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Review

Mechanical and neuroendocrine regulation of the endocrine heart

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Abstract

The cardiac natriuretic peptides (NP)—atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP)—are polypeptide hormones produced by cardiocytes in the atria of mammals. ANF and BNP are continuously released from the heart, but appropriate mechanical or neuroendocrine stimuli increase their rate of release with or without a concomitant increase in synthesis. The results of our investigations lead us to propose that the endocrine response of the heart to pressure or volume load varies in relation to whether the challenge is acute, subacute or chronic. The acute response to stretch is based on a phenomenon referred to as "stretch–secretion coupling" which results in enhanced secretion of NP stored in the atria. NP release following stretch is made at the expense of a depletable NP pool with no apparent effect on synthesis. The stimulation of NP production that is seen during mineralocorticoid escape is referred to as "subacute" and is characterized by stimulation of atrial ANF and BNP gene transcription secondary to volume overload in which plasma ANF, but not plasma BNP, is significantly elevated. With chronic stimulation, as seen in DOCA-salt treatment at the hypertensive stage, activation of the cardiac fetal program in ventricle is seen together with a stimulation of ANF and BNP production in both atria and ventricles. However, the activation of NP gene expression in the atria is not necessarily associated with fetal isogene expression even though the ventricular hypertrophic process is characterized by the expression of fetal isogenes, including ANF and BNP, that are normally expressed in the fetal ventricle. It seems likely that the acute stimulation of NP release is based on an electromechanical coupling. However, protracted stimulation of release is seen in situations in which profound neuroendocrine changes have taken place, thus suggesting that the primary stimulus for chronically enhanced NP gene expression and NP release is based on changes in the hormonal environment of the atrial cardiocyte. It is concluded that the endocrine heart responds to changes in hemodynamic load with specific changes in translational, post-translational and storage processes for ANF and BNP following acute or chronic stimulation. As a result, plasma levels of ANF and BNP may be used as indicators of the degree of atrial hemodynamic overload and ventricular hypertrophy, respectively. It may be advanced that the endocrine heart differentiates and responds to different hemodynamic challenges in either acute or chronic conditions with specific changes in transcription, translation, post-translational processing, storage, and release of ANF and BNP. We propose that this differentiation is part of the reason for the heart to produce two hormones with similar spectra of activity. This paradigm warrants further investigation.

Keywords: ANF (atrial natriuretic factor); Stretch; BNP (brain natriuretic peptide)

1. Introduction

The cardiac natriuretic peptides (NP) — atrial natriuretic factor (ANF) [1,2] and brain natriuretic peptide (BNP) [3] — are polypeptide hormones produced by cardiac muscle cells (cardiocytes) in the atria of mammals. Thus, these cells are involved in both the mechanical activity of the atria and the endocrine function of the heart. In fact, phenotypically, the bulk of mammalian atrial cardiocytes express elements common to muscle cell as well as endocrine cells [2].

ANF and BNP are continuously released from the heart, but appropriate mechanical or neuroendocrine stimuli increase their rate of release with or without a concomitant increase in synthesis. ANF and BNP have similar and remarkably wide spectra of biological properties which are predominantly mediated through increases of cGMP in target cells. Intracellular cGMP targets include cGMP-dependent protein kinases, cGMP-gated ion channels and cGMP-regulated cyclic nucleotide phosphodiesterases [4]. These properties give ANF and BNP the potential to interact in various manners at the integrative level. Indeed,

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the endocrine heart interacts with fast-responding as well as slow-onset mechanisms involved in cardiovascular homeostasis such as those based on modulation of the responses of the autonomic or central nervous system and those based in slower responding mechanisms such as the renin–angiotensin–aldosterone system. Further, it appears that the function of the endocrine heart is particularly relevant during deviations from homeostasis as demonstrated by recent investigations using the specific guanylyl-cyclase-coupled receptor blocker, HS-142-1 [5]. Blockade of guanylyl-cyclase-coupled NP receptors results in impairment of the ability of the cardiorenal axis to maintain cardiovascular homeostasis [6,7].

The dual contractile-secretory nature of atrial cardiocytes suggests *a priori* that the control of cardiac NP release may be related to atrial muscle mechanics. Stimulation of ANF release by atrial muscle stretch is a well-documented phenomenon demonstrable both in vivo and in vitro [8–12]. The increase in plasma ANF observed in head-out water immersion studies [9,10,13], is consistent with the view that an increase in atrial stretch is a physiological mechanism promoting ANF release. The exact nature of the stimulus sensed by the atrial cardiocytes with the consequent transduction of the stimulus (stretch–secretion coupling [11,12]) which results in an increased rate of ANF release remains to be elucidated and much controversial literature exists on the subject. Part of this controversy appears to arise from the different experimental systems used in the past to study the phenomenon of stretch–secretion coupling. For this reason, this review is limited largely to studies on whole tissue, relying heavily on our experience with the spontaneously beating, isolated rat atria preparation [11].

2. Influence of cationic environment on basal ANF release

The crucial role played by Ca^{2+} in the excitation–contraction coupling in muscle and in the stimulus–secretion coupling of most endocrine and neural cells led us to investigate possible relationships between the mechanical and endocrine function of atrial cardiocytes as possibly mediated by Ca^{2+}. Surprisingly, in investigations using the isolated perifused atrial preparation we found that basal ANF release is augmented in Ca^{2+}-free medium [14], a phenomenon that was rapidly reversed upon reintroduction of this ion. Further, increasing the plasmalemmal Ca^{2+} influx through the opening of voltage-sensitive Ca^{2+} channels by perifusion with media containing 49 mM K^+ reduced ANF release in nominally Ca^{2+}-free media and in media containing 0.625 mM Ca^{2+} while no changes in ANF release were observed at 1.25 and 2.5 mM Ca^{2+} despite the cessation of atrial contractility. These observations are at variance with those made for most other endocrine or neuroendocrine tissues under the same experimental conditions. We postulated that K^+ depolarization could conceivably exert a similar effect to re-introduction of Ca^{2+} in nominally Ca^{2+}-free medium or in 0.625 mM Ca^{2+} through an increase in cytosolic Ca^{2+} due to mobilization of intracellular stores. A similar effect has previously been observed in juxtaglomerular cells [15]. It is possibly of more than passing interest that both the atrial cardiocyte and the juxtaglomerular cell respond similarly to changes in their Ca^{2+} environment and that they both are modified muscle cells. At variance with these observations, it has been reported that basal ANF release was not affected by 8 mM K^+ in the Langendorff preparation [16], but others [17], using the same preparation, reported that 50 mM K^+ significantly reduced ANF release. Potassium depolarization has been reported not to increase ANF release in static atrial tissue incubations [18] but in cultured rat atrial cardiocytes, high K^+ is reported to stimulate ANF release [19–22] or to not affect it [23].

Further insight into the relevance of the ionic environment to basal ANF release was obtained by substituting Ca^{2+} by Ba^{2+}, Sr^{2+} or La^{3+} in the perifusion media [12]. When the response of atrial cardiocytes to these changes is compared to other endocrine cells under the same conditions, it becomes clear that the mechanical activity of the atrial cardiocyte is largely dissociated from its endocrine function. Our study showed that ANF release responded uniquely to Sr^{2+} and Ba^{2+}. Ba^{2+} perifusion did not alter the pattern of ANF release, but perifusion within Sr^{2+}-containing media induced a gradual increase in ANF release starting at 30 min of Sr^{2+} perifusion, although this did not reach significant values. This is an effect similar to that obtained by Ca^{2+} removal from the perifusion medium and shows that Sr^{2+} is not as effective as Ca^{2+} to tonically inhibit ANF release. La^{3+} prevented the sharp increase in the rate of ANF release expected in perifusion media with no Ca^{2+} added, suggesting that La^{3+} might be blocking the Ca^{2+} fluxes by displacement of Ca^{2+} from the cell membrane, leading to an increase in cytosolic Ca^{2+} concentrations.

A most valuable insight from these studies was the finding that although the different cations used to replace Ca^{2+} exerted different effects on atrial ANF release, they all inhibited atrial beating and developed tension, suggesting that, in general, ANF release is independent of atrial contraction. We obtained evidence in support of this view in studies with rat atria in vitro [24,25] in which it was demonstrated that the rates of basal ANF release do not differ in electrically driven or quiescent atrial muscle. Moreover, we did not observe changes in ANF release from either the rat left auricle or human atrial trabeculae subjected to different pacing frequencies even though the tissues showed their characteristic positive and negative (staircase) changes in developed tension with increasing pacing frequency. This is supported by other reports in which changes in pacing frequency had no effect on ANF release from perfused rat hearts [26] or rabbit atria [27]. Other investigators have found a direct correlation between

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frequency of contraction and ANF release from atria in vitro [17,28,29] or in rapidly paced animals [30–32]. The increased plasma ANF observed in chronotropic stimulation in vivo is accompanied by an increase in intra-atrial pressures [30,31] and pulmonary wedge pressures [31], which could be responsible for the increased secretion of ANF. In fact, increased ANF plasma levels in rapidly paced dogs is dependent mostly on the increase in passive atrial diastolic stretch [33]. In addition, rapid pacing cannot sustain elevated ANF plasma levels [32]. It has also been reported [34] that in humans with atrial paralysis and in patients with normal atrial activity paced at 70 beats/min in VVI mode for complete heart block, atrial contraction is not necessary for ANF release. Further, in humans, atrial pacing did not increase atrial pressure or circulating ANF [35]. However, ventricular pacing resulted in a significant increase in atrial pressure, resulting in increased circulating ANF levels. These findings suggest that atrial tachycardia *per se* does not stimulate ANF release.

Manipulations affecting Ca^{2+} release and uptake from the sarcoplasmic reticulum confirmed the view that basal ANF release and excitation–contraction coupling do not share a common regulator in Ca^{2+}. Caffeine increased resting tension and beating rate but did not affect peptide release [12]. Ryanodine did not change the basal rate of ANF release in the same preparation, but the magnitude of the release response to atrial stretch appeared reduced [12,36,37]. Therefore, unlike basal release, stretch-induced ANF release seems to partially depend upon a Ca^{2+} pool that is sensitive to ryanodine [12,36,37].

Taken together, the above results point to a dissociation of basal ANF release from excitation–contraction coupling in atrial cardiocytes, and supports the view that fluctuations in cytosolic Ca^{2+} concentrations are not a prerequisite for basal ANF release or even for stretch-induced ANF release [14]. The latter is in contrast to the known controlling role of Ca^{2+} in most secretory processes. It has come to light that Ca^{2+} also negatively modulates the basal expression of the ANF gene [38], thus further supporting the proposed role of Ca^{2+} as a negative modulator of ANF synthesis and secretion. Recently, it has been proposed that Ca^{2+}, while exerting a negative modulatory role for ANF under basal conditions as reported by us, is necessary for agonist-mediated release [39]. However, as no physiological condition is expected to entail a very low or non-Ca^{2+} environment, our findings should only be taken as indicative of differences between atrial cardiocytes and most other endocrine cells. Above all, the findings show a dissociation between the mechanical activity of the atria and its secretory function.

3. Stretch and modulation of NP secretion and synthesis

Atrial muscle stretch has been repeatedly shown to stimulate ANF release from atrial cardiocytes both in vivo and in vitro [8–11,13,40–42]. Initial speculation about the in vivo mechanism of release centred on the theory that atrial pressures were important in determining release of the peptide, but experiments in which atrial transmural pressure was kept constant pointed out that it was volume-load-induced stretch of the muscle that was the major determinant of ANF release in vivo [43]. These observations were reinforced by studies in humans with cardiac tamponade, in which the high pressure and low volume in the atria resulting from pericardial blood or effusion is associated with low circulating ANF levels, and relief of this situation results in greater plasma ANF levels [44,45]. This clearly shows that it is an increase in atrial dimensions (and therefore stretch) which is the main stimulus for increased ANF release, and not changes in atrial pressure.

Athough atrial muscle stretch is widely believed to be the main stimulus for ANF release, we and others have observed that even when atrial stretch is maintained or repetitive stretch is used, the acute increased in ANF release observed decays to baseline values within minutes both in vivo and in vitro [8,11,41,42,46]. The rapid decline in ANF output observed after acute atrial muscle stretch suggests the existence within atrial cardiocytes of an acutely releasable hormone pool that is exhausted within minutes after the initial stretch stimulus. We tested this hypothesis using the isolated rat atria preparation and a double isotope labelling pulse-chase protocol [47]. We found that, in the basal condition (0.2 g load), a portion of ANF is immediately and preferentially released following synthesis, and the rest is taken into tissue stores and released from them at a lower rate. The stretch-stimulated ANF released that follows an increase in load to 5 g is from newly synthesized ANF and there is no change in older, stored ANF. These data suggest that stretch may play a role in increasing the translocation of newly synthesized ANF into a stretch-sensitive pool.

In order to better understand the depletable nature of the stretch-sensitive atrial ANF pool, we have investigated whether atrial stretch is accompanied by changes in ANF gene expression [48]. It is of interest to note that a link between atrial stretch and changes in ANF gene expression has been inferred from in vivo studies [49–51], but no direct evidence exists to support this notion. Stretch did not alter ANF mRNA levels over a 4-hour period of stimulation. In cultured neonatal cardiocytes of atrial or ventricular origin, stretch-induced changes in ANF gene expression occur after 24 hours [52,53]; therefore it is likely that more time is required for stretch to effect changes in ANF mRNA levels in atrial tissue. However, a rapid induction of ANF gene expression by stretch has been observed in papillary muscle strips [54]. We also studied the response of BNP to stretch [48]. Surprisingly, neither BNP secretion nor gene expression was significantly stimulated by stretch. However, stretch did enhance the expression of the early response genes *c-fos*, *Egr-1* and *c-myc* [48], as has been also shown in ventricular cardio-

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cytes [53]. Others have observed stretch-induced increases in BNP secretion and gene expression, using a modified Langendorff preparation [55]. In an in vivo model, infusion of phenylephrine or AVP caused a rapid increase in BNP plasma levels, and increased BNP tissue content and mRNA levels in atria and ventricles within 4 hours [56], presumably due to the alteration in hemodynamics caused by the pressor agents. There exist no data in the literature that addresses the possibility that increases in NP translation may occur independently from changes in NP mRNA levels, so it is considered that changes in NP mRNA levels reflect an increase in NP synthesis.

The mechanism underlying the mechanotransduction process responsible for stretch-induced ANF release remains to be elucidated. One of the possible mechanisms proposed as an initiator of the stretch-induced increase in ANF secretion is the action of mechanosensitive ion channels. Supporting this claim, a recent report [57] has shown that gadolinium (Gd^{3+}), an inhibitor of stretch-activated ion channels in *Xenopus* oocytes [58] and chick ventricular cardiocytes [59], can inhibit stretch-induced ANF release from perfused atria. Mechanosensitive K^+-selective [60], cation-selective [61,62] and anion-selective [63] channels have been described in rat atrial cardiocytes; however, these channels are insensitive to Gd^{3+}. Therefore, the effects of Gd^{3+} on ANF secretion either might occur through an undefined Gd^{3+}-sensitive channel or are due to effects of Gd^{3+} that are distinct from their effects on mechanosensitive ion channels. It has been pointed out, however [57], that the mechanosensitive ion channels may be in atrial cells other than the cardiocytes, such as endothelial cells [64], and their activation might lead to the release of a factor that would influence ANF secretion. A recent preliminary report has suggested that paracrine actions of endothelin in atria mediate stretch-induced ANF secretion [65].

Mechanosensitive ion channels have also been conjectured as mediators of stretch-induced changes in ANF gene expression. Linear stretch of ventricular cardiocytes plated on silicone membranes results in increased ANF mRNA levels, as well as increased early-response gene, β myosin heavy chain, and α skeletal actin gene expression [53]. A Gd^{3+}-sensitive stretch-activated ion channel has been characterized in this model, but blocking it with Gd^{3+} is ineffective in inhibiting the stretch-induced increase in early-response gene mRNA levels [66]. It has since been shown that the stretch-induced increase in ANF mRNA levels is due to autocrine release of angiotensin II from the ventricular cardiocytes [67]. Cultured neonatal atrial cardiocytes stretched by hypotonic swelling [68] or by cyclical stretching on flexible membranes [52] have increased ANF mRNA levels; however, atrial cardiocytes do not appear to synthesize angiotensin II [67], thus eliminating this as a possible mediator of stretch-induced changes in ANF gene expression in atrial cardiocytes. The mechanosensitive channels described in atrial cardiocytes might be involved in regulating stretch-induced changes in ANF gene expression. However, until specific blockers are available for these channels, this cannot be conclusively demonstrated. Therefore, the involvement of stretch-activated ion channels and the mechanisms involved in modulating stretch-induced ANF gene expression in atrial cardiocytes are still unclear.

4. Neuroendocrine modulation of NP secretion and synthesis

Since stretch appears to effect only transient changes in NP secretion, it is probable that neural or endocrine factors are involved in stimulating NP secretion. Several such factors have been proposed: ANF release is believed to be effected by adrenergic agonists [69,70] endothelin-1 (ET-1) [71], glucocorticoids [72], acetylcholine [73], Na-K-ATPase inhibitors [74–76], AVP [56,77,78], prostaglandins [79], thyroid hormone [80], and angiotensin II [81].

Of these, the vasoconstrictor ET-1 is the most potent stimulator of ANF secretion. ET-1 rapidly stimulates ANF release from isolated atria [48,71,82] and perfused hearts [83]. ET-1 also stimulates BNP secretion from atria [48] and atrial cardiocytes in culture [84,85]. Although it is more sustained than that effected by stretch, ET-1-stimulated increases in NP secretion are transient [48], which is probably due to desensitization of ET-1 receptor binding, and the subsequent down-regulation of phospholipase C activity [86]. The time course of ET-1-stimulated BNP secretion from atrial tissue has different release kinetics from that observed for ANF release [48], suggesting that each peptide utilizes a different secretory pathway. Since ET-1 is produced in endothelial [87], and mesothelial cells [88] that are in close proximity to the cardiocytes, it is possible that ET-1 acts in a paracrine fashion to modulate ANF secretion. This theory has recently been supported by preliminary observations that the ET_A receptor antagonist BQ123 can inhibit stretch-induced ANF release from isolated atria [65].

Conflicting evidence has arisen from experiments attempting to define the secretory effects of adrenergic agonist stimulation of atrial cardiocytes. Norepinephrine has been shown to increase ANF secretion from isolated rat atria [69], and cultured atrial cardiocytes [89], although other reports show that it had no effect on ANF secretion from cultured neonatal atrial cardiocytes [90], and that it decreased ANF secretion from perifused atrial strips [91]. Epinephrine increased ANF secretion from freshly isolated adult atrial cardiocytes [90] and perifused atria [92], but decreased ANF release from perifused atrial strips [91]. Norepinephrine in the presence of the β-adrenergic receptor, propranolol, increased ANF secretion from cultured adult atrial cardiocytes, suggesting that α_1-receptor activation is mainly responsible for norepinephrine-stimulated

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ANF release [89]. ANF secretory rates are also rapidly increased with α_1-specific drugs [19,69,70,93,94]. The β-adrenergic agonist, isoproterenol, has been shown to increase ANF secretion rates from freshly isolated adult atrial cardiocytes [90] and perifused atria [69,92,95] but had no effect on ANF release from a heart–lung preparation [93] or cultured neonatal atrial cardiocytes [19], and decreased ANF release from perifused atrial strips [91]. Isoproterenol also reduced phenylephrine-stimulated ANF secretion from cultured neonatal or adult atrial cardiocytes [70,89]. It is unclear from these results which receptor is mainly responsible for adrenergic effects on ANF secretion. However, most evidence is in favour of a predominant effect of the α_1-adrenergic receptor subtype. The secretion of BNP from cultured neonatal ventricular cardiocytes is also stimulated by α_1-adrenergic stimulation, and not by isoproterenol [96]. We have recently shown that phenylephrine can also stimulate the secretion of BNP from atrial tissue [94]. We observed that the stimulated secretion of BNP was more sustained than that of ANF, further supporting the concept that different pathways of secretion are used by each NP.

The involvement of the autonomic nervous system in the control of ANF secretion has also been suggested. It was initially shown that hypothalamic lesions reduced basal ANF secretion [97]. Subsequently, the same researchers performed lesions in the hypothalamus or pituitary, which blunted volume-expansion-induced ANF secretion [98]. It was suggested that muscarinic and α_1-adrenergic receptors were involved in this process [99]. The involvement of the ascending serotonergic system in controlling basal and stimulated ANF secretion has also been proposed [100]. These authors put forth the theory that distension of baroreceptors of the atria, carotid and aortic sinuses; and kidney, results in alterations in the afferent output to the brainstem noradrenergic neurons, which by the means of axons projecting into the AV3V region of the brain, activated cholinergic neurons via an α_1-adrenergic synapse. It is proposed that the loop makes its way back to the heart to effect release of ANF from the atria. This has gained support from the observations that stimulation of cardiac nerves in perfused heart stimulates ANF secretion, and that chemical sympathectomy abolished this response [101]. The involvement of the pituitary in controlling ANF secretion has also been proposed following the observations that pituitary lesions caused a decrease in ANF plasma levels, and that this could be reversed by reintroducing the resected tissue [102].

The involvement of neuroendocrine agents in the modulation of NP gene expression has mostly been studied in cultured cardiocytes, in which it was found that ANF gene expression can be stimulated by ET-1 [103–105], glucocorticoids [106], thyroid hormone [107], growth factors [108,109], α_1-adrenergic agonists [110], angiotensin II [111], prostaglandins [79], and thrombin [112]. Hydroxyvitamin D_3 negatively regulates the transcription of the ANF gene [113]. BNP gene expression in cultured neonatal atrial cardiocytes is also increased by ET-1 [84], and α_1-adrenergic agonists stimulate its expression in cultured neonatal ventricular cardiocytes [96]. The observations on the effects of thyroid hormone and glucocorticoids have been extended to in vivo studies [107,114,115].

We have studied the response in adult atrial tissue of both ANF and BNP to ET-1 [48]. Acute stimulation of ANF secretion for up to 4 hours by ET-1 was not accompanied by changes in synthesis as measured by steady-state mRNA levels. ET-1 transiently stimulated BNP gene expression, suggesting that ANF and BNP gene expression is modulated by ET-1 through partially independent processes, as is their secretion. We have also shown that phenylephrine stimulates ANF gene expression in atrial tissue after 6 hours; BNP mRNA levels were also enhanced at the same time point, and remained elevated after 8 hours of stimulation [94]. The time course in phenylephrine-stimulated NP gene expression is distinct from that of stimulated NP secretion, which indicates that in an acute setting, NP synthesis and secretion are not intimately linked. From these observations the notion arises that the endocrine heart differentiates between types of stimuli by differentially controlling production of one or the other NP. This differentiation extends to the expression of the early-response genes *c-fos*, *Egr-1* and *c-myc* that were all stimulated by stretch, while ET-1 strongly induced *Egr-1* expression only [48]. Phenylephrine stimulated *Egr-1* and *c-myc* gene expression, albeit following a time course that differed from that elicited by stretch [94]. The changes in NP and early-response gene expression found appear specific enough to advance the idea that atrial gene expression is differentially modulated by mechanical or neuroendocrine stimuli.

5. Chronically enhanced NP synthesis and secretion

It is generally believed that chronically enhanced release of ANF and BNP is mediated by hemodynamically-induced muscle stretch. The mechanical stimulus brought about by changed hemodynamics is translated into changes in the circulating levels of peptides. As discussed above, ANF acutely released through this mechanism arises from a rapidly depleting pool of newly synthesized peptide, and therefore it does not appear that sustained demand may be met simply by muscle stretch.

Chronic elevations in circulating NP, unlike acute ones, are seen together with the cardiac gene re-programming associated with cardiac hypertrophy [116] and with drastic changes in ventricular NP synthesis, storage, and release [117–122]. The mechanisms underlying the re-expression of fetal isogenes, including the increased expression of ventricular ANF, BNP or β myosin heavy chain (MHC) are far from clear, but these processes hint at a relationship between the control of NP genes and cardiac hypertrophy

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and, hence, to a number of growth-related entities such as proto-oncogene products and growth factors [108,116]. In fact, there exists clear experimental evidence for functional interactions between proto-oncogene products and the ANF gene promoter [123–125]. However, while it is true that c-fos and c-jun interacts with the ANF promoter, it remains to be determined if this mechanism is operative in vivo. Therefore, it is not possible to directly correlate changes in proto-oncogene expression and changes in NP gene expression.

The possible relationship between chronically enhanced NP production by the heart, hemodynamic overload, and cardiac growth and hypertrophy as discussed above does not indicate whether these processes are coordinately regulated. Indeed, as mentioned above, we have been able to dissociate cardiac muscle mechanical activity from NP release [12]. In recent investigations [119], we characterized the transcriptional and post-translational changes of cardiac ANF and BNP as well as MHC isoform expression in deoxycorticosterone acetate (DOCA)-salt-treated rats. We had previously demonstrated [126] that DOCA-salt treatment leads to a powerful stimulation of cardiac NP that is manifested by significant changes in the number of specific atrial granules as determined by morphometry. Short-term experiments with the same model have shown that DOCA-salt treatment causes increases in ANF mRNA levels in all chambers of the heart [49,127]. In another model characterized by volume overload followed by hypertrophy (aortocaval fistula), it was shown that ANF [49,127] and BNP [127] mRNA levels were increased in atria [49,127] and ventricular [49] tissues.

After DOCA-salt treatment, we found changes in NP production that were specific for either the pre-hypertensive (1 week treatment) or the hypertensive stage of the model (5 weeks treatment) but dissimilar for ANF and BNP [119]. After the first week of DOCA-salt treatment we found a partial depletion of ANF atrial content and no increase in ANF mRNA together with elevated ANF plasma levels, suggesting increased demand over supply. These findings are in line with the studies using the isolated atria preparation which, as discussed above, showed no evidence of increased peptide synthesis following muscle stretch. We also found in the in vivo model that atrial BNP stores and BNP plasma levels remained unaffected even though changes in these parameters were observed for ANF [119]. This occurred even though at least a portion of atrial BNP is released via a regulated pathway as shown by the above-mentioned investigations using ET-1 and phenylephrine in the isolated atrial preparation [48,94] and that BNP is partially co-stored with ANF in the specific atrial granules [128,129]. However, total BNP content may be a poor measure of the pool of BNP that responds to DOCA-salt treatment, because although the total tissue content of atrial BNP did not change after 1 week of treatment, a fraction of purified granules isolated from atrial tissue of treated animals showed a 35% decrease in BNP content. This suggests that the fraction of BNP that is stored in granules and probably destined for regulated release is sensitive to DOCA-salt treatment. These observations are compatible with our previous findings in normal atrial tissue from which 40% of total ANF and only 8% of the total BNP were recovered in the purified granule fraction following tissue fractionation (our unpublished data). This suggests that, compared to ANF, a smaller proportion of BNP proceeds to the storage pool after synthesis and that the bulk of BNP may be exported from the cell via constitutive secretory vesicles. Therefore, changes in BNP stored in granules contribute less significantly to changes in total NP content than changes in stored ANF content do. If most of the BNP is secreted via a constitutive pathway,and given that ANF and BNP circulate as processed peptides ($ANF_{99\text{-}126}$ and BNP_{51-95}, respectively) [130,131], it is possible that BNP is processed before proceeding to storage, while ANF is processed co-secretionally as the predominant form of tissue ANF is proANF (ANF_{1-126}) [132] while a significant proportion of tissue BNP is BNP_{51-95} [133].

After 5 weeks of DOCA-salt treatment both ANF and BNP mRNAs were significantly augmented in the left atrium and the plasma levels of both peptides were clearly increased [119]. By this time, hypertrophy of this chamber had taken place and ANF content remained significantly depressed while that of BNP tended to be higher. This is consistent with observations in the aortocaval fistula model, in which increased atrial ANF and BNP mRNA levels were associated with atrial hypertrophy [127]. Increased atrial ANF mRNA levels have also been observed in spontaneously hypertensive rats (SHR) [134,135], in dogs subjected to rapid ventricular pacing [136], and in humans with various types of disease in which overload of the atrium occurred [50,51,137]. Atrial BNP gene expression is also increased in SHR and SHR-stoke-prone rats [135,138], and in humans with congestive heart failure [137]. These findings suggest that increased BNP release depends on increased steady-state levels of BNP mRNA and likely reflect the mainly constitutive nature of BNP release referred to above. Increased ANF release, on the other hand, may be supported at least in part through an increased utilization of the ANF present in the atrial granule storage pool and in part by an increased rate of peptide synthesis.

It is important to note that DOCA-salt treatment induces a significant LV hypertrophy after 1 week of treatment without blood pressure elevation, suggesting that blood pressure, a determinant of left ventricular wall tension, is not a primary trigger in the initiation of left ventricular hypertrophy in this model. We found that both ANF and BNP peptide and mRNA left ventricular levels in the DOCA-salt rats were comparable to those found in controls after 1 week, indicating that neither ANF nor BNP gene transcription is enhanced in the early phase of left ventricular hypertrophy [119]. In contrast, in the SHR, BNP mRNA and peptide levels were significantly in-

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creased in the pre-hypertensive phase [122,135], while ANF mRNA levels were comparable to those of control Wistar-Kyoto rats [135]. These findings demonstrate significant differences between non-genetic and genetic models of ventricular hypertrophy for BNP gene expression.

The degree of change in NP mRNA levels usually correlates with the degree of hypertrophy [116,139–143]. For example, LV hypertrophy induced by aortic co-arctation or experimental infarct raises ANF mRNA to levels higher than those induced by volume load [142]. In genetically hypertensive rats [135,138] or humans with congestive heart failure [137,144], the BNP gene is strongly induced in the ventricle, to a degree far greater than in the atria. It has been suggested that BNP is more of a ventricular hormone, since it is so strongly recruited in overloaded ventricular tissue. A significant ANF and BNP mRNA increase was detected in the left ventricle of rats treated for 5 weeks with DOCA-salt [119]. These animals exhibited pronounced left ventricular hypertrophy. Thus, in the DOCA-salt model, up-regulation of ANF and BNP genes appears to be linked with the hypertrophic process only in a late phase of hypertrophy.

Findings obtained during the time course of DOCA-salt treatment in the different cardiac chambers showed that anatomical hypertrophy, stimulation of NP production and MHC isoform expression are processes that may be disassociated from each other. In rodents, the increased expression of βMHC relative to the adult αMHC isoform and the increased expression of ANF and BNP, are hallmark phenotypic changes of the hypertrophied ventricle [53,145,146]. However, it is not known whether there is a necessary or a coincidental association between βMHC and increased NP expression in the hypertrophied ventricle. Our studies [119] showed that in the left atria, increased NP production occurs in the presence of anatomical hypertrophy but without induction of MHC isoform switch. These results suggest that the stimuli for rat atrial cardiocyte growth do not active MHC isoform transition. The hypertrophied left ventricle in 5-weeks-treated DOCA-salt rats, unlike the atria, exhibited a significant degree of MHC isoform switch. In the same model, α skeletal actin mRNA has been reported to be re-expressed in the hypertrophied left ventricle [147]. Therefore, the ANF and BNP genes would appear to share sensitivity to a common set of regulatory stimuli with the structural contractile protein genes. However, our studies show that MHC isoform switch also occurs in the non-hypertrophied right ventricle and that it occurs to an equal extent to that seen in the hypertrophied left ventricle.

The studies above showed that plasma BNP is not elevated in the early stage of hypertrophy but is increased in moderate or severe stage of left ventricular hypertrophy. In contrast, plasma ANF in DOCA-salt rats are increased after 1 weak, possibly reflecting volume overload, and are maintained elevated at 5 weeks through atrial and ventricular neosynthesis and secretion [119,120]. This would suggest that ANF and BNP in plasma may be used as indicators of the degree of atrial hemodynamic overload and ventricular hypertrophy respectively.

The mechanisms involved in eliciting chronic changes in NP synthesis and secretion are unclear. The involvement of the growth-promoting renin–angiotensin system (RAS) and ET-1 have been addressed in in-vivo studies using blockers of AII [67,148,149] and ET-1 receptors [150], as well as angiotensin-converting enzyme (ACE) blockade [149,151]. The conclusion derived from these studies is that, as suggested from in vitro studies [104,111], these trophic factors are at least partly involved in modulating the hypertrophic response of the ventricles to hemodynamic overload, as well as the associated increases in ANF production and secretion. However, it is unclear whether it is the hemodynamic effects of these factors or their direct effect on cardiocyte growth that is responsible for the changes in NP production. In SHR rats, for example, NP synthesis is increased before elevation of blood pressure takes place [135], and in DOCA-salt-treated rats [119] increased NP synthesis occurs coincidentally with both hypertension and hypertrophy. We have taken advantage of the fact that in aortic banded rats, a low dose of the ACE inhibitor, ramipril, can prevent or regress hypertrophy without affecting blood pressure, while a higher dosage can normalize both parameters [152]. From these experiments [153] we have discovered that there are two distinct components that affect NP plasma levels and ventricular synthesis: one that is dependent on anatomical hypertrophy, and a more important one that is dependent on hemodynamic load. On the other hand, MHC isoform expression in this model is dependent only on hemodynamic load [153]. These results support the notion that ventricular NP synthesis is not necessarily associated with cardiac hypertrophy, and can be dissociated from MHC isoform expression.

An intermediate type of response of the endocrine heart to hemodynamic overload may be discerned in experiments dealing with the mineralocorticoid escape [7]. These experiments were carried out to demonstrate that cardiac ANF contributes significantly to the ability of the kidney to "escape" the continued sodium-retaining effects of mineralocorticoid (DOCA) excess. This investigation also provided useful information regarding the regulation of cardiac NP production. During mineralocorticoid escape, plasma ANF rose significantly in response to daily DOCA injection, but plasma BNP did not change significantly. This is similar to observations made in volume-loaded humans, in which ANF plasma levels were increased, but BNP secretion was unaffected [154]. Atrial ANF content decreased significantly after 24 hours of DOCA treatment and continued to decline by 48 and 72 hours. ANF and BNP transcript levels increased by the third day. Unlike the findings after 5 weeks DOCA-salt treatment above, no significant changes in peptide or mRNA content for ANF or BNP were observed in the ventricles of DOCA-treated

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animals. We suggested that extracellular volume expansion due to mineralocorticoid excess results in increased atrial ANF synthesis and release. The less pronounced stimulation of plasma BNP levels as compared to ANF during DOCA treatment could be related to the lack of stimulation of ventricular BNP synthesis.

6. Conclusions

The results of all of the above investigations lead us to propose that in terms of the endocrine response of the heart to changes in pressure or volume load, three types of responses may be differentiated. Firstly, the acute response to stretch is based on stretch–secretion coupling, resulting in enhanced secretion of ANF stored in the atria sufficient to increase plasma levels of the hormone. BNP, which undoubtedly is released in increased amounts from the granules together with ANF, is only 1–2% of ANF levels in tissue, and, therefore, is not released in sufficient amounts to significantly alter plasma levels. The release in the acute situation is made at the expense of a depletable pool, and synthesis of the NP is not affected. Secondly, there exists an intermediate response, which is referred to as a "subacute" type of stimulation of NP production, and which is seen during the mineralocorticoid escape phenomenon. This stimulation is characterized by stimulation of ANF and BNP gene transcription secondary to volume overload in which plasma ANF but not plasma BNP are significantly elevated. Neither ANF nor BNP gene expression is stimulated in ventricles. Thirdly, with chronic stimulation, as seen in the DOCA-salt model at the hypertensive stage, the cardiac fetal gene program is activated and stimulation of ANF and BNP takes place in both atria and ventricles. However, the activation of NP gene expression is not necessarily associated with fetal isogene expression in atria even though the ventricular hypertrophic process is characterized by the expression of fetal isogenes including ANF and BNP that are normally expressed by the bulk of ventricular cardiocytes during fetal life.

It seems likely that the acute stimulation of NP release is based on an electrochemical coupling. However, protracted stimulation of cardiac NP gene expression and release in seen in situations in which profound neuroendocrine changes have taken place, thus suggesting that the primary stimulus for chronically enhanced NP gene expression and NP release is based on changes in the hormonal environment of the atrial cardiocyte.

From the above studies it may be advanced that the endocrine heart differentiates and responds to different hemodynamic challenges in either acute or chronic conditions with specific changes in transcription, translation, post-translational processing, storage, and release of ANF and BNP. We propose that this differentiation is part of the reason for the heart to produce two hormones with similar spectra of activity. This paradigm warrants further investigation.

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