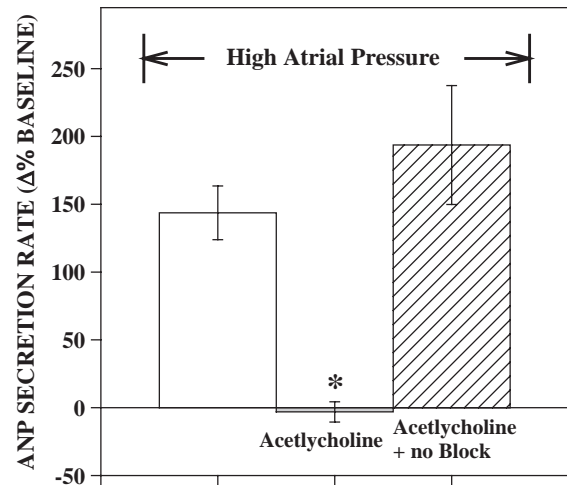


Fig. 5. In this experiment in perfused rat atria, high atrial pressure increased ANP secretion by 150%. Acetylcholine inhibited this response but not when nitric oxide (NO) was blocked with a combination of L-NAME (30 μ M) and hydroquinone (10 μ M), (from Skvorak and Dietz [47], used with permission).

methyl ester (L-NAME), augments ANP secretion in response to blood volume expansion [45]. Furthermore, inhibition of NO with methylene blue augments ANP secretion from isolated rat atria [46].

Experiments from our laboratory in the isolated perfused rat atria helped to confirm the hypothesis that NO inhibits ANP secretion [47]. We found (Fig. 5) that increasing atrial pressure from 2 to 10 mmHg stimulated ANP secretion by 150% (Fig. 5, open bar). Adding acetylcholine to the bath, which is known to stimulate NO synthesis, markedly attenuated the stretch-induced increase in ANP secretion [47]. When L-NAME and hydroquinone were added to block NO synthesis, acetylcholine no longer inhibited ANP secretion. In the same study [47], acetylcholine did not depress basal ANP secretion in the isolated atria at low atrial pressure (unstretched). Taken together, these experiments clearly show that NO has an inhibitory effect on ANP secretion mediated by cGMP and that atria stretch leads to a decrease in NO production resulting in augmented ANP release.

4.3. Stimulation of ANP Secretion by Angiotensin II, Vasopressin and Adrenergic agonists

There is some controversy as to whether these important regulatory hormones have significant direct effects on ANP secretion or alter secretion indirectly through their ability to produce vasoconstriction and affect venous return or cardiac afterload. Ruskoaho et al., [48], have detailed several key points in this controversy in a review. Previous experiments from the author's laboratory using an isolated rat heart–lung preparation clearly showed that increasing venous return (preload) or increasing aortic pressure (afterload) would stimulate ANP secretion from the heart [49]. Katsube et al. [50] infused angiotensin, vasopressin and phenylephrine in

anesthetized rats and found that changes in the plasma concentration of ANP correlated directly with changes in atrial pressure. However, Lachance and Garcia, [51] found an increase in plasma ANP in rats with angiotensin infusion at doses that did not alter atrial or left ventricular end-diastolic pressures. In vitro studies have also produced contradictory results. Several investigators failed to find any effect of angiotensin on ANP secretion in isolated tissues or primary cell cultures [52–54] while others have shown stimulation of ANP secretion by angiotensin [55–59]. There have been similar disagreements in regard to the effects of antidiuretic hormone (vasopressin) on ANP secretion in vitro. Sonnenberg and colleagues [55,60,61] initially reported that vasopressin increased ANP secretion in cell cultures but later reported [51] that vasopressin inhibited ANP secretion from isolated rat hearts. Our laboratory [52] found no significant effect of vasopressin on ANP secretion in the isolated rat heart–lung preparation. Clearly, several vasoconstrictor hormones including norepinephrine, epinephrine, angiotensin II and vasopressin can increase ANP secretion by indirect mechanisms related to vasoconstriction and increased atrial and ventricular stretch. However, it has not been established conclusively whether these hormones possess direct inhibitory or stimulatory effects on ANP secretion. Thus, the physiological role of vasoconstrictors modulating ANP secretion is unclear and requires additional study.

4.4. Stimulation of ANP Secretion by other neurohumoral factors

Opioids have been shown to increase ANP secretion in vivo and in isolated atria [62,63]. Corticotropin releasing factor also stimulates ANP secretion by what appears to be a direct receptor-mediated mechanism [64]. The effects of Calcitonin gene-related peptide (CGRP) on ANP secretion are less clear. CGRP was first shown to inhibit ANP secretion in isolated rat atria [65] but more recently another laboratory has found that CGRP suppressed ANP secretion in vitro [66]. Although the precise physiological role for these hormones in regulating ANP release is not known, they could likely contribute to the marked stimulation of ANP secretion seen in ischemia and myocardial infarction.

5. Cellular mechanisms for ANP secretion

Since atrial muscle stretch has been determined to be an important physiological mechanism controlling the secretion of ANP, the cellular mechanisms that transduce this response have been a topic of intensive study [48]. These lines of investigation have led to many seemingly contradictory results. However, what must be considered is the fact that the secretory process for ANP clearly involves at least four broadly defined steps: transduction of the signal at

the cell membrane, integration of several intracellular messengers, processes involved in hormone packaging, trafficking and release and finally, extracellular fluid transport of the peptide. All of these processes can potentially be affected by a given stimulus, for example, calcium or cyclic nucleotides.

Laine et al. [67,68] found that gadolinium, a blocker of stretch-activated ion channels, attenuated stretch-induced ANP secretion from isolated perfused rat atria but did not suppress basal ANP secretion. Blockers of L-type and T-type calcium channels (using diltiazem and NiCl_2 , respectively) had no effect on stretch-induced ANP release. Recent evidence suggests that G proteins may act as important transducers of stretch activated ANP secretion. Bensimon et al. [69] showed that mastoparan-7, a $\text{G}_{i/o}$ agonist, increased basal ANP secretion in perfused rat atria even in the absence of atrial stretch. Furthermore, they demonstrated that inhibition of $\text{G}_{i/o}$ with pertussis toxin completely blocked stretch-induced ANP release. However, pertussis toxin did not block endothelin induced ANP secretion, which seems plausible since endothelin-1 is hypothesized to exert its effects on ANP secretion through a different G protein.

Calcium clearly plays a central role in secretion mechanisms of many hormones but the evidence supporting a role for calcium in the secretion of ANP appears to be complex and not without significant contradictions. De Bold and De Bold [70] demonstrated that ANP secretion was independent of changes in extracellular calcium even in the presence of EGTA, a calcium chelator. Interestingly, BAY K 8644, a calcium channel agonist that increases calcium entry into cardiac myocytes and increases cardiac contractility was found to stimulate ANP secretion in atrial myocyte cultures [71]. However, BAY K 8644 inhibited ANP secretion in perfused rat hearts [72] and rabbit atria [73]. However, ryanodine, which inhibits calcium release from the sarcoplasmic reticulum, inhibited stretch-induced ANP secretion in atrial myocytes [74] and in the perfused rat atria [67] but did not affect basal release. Laine et al. [67,68] used gadolinium to block voltage gated calcium channels and showed a decrease in stretch activated ANP release which was not affected by the L-type calcium channel blocker diltiazem. Thapsigargin, which inhibits sarcoplasmic reticulum calcium adenosine triphosphate and depletes intracellular calcium stores, does not alter basal ANP secretion but blocks stretch induced ANP release [75]. Based on their experiments in cultured rat atrial myocytes, Doubell and Thibault [76] postulated that calcium has a negative effect on ANP secretion under basal conditions but a positive modulatory role under conditions of stimulated sustained release. Calcium depletion has been shown to decrease the stimulation of ANP secretion by phenylephrine and endothelin [54,77] in cultured myocytes but also attenuated the ANP inhibitory response to C-type natriuretic peptide and cGMP in vitro [44].

Calcium could potentially be involved at several points in the secretory process including transduction of the stretch

signal, integration of multiple stimuli, granule formation, translocation, docking or release, prohormone processing and finally extracellular translocation of the hormone. Further studies will be helpful in resolving the complex role of calcium in ANP release. It seems likely that basal ANP secretion may be regulated by calcium in a negative fashion as has been most clearly demonstrated for renin secretion from the kidneys [78]. Supporting this idea are two recent studies by Kim et al. who found that calcium depletion augmented stretch-induced ANP secretion [79] and negated the inhibition of ANP secretion by the K_{ATP} channel inhibitor glibenclamide [80].

Cyclic nucleotides act as important cellular messengers in the release of many hormones and in several cases cAMP and cGMP have opposing effects. However, for ANP secretion both cAMP and cGMP appear to be inhibitory. Forskolin, which increases cellular cAMP, inhibits ANP secretion in atrial myocytes [81,82]. A number of humoral factors appear to inhibit ANP secretion by stimulating cAMP including adrenomedullin [28], α -adrenergic agents such as isoproterenol [82], histamine [83], and phosphodiesterase-3 inhibitors [84]. Also, cGMP inhibits ANP and appears to mediate the actions of nitric oxide and C-type natriuretic peptide on ANP secretion [44].

Gardner et al., [85,86] demonstrated that prostaglandins play a critical role in the stimulus secretion mechanism for ANP secretion. $PGF_2\alpha$ and PGE_2 but not PGI_2 stimulated ANP synthesis and secretion in rats in vivo and in cultured rat atrial myocytes [85]. Furthermore, they showed that both atrial stretch and $PGF_2\alpha$ markedly stimulate ANP secretion but combining stretch with $PGF_2\alpha$ together had no greater effect than the individual stimuli. This suggested that $PGF_2\alpha$ may act as a direct mediator of the stretch-induced ANP response. Others have reported that prostaglandins are a potent stimulator of ANP secretion in rat ventricular myocytes [87,88] and rabbit atria sections [89]. Calmidazolium, a calmodulin inhibitor, suppresses both basal and stretch-stimulated ANP secretion [86] while other calmodulin inhibitors attenuated ANP stimulation by $PGF_2\alpha$ [89]. The cyclooxygenase inhibitor, indomethacin, inhibits prostaglandin synthesis in vitro and reduces basal ANP secretion [61,90]. Another prostaglandin synthesis inhibitor, meclofenamate, had very similar inhibitory effects on ANP release [31,91]. Our laboratory demonstrated that indomethacin nearly completely blocked the ANP responses to ischemia and endothelin [33]. In a recent study, inhibition of cytochrome P450 arachidonate metabolites decreased basal ANP secretion in isolated perfused rat atria and inhibited endothelin stimulated ANP secretion in cultured rat myocytes [92]. Clearly, arachidonic acid metabolites play a key role in stimulus secretion coupling for ANP secretion.

Several protein kinases have been implicated in the stimulus-secretion coupling for ANP secretion. Ruskoaho and Colleagues demonstrated that phorbol esters, which increase the cellular concentration of protein kinase C

(PKC), stimulate ANP secretion from the isolated rat heart [72]. Staurosporine, a protein kinase inhibitor, blocked Forskolin-induced inhibition of ANP secretion in the perfused rabbit atria [84]. Taskinen et al. found that the tyrosine kinase inhibitor, lavendustin, blocked stretch-induced ANP release [93]. However, in an earlier study they showed that another tyrosine kinase inhibitor, genistein, stimulated ANP release [94]. Studies suggest that calmodulin kinase is involved in a positive fashion in the secretory pathway [71,84].

5.1. Storage of ANP and Release into the Circulation

ANP is stored as a 126 amino acid prohormone, proANP 1–126 [25]. Our laboratory showed that several peptides

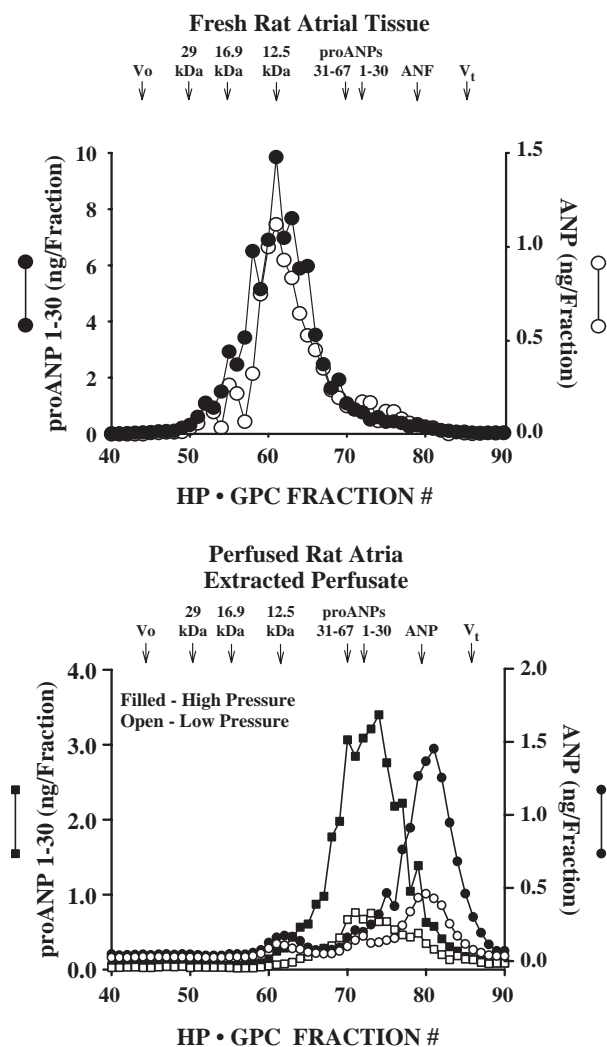


Fig. 6. High performance gel-permeation chromatography (HP-GPC) profiles of proANP 1–126, ANP and proANP 1–30 immunoreactivity in extracts of atrial tissue. Arrows indicate the elution positions of molecular weight calibrators, void (V_o) and total volume (V_t). Each value represents the average of three experiments, (from Dietz et al. [97], used with permission).

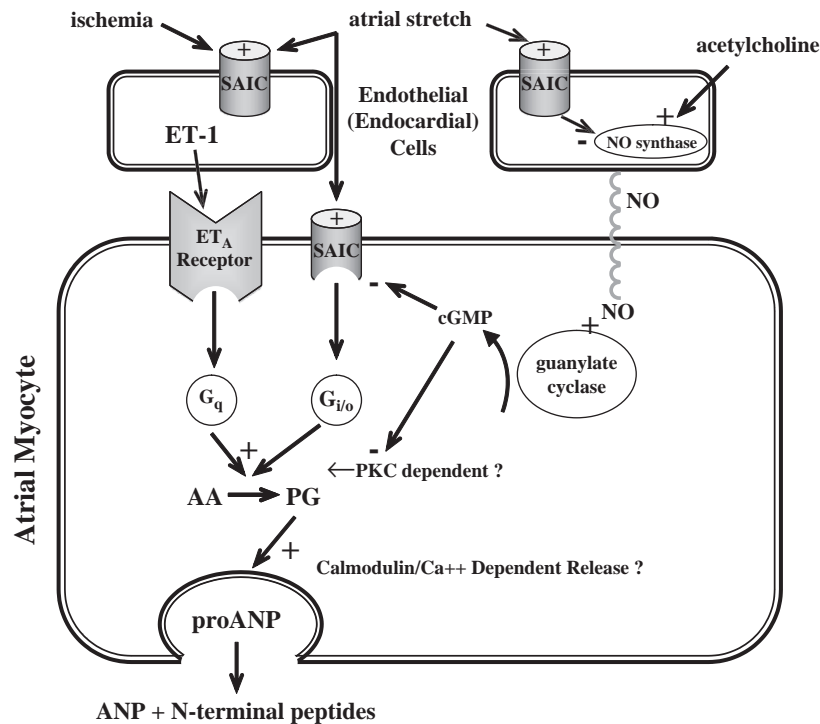


Fig. 7. Proposed scheme for stimulation of ANP secretion by stretch and endothelin and inhibition by acetylcholine. SAIC=stretch activated ion channel; ET-1=endothelin-1; NO=nitric oxide; ET_A=endothelin type A receptor; AA=arachidonic acid; PG=prostaglandins; ANP=atrial natriuretic peptide.

derived from the prohormone are secreted by isolated rat atria including ANP (proANP 99–126), proANP 1–30 and proANP 31–37 [95]. In a subsequent study we compared the stored versus the secreted forms of ANP in isolated atria using a “sandwich” radioimmunoassay which measures the C-terminus and N-terminus of the ANP prohormone combined with gel permeation chromatography [96,97]. In Fig. 6, in the right upper panel, fresh atria were extracted and the eluted fractions showed only one major peak indicated by both the C-terminal and N-terminal assays at a molecular weight of approximately 10 kD. This confirms that the major storage form of ANP is proANP 1–126. The lower panel of Fig. 6 shows the same measurements on extracted perfusates from isolated rat atria in vitro under both stretched and unstretched conditions. Two peaks are clearly shown that correspond to ANP and proANP 1–30. Thus, conversion of proANP to the various smaller peptides, including ANP, takes place during the secretion process and not at some peripheral site. In addition, there appears to be no significant differences in the processing of proANP into the smaller active peptides under either stretched or unstretched conditions [97].

6. Summary and conclusions

Numerous studies focusing on the mechanisms regulating ANP secretion clearly show that, from a physiological standpoint, the most important factor affecting ANP

secretion from the atria is mechanical stretch, which normally occurs when extracellular fluid volume or blood volume is increased. A scheme for the stimulation of ANP secretion by atrial stretch or endothelin and inhibition of ANP secretion by nitric oxide is shown in Fig. 7 below. Increases in atrial volume stimulate stretch-activated ion channels, which are most likely linked to a G_o regulatory protein. Endothelin, a potent vasoconstrictor, stimulates ANP secretion and augments stretch induced ANP secretion. One of the most dramatic increases in ANP release from the heart can be seen with cardiac ischemia, which appears to be mediated in part by endothelin-1. Nitric oxide (NO), an important vasodilator, is also produced by endothelial cells and inhibits ANP secretion acting through cGMP. Both cGMP and cAMP appear to have inhibitory effects on ANP release. The role of cell calcium on ANP secretion is less straightforward but basal ANP secretion appears to be inversely coupled to intracellular calcium release. A more complete picture of the mechanical and cellular events regulating the release of ANP has provided a better understanding of its role in disease states such as cardiac ischemia and heart failure.

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