ROLE OF ATRIAL NATRIURETIC FACTOR IN EXPERIMENTAL HIGH-OUTPUT HEART FAILURE

by

Gefei Qing

A Thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of Master of Sciences

Gefei Qing, October 1992

Department of Medicine
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ABSTRACT

Atrial natriuretic factor (ANF), produced mainly by mammalian atria, has been shown to exert potent diuretic, natriuretic, vasodilator, and renin-aldosterone suppressing activities. Atrial stretch induced by either a chronic preload or afterload stimulates ANF release. Therefore, ANF may be involved in the regulation of blood pressure and body fluid as well as electrolyte balance in physiological and pathophysiological states.

Neurohormonal vasoconstrictor systems are frequently activated during the development of chronic heart failure (CHF), including increased activity of the sympathetic nervous and renin-angiotensin systems (RAS) and vasopressin, which together evoke such characteristic CHF abnormalities as vasoconstriction, changes in cardiac performance, and sodium retention.

The characterization of pathological alterations of CHF seems incompatible with the physiological actions of ANF. Thus, it has been hypothesized that the pathophysiology of CHF may be due to a relative deficiency of ANF and/or blunted responsiveness to ANF in this state. The work presented in this thesis evaluates the role of ANF in rats with chronic moderate high-output heart failure by (1) characterizing plasma and tissue ANF levels, hemodynamics and renal function at different stages of the development of heart failure; (2) assessing the contribution of the atria and ventricles to plasma ANF levels; and (3) investigating the role of ANF and RAS in rats with aorto-caval (A-C) shunts.

Chronically increased cardiac filling pressure stimulated not only ANF release but also ANF synthesis in each cardiac chamber. This in turn contributed to elevated plasma ANF levels in A-C shunt rats. An attenuated renal response to endogenous ANF and sodium and water retention were apparent in A-C shunt rats. Under inhibition of RAS, plasma ANF may exert its actions more effectively. Thus, chronic ACE inhibition and ANG II receptor antagonism improved hemodynamic conditions,

diminished water retention, reversed cardiac hypertrophy, and restored plasma and tissue ANF to more "normal" levels in rats with moderate high-output heart failure.

Taken together, elevated plasma ANF levels may play an important role in maintaining hemodynamic and body fluid homeostasis by opposing the neurohormonal vasoconstrictor systems in A-C shunt rats. However, a relative deficiency of plasma ANF and/or an attenuated response to endogenous ANF, mediated by activated neurohormonal vasoconstrictor systems, may contribute to the pathophysiology and development of heart failure at different stages.

RESUME

Il a été démontré que le facteur natriurétique de l'oreillette (FNO), produit principalement par les oreillettes de mammifères, exerce de puissantes activités diurétiques, natriurétiques et vasodilatatrices ainsi qu'une répression du système rénine-angiotensine. L'étirement des oreillettes, qu'il soit provoqué par une précharge ou une postcharge chronique, stimule la relâche du FNO. Par conséquent, le FNO pourrait être impliqué dans la régulation de la pression sanguine, des fluides corporels et de la balance électrolytique lors d'états physiologiques et pathophysiologiques.

Les systèmes vasoconstricteurs neurohormonaux sont fréquemment activés au cours du développement de l'insuffisance cardiaque chronique (ICC). Ainsi l'augmentation de l'activité du système sympathique nerveux, du système rénine-angiotensine (RAS) et de la vasopressine évoquent des anomalies caractéristiques de l'ICC telles la vasoconstriction, la rétention de sodium et des changements de la performance cardiaque.

La caractérisation des altérations pathologiques associées à l'ICC semble démontrer une incompatibilité avec les actions physiologiques du FNO. Il a donc été suggéré que la pathophysiologie de l'ICC pourrait être due à une déficience relative de FNO et/ou à la réactivité affaiblie au FNO rencontrée dans cet état. Le travail présenté dans ce mémoire évalue le rôle du FNO lors d'une ICC modérée à haut débit cardiaque (induite par un shunt aorto-caval (A-C)) chez le rat par 1) la caractérisation des niveaux plasmatiques et tissulaires du FNO, de l'hémodynamique et de la fonction rénale à différentes étapes du développement de l'insuffisance cardiaque; 2) l'évaluation de la contribution des oreillettes et des ventricules aux niveaux plasmatiques du FNO; et 3) l'investigation du rôle du FNO et du RAS ainsi que les effets du losartan lors de l'insuffisance cardiaque.

La pression cardiaque de remplissage chroniquement élevée stimule non seulement la relâche du FNO, mais aussi la synthèse du FNO aux niveaux de chacune des chambres cardiaques. Ceci, par la suite, contribue à élever les niveaux plasmatiques

de FNO chez les rats possédant un shunt A-C. On retrouve une réponse rénale atténuée au FNO endogène ainsi qu'une rétention hydro-sodée chez les rats possédant un shunt A-C. Lors de l'inhibition du RAS, le FNO plasmatique semble exercer ces actions plus efficacement. Ainsi, l'inhibition chronique de l'enzyme de conversion de l'angiotensine II ou l'antagonisme des récepteurs de l'angiotensine II améliore les conditions hémodynamiques, diminue la rétention d'eau, renverse l'hypertrophie cardiaque et rétablie le FNO plasmatique et tissulaire à des valeurs plus «normales» chez des rats ayant une insuffisance cardiaque modérée à haut débit cardiaque.

Les niveaux élevés de FNO plasmatiques semblent jouer un rôle important dans le maintien de l'hémodynamique et de l'homéostase des fluides corporels en s'opposant aux systèmes vasoconstricteurs neurohormonaux des rats possédant un shunt A-C. Cependant, une déficience relative du FNO plasmatique et/ou une réponse atténuée au FNO endogène, due à l'activation des systèmes vasoconstricteurs neurohormonaux, peuvent contribuer à la pathophysiologie et au développement de l'insuffisance cardiaque.

PREFACE

In writing this thesis, the author has taken advantage of the option provided by the regulations of the Faculty of Graduate Studies and Research which allows for the inclusion of original papers, suitable for submission to learned journals for publication, as part of the thesis.

The guidelines read as follows:

Candidates have the option, **subject to the approval of their Department**, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provided that these copies are bound as an integral part of the thesis.

- If this option is chosen, **connecting texts, providing logical bridges between the different papers, are mandatory**.
- The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published. **The thesis must included, as separate chapters or sections:** (1) a Table of Contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and /or summary.
- Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.
- In the case of manuscripts co-authored by the candidate and others, **the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent;** supervisors must attest to the accuracy of such claims at the

Ph.D. Oral Defense. Since the task of the Examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers.

Chapter I is a general introduction. Chapter II and III are scientific articles in their original form. Chapter II is in press in "Cardiovascular Research". Chapter III is published in the "American Journal of Physiology" (263: H833-H840, 1992) Chapter IV includes a general discussion with claims to originality. A description of the prospect of future studies on the role of ANF in CHF is also included A general bibliography follows chapter IV and includes the references cited in the general introduction and discussion. References for each individual article (Chapters II and III) are included within each respective chapter. The terms "we" and "our" refer to myself and my thesis supervisor, Dr. Raul Garcia.

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ABBREVIATIONS

ACE:	angiotensin converting enzyme
A-C shunt:	aortocaval shunt
ANF:	atrial natriuretic factor
ANG II:	angiotensin II
cGMP:	3',5'-cyclic guanosine monophosphate
CHF:	chronic heart failure
dP/dt max:	first derivative of left ventricular pressure
FNO:	facteur natriurétique de l'oreillette
ICC:	insuffisance cardiaque chronique
LVEDP:	left ventricular end-diastolic pressure
MAP:	mean arterial pressure
PRA:	plasma renin activity
RAP:	right atrial pressure
RAS:	renin-angiotensin system
RIA:	radioimmunoassay
UNaV:	urinary sodium excretion
UV:	urinary volume

CHAPTER I

GENERAL INTRODUCTION

1.1 The history about the discovery of ANF

For a long time, the heart has been recognized as a pump which pushes blood throughout the circulation. Now, it is well known that heart is not only a pump, but an endocrine organ, secreting a hormone, atrial natriuretic factor (ANF), which is involved in the regulation of blood pressure (BP), fluid as well as electrolyte balance and interaction with other hormones. It has, however, taken a long time and years of laborious work to confirm that the heart is, indeed, an endocrine gland. Earlier work in the discovery of hormones released from the heart go back to the 1950s and involve two field of studies: anatomical and physiological.

1.1.1 Anatomical studies

In 1956, Kisch first described that granules were present in atrial but not in ventricular cardiocyte of guinea pigs (55). The presence of these storage granules were further confirmed in mammalian atrial cardiocytes by Jamieson and Palade (53, 92), who characterized these granules to be secretory granules in nature (53). The degree of granulation was higher in the right atrium than in the left (71). The number of atrial granules was increased after the administration of ß-blockers (90), but decreased after the production of aorto-caval fistula in the rats (10) and in presence of reserpine (92) or atropine (45). De Bold (20) reported that the number and density of atrial granules changed with alterations in salt and water intake, implying that these granules must store some substance involved in a functional role in the control of sodium and water balance.

1.1.2 Physiological studies

Shortly after Kisch described the presence of granules in the atria, Henry et al. (48) established experimental evidence that cardiac atria possessed the capacity to "sense the fullness of the blood stream" and to modulate renal function in response to increased atrial pressure. They demonstrated that balloon inflation in the left atrium

resulted in atrial distention and reproducible increases in urine flow. These results suggested that stretch receptors were present in the atria, linking changes in circulating blood volume with homeostatic responses of the kidney. Moreover, diuresis, in response to an increased atrial pressure, was also observed in animals with a denervated heart or denervated kidney, suggesting a hormonal pathway between the heart and the kidney (66).

de Wardener et al. (22) further demonstrated that an unidentified hormonal factor, other than increased glomerular filtration rate (GFR) or decreased aldosterone, was involved in natriuretic response to acute volume expansion in dogs. Thus, this unknown hormonal substance was named as a "third factor". The search for this "third factor" soon focused on a possible hormonal mediator that came to be known as "natriuretic hormone". Despite an intensive search for the "third factor" or "natriuretic hormone", its nature and source remain unclear.

1.1.3 ANF discovery

Earlier anatomical and physiological studies which searched for "third factor" or "natriuretic hormone" led DeBold et al. to perform elegant animal studies (21) which demonstrated that crude extracts of rat atria contain a potent diuretic and natriuretic substance, now termed atrial natriuretic factor (ANF). Thus, ANF plays a fundamental role in the cardiorenal sensor-effector system of regulating body fluid balance. It may be an answer for the search of the "third factor" or "natriuretic hormone".

Since the initial identification of ANF in 1981, there has been an explosion of information on the molecular biology, biochemistry, and pharmacology of this hormone. The genes encoding ANF from many diverse species have been cloned and characterized. These studies have not only characterized a novel class of hormones with important physiological function, but have also provided insights and posed questions regarding the physiological and pathophysiological functions of the heart.

1.2 Natriuretic peptide family: atrial natriuretic factor, brain natriuretic peptide, C-type natriuretic peptide and urodilatin

Following the discovery of atrial natriuretic factor (ANF), an increasing number of peptides with vasorelaxant and natriuretic activities have been isolated from various mammalian organs. They include brain natriuretic peptide (BNP), detected in the porcine brain and heart (75, 115); type C natriuretic peptide (CNP), found in the porcine brain (116), and urodilatin, isolated from urine (106). Thus, these peptides constitute a natriuretic peptide family. Though the peptides in this family share common characterizations in structure and bioactivity, each has its specific properties.

1.2.1 ANF

The gene sequence for human ANF precursor has been established and the gene has been located on the short arm of chromosome 1 in human (85) and chromosome 4 in mouse. ANF is synthesized mainly in mammalian atria as a 151 amino acid pre-prohormone (83, 114), sharing strong homology between human, rat, dog, and rabbit (9, 68, 89). Cleavage of the signal peptide from preproANF produces proANF-(1-126), which is a storage form of ANF (9). Upon appropriate stimulation, such as atrial stretch, the prohormone is cleaved into a N-terminal and C-terminal fragment. Processing of proANF to ANF certainly occurs immediately before and possibly even soon after secretion from the myocyte, resulting in ANF-(99-126) as the predominant form entering the coronary sinus blood (6, 39, 52, 124, 130). Bioactive ANF-(99-126), having strong homology between different species, is derived from the carboxy-terminus of proANF-(1-126). A central ring structure formed by a disulfide bridge between cysteine residues at positions 7 and 23 (78), and the three carboxy-terminal residues Phe-Arg-Tyr of ANF are very important for the biological action of ANF (79). Disruption of this ring structure by cleavage of the disulfide bond or deletions of the three carboxy-terminal residues of ANF leads to a loss of bioactivity or markedly reduces natriuretic and vasorelaxant potency (78, 79).

1.2.2 BNP

BNP is a novel natriuretic peptide recently isolated from the brain (115) and the heart (75). BNP has a remarkable sequence homology to ANF with only 4 amino acids being different in the 17 amino acid ring structure common to both peptides (115). Similar to ANF, BNP has potent diuretic-natriuretic and vasorelaxant activities and appears to play a significant role in fluid-electrolyte homeostasis. Although BNP was first isolated from the brain, it appears that there is actually more BNP in the heart than in the brain (75). However, the amount of BNP in the atria is only 1-3% of that of ANF (75, 82, 102). The mRNA levels for BNP in the ventricle are less than half of that found in the atrium, but taking into account ventricular mass, muscle total mRNA levels are higher in the latter. Moreover, the abundance of BNP is similar to that of ANF in cardiac ventricles (82). These data suggest that the ventricles may be the major source of circulating BNP (82), which has been confirmed by sampling blood from different cardiac sites. BNP binds to ANF R1-A receptor in a similar manner as ANF, but with less affinity (41, 49, 114). It has been suggested that BNP probably works through the ANF R1-A receptor in the kidney. ANF and BNP interact with the same receptor (A-type receptors) and stimulate the intracellular production of cGMP, but they do not significantly stimulate B-type receptors (12, 57, 107). Thus, the physiological effects of ANF and BNP may be mediated through the same receptor, the ANF R1-A receptor (57).

Circulating levels of BNP in normal subjects were only one twentieth of ANF (102). However, in heart failure, plasma BNF levels increased markedly and were found to be proportional to the degree of severity of failure (82, 102), as was ANF. The levels of mRNAs encoding both BNP and ANF were significantly augmented in the left ventricular myocardium in most patients with end-stage heart failure (122). There was a significant positive correlation between the levels of BNP and ANF mRNAs in the left ventricular myocardium of patients (122). Moreover, it has been

shown that cardiac performance was improved after an intravenous injection of BNP in patients with heart failure (132). These preliminary results suggest that BNP may be less important in physiological states than ANF for the maintenance of daily sodium and water metabolism, but may play a critical compensatory role for ANF in pathophysiological states, such as CHF. The degree to which these natriuretic peptides contribute to control of sodium and water metabolism in physiological and pathophysiological states, however, has not been determined.

1.2.3 C-type natriuretic peptide (CNP)

CNP is the third member of the natriuretic peptide family identified after ANF and BNP. Two related CNPs, a 22-residue peptide (CNP-22) and its N-terminally elongated peptide (CNP-53), have been identified and found to be major molecular forms of CNP in the brain (76, 116). Unlike ANF and BNP, CNP ends at the second cystine residue and lacks further C-terminal extension from the ring structure. Thus, CNP shows much less potent diuretic-natriuretic and vasorelaxant activities than ANF or BNP (116) and is not detectable at significant levels in cardiac tissue (56, 77). The CNP mRNA is also undetectable by the Northern blot analysis in either the normal, fetal, or diseased ventricular myocardium (118). There has been no report that circulating CNP level is elevated, either in experimental cardiac overload or in patients with heart failure. Thus, it is possible that the primary biological activity of CNP is not as a classic natriuretic peptide with peripheral sites of actions. Preliminary experiment on the distribution of CNP mRNA by Northern analysis in situ suggests that CNP is limited primarily to the nervous system (56), indicating that CNP may be responsible for most of the central actions of the natriuretic peptide family. In contrast to ANF and BNP, CNP potently and selectively activates B-type receptor but has little effect on A-type receptors (57).

1.2.4 Urodilatin (Renal natriuretic peptide)

In 1988, a new and unique 32 residue natriuretic peptide was isolated from human urine (106) and named as urodilatin or renal natriuretic peptide. It has a four-residue NH_2-terminal extension of the ANF peptide and is identical in structure to the α-human ANF-(95-126). This newly discovered peptide is produced solely by the kidney and is not found in plasma. Urodilatin is probably produced by same gene coding ANF in the heart but with different posttranslational processing (106). Because urodilatin has exactly the same structure (and amino acids) as ANF with a 4 amino acid extension, one would suspect it would have actions nearly identical to ANF. It has been shown that urodilatin binds to the ANF receptor with a higher affinity than ANF itself and stimulates particulate guanylate cyclase to the same extent as ANF (46). This may be due to the N-terminal "tail" on this peptide. Urodilatin binds to receptors in the distal nephron and inhibits the reabsorption of sodium and chloride at this site, raising the possibility that the primary physiological ligand for some of these receptors, such as those located in inner medullary collecting ducts, may be urodilatin rather than ANF. Feller et al. speculated that urodilatin, rather than blood-derived ANF, could have a major role in the regulation of natriuresis and diuresis under physiological conditions (29). It has been reported that urodilatin has a greater natriuretic potency than ANF when given intravenously (97, 104, 129), which may be due to urodilatin's resistance to degradation by the brush border of renal cells (33).

1.2.5 Structure similarity of natriuretic peptide family

These natriuretic peptides have been shown to be coded by distinct genes (111, 121), and have a common ring consisting of 17 amino acids bound by intramolecular disulfide bonds. Only four amino acids in the ring structure of these natriuretic peptides are different from each other, whereas the NH_2- and COOH-terminal vary in both amino acid composition and length (75, 106, 116). Among different species, the structure of both ANF and CNP are highly conserved (56), whereas the amino acid

sequence of BNP varies by as much as 50% (115). As a result of the similarity of structure between these peptides, they exhibit similar functions with different potencies. However, the diversity of structure and distribution of these natriuretic peptides show that they may serve appreciably different functions. It is possible that these natriuretic peptides work together to regulate blood pressure and body fluid in different ways or places under physiological and pathophysiological states.

1.3 Regulation of ANF secretion

1.3.1 Atrial stretch

As with many endocrine systems, there may be a variety of ways by which the release of ANF is controlled. The most effective and best documented stimulus for ANF release is the atrial stretch, induced by an increase in preload or afterload (60, 69). This phenomenon was first evidenced by Henry et al (48), who demonstrated the inflation of a balloon in the left atrium induced a marked increase in urine flow. In 1984, Dietz confirmed that atrial stretch resulted in a release of ANF directly from isolated perfused rat heart in vitro (23). Several physiological states have also produced increments in plasma ANF levels, including head-out immersion (28) and changes in body posture (50). In addition, acute as well as chronic volume loading and high salt intake have been potent stimuli for ANF secretion (60, 99). On the other hand, a blunted natriuresis and attenuated release of ANF following volume expansion have also been observed in rats with partial right atrectomy (108). Moreover, plasma ANF levels have been closely correlated with cardiac filling pressures in patients with various cardiovascular diseases (93). Taken together, these results suggest that atrial stretch is a major stimulus for the release of ANF. However, cellular processes involved in linking mechanical distension to ANF release remain unclear.

1.3.2 Hormone-induced release of ANF

Plasma ANF levels have been reported to change in response to a number of other peptides circulating in the blood. Vasoconstrictor agents, such as vasopressin, ANG II, and phenylephrine, have been shown to increase levels of plasma ANF. The mechanisms by which vasoconstrictor agents induce ANF release may be secondary to hemodynamic changes induced by their vasoconstrictor properties (69). The direct actions, however, cannot be fully excluded.

1.3.3 ANF release and nervous reflexes

Moderate sympathetic or vagal stimulation does not change plasma ANF levels (94). The increased ANF release with volume expansion is not affected by cardiac denervation (86) and atrial stretch results in the release of ANF directly from a rat heart-lung preparation (23). These results suggest that atrial distension is the primary stimulus of ANF release and that this effect does not require any mediation by cardiac nerves.

1.3.4 Patterns of ANF release

It seems that there are at least two patterns involved in regulation of ANF secretion, although the cellular processes involved in linking mechanical atrial distension to ANF release are not fully understood. Acute stimulation probably results in a rapid conversion of proANF to ANF and an increase in the release of ANF. This response decays over a period of minutes, indicating that the mechanism of ANF release in response to acute or chronic stimulation may be different. Chronic increases in the rate of ANF release appear to be related to the increase in ANF gene expression. It has been observed that atrial ANF mRNA levels rise sharply with changes in salt and water intake (119). Furthermore, in adult animals and humans, ventricles "under stress" express specific ANF mRNA or immunoreactive ANF, suggesting a recruitment of these cell to secrete ANF in pathophysiological states (73). Cellular or molecular

mechanisms underlying ANF release in both acute and chronic conditions, however, are not well understood.

1.4 Mechanism of ANF action: ANF receptors and its second messenger

As with other peptide agonists, ANF must first bind to specific glycoprotein receptors in the plasma membrane of target cells in order to mediate physiological effects. ANF receptors have been identified in all ANF target tissues by radioligand binding and autoradiographic techniques. It is clear that there are at least two classes of ANF membrane surface receptors, one coupled to particulate guanylate cyclase (ANF R1 receptor), and the other devoid of guanylate cyclase activity (ANF R2 receptor). ANF exerts its physiological actions by binding to the ANF R1 receptor and activating its guanylate cyclase. Recently, the ANF R1 receptor has been reclassed into R1-A and R1-B receptors. The functional and physiological role of the R2 receptor has not been elucidated, but it has been postulated to be a clearance receptor for removal of ANF from circulation.

1.4.1 ANF R1-A receptor

ANF R1-A receptor is a 120-140 kDa transmembrane protein that mediates the cGMP generating effect of ANF via the activation of particulate guanylate cyclase (64, 83, 109, 117). It has been shown (14) that the ANF R1-A receptor possesses a single transmembrane domain, an amino-terminal extracellular ANF-binding domain, and a carboxyl-terminal intracellular catalytic domain. The intracellular domain is homologous with protein kinase and to a subunit of the soluble form of guanylate cyclase, whereas the extracellular domain shows 33% sequence homology with the ANF C receptor. The ANF R1-A receptor binds ANF specifically and with a high affinity. Therefore, ANF is the most potent hormone for stimulating ANF R1-A guanylate cyclase; BNP is as efficacious, but only at approximately a tenfold higher concentration (107). The ANF R1-A receptor does not bind several ANF synthetic

analogues, such as the internally deleted and truncated des [Gln19, Ser19, Gly20, Leu21, Gly 22] ANF_{4-23} (c-ANF) and the internally intact and truncated ANF_{5-25} (67, 105). Moreover, ANF_{7-28} competes with ANF for ANF binding sites in glomerular and vascular smooth muscle cells, indicating that the amino terminus of ANF up to the disulfide bridge is not required for the binding activity (7). These data suggest that the carboxyl terminal phenylalanine-arginine (phe-arg) and ring structure of ANF seem to play an important role in binding to the ANF A receptor, and is required for the biological activity of ANF. However, the C-terminal phenylalanine-arginine (phe-arg) and the ring structure of ANF are not required for binding to the ANF R2-C receptor. ANF activates particulate guanylate cyclase and increases the intracellular concentration of cGMP, but ANF has no effect on soluble guanylate cyclase.

1.4.2 ANF R1-B receptor

The ANF R1-B receptor is also a transmembrane protein with the activity of guanylate cyclase, as is the ANF R1-A receptor (12). The ANF R1-B receptor has a low affinity for all tested peptides but binds CNP with higher affinity and is preferentially activated by CNP rather than by ANF or BNP (57). The extracellular domain of the ANF R1-B receptor has a 44 % amino acid homology with the ANF R1-A receptor, but the binding region has a 79% sequence identity between the ANF R1-A and R1-B receptors. There is 74% identity between the intracellular domains of the ANF R1-B receptor and the ANF R1-A receptor (12).

1.4.3 ANF R2-C receptor

The ANF R2-C receptor is a homodimer of a 64-kDa transmembrane protein with a short 37-amino acid cytoplasmic tail, which does not couple with guanylate cyclase (32, 64). ANF R2-C receptor does not mediate any of the known renal and vascular actions of ANF and may have a primary role in the removal of ANF from the

circulation (67). Therefore, this receptor is also termed a clearance receptor, which may function through intermediate G proteins to inhibit adenylate cyclase or activate the phosphoinositol pathway but not be signaled through the activation of guanylate cyclase (3). Whereas ANF R1-A or R1-B receptors display a high degree of selectivity for ANF, BNP, and CNP, ANF R2-C receptor recognizes all the natriuretic peptides, ANF fragments and ring-deleted ANF analogues with high affinities (67); however, its affinity for CNP is fivefold lower than that of the ANF R1-B receptor (57). Consistent with its "clear" function, it has been found that there is an abundance of ANF R2-C receptors on vascular endothelial cells (63, 67).

1.4.4 Second messenger of ANF

ANF stimulates cGMP accumulation by activating plasma membrane-associated guanylate cyclase (42, 83). Bioavailable analogues of cGMP mimic the physiological actions of ANF, including smooth relaxation and the inhibition of renal tubule sodium transport. This confirms cGMP's role as a second messenger for ANF action (83, 133). Increments in intracellular cGMP levels result in the activation of cGMP-dependent protein kinases and the phosphorylation of a number of intracellular proteins (83), which appear to mediate the actions of ANF. Currently, cGMP is believed to be a marker and mediator of ANF's biological activity (43).

1.5 Physiological function of ANF

1.5.1 Renal actions of ANF

The kidney is a major site for ANF activity. Consistent with its renal effects, ANF receptors coupled to guanylate cyclase have been observed in renal microvessels, glomeruli and renal tubule, raising the possibility that there may be a multiplicity of ANF actions in the kidney. ANF may produce afferent arteriolar vasodilation and efferent arteriolar vasoconstriction (26), serving to augment glomerular capillary hydraulic pressure. ANF may also relax glomerular mesangial cells (112), thereby

increasing the filtration surface area and the glomerular capillary ultrafiltration coefficient, Kf.. These results indicate that most of the renal effects of ANF may be mediated by hemodynamic changes through blood vessels and glomerular alterations (16, 51). It has been found that ANF inhibits angiotensin-stimulated NaCl and fluid absorption in the proximal tubule (38, 44), increasing sodium delivery to the descending limb of long-looped nephrons in rats (98). However, ANF receptors have not been demonstrated in proximal tubule segments. The renal inner medullary collecting duct (IMCD) plays an important role in regulating the volume and composition of urine. Increased blood flow in the renal medulla induced by ANF (14) can wash out medullary solutes, thus acting to sustain natriuretic responses. It has also been reported that ANF inhibits the amiloride-sensitive cation (Na+) channel in the inner medullary collecting duct (IMCD), thus reducing Na+ flux across the conductive cation channels (65). ANF is also suggested to have indirectly involved in the fine regulation of sodium and water transport in the collecting duct through the regulation of renin (11) and aldosterone (5, 13, 27) secretion or through the inhibition of AVP induced hydraulic conductivity (24).

1.5.2 Actions of ANF on the cardiovascular system

ANF may have diverse effects on the cardiovascular system depending on a number of factors, such as autonomic tone, volume status, circulating ANG II and norepinephrine levels. However, dose, duration, and the mode of administration of ANF are also important in determining cardiovascular responses to this peptide.

ANF has been demonstrated to have potent vasorelaxant effects on preconstricted vascular smooth muscle by norepinephrine (NE) or ANG II *in vitro* (34). ANF has a greater effect on the aorta and its main branches than on smaller, more distal arteries. Small renal preglomerular arteries are an exception in that they relax to ANF with an IC_{50} of about 8×10^{-9} M, whereas similarly sized arteries isolated from the mesenteric, femoral, cerebral, and coronary beds do not respond (1, 91).

The acute administration of ANF to humans or animals as intravenous bolus or continuous infusion in pharmacological doses lowers arterial blood pressure. Moreover, chronic infusions of ANF at levels achieved by endogenous peptide release also lower blood pressure (35). Hypotensive response to ANF may be associated with diminished cardiac output, reductions in peripheral vascular resistance, and decreased intravascular volume. Diminished cardiac output is likely due to its action in the peripheral circulation to decrease venous return (126). It has been noted that the administration of ANF leads to increases in hematocrit and plasma protein concentrations, indicating the transfer of plasma from intravascular to extravascular sites (30) A decrease in total peripheral resistance is observed in normotensive rats after a bolus injection of ANF (125). Another most consistent effect of ANF is to decrease cardiac filling pressure, which occurs in human and animal under a variety of conditions, including consciousness, anesthesia, hypertension, and heart failure (8, 15, 17, 100, 126).

There is a striking difference in the hemodynamic response to ANF administration between patients with CHF and healthy subjects. Unlike the decreased cardiac output often observed in healthy experimental animals (126), increased cardiac output has been often observed in patients with CHF after ANF administration (15, 17, 95, 100). An activated neurohormonal vasoconstrictor system, as occurring in CHF, might predispose the cardiovascular response to ANF. Therefore, ANF may have a prominent effect on afterload in these patients and thus improve cardiac performance by reducing peripheral vascular resistance.

1.5.3 Effects of ANF on other endocrine hormones

Another intriguing aspect of ANF is its interactions with other endocrine systems involved in the maintenance of blood pressure and electrolyte homeostasis, especially with the renin-angiotensin-aldosterone system (RAS). The infusion of ANF markedly lowers renin secretion and plasma renin concentrations (11). The administration of ANF antiserum, however, reduces urinary sodium excretion and

increases plasma renin activity (PRA) in rats (84). These results suggest that ANF has a physiological role on inhibiting renin release and increasing natriuresis. It has been demonstrated that ANF inhibits renin secretion by increasing sodium delivery to the *macula densa*. ANF supposedly enhances the delivery of sodium chloride to the *macula densa* by increasing the filtered load of sodium and/or decreasing proximal tubular reabsorption, as estimated from the increased fractional lithium excretion (109). It is well known that increased tubular delivery of sodium chloride to the *macula densa* decreases renin release from juxtaglomerular cells (18, 54). But, ANF inhibits both basal renin secretion (87) and renin release induced by isoproterenol, forskolin, or dibutyril cAMP (87, 47) from rat kidney slices *in vitro* in a dose-dependent fashion, suggesting a direct renin-inhibitory action. The direct renin-inhibitory action of ANF may be mediated by a cGMP dependent mechanism (59). ANF may also cause the inhibition of sympathetic stimulation to the kidney, reducing the stimulus for renin secretion. Thus, the mechanism of inhibiting renin release by ANF probably involves multiple pathways, both direct and indirect.

ANF inhibits aldosterone secretion both by reducing renin release and directly acting on the zona glomerulosa of the adrenal cortex to inhibit adrenal aldosterone secretion (5, 13, 27). It has been reported that ANF inhibits ACTH, K^+, and ANG II-stimulated aldosterone release (5, 13, 58). Thus, ANF suppresses the entire RAS by inhibiting both renin and aldosterone secretions. The administration of ANF has been reported to inhibit AVP secretion induced by dehydration and hemorrhages (103).

1.6 Pathophysiological role of ANF in chronic heart failure (CHF)

ANF, produced mainly by mammalian atria, has been shown to exert potent diuretic, natriuretic, vasodilator, and renin-aldosterone suppressing activities. The location and structure of atria make them ideally suited to sense alteration in intravascular volume and to respond by the secretion of ANF. Therefore, ANF may

play a critical pathophysiological role in diseases associated with abnormalities of blood pressure, body fluid, as well as electrolyte metabolisms.

1.6.1 The definition of heart failure

Chronic heart failure (CHF) is a common clinical syndrome. It is defined as the heart's inability to deliver sufficient blood to meet the metabolic needs of the peripheral tissues. The etiology of underlying CHF is varied. It may be due to increased pressure (hypertension, valvular stenosis) or volume (valvular regurgitation, aorto-vacal [A-V] shunt, thyrotoxicosis) loads, cardiac ischemia (infarction), and myocardiopathy. Although the etiology of CHF is varied, CHF has some common characteristics in pathophysiological changes during the development of CHF.

1.6.2 Characterization of CHF

CHF is characterized by an increase in cardiac filling pressure and volume overload, which is associated with sodium retention and activation of the neurohormonal vasoconstrictor systems. This includes the sympathetic nervous and RAS systems as well as arginine vasopressin, which makes important contributions to characteristic abnormalities in CHF. Moreover, alterations in renal functions and peripheral vasculature, in part mediated by activated neurohormonal vasoconstrictor systems, can have profound effects on overall cardiac performance in CHF, leading further to the development of CHF.

In experimental and human CHF, ANF is also activated (37, 96, 123, 128). Plasma ANF levels are elevated in both experimental and human CHF directly related to the severity of the disease (123). ANF exhibits its many potent physiological effects, which *in vitro* and *in vivo* oppose the main actions of the neurohormonal vasoconstrictor systems, at different levels and location. Therefore, it is believed that ANF may play an important role to counterbalance the neurohormonal vasoconstrictor systems and to contribute to the regulation of body fluids and cardiovascular

homeostasis in CHF. However, its role in the pathophysiology of CHF remains to be better understood.

1.6.3 ANF synthesis and release in CHF

Despite the increased levels of plasma ANF and its possible functional role in CHF, it remains unclear whether or not ANF secretion is appropriate for the magnitude of increased atrial and ventricular filling pressure. It has been observed that atrial content of ANF in CHF is deficient. This may be secondary to an increased release of ANF and may also reflect that atria can not appropriately enhance ANF synthesis during this pathological state. The synthesis and secretion of ANF are found to be markedly augmented in the ventricles of humans (101, 120, 127) and animals (4, 31, 61, 122) with ventricular hypertrophy or failure. Circulating ANF in normal humans or animals is produced almost exclusively by atria. Elevated plasma ANF levels in experimental and human CHF, however, may come from an increased synthesis and release of ANF in both atria and ventricles. Thus, an enhanced synthesis and release of ANF in the ventricles of CHF may be a critical compensatory source for the elevated plasma ANF. The profile of plasma and tissue ANF changes and interaction between plasma ANF and alterations in hemodynamics and renal function during the development of CHF are unclear. Moreover, the contribution of the atria and ventricles to elevated plasma ANF in CHF needs to be better defined.

1.6.4 Pathophysiological role of ANF in CHF

The precise role of elevated levels of plasma ANF in the regulation of hemodynamics, renal function and volume homeostasis in CHF is not well defined. The administration of specific anti-ANF antibodies significantly increases right atrial pressure, left ventricular end-diastolic pressure, and systemic vascular resistance, and reduces urine volume and Na^+ excretion in rats with CHF induced by myocardial infarction but not in a control group (6, 25). Recently, Lee et al. demonstrated that

elevated endogenous ANF in acute CHF may serve to limit activation of RAS and to maintain sodium excretion (62). Further increased circulating ANF levels by administration of exogenous ANF in patients with CHF lead to favorable hemodynamic, renal, and hormonal responses (15, 100). These results suggest that endogenous circulating ANF may have important physiological actions in maintaining sodium excretion and limiting activation of RAS, as well as opposing the vasoconstrictor stimuli in CHF. But plasma ANF may not be enough to normalize abnormalities of hemodynamics and renal parameters in CHF. Thus, ANF secretion in CHF may not be appropriate for the magnitude of increase in atrial and ventricular filling pressure, i.e. the relative deficiency of ANF may contribute to the development of CHF.

Despite the beneficial compensatory action of ANF in CHF, natriuretic and diuretic responses to exogenously administered ANF are attenuated in animals and humans with CHF. The mechanism of the attenuated natriuretic response to ANF in CHF may be multifactorial. Activated neurohormonal vasoconstrictor systems, which occurred in CHF, may contribute to hyporesponsiveness to ANF in CHF. The RAS appears to play a key role in this regard because of its interaction with ANF at multiple sites. It has been observed that angiotensin II decreases intracellular cGMP accumulation in culture vascular smooth muscle cells by stimulating its hydrolysis (113). This augmented hydrolysis of cGMP appears to be mediated via a Ca^{2+}activated cGMP phosphodiesterase. Wilkins et al. (131) reported that cGMP phosphodiesterase inhibitor (M+B 22948) enhanced the natriuretic and cGMP response to volume expansion, an effect that was attenuated by a monoclonal antibody to ANF. Thus, it appears that ANG II, by stimulating phosphodiesterase activity and increasing cGMP hydrolysis, may contribute to the blunted renal response to ANF during CHF. Moreover, ANG II may also mediate blunted natriuretic and diuretic responses to ANF by stimulating aldosterone secretion and an alteration of renal hemodynamics. ACE inhibitors have been shown to improve the survival and symptomatology of patients

with CHF, suggesting that the RAS plays a critical role in the pathophysiology of CHF. The increased activities of the sympathetic nervous system may also mediate a blunted renal response to ANF by direct renal action or the enhancement of intrarenal ANG II. This hyporesponsiveness to ANF may also be secondary to downregulation of renal ANF receptors, as it has been reported in the platelets of patients with CHF, and in the inner medulla of rats with CHF induced by myocardial infarction (128). Consistent with the downregulation of ANF receptors, an attenuated renal cGMP production has been observed in dogs with rapid ventricular pacing (70) and in rats with A-V fistula (2). Decreased renal perfusion pressure, occurring in CHF, may also contribute to the blunted renal responses to ANF.

1.7 Rational for present experiments

During CHF, both ANF and neurohormonal vasoconstrictor systems may be activated. They may reach a new balance at a higher steady state. However, their degree of participation is far from being established. This new balance may be altered depending on the changes in hemodynamics and body fluid hemostasis during the development of heart failure. The modification of ANF and the neurohormonal vasoconstrictor systems will also influence hemodynamics and renal function, which in turn impacts on the development of heart failure.

The characterization of pathological alterations of CHF seems incompatible with ANF physiological actions. Thus, it has been hypothesized that the pathophysiology of CHF might be due to a relative deficiency of ANF or a blunted responsiveness to ANF in this state. Attenuated effects of ANF may be mediated by (a) increased activities of neurohormonal vasoconstrictor systems, such as RAS, AVP, sympathetic never system; (b) functional ANF receptor downregulation. In this regard, because of its multiple biological activities and its presence in tissue, the RAS appears to play a key role.

Thus, in order to understand the role of ANF in CHF, it is very important to characterize the profile of plasma and tissue ANF changes, and interaction between the

plasma ANF levels and alteration in hemodynamics and renal function during the development of CHF. It is also quite critical to investigate the mechanism of attenuated response to ANF and the interaction between ANF and RAS during the development of CHF.

In our experiments, we chose rats with high-output heart failure induced by producing an aorto-caval shunt. This is a reproducible, simple, and rapid method which was devised by our laboratory (36).

Therefore, the present experiments are designed to investigate the role of ANF in experimental high-output heart failure by:

1. Characterizing plasma and tissue ANF levels, hemodynamics, and body fluid hemeostasis, and assessing the contribution of atria and ventricles to plasma ANF levels during the development of high-output heart failure.
2. Investigating the role of ANF and RAS by using losartan, a new, potent, orally active, and highly specific nonpeptide AT1 receptor antagonist in rats with A-C shunts.

Chapter II

CHARACTERIZATION OF PLASMA AND TISSUE ANF CONTENT DURING THE DEVELOPMENT OF MODERATE HIGH-OUTPUT HEART FAILURE IN RATS

2.1 ABSTRACT

Study objective - The aims of the present studies were (1) to characterize plasma and tissue atrial natriuretic factor (ANF) levels, hemodynamics and renal function at different stages of moderate chronic high-output heart failure in the rat; and (2) to assess the contribution of the atria and ventricles to plasma ANF levels.

Design - Plasma and tissue ANF levels, hemodynamics and renal function were evaluated at 1,2,4,8 and 16 weeks after the development of aorto-caval (A-C) shunts. Sham-operated animals served as controls at identical time points.

Experimental material - Male Sprague-Dawley rats weighing 225-275 g were used in all experiments.

Measurements and main results - Mean arterial blood pressure (MAP) was lower and right atrial pressure (RAP) was higher in the A-C shunt groups than in sham-operated controls at time periods. Left ventricular end-diastolic pressure (LVEDP) was increased significantly in A-C shunt rats at 1, 2 and 4 weeks when compared with their control counterparts. Plasma COOH- and NH_2-terminal ANF levels were elevated significantly in A-C shunt animals. A positive correlation between plasma ANF and RAP or LVEDP was observed in A-C shunt rats but not in sham-operated controls. A-C shunt animals also presented marked cardiac hypertrophy with decreased atrial ANF concentrations but not content and increased ventricular ANF content and concentrations. Despite high plasma ANF levels, A-C shunt rats had lower hematocrit at all observation periods and reduced urinary sodium excretion ($U_{Na}V$) as well as urinary volume (UV) at 1 and 2 weeks with a tendency toward diminished $U_{Na}V$ and UV at 4, 8 and 16 weeks. Moreover, body weight was higher in A-C shunt animals at 16 weeks than in sham-operated controls.

Conclusion - (1) Chronically increased cardiac filling pressure stimulated not only ANF release but also ANF synthesis in each cardiac chamber, which in turn contributed to elevated plasma ANF levels in A-C shunt rats; (2) an attenuated renal response to end ogenous ANF and sodium and water retention were apparent in A-C

shunt rats. Activation of neurohormonal vasoconstrictor systems and gradually decreased plasma ANF levels may contribute to sodium and water retention at different stages of this experimental model of heart failure.

2.2 INTRODUCTION

Atrial natriuretic factor (ANF), produced mainly by mammalian atria, has been shown to exert potent diuretic, natriuretic, vasodilator, and renin-aldosterone suppressing activities (1, 2). Atrial stretch induced by either a chronic preload or afterload stimulates ANF release (3, 4). Therefore, ANF may be involved in the regulation of blood pressure and fluid as well as electrolyte balance in physiological and pathophysiological states.

Several compensatory mechanisms can be activated during the development of heart failure, including increased activity of the sympathetic nervous system, renin-angiotensin system (RAS) and vasopressin, which together evoke sodium and water retention as well as vasoconstriction (5-8). On the other hand, ANF is also activated and is believed to counterbalance the RAS and sympathetic nervous system (9). Thus, during chronic heart failure (CHF), both ANF and the neurohormonal vasoconstrictor system may reach a new balance at a higher steady state. However, this new balance may be altered depending on changes in hemodynamics and body fluid homeostasis during the development of heart failure. On the other hand, modifications of ANF and the neurohormonal vasoconstrictor system will also influence hemodynamics and renal function, which will in turn impact on the development of heart failure Thus, it is important to characterize the changes induced in ANF, hemodynamics, and body fluid hemeostasis during the development of heart failure. Little information is available about alterations in these parameters. The present studies therefore evaluated plasma and tissue ANF levels, as well as hemodynamics and renal function during the development of chronic high-output heart failure in the rat.

2.3 MATERIALS AND METHODS

2.3.1 Animals

Male Sprague-Dawley rats (Charles River, St-Constant, Quebec) weighing 225-275 g were kept at controlled room temperature under a 6 a.m. to 6 p.m. light regime, and fed regular pelleted rat chow (Purina, Richmond, IN) and tap water ad libitum. These studies respected the guidelines of the Canadian Council on Animal Care.

2.3.2 Aorto-caval (A-C) shunts

The method used to produce A-C shunts has been detailed elsewhere (10). Briefly, on the day of the surgery, the animals were anesthetized with ether, and the vena cava and abdominal aorta were exposed by opening the abdominal cavity via a midline incision. The aorta was punctured at the union of segment two-thirds caudal to the renal artery and one-third cephalic to the aortic bifurcation with an 18 gauge disposable needle (Becton-Dickinson, Rutherford, NJ), held with a plastic syringe. The needle was advanced into the aorta, perforating its adjacent wall and penetrating the vena cava. At this moment, a bulldog vascular clamp was placed across the aorta caudal to the left renal artery. Once the aorta was clamped, the needle was fully withdrawn and a drop of cyanoacrylate glue (Krazy Glue, TM Dupont, Atlantic Promotions Inc., Longueuil, Quebec) was used to seal the aorta puncture point. The clamp was removed 30 sec later, and the patency of the shunt was verified visually by vena cava swelling and the mixing of arterial and venous blood. The whole surgical procedure took less than 10 min. Sham-operated animals, in which the abdominal cavity was opened and the vena cava and aorta exposed, served as controls. Previous experience from our laboratory has shown that patent A-C shunts are always accompanied by elevated plasma ANF-(1-98) levels. Hence, all A-C shunted animals with normal plasma ANF-(1-98) levels were not included in our experiments.

2.3.3 Hemodynamic measurements

At different time periods (1, 2, 4, 8 and 16 weeks) after the A-C shunts were created, the animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.), and their femoral artery, right atrium (through the right jugular vein) and left ventricle (through the right carotid artery) were cannulated with polyethylene catheters (PE 50) to measure mean arterial (MAP), right atrial (RAP) and left-ventricular end-diastolic (LVEDP) pressures, respectively. MAP, RAP and LVEDP were monitored with Gould Statham P23ID transducers (Gould Inc., Oxnard, CA) and recorded on a Grass Model 7D polygraph (Grass Instruments, Quincy, MA). A bladder catheter was installed for urine collection. Heart rate was measured through a Grass Model 7P44 Tachograph preamplifier. The amplifier receiving the signal from the ventricle was connected by a bridge to a Grass Model 7P20C polygraph differentiator for registration of the first derivative of left ventricular pressure (dP/dt max). The rats were then installed in restriction cages and allowed to regain consciousness and to stabilize their hemodynamic parameters before the experiments began. Pressures, heart rate and dP/dt max were recorded for 20 min after the animals regained consciousness. Urine was also collected during this period. At the end of the experiments, blood was withdrawn from the femoral artery for the measurements of hematocrit, plasma ANF, osmolality and sodium. The hearts and lungs were excised and weighed. Both atria and ventricles were dissected, weighed separately and kept at -70^{o}C until assayed.

2.3.4 Biochemical methods

Blood for ANF measurement was collected in tubes containing 10^{-5} M EDTA. The samples were immediately centrifuged at 3,000 rpm for 10 min at 4^{o}C. ANF-(99-126) was extracted from plasma with Vycor glass beads (Corning Glass Works, Corning, NY) and measured by radioimmunoassay (RIA), as described elsewhere (11). Plasma samples were directly assayed for ANF-(1-98) by RIA (12). The detection limit

of the assay was 0.75 pg/tube. The interassay and intra-assay coefficient of variance was below 14%, and the recovery of [^{125}I]ANF was 80%.

Tissue ANF content was also measured by RIA (11). Briefly, the atria were homogenized for 30 sec with a Polytron (setting 7; Kinematica, Lucerne, Switzerland) in 2 ml 0.1 M acetic acid containing the following final concentrations of protease inhibitors: 1 x 10^{-5} mol/l EDTA, 5 x 10^{-6} mol/l pepstatin, and 3 x 10^{-5} mol/l phenylmethylsulfonyl fluoride (PMSF). The ventricles were carefully dissected, minced, and boiled in 1 M acetic acid for 5 min (10 ml acetic acid/g tissue), then cooled to room temperature and homogenized in a Polytron (30 sec, set at 7). The homogenates were centrifuged at 15,000 rpm for 10 min, and the supernatant was applied to a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA) that had been previously activated with 5 ml 100% acetonitrile (CH_3CN). The cartridge was washed twice with 5 ml 0.1% trifluoroacetic acid in H_2O, and the ANF absorbed on columns was eluted with 80% CH_3CN in 0.1% trifluoroacetic acid. The material was lyophilized in a Speed-Vac (Savant Instruments Inc., Farmingdale, NY) and kept at -70^{o}C until ANF was measured by RIA (11). Atrial and ventricular protein content was quantified by a modification of Bradford's method (13). Plasma ANF levels and tissue ANF content were not corrected for recovery. Plasma osmolality was measured by the depression of freezing point in an osmometer (Advanced Instruments Inc., MA) and hematocrit packed cell volume with capillary tubes in a single-speed capillary centrifuge (Model MB, International Equipment Co., Mass) 6 min. UV was calculated by weight. Plasma and urinary sodium was assessed by flame photometry.

2.3.5 cGMP

Urine samples were kept at -70^{o}C until analysis. Aliquots of 50 μl urine were acetylated for 3 minutes with 12.5 μl of a mixed acetylation solution (1:2 aceticanhydrid acid and triethylamine) and the reaction stopped by dilution to 1/5,000 with Na^+ acetate buffer (pH 6.2). One hundred μl standard cGMP (10 pmol) was

acetylated for 3 minutes with same acetylation solution and the reaction stopped with 900 μl Na^+ acetate buffer. Then, acetylated standard cGMP was diluted in 1:2 serial until reaching 1.95 fmol/100 μl. The detection limit was 1.95 fmol per assay tube. One hundred μl acetylated urine or standard were incubated overnight at 4^{o}C with 100 μl rabbit anti cGMP antibodies raised in our institution (1:2,000 dilution) and 100 μl ^{125}I-cGMP (6,000 cpm/100 μl). On the second day, 100 μl goat anti-rabbit immunoglobulin G (1/25 dilution) (Immunocorp Sciences Inc., Montreal, Quebec) was added and incubated for 2 hours at room temperature. Antibody bound ^{125}I-labelled cGMP in sample or standard was precipitated by adding 1 ml 6.25% polyethylene glycol and centrifugated at 3,000 rpm for 20 min, and counted in a Gamma counter. All samples were made in duplicate. The interassay and intraassay coefficients of variance were $< 15\%$.

2.3.6 Statistical analysis

The data, expressed as means $\pm$ SE, were evaluated by two-way analysis of variance (ANOVA). Whenever the overall F test was significant, an a posteriori contrast test was applied according to the Bonferroni method (14). Correlations between plasma COOH- and NH_2-ANF and RAP or LVEDP were analyzed by multiple regression.

2.4 RESULTS

2.4.1 Hemodynamic changes

Table 2.1 shows the time course of hemodynamic changes in A-C shunt rats and their respective sham-operated controls. MAP was lower and RAP was higher in A-C shunt animals in all observation periods. LVEDP was significantly higher in A-C shunt

rats at 1, 2 and 4 weeks than in sham-operated controls. No significant differences were observed in either dP/dt max or heart rate between groups.

2.4.2 Plasma and tissue ANF levels

Plasma COOH- and NH_2-terminal ANF levels (Fig. 2.1A and 2.1B respectively) were significantly higher in A-C shunt rats at all time-points examined when compared with sham-operated controls. However, plasma COOH- and NH_2-terminal ANF levels in A-C shunt animals were lower at 2, 4, 8, and 16 weeks in comparison with 1 week values. A positive correlation between RAP or LVEDP and plasma COOH- ($r=0.49$, $p<0.01$ vs $r=0.39$, $p<0.05$) and NH_2- ($r=0.44$, $p<0.01$ vs $r=0.38$, $p<0.05$) terminal ANF was observed in A-C shunt rats but not in sham-operated controls. Left atrial ANF concentrations (Fig. 2.2A) were lower at 1 week and total left atrial ANF content (Fig. 2.2B) was higher at 8 and 16 weeks in the A-C shunt groups than in sham-operated animals. Right atrial ANF concentrations (Fig. 2.3A) were decreased significantly at 1, 2 and 4 weeks in A-C shunt rats when compared with their controls. This parameter (Fig. 2.3A) tended to be lower in A-C shunt animals at 8 and 16 weeks than in sham-operated controls. No differences in total right atrial ANF content (Fig. 2.3B) were observed at any time-period between groups. ANF concentrations (Fig. 2.4A) in either ventricle were much higher in A-C shunt rats than in their sham-operated controls at all times. Total ANF content (Fig. 2.4B) in both ventricles was also increased significantly in all observation periods except at 1 week in A-C shunt animals when compared with their controls.

2.4.3 Body and tissue weights

Table 2.2 summarizes the changes in body and tissue weights in A-C shunt and sham-operated rats at different stages of heart failure. Absolute and relative heart weights were much higher in each time period in A-C shunt animals than in the controls. A-C shunt rats also showed a large increase in the absolute and relative

weights of each chamber when compared with their sham-operated counterparts in all observation periods. The ratio between right and left ventricles in A-C shunt animals at 4, 8 and 16 weeks was increased significantly in comparison with the controls. At 1 and 2 weeks, A-C shunt rats tended to have a higher ratio between their right and left ventricles. No differences in ratio between the right and left atria were observed in the A-C shunt and sham-operated groups. A-C shunt animals at 2, 4, 8, and 16 weeks showed a significant increase in absolute lung weight when compared with their sham-operated controls. Relative lung weights in the A-C shunt groups were also increased significantly in comparison with sham-operated rats. Body weight was greater in A-C shunt than in sham-operated animals at 16 weeks.

2.4.4 Urinary parameters

UV (Fig. 2.5A) and urinary sodium excretion ($U_{Na}V$) (Fig. 2.5B) were reduced significantly in A-C shunt rats at 1 and 2 weeks. At 4, 8 and 16 weeks, A-C shunt animals tended to have lower UV and $U_{Na}V$ but no significant differences were found. Urinary cGMP excretion was higher and hematocrit was lower in A-C shunt rats in at all observation periods when compared with their sham-operated controls (Figs. 2.5C and 2.6 respectively). No differences were noted in plasma sodium and osmolality between groups (data not shown).

2.5 DISCUSSION

In the present studies, we have characterized changes in plasma and tissue ANF levels, hemodynamics and renal sodium, water and cGMP excretion in rats during the development of heart failure induced by A-C shunt. Atrial stretch is known to be a potent stimulus of ANF release (3, 4). Our results showed that animals with A-C shunts had elevated RAP in all observation periods and heightened LVEDP at 1, 2 and 4 weeks, suggesting an increased cardiac preload. A-C shunt rats also presented decreased MAP in each time period, which was probably due to reduced total

peripheral resistance. As reported previously in both human and experimental CHF (15-18), plasma COOH- and NH_2-terminal ANF levels were greatly elevated at different stages of heart failure in the A-C shunt groups. A positive correlation between plasma ANF and RAP or LVEDP was observed in A-C shunt rats but not in sham-operated animals, which suggests that chronically increased atrial filling pressure, induced by augmented cardiac preload, may chronically elicit ANF secretion. Therefore, our present results further confirm previous reports that chronically increased cardiac filling pressure is a sustained stimulus of ANF release (3).

Although plasma COOH- and NH_2-terminal ANF levels were higher in A-C shunt rats than in sham-operated controls in all observation periods, they tended to decrease from the second week. Similar changes in the plasma ANF profile, increasing and peaking early and then declining with time during the development of heart failure, have been reported in Syrian cardiomyopathic hamsters (16), in dogs with cardiac failure produced by chronic rapid ventricular pacing (19, 20) and in myocardial infarcted rats (21). However, Hartter et al. (22) noted that some patients with heart failure had normal ANF levels despite of very high RAP. These observations indicate a relative deficiency of plasma ANF in the later stages of heart failure.

The mechanism of decreasing plasma ANF is unclear. It may be due to reduced atrial sensitivity after longterm stretch (23,24). Similarly, attenuated ANF secretion in response to volume loading has been noted in rats with severe but not with moderate myocardial infarction (25).

Another cause of declining plasma ANF may be the exhaustion of atrial ANF storage. Previous histologic studies on atria have indeed shown that granules in atria are not restored under longterm stimulation (26). However, our results revealed that ANF concentrations in the left atrium were lower in the early but not in the later stages of heart failure. Furthermore, right atrial ANF content was not different between groups. Decreased atrial ANF concentrations but not content (which was even increased) in A-C shunt rats indicate that elevated cardiac filling pressure stimulates not

only ANF release but also ANF synthesis, which prevent the depletion of cardiac tissue ANF stores. On the other hand, lower tissue ANF concentrations may be a reflection of cardiac hypertrophy and consequently an augmentation of total cardiac protein content.

ANF concentrations and content in either ventricle of A-C shunt rats were also higher than in sham-operated controls, suggesting that ANF synthesis was increased in both ventricles of these animals. In agreement with increased ANF synthesis in atria and ventricles, it has been reported that ANF mRNA is elevated in atria and ventricles in response to various volume or pressure overloads, including rats with myocardial infarctions (25) or A-C shunts (27).

At 8 and 16 weeks, LVEDP tended to be higher in A-C shunt rats, but was not significantly different from control values, suggesting that a decrease in cardiac filling pressure may have occured during these time periods. Taken together, our results indicate that the increased ANF synthesis and release by each cardiac chamber may have contributed to elevated plasma ANF levels in A-C shunt rats and that decreased plasma ANF levels in later stages may have been due to either reduced sensitivity to atrial stretch or to declining cardiac filling pressure but not to exhausted ANF storage.

The ratio of right to left ventricular weights in A-C shunt animals was much higher than in sham-operated controls from 4 weeks, suggesting that hypertrophy was greater in the right than in the left ventricle. This result may be associated with our experimental model, reflecting a larger increase in right than in the left ventricle preload. RAS stimulation, and an augmented cardiac preload may contribute to hypertrophy in this animal model (15). The increased absolute and relative lung weights in A-C shunt rats indicate either an enhanced pulmonary circulation (because of shunt flow) or pulmonary congestion. Significant increases in absolute but not in relative lung weight in A-C shunt rats at 16 weeks may be due to significantly augmented body weight, suggesting the presence of edema in these animals at this time.

A-C shunt rats had lower hematocrit at all time periods and lower $U_{Na}V$ and UV at 1 and 2 weeks despite higher plasma ANF levels than their sham-operated controls. They also tended to have lower $U_{Na}V$ and UV at 4, 8 and 16 weeks, indicating sodium retention and increased plasma volume. The time course of the renal response after A-C shunt creation was similar to that reported by Winaver et al. (18), who found that about 60% of arteriovenous (A-V) rats with fistulas had significantly lower $U_{Na}V$ in the 1st week after the operation when compared with sham-operated controls. However, $U_{Na}V$ in these animals increased progressively and returned to control levels 1-2 weeks after the operation, although they showed a patent A-V fistula and cardiac hypertrophy when killed 8-10 weeks after the operation. On the other hand, about 40% of rats with A-V fistulas displayed progressive sodium retention and died within 7-12 days. The different patterns of development of animals with an A-V shunts induced by microvascular surgery (18) may be due to variations in fistula size. We did not find obvious differences in $U_{Na}V$ and UV in A-C shunt rats because the fistulas, when produced with an 18-gauge needle, were always of the same caliber.

Increased plasma ANF levels and decreased $U_{Na}V$ and UV in the early stages of heart failure (1 and 2 weeks) or elevated plasma ANF levels and a relatively "normal" sodium balance in later stages (4, 8 and 16 weeks) suggest resistance to the renal action of ANF in A-C shunt rats and a new steady state. This apparent renal hyporesponsiveness to endogenous ANF correlates well with the blunted renal reaction to exogenously administered ANF in both human and experimental models of CHF (17,28,29). The attenuated renal response to ANF may be secondary to renal ANF receptor downregulation (30) and/or the activation of neurohormonal vasoconstrictor system as well as renal hemodynamic abnormalities (31). Recent results indicate that the RAS is involved, at least partially, in the blunted renal response to ANF in heart failure despite the normalization of circulating renin-angiotensin in rats (15) or dogs (32) with A-C shunts. Our present data show that $U_{Na}V$ and UV were partially

restored from 4 weeks after the creation of A-C shunts, perhaps due to partial restoration of MAP or systemic renin-angiotensin (18).

In our experiments, urinary cGMP excretion was much higher in A-C shunt rats than in their sham-operated counterparts in all observation periods, suggesting that there may be a kinetic balance between plasma ANF concentrations and functional ANF receptors. Increased urinary cGMP excretion is inconsistent with attenuated renal function, suggesting that factors other than circulating ANF cause a marked reduction in ANF's biologic action. Our results cannot exclude a downregulation of functional ANF receptors in some specific organ, such as the kidney, which may contribute to this attenuated renal response. Tsunoda et al. (30) have reported ANF downregulation receptor in the renal medulla of rats with CHF, which is consistent with attenuated renal cGMP production in dogs with rapid ventricular pacing (33) and in rats with A-V fistulas (34).

In summary, the present studies demonstrate lower MAP and higher RAP and LVEDP, marked hypertrophy of each cardiac chamber and elevated plasma ANF levels in each time-period in A-C shunt rats. The increased plasma ANF levels are presumably caused by elevated cardiac filling pressure, which stimulates ANF secretion and synthesis in atria and ventricles. Heightened plasma ANF levels may play an important role in the maintenance of sodium and body fluid homeostasis by opposing the neurohormonal vasoconstrictor system in A-C shunt rats. However, a relative deficiency of plasma ANF or an activated neurohormonal vasocontrictor system may contribute to attenuated renal function in different stages of this experimental model of heart failure. The decreased plasma ANF levels in later stages in A-C shunt rats may be due to reduced sensitivity to atrial stretch.

TABLE 2.1

MEAN ARTERIAL, RIGHT ATRIAL AND LEFT VENTRICULAR END-DIASTOLIC PRESSURES, HEART RATE AND FIRST DERIVATIVE OF LEFT VENTRICULAR PRESSURE DURING THE DEVELOPMENT OF HEART FAILURE

	1 week		2 weeks		4 weeks	
	Sham (n=13)	Shunt (n=12)	Sham (n=10)	Shunt (n=10)	Sham (n=10)	Shunt (n=9)
MAP (mmHg)	98.79 ± 3.19	75.89 ± 3.16**	105.80 ± 3.82	81.83 ± 2.70**	111.30 ± 3.31	91.59 ± 3.15**
RAP (mmHg)	0.16 ± 0.41	3.87 ± 0.70**	0.72 ± 0.54	3.15 ± 0.46**	0.00 ± 0.39	2.61 ± 0.44**
LVEDP (mmHg)	2.21 ± 1.75	14.38 ± 3.27**	3.18 ± 1.52	10.53 ± 2.36*	3.00 ± 1.57	10.56 ± 1.94*
dP/dt (mmHg/sec)	2822.2 ± 180.0	2043.4 ± 181.5	3100.0 ± 271.0	2361.1 ± 204.5	4125.0 ± 227.5	3500.00 ± 326.1
Heart rate (beats/min)	392.7 ± 7.1	397.1 ± 11.3	381.7 ± 8.2	396.7 ± 10.8	394.5 ± 9.0	395.6 ± 9.3

TABLE 2.1 (CONTINUED)

	8 weeks		16 weeks	
	Sham (n=11)	Shunt (n=11)	Sham (n=11)	Shunt (n=10)
MAP (mmHg)	108.29 ± 4.05	97.30 ± 5.47*	112.93 ± 3.38	93.63 ± 3.14**
RAP (mmHg)	0.79 ± 0.54	3.20 ± 0.69**	0.31 ± 0.38	1.83 ± 0.68*
LVEDP (mmHg)	0.34 ± 1.43	5.57 ± 1.62	0.33 ± 3.04	5.19 ± 2.23
dP/dt (mmHg/sec)	3453.1 ± 176.7	3046.9 ± 163.6	4020.8 ± 223.4	3414.1 ± 333.5
Heart rate (beats/min)	380.9 ± 5.9	364.1 ± 9.0	387.7 ± 6.4	371.5 ± 7.5

Values are means ± SE; MAP=mean arterial pressure; RAP=right atrial pressure; LVEDP=left ventricular end-diastolic pressure; dP/dt max=1st derivative of left ventricular pressure; *p<0.05 vs sham; **p<0.01 vs sham

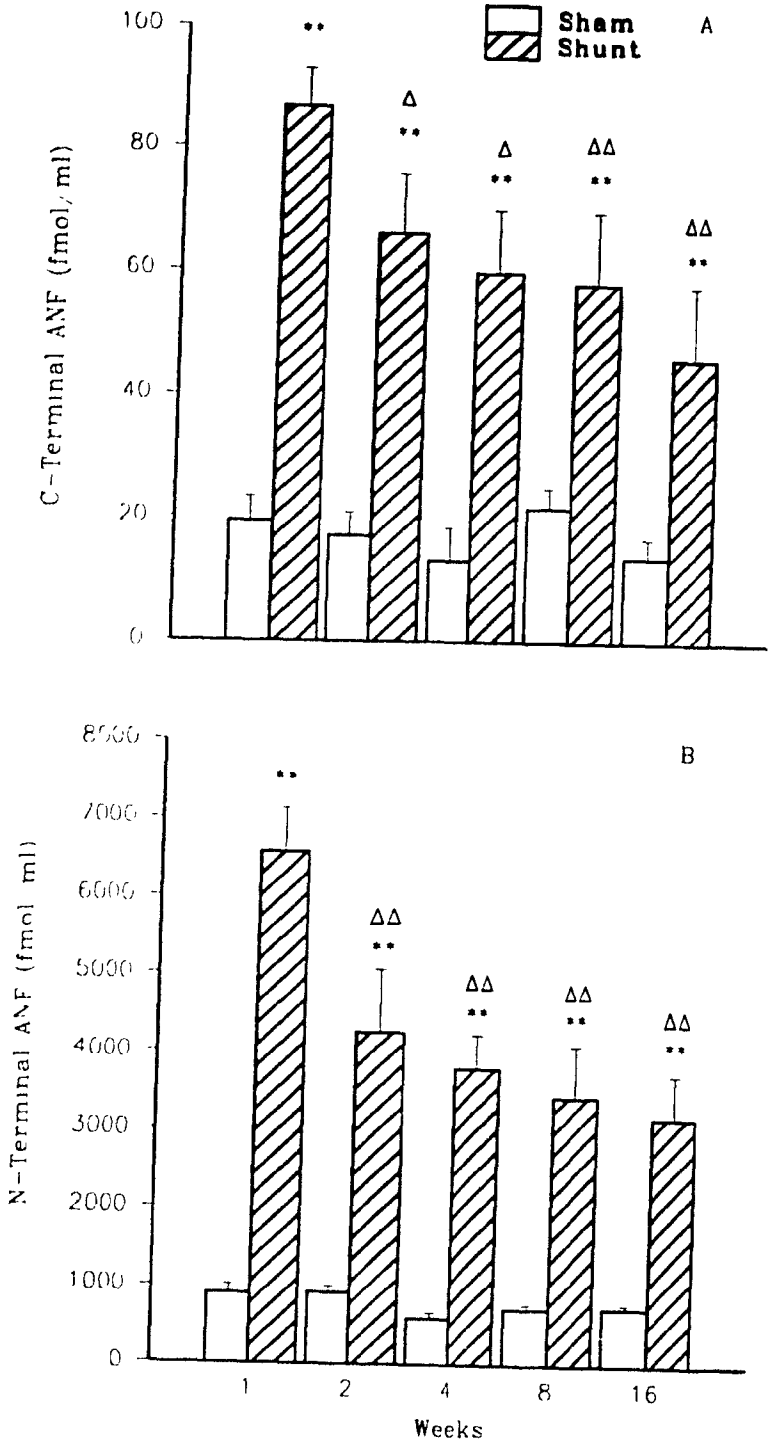

Fig. 2.1: Plasma (A) COOH- and (B) NH_2- terminal ANF levels during the development of heart failure.

**p<0.01 vs sham; Δp<0.05 vs 1 week; ΔΔp<0.01 vs 1 week.

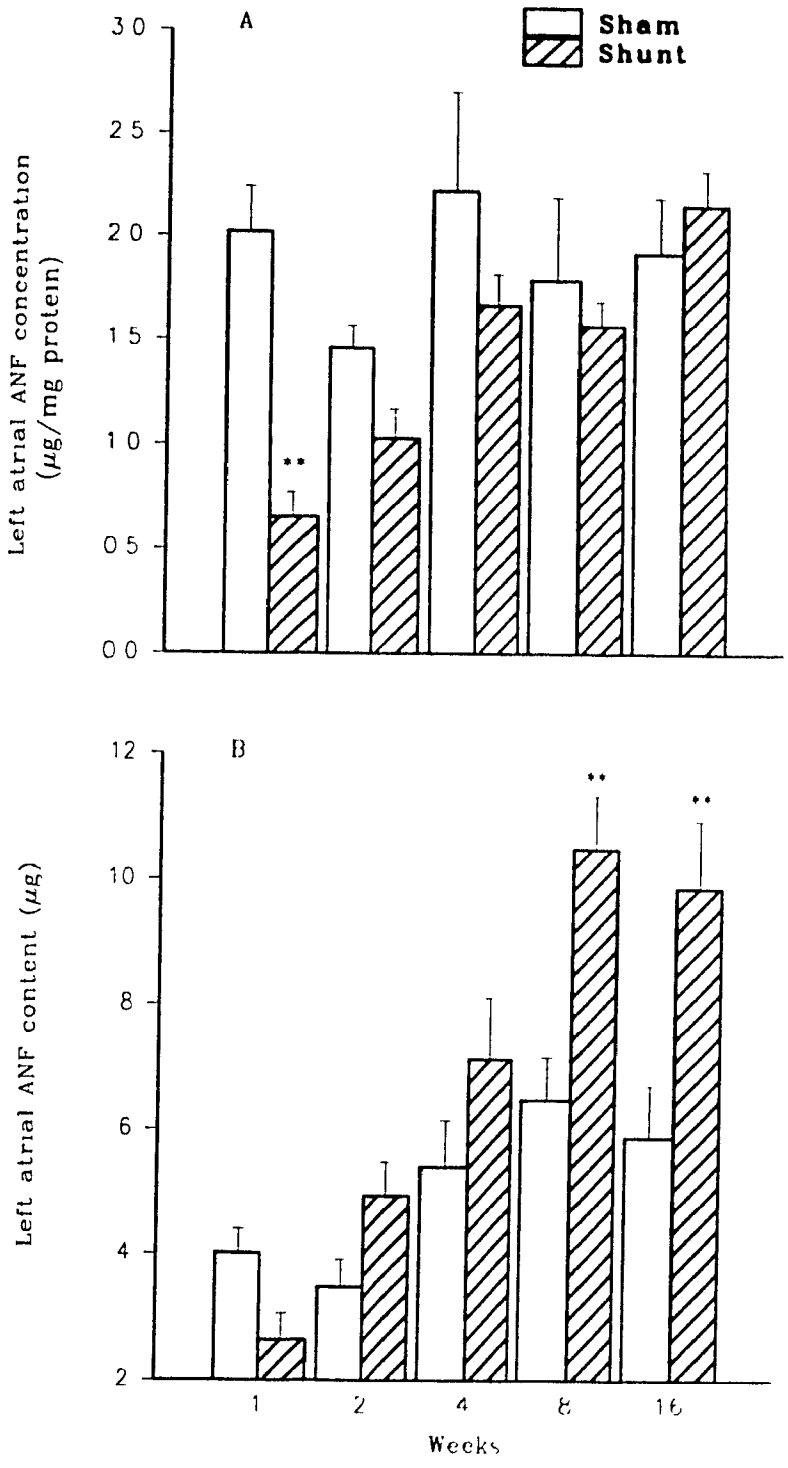

Fig. 2.2: Left atrial ANF concentrations (A) and total ANF content (B) during the development of heart failure. **$p < 0.01$ vs sham.

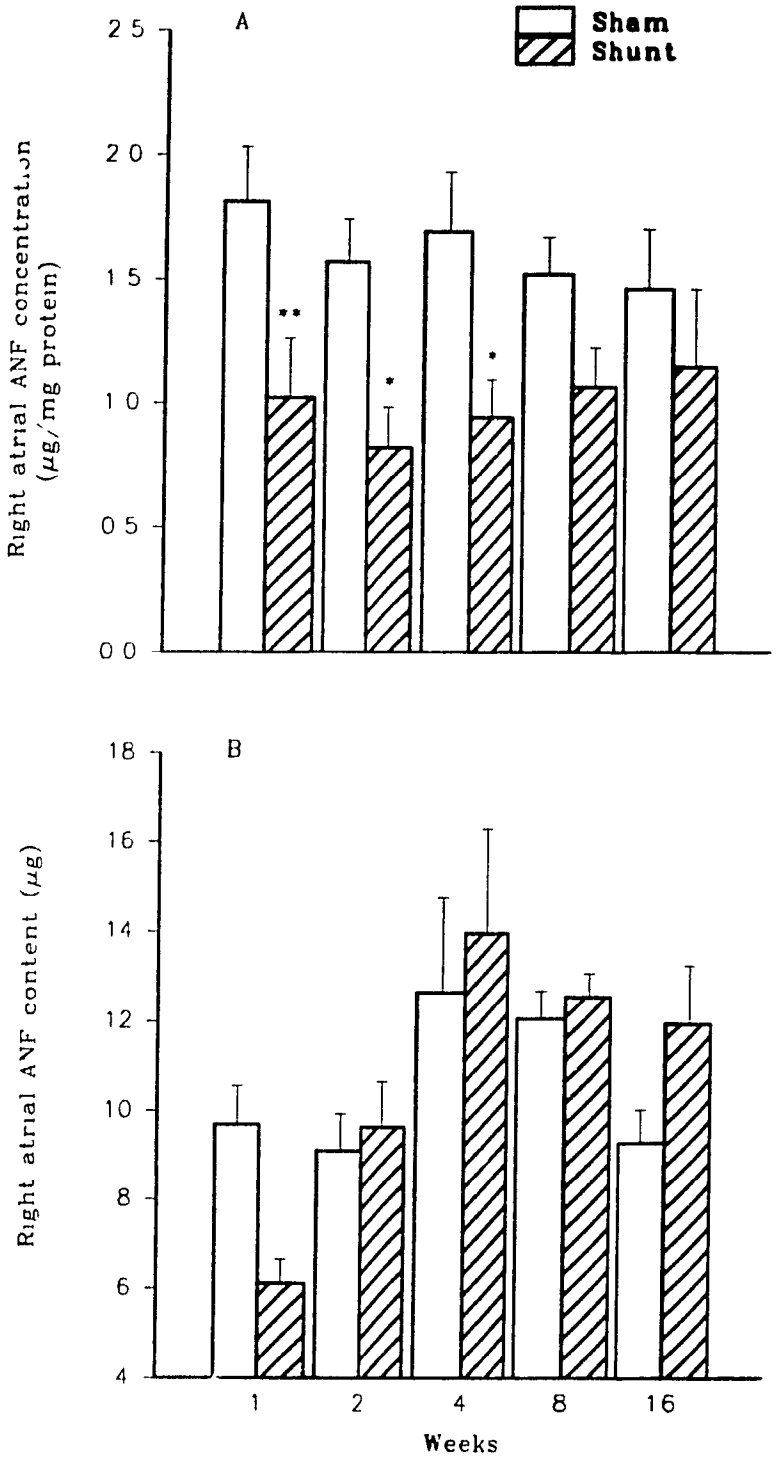

Fig. 2.3: Right atrial ANF concentrations (A) and total ANF content (B) during the development of heart failure.

*$p < 0.05$ vs sham; **$p < 0.01$ vs sham.

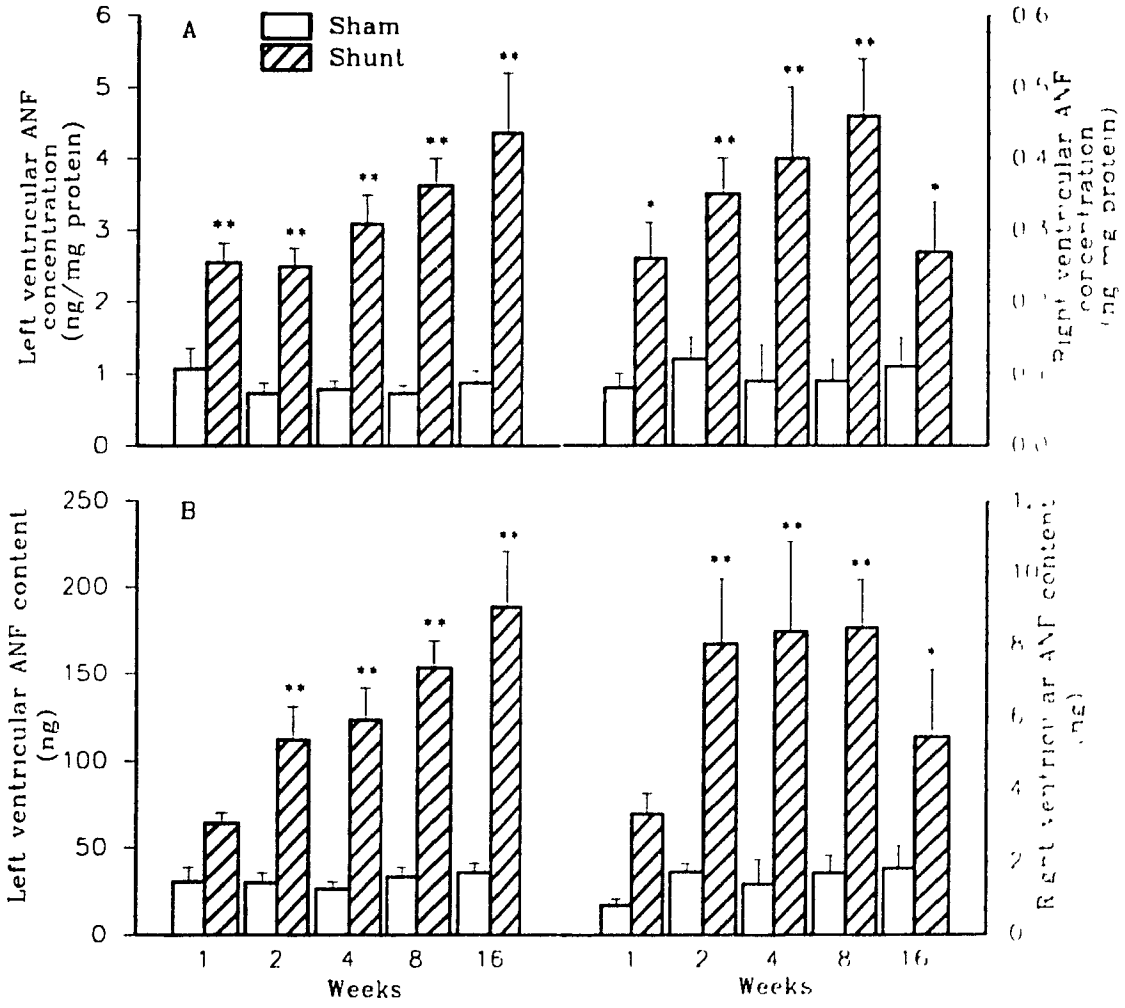

Fig. 2.4: Left and right ventricular ANF concentration s(A) and total ANF content (B) during the development of heart failure.

*p < 0.05 vs sham; **p < 0.01 vs sham.

TABLE 2.2

BODY AND TISSUE WEIGHTS DURING THE DEVELOPMENT OF HEART FAILURE

	1 week		2 weeks		4 weeks	
	Sham (n=13)	Shunt (n=12)	Sham (n=10)	Shunt (n=10)	Sham (n=10)	Shunt (n=9)
BW (g)	302 ± 5.4	292.8 ± 6.7	335.8 ± 7.1	343.8 ± 6.1	392.2 ± 9.3	389.3 ± 10.8
HW (mg)	992.5 ± 24.9	1355.5 ± 42.5**	1014.7 ± 36.3	1561.9 ± 49.7**	1172.0 ± 27.5	1956 ± 71.9**
HW ($mg.100g^{-1}$ B.W.)	329.1 ± 7.8	463.1 ± 10.9**	302.0 ± 8.6	454.1 ± 10.8**	299.4 ± 5.9	504.9 ± 21.7**
RVW (mg)	148.3 ± 4.4	204.2 ± 6.3*	169.4 ± 8.1	250.3 ± 12.1**	191.2 ± 9.5	319.9 ± 26.9**
RVW ($mg.100\ g^{-1}$ B.W.)	49.1 ± 1.2	69.9 ± 2.1**	50.6 ± 1.9	72.4 ± 2.8**	48.8 ± 2.2	82.5 ± 7.3**
LVW (mg)	669.4 ± 16.1	838.3 ± 30.0**	675.1 ± 31.0	977.2 ± 29.6**	798.4 ± 17.1	1176.4 ± 65.6**
LVW ($mg.100\ g^{-1}$ B.W.)	221.9 ± 4.5	285.8 ± 5.7*	201.8 ± 7.5	282.7 ± 4.9*	203.9 ± 3.2	301.9 ± 15.0*
RAW (mg)	74.9 ± 3.3	131.8 ± 5.1**	82.0 ± 4.2	137.0 ± 14.2**	81.5 ± 3.3	159.9 ± 12.7**
RAW ($mg.100\ g^{-1}$ B.W.)	24.9 ± 1.1	45.2 ± 1.8**	24.6 ± 1.3	39.6 ± 3.8**	20.8 ± 0.8	40.9 ± 3.1**
LAW (mg)	24.9 ± 1.3	52.3 ± 2.4**	30.2 ± 2.0	54.2 ± 6.7**	30.4 ± 1.9	59.4 ± 4.0**
LAW ($mg.100\ g^{-1}$ B.W.)	8.25 ± 0.4	17.9 ± 0.7**	9.01 ± 0.5	15.6 ± 1.8**	7.8 ± 0.5	15.1 ± 1.0**
RVW/LVW	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.23 ± 0.00	0.27 ± 0.4*
LW (mg)	1405.8 ± 63.3	1619.0 ± 97.4	1442.2 ± 49.4	1793.7 ± 57.3*	1508.3 ± 73.1	1853.0 ± 78.3*
LW ($mg.100^{g-1}$ B.W.)	467.3 ± 22.5	553.5 ± 31.4*	426.9 ± 16.9	516.8 ± 17.2*	386.7 ± 21.5	477.2 ± 20.3*

TABLE 2.2 (CONTINUED)

	8 weeks		16 weeks	
	Sham (n=11)	Shunt (n=11)	Sham (n=11)	Shunt (n=10)
BW (g)	479.5 ± 18.0	468.8 ± 16.0	502.7 ± 10.3	567.4 ± 12.8**
HW (mg)	1389.6 ± 69.5	2237.5 ± 149.5**	1370.8 ± 37.0	2366.1 ± 61.6**
HW ($mg.100\ g^{-1}$ B.W.)	288.6 ± 5.4	476.9 ± 24.9**	273.1 ± 6.7	419.8 ± 17.6**
RVW (mg)	222.0 ± 11.2	384.2 ± 23.4**	224.5 ± 10.7	406.9 ± 36.1**
RVW ($mg.100\ g^{-1}$ B.W.)	46.2 ± 1.1	83.9 ± 5.8**	44.8 ± 2.4	72.6 ± 7.8**
LVW (mg)	962.5 ± 47.1	1388.3 ± 76.4**	926.3 ± 24.0	1532.9 ± 19.9**
LVW ($mg.100\ g^{-1}$ B.W.)	200.1 ± 4.5	296.3 ± 12.3*	184.5 ± 4.2	271.1 ± 5.9*
RAW (mg)	94.4 ± 5.6	188.8 ± 20.1**	101.6 ± 6.1	187.0 ± 12.9**
RAW (mg.100 g B.W.)	19.6 ± 0.6	40.3 ± 3.9**	20.3 ± 1.2	33.4 ± 2.9**
LAW (mg)	38.0 ± 3.1	62.0 ± 4.8**	34.6 ± 1.6	65.2 ± 5.5**
LAW ($mg.100\ g^{-1}$ B.W.)	8.1 ± 0.5	13.3 ± 0.9**	6.9 ± 0.3	11.5 ± 0.9**
RVW/LVW	0.23 ± 0.01	0.28 ± 0.01**	0.24 ± 0.01	0.27 ± 0.02*
LW (mg)	2032.6 ± 115.0	2389.5 ± 137.6 **	2115.1 ± 119.9	2591.3 ± 195.0**
LW ($mg.100\ g^{-1}$ B.W.)	426.1 ± 24.9	514.3 ± 31.8**	413.1 ± 26.7	463.4 ± 43.7

Values are means ± SE; BW=body weight; HW=heart weight; LW=lung weight; RVW=right ventricular free wall weight; LVW=left ventricular free wall + septum weight; LAW=left atrial weight; RAW=right atrial weight; *p<0.05 vs sham; **p<0.01 vs sham

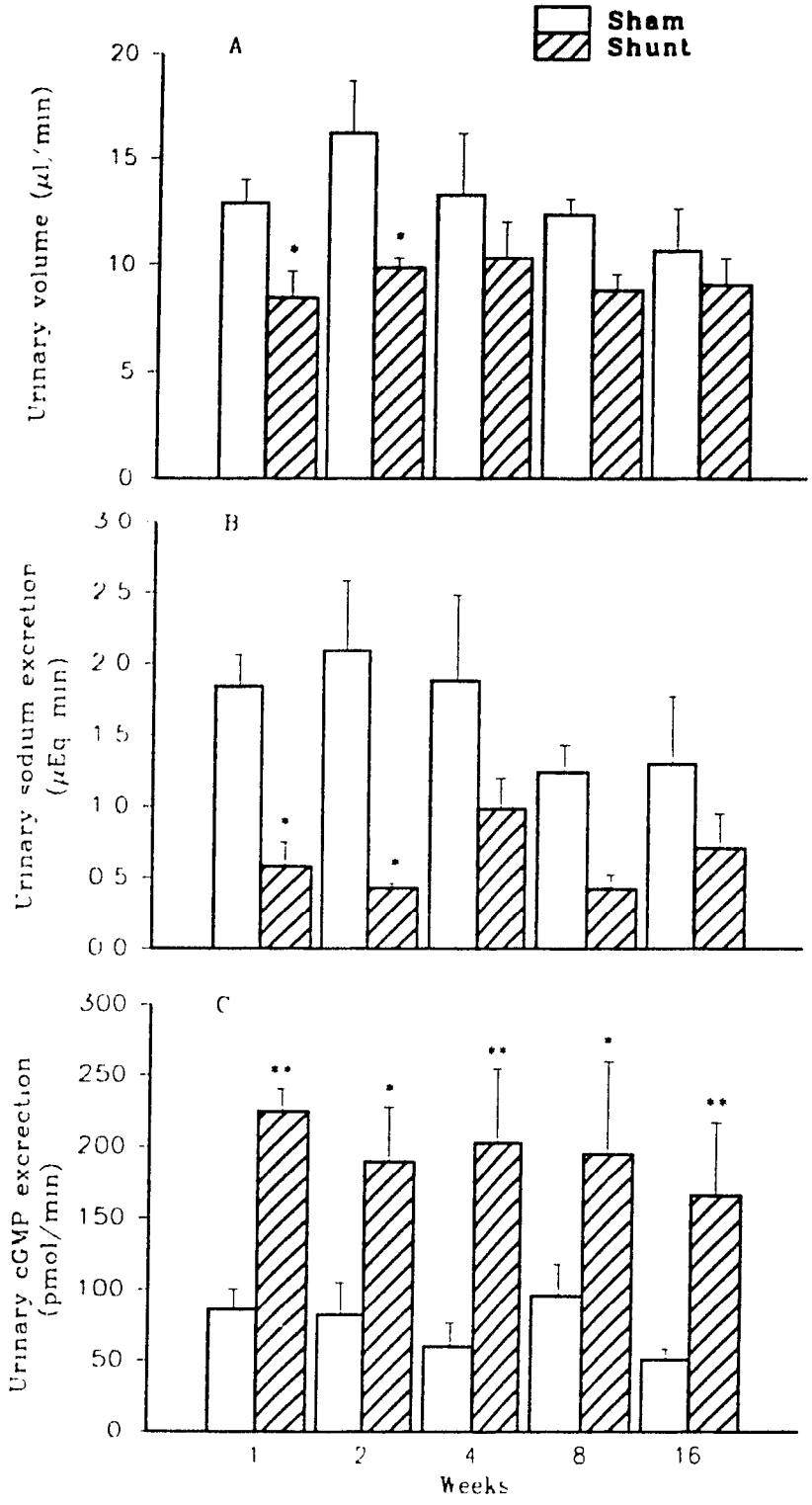

Fig. 2.5: Urinary volume (A), urinary sodium excretion (B), and urinary cGMP excretion (C) during the development of heart failure.

*$p < 0.05$ vs sham.

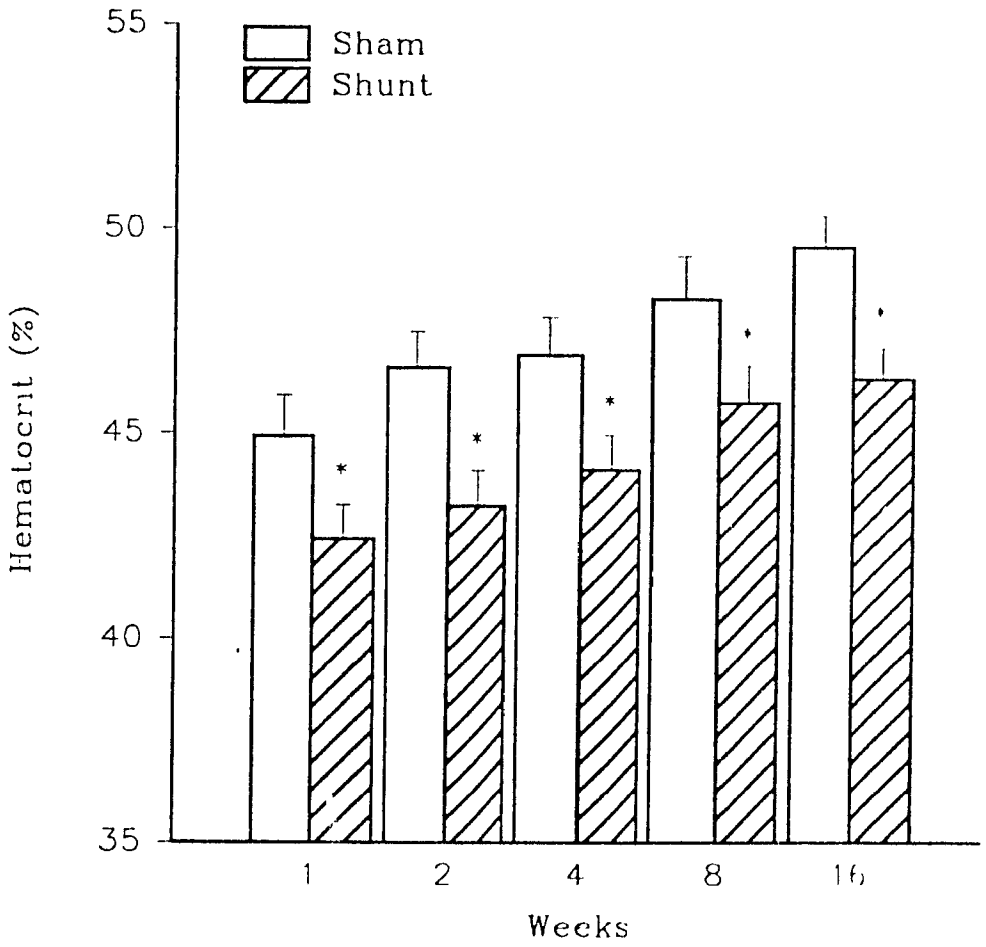

Fig. 2.6: Hematocrit during the development of heart failure.

*$p < 0.05$ vs sham.

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2.7 REFERENCES

1. Genest J, Cantin M. Atrial natriuretic factor. Circulation 1987;75:I118-I124.
2. Kuchel O., Debinski W, Racz K, Buu N.T., Garcia R, Cusson J.R., Larochelle P, Cantin M, Genest J. An emerging relationship between peripheral sympathetic nervous activity and atrial natriuretic factor. Life Sci. 1987;40:1545-1551.
3. Burnett JC Jr, Kao PC, Hu DC, Heser DW, Heublin D, Granger JP, Opgenorth TJ, Reeder GS. Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 1986;231:1145-1147.
4. Manning PT, Schwartz D, Katsube NC, Holmberg SW, Needleman P. Vasopressin-stimulated release of atriopeptin: endocrine antagonists in fluid homeostasis. Science 1985;229:395-397.
5. Francis GS. Neurohormonal mechanisms involved in congestive heart failure. Am. J. Cardiol. 1985;55:15A-21A.
6. Hostetter TH, Pfeffer JM, Pfeffer MA, Dworkin LD, Braunwald E, Brenner BM. Cardiorenal hemodynamics and sodium excretion in rats with myocardial infarction. Am. J. Physiol. 1983; 245 (Heart Circ. Physiol.14):H98-H103.
7. Ichikawa I, Pfeffer JM, Pfeffer MA, Hostetter TH, Brenner BM Role of angiotensin II in the altered renal function of congestive heart failure. Circ. Res. 1984;55:669-675.
8. Kon V, Yared A., Ichikawa I. Role of the renal sympathetic nerves in mediating hypoperfusion of renal cortical microcirculation in experimental congestive heart failure and acute extracellular fluid volume depletion. J. Clin. Invest. 1985;76:1913-1920.
9. Riegger AJG. The role of the atrial natriuretic factor in congestive heart failure. Prog. Pharmacol. and Clin. Pharmacol. 1990;713:101-115.

10. Garcia R, Diebold S. A simple, rapid and effective method of producing aortocaval shunts in the rat. Cardiovas. Res. 1990;24:430-432.

11. Gutkowska J, Genest J, Thibault G, Garcia R, Larochelle P, Cusson JR, Kuchel O, Hamet P, De Léan A, Cantin M. Circulating forms and radioimmunoassay of atrial natriuretic factor. In: Rosenblatt, M., Jacobs, J. W. (eds): Endocrinology and Metabolism Clinics of North America, Atrial Natriuretic Factor, Philadelphia, W.B. Saunders Co., 1987, p. 183.

12. Thibault G, Murthy KK, Gutkowska J, Seidah NG, Lazure C, Chrétien M, Cantin M. NH2-terminal fragment of rat pro-atrial natriuretic factor in the circulation: Identification, radioimmunoassay and half-life. Peptides 1988;9:47-53.

13. Spector T. Refinement of a Coomassie blue method of protein quantification. Anal Biochem. 1978; 86:142-146.

14. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. Circ. Res. 1980;47:1-9.

15. Gefei Q, Garcia R. Chronic captopril and losartan (DuP 753) administration in rats with high-output heart failure. Am. J. Physiol., in press.

16. Ding J, Thibault G, Gutkowska J, Garcia R, Karabatsos T, Jasmin G, Genest J, Cantin M. Cardiac and plasma atrial natriuretic factor (ANF) in experimental congestive heart failure. Endocrinology 1987;121:248-257.

17. Cody RJ, Atlas SA, Laragh JH, Kubo SH, Covit AB, Pyman KS, Shaknovich A, Pindolfino K, Clark M, Camargo MJF, Scarborough RM, Lewicki JA. Atrial natriuretic factor in normal subjects and heart failure patients. J. Clin. Invest. 1986;78:1362-1374.

18. Winaver J, Hoffman A, Burnett J C Jr, Haramati A. Hormonal determinants of sodium excretion in rats with experimental high-output heart failure. Am. J. Physiol. 1988;254:R776-R784, 1988.

19. Moe GW, Stopps TP, Angus C, Forster C, de Bold AJ, Armstrong PW. Alteration in serum sodium in relation to atrial natriuretic factor and other neuroendocrine variables in experimental pacing-induced heart failure. J..Am. Coll. Cardiol. 1989;13:173-179.

20. Stopps TP, de Bold AJ, Forster C, Moe G, Armstrong PW. Experimental heart failure: atrial natriuretic factor and other neuroendocrine changes during rapid ventricular pacing. Clin Res 1986;34:347A.

21. Wang YT, Uematsu T, Sato R, Hayashi Y, Nakashima M. Changes in plasma level of alpha-atrial natriuretic polypeptide (alpha-ANP) and responsiveness of the aorta to exogenous alpha-ANP subsequent to myocardial infarction in rats. Jpn. J. Pharmacol. 1989;49:463-469.

22. Hartter E, Weissel M, Punzengruber C, Burghuber OC, Wolosz̀czuk W. Interpretation of plasma concentrations of human atrial natriuretic peptide (hANP) in congestive heart failure. AMAJ 1990; 17 (4):71-77.

23. Zucker I, Earle AM, Gilmore JP. The mechanism of adaptation of left atrial stretch receptors in dogs with chronic congestive heart failure. J. Clin. Invest. 1977;60:323-331.

24. Greenberg TT, Richmond WH, Stocking RA, Gupta PD, Meehan JP, Henry JP. Impaired atrial receptor responses in dogs with heart failure due to tricuspid insufficiency and pulmonary artery stenosis. Circ Res 1973;32:424-433.

25. Drexler H, Hanze J, Finckch M, Lu W. Just H, Lang RE. Atrial natriuretic peptide in a rat model of cardiac failure. Atrial and ventricular mRNA, atrial content, plasma levels, and effect of volume loading. Circulation 1989;79:620-633.

26. Marie JP, Guillemot H, Hyatt PY. Le degré de granulation des cardiocyte auriculaires: Etude planimetrique au cours de differents apports d'eau et de sodium chez le rat. Path. Biol. 1976;24:549-554.

27. Lattion AL, Michel JB, Arnauld E, Corvol P, Soubrier F. Myocardial recruitment during ANF mRNA increase with volume overload in the rat. Am. J. Physiol. 1986;251:H890-H896.

28. Kohzuki M, Hodsman GP, Johnston CI. Attenuated response to atrial natriuretic peptide in rats with myocardial infarction. Am. J. Physiol. 1989;256:H533-H538.

29. Hoffman A, Burnett JC Jr, Haramati A, Winaver J. Effects of atrial natriuretic factor in rats with experimental high-output failure. Kidney Int. 1988;33:656-661.

30. Tsunoda K, Mendelsohn FAO, Sexton PM, Chai SY, Hodsman GP, Johnston CI. Decreased atrial natriuretic peptide binding in renal medulla in rats with chronic heart failure. Circ. Res. 1988;62:155-161.

31. Cody RJ. Sodium and water retention in congestive heart failure - The pivotal role of the kidney. Am. J. Hypertens. 1988;1:395S:401S.

32. Abassi Z, Haramati A, Hoffman A, Burnett JC Jr, Winaver J. Effect of converting enzyme inhibition on renal response to ANF in rats with experimental heart failure. Am. J. Physiol. 1990;259:R84-R89.

33. Margulies KB, Heublein DM , Perrella MA, Burnett JC Jr. ANF-mediated renal cGMP generation in congestive heart failure. Am. J. Physiol. 1991;260:F562-F568.

34. Abassi Z, Burnett JC Jr., Grushka E, Hoffman A, Haramati A, Winaver J. Atrial natriuretic peptide and renal cGMP in rats with experimental heart failure. Am. J. Physiol. 1991;261:R858-R864.

Chapter III

CHRONIC CAPTOPRIL AND LOSARTAN (DuP 753) ADMINISTRATION IN RATS WITH HIGH-OUTPUT HEART FAILURE

3.1 ABSTRACT

We investigated the role of atrial natriuretic factor (ANF) and the renin angiotensin system (RAS) as well as the effects of losartan in rats with aorto-caval (A-C) shunts. Right atrial (RAP) and left ventricular end-diastolic pressures (LVEDP) were higher, and mean arterial blood pressure (MAP) was lower in A-C shunt animals than in their controls A-C shunt rats presented marked cardiac hypertrophy, decreased right atrial ANF concentration and increased ventricular ANF content and concentration. Plasma ANF levels were elevated, and hematocrit was lower in A-C shunt animals than in the controls. Captopril or losartan treatment decreased MAP and returned LVEDP to sham-operated control values. A clear regression of cardiac hypertrophy was evident in both treated A-C shunt groups with plasma ANF levels tending to follow those in sham-operated rats. Plasma COOH-terminal ANF levels were decreased and urinary volume and hematocrit were increased in losartan-treated A-C shunt animals. We conclude that chronic ACE inhibition and ANG II receptor antagonism improved hemodynamic conditions, diminished water retention, reversed cardiac hypertrophy, and restored plasma and tissue ANF to more "normal" levels in rats with moderate high-output heart failure.

3.2 INTRODUCTION

Neurohormonal vasoconstrictor systems are frequently activated in patients with chronic heart failure (CHF). These include the sympathetic nervous and renin angiotensin systems (RAS) as well as arginine vasopressin, which make important contributions to such characteristic CHF abnormalities as vasoconstriction, changes in cardiac performance, and sodium retention (5,9,18). The RAS appears to play a key role in this regard because of its multiple biological activities and its presence in tissue. Plasma atrial natriuretic factor (ANF), which is involved in the regulation of body fluids and blood pressure, is elevated in both experimental and human CHF. However, the physiological actions of exogenous ANF are blunted in CHF.

The use of angiotensin converting enzyme (ACE) inhibitors in congestive heart failure is now established therapy. It has been shown that ACE inhibitors improve the survival and symptomatology of CHF patients (5). However, the mechanism underlying these improvement has not been fully elaborated. Their longterm effects on RAS markers, and their interactions with ANF are also poorly defined.

Because of the beneficial consequences of ACE inhibitors in essential hypertension and congestive heart failure, angiotensin II (ANG II) receptor antagonists should also be therapeutically useful against these disorders. Losartan, a new, potent, orally active, and highly specific nonpeptide AT_1 receptor antagonist, is an antihypertensive agent without partial agonistic or bradykinin-potentiating effects (4,31). Its chronic therapeutic efficacy in CHF has not yet been evaluated. Therefore, the present studies were designated to comparatively investigate the chronic hemodynamic, hormonal and renal effects of ACE inhibition with captopril and ANG II receptor antagonism with losartan as well as RAS and ANF interactions in rats with a moderate degree of high-output heart failure.

3.3 MATERIALS AND METHODS

3.3.1 Animals

Male Sprague-Dawley rats (Charles River, St-Constant, Quebec) weighing 225-275 g were kept at controlled room temperature under a 6 a.m. to 6 p.m. light cycle on regular pelleted rat chow (Purina, Richmond, IN) and tap water ad libitum.

3.3.2 Aorto-caval (A-C) shunts

The method of producing A-C shunts has been detailed elsewhere (12). Briefly, on the day of surgery, the animals were anesthetized with ether, and the vena cava and abdominal aorta were exposed by opening the abdominal cavity via a midline incision. The aorta was punctured at the union two-thirds caudal to the renal artery and one-third cephalic to the aortic bifurcation with an 18 gauge disposable needle (Becton-Dickinson, Rutherford, NJ) held with a plastic syringe. The needle was advanced into the aorta, perforating its adjacent wall and penetrating the vena cava. At this moment, a bulldog vascular clamp was placed across the aorta caudal to the left renal artery. Once the aorta was clamped, the needle was fully withdrawn, and a drop of cyanoacrylate glue (Krazy Glue, TM DuPont, Atlantic Promotions Inc., Longueuil, Quebec) was used to seal the aorta puncture point. The clamp was removed 30 sec later. The patency of the shunt was verified visually by vena cava swelling and mixing of arterial and venous blood. The entire surgical procedure took less than 10 min. Sham-operated rats, in which the abdominal cavity was opened and the vena cava and aorta exposed, served as controls.

3.3.3 Captopril and losartan administration

Three weeks after surgery, four experimental groups were established: A-C shunt and sham-operated controls, and A-C shunt rats treated with either captopril or losartan. The animals were installed in metabolic cages and allowed to become accustomed to their new environment before drug administration was begun. They

were kept on regular rat chow and tap water ad libitum. Water and food intake and urinary volume were measured daily during treatment. Captopril was given via drinking water at a dose level of 1 g/l. Losartan potassium was administered p.o. at 10 mg/kg twice a day (the dose was calculated according with reference # 31. Four to 6 animals from each group were killed by decapitation at the last treatment day and trunk blood collected for plasma renin activity measurement. Captopril and losartan were generous gifts from E.R. Squibb and Sons Inc. and The DuPont Merck Pharmaceutical Co., respectively.

3.3.4 Measurement of hemodynamic parameters

Seven to 8 days after treatment was started, the animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.), and their femoral artery, right atrium (through the right jugular vein) and left ventricle (through the right carotid artery) were cannulated with polyethylene catheters (PE 50) to measure mean arterial (MAP), right atrial (RAP) and left-ventricular end-diastolic (LVEDP) pressures, respectively. These parameters were monitored with Gould Statham P23ID transducers (Gould Inc., Oxnard, CA) and recorded on a Grass model 7D polygraph (Grass Instruments, Quincy, MA). Heart rate was measured through a Grass Model 7P44 Tachograph preamplifier. The amplifier receiving the signal from the ventricle was connected by a bridge to a Grass Model 7P20C polygraph differentiator for registration of the first derivative of left ventricular pressure (dP/dt max). The rats were then installed in restriction cages and allowed to regain consciousness and to stabilize their hemodynamic parameters before the experiment commenced Pressures, heart rate and dP/dt max recordings for 20 min were started 2 h after the animals regained consciousness. At the end of the experiment, blood was withdrawn from the femoral artery for measurement of hematocrit, plasma ANF, osmolality and sodium. The hearts and lungs were excised and weighed. Atria and ventricles were dissected, weighed separately and kept at -70^{o} until assayed.

3.3.5 Biochemical methods

Blood for ANF and plasma renin activity (PRA) measurements was collected in tubes containing 10^{-5} M EDTA. The samples were immediately centrifuged at 3,000 g for 10 min at 4^{o}C. ANF (99-126) was extracted from plasma with Vycor glass beads (Corning Glass Works, Corning, NY) and measured by radioimmunoassay (RIA), as described elsewhere (13). Plasma samples were directly measured for ANF (1-98) by RIA (27). PRA was assessed by RIA of angiotensin I (ANG I) generation.

Tissue ANF content was also measured by RIA (13). Briefly, the atria were homogenized for 30 sec with a Polytron (setting 7; Kinematica, Lucerne, Switzerland) in 2 ml 0.1 M acetic acid containing the following final concentrations of protease inhibitors: 1 x 10^{-5} mol/l EDTA, 5 x 10^{-6} mol/l pepstatin, and 3 x 10^{-5} mol/l phenylmethylsulfonyl fluoride (PMSF). The ventricles were carefully dissected, minced, and boiled in 1 M acetic acid for 5 min (10 ml acetic acid/g tissue), then cooled to room temperature and homogenized in a Polytron (30 sec, set at 7). The homogenates were centrifuged at 15,000 rpm for 10 min, and the supernatant was applied to a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA) that had been previously acti ated with 5 ml 100% acetonitrile. The cartridge was washed twice with 5 ml 0.1% trifluoroacetic acid in H_2O, and ANF absorbed on the columns was eluted with 80% CH_3CN in 0.1% trifluoroacetic acid. The material was lyophilized in a Speed-Vac (Savant Instruments Inc., Farmingdale, NY) and kept at -70^{o}C until ANF was measured by RIA (13). Atrial and ventricular protein content was quantified by a modification of Bradford's method (25). Plasma ANF levels and tissue ANF content were not corrected for recovery. Plasma osmolality was measured by the depression of freezing point in an osmometer (Advanced Instruments Inc., MA) and packed cell volume with capillary tubes in a single speed capillary centrifuge (Model MB, International Equipment Co., MA) 6 min. Plasma and urinary sodium was assessed by flame photometry.

3.3.6 Statistical analysis

The data, expressed as means ± SE, were evaluated by one-way analysis of variance (ANOVA). Whenever the overall F test was significant, an a posteriori contrast test was applied according to the Bonferroni method. One-way ANOVA with repeated measures on one factor (time) was sometimes used to test the time effect within each separate group.

3.4 RESULTS

3.4.1 Hemodynamic effects of ACE inhibition and AT_1 receptor antagonism

MAP was lower and RAP and LVEDP were higher in A-C shunt rats than in their sham-operated controls (Table 3.1). Captopril and losartan reduced MAP further when compared with untreated A-C shunt animals. Both agents tended to decrease RAP in A-C shunt rats but their values were nevertheless higher than in sham-operated controls. LVEDP was significantly lower in captopril-treated animals and tended to be reduced in A-C shunt rats given losartan when compared with untreated A-C shunt controls. Both treatments suppressed the LVEDP elevations observed in A-C shunt animals to values not different from those of sham-operated controls. No significant differences were found in either dP/dt max or heart rate between groups.

3.4.2 Effects of captopril and losartan on tissue weights and cardiac hypertrophy

Absolute and relative heart and lung weights were much greater in A-C shunt rats than in sham-operated controls (Table 3.2). A-C shunt animals also showed a large increase in the absolute and relative weights of each heart chamber when compared with their sham-operated controls. Furthermore, the ratio of right to left ventricular weight was significantly increased in A-C shunt versus sham-operated rats. However, the ratio of right to left atrial weight in A-C shunt animals was not significantly different from that in sham-operated controls.

Compared with untreated A-C shunt rats, captopril and losartan significantly reduced absolute and relative heart weights. The decreases in absolute and relative heart weights were 15.62% and 12.48% in losartan-treated and 12.86% and 12.38% in captopril-treated A-C shunt animals respectively. However, absolute and relative heart weights in both treated A-C shunt groups were still higher than in the sham-operated controls. Except for relative right ventricular weight, losartan significantly decreased the absolute and relative weights of each cardiac number chamber. The reductions of relative left and right atrial and ventricular weights in A-C shunt rats treated with losartan were 12.72%, 13.83%, 8.78%, and 9.42%, respectively. The absolute and relative atrial and left ventricular weights were also lower in A-C shunt rats treated with captopril than in A-C shunt controls. The decreases in relative left and right atrial and ventricular weights in A-C shunt rats treated with captopril were 16.63%, 15.62%, 8.21%, and 2.93%, respectively. Neither treatment reduced cardiac chamber weights to sham-operated control values. Neither captopril nor losartan changed the ratio of right to left ventricular weights, which was higher than in sham-operated rats. No significant differences were noted in the ratio of right to left atrial weights between groups. Both treatments tended to decrease absolute and relative lung weight in A-C shunt rats; absolute lung weight was not different in losartan-treated animals compared to sham-operated controls. No difference was observed in body weight between groups.

3.4.3 Effects of captopril and losartan on plasma and tissue ANF levels and PRA

Plasma COOH- and NH_2-terminal ANF levels (Fig. 3.1A and 3.1B) in A-C shunt rats were increased significantly when compared with sham-operated controls ($p<0.01$). Treatment with the ACE inhibitor or AT_1 receptor antagonist significantly reduced plasma COOH-terminal ANF ($p<0.05$) when compared with A-C shunt controls, but these values were still higher than those of sham-operated rats ($p<0.05$). Plasma NH_2-terminal ANF levels in A-C shunt animals receiving either agent were similar to those in untreated A-C shunt rats, and the latter were higher than those in

sham-operated controls ($p < 0.01$).

No differences in left atrial ANF concentration were observed between groups (Fig. 3.2). Total left atrial ANF content was higher ($p < 0.01$) and right atrial ANF concentration was lower ($p < 0.01$) in A-C shunt rats than in sham-operated controls. Total right atrial ANF content was similar in A-C shunt and sham-operated animals. Captopril or losartan administration had no effect on total ANF content and ANF concentration in either atrium. Total ANF content and ANF concentration in either ventricle were much higher in A-C shunt rats than in sham-operated controls ($p < 0.01$) (Fig. 3.3). Captopril and losartan reduced ANF concentration in both ventricles ($p < 0.01$ and $p < 0.05$ respectively). Total ANF content was diminished in the left ventricle by both treatments, but only captopril decreased it, albeit slightly, in the right ventricle. Total ANF content and ANF concentration in the left ventricle and total right ventricular ANF content were still higher in each treated A-C shunt group than in the sham-operated controls ($p < 0.01$ and $p < 0.05$ respectively). PRA was 4.77 ± 0.90, 5.12 ± 1.38, 34.43 ± 3.61 and 27.63 ± 2.87 (ANG I ng/ml.h) in sham, A-C shunt, A-C shunt + losartan and A-C shunt + captopril groups respectively. The values were significantly higher ($p < 0.01$) in both treated than in untreated groups.

3.4.4 Effects of captopril and losartan on body fluid hemeostasis:

Urinary volume was significantly increased in A-C shunt rats from the 1st and 2nd day of captopril or losartan treatment respectively ($p < 0.01$ and $p < 0.05$) (Fig 3.4). When compared with the A-C shunt or sham-operated controls, urinary volume in treated A-C shunt animals was significantly different from the 3rd day of treatment ($p < 0.05$ or $p < 0.01$). No differences in urinary sodium excretion were observed between groups (data not shown). Captopril increased water intake during the last 3 days (Fig. 3.5A). On the other hand, losartan decreased food intake, also during the last 3 days of treatment (Fig. 3.5B). Urinary GMP excretion was much higher in treated A-C shunt rats and in untreated A-C shunt controls than in sham operated

animals ($p<0.01$ or $p<0.05$). Losartan resulted in a significant decrease of urinary cGMP from the 3rd day of treatment when compared with the period prior to treatment or with A-C shunt controls ($p<0.01$ or $p<0.05$) (Fig. 3.6). Captopril tended to depress urinary cGMP excretion but not significantly. Hematocrit was lower in A-C shunt rats than in sham-operated controls ($p<0.01$). Losartan elevated hematocrit in A-C shunt animals ($p<0.05$) to levels seen in the sham-operated controls. Captopril had no effect on hematocrit, which was lower than in the sham-operated group ($p<0.01$) (Fig. 3.7). No differences were observed in plasma sodium and osmolality between groups (data not shown).

3.5 DISCUSSION

Several compensatory mechanisms can be activated during the development of heart failure. These include increased activity of the sympathetic nervous system and RAS and changes in a variety of other neuroendocrine functions, which together evoke sodium and water retention as well as vasoconstriction (10,15,18). On the other hand, ANF is also activated and is believed to counterbalance the RAS and sympathetic nervous system (23). Thus, during CHF, both ANF and neurohormonal vasoconstrictor systems may reach a new balance at a higher steady state. However, their degree of participation is far from being established. Recently, it has been demonstrated that, in addition to the circulation, the RAS also exists in various tissues, including the heart, vasculature, and kidney (7,8,19). The RAS in tissue may also contribute to the pathophysiology of heart failure via the vascular, renal, and cardiac effects of local ANG II (7,8,19). Thus, the RAS may be especially important in cardiovascular homeostasis under CHF. The use of specific agents, such as ACE inhibitors and/or ANG II receptor antagonists, is an important method of investigating the specific role of a hormone under a particular condition. The physiological and pathophysiological changes that may occur after treatment with an antagonist may provide further insight into the mechanism of the disease process itself.

The present study, by blocking the RAS at the ANG II receptor level or by inhibiting ANG II production, evaluates the role of the RAS and ANF systems in rats with moderate high-output heart failure. We believe this is the first time that the chronic effect of losartan, a new non-peptide AT_1 receptor antagonist, has been investigated in rats with moderate high output heart failure. The hemodynamic responses to this agent and to captopril were similar; both significantly lowered MAP. Although RAP was still higher in A-C shunt rats treated with captopril or losartan than in sham-operated animals, both drugs tended to reduce it when compared with untreated A-C shunt controls. LVEDP was lower in captopril-treated A-C shunt rats than in the untreated A-C shunt controls. Furthermore, LVEDP in both treated A-C shunt groups was not different from that in sham-operated animals despite only a tendency towards decreased LVEDP noted in the losartan-treated A-C shunt group when compared with untreated A-C shunt controls. The reductions in MAP, RAP and LVEDP suggest a fall in both pre- and afterload in treated A-C shunt rats. These beneficial hemodynamic effects are similar to those reported by other investigators during ACE inhibition (2,22).

Left ventricular hypertrophy is an independent risk factor for a number of severe cardiac dysfunctions (20). The causes of cardiac hypertrophy are complex. Although an increase in pre- or afterload may be a major factor, the sympathetic nervous system and RAS have emerged as major candidates in cardiac hypertrophy. Furthermore, local active RAS and ANG II receptors have been demonstrated to exist in the heart (24). Recent experimental studies suggest the intriguing possibility that ANG II may exert a major influence, stimulating the proliferation of cardiac myocytes (16). Therefore, ACE inhibitors or ANG II receptor antagonists are capable of preventing or reversing cardiac hypertrophy in experimental animals as well as in humans; their antiproliferative effects, however, are difficult to dissociate from their concomitant *in vivo* systemic hemodynamic actions

Our present studies reveal hypertrophy of each cardiac chamber in A-C shunt rats. Furthermore, the ratio of right to left ventricular weight in A-C shunt rats is much higher than in sham-operated controls, indicating that hypertrophy is greater in the right than in the left ventricle. This result may be associated with the experimental model used, reflecting a larger increase in loading in the right ventricle than in the left. Another possibility is that local RAS activity could be higher in the right than in the left ventricle, which has been demonstrated recently (19). Probably both mechanisms play a role in biventricular hypertrophy.

Treatment with an ACE inhibitor or AT_1 receptor antagonist partially reverses cardiac hypertrophy in A-C shunt rats after 7 days of drug administration. The degree of regression of cardiac hypertrophy with both treatments is similar. Both agents increase urinary volume and hematocrit (although the latter is not significantly changed in A-C shunt rats treated with captopril) suggesting a decrease in blood volume. In the case of captopril, the increase in urinary volume could be partly secondary to augmented water intake, which is not observed in losartan-treated animals.

As a result of a decrease in blood volume (preload) and possibly in vascular peripheral resistance (afterload), a reduction of RAP and LVEDP is seen in A-C shunt rats. Treatment with captopril or losartan lowers blood pressures to similar degrees. Therefore, the reversal of cardiac hypertrophy may be due to reductions of both pre- and afterloading imposed on the heart. However, our results also show that regression of right atrial hypertrophy is the most pronounced among all chambers although the decrease in RAP is small and not significant, suggesting that factors other than loading may contribute to cardiac hypertrophy.

It has been demonstrated that the highest concentrations of ANG I and ANG II are in the right cardiac atrium (19). Recent data indicate that ACE is distributed throughout cardiac tissue but may be greater in the atria compared to ventricles and higher in the right atrium compared to the left (9). It has also been found that the cardiac expression of ACE and angiotensinogen is increased in rats with experimental

myocardial infarction (14). Our results indicate then that captopril and losartan reverse cardiac hypertrophy in A-C shunt rats by inhibiting local cardiac ANG II production or antagonizing the action of ANG II at its receptors, or by decreasing cardiac pre- and afterload.

As reported previously in both human and experimental CHF (11,29), plasma COOH- and NH_2-terminal ANF levels were much higher in A-C shunt rats than in sham-operated controls. Because atrial stretch is the main stimulant of ANF release by the atria, our results suggest that chronically elevated atrial filling pressure, induced by heightened cardiac preload, may chronically elicit ANF secretion. Immunoreactive ANF concentration in the right atrium was lower and total right and left atrial ANF content was higher in A-C shunt rats than in sham-operated controls. The decrease in atrial immunoreactive ANF concentration and the increase in total atrial immunoreactive ANF content indicate that enhanced ANF release and synthesis observed in both atria contribute to elevated plasma ANF levels in rats with moderate high-output heart failure. It has been suggested that the ventricles may be an important source of ANF synthesis and release in CHF (28). In our experiments, both total ANF content and ANF concentration in right and left ventricles were higher in A-C shunt rats indicating that the ventricles may contribute to basal plasma levels since ventricular

myocytes seem to release ANF by a "constitutive" rather than by a "regulated" pathway (1).

Treatment with captopril or losartan reduced but did not normalize plasma COOH-terminal ANF levels, which may have resulted in part from LVEDP normalization (and, therefore, decreased left atrial distension) but not RAP normalization. This finding is similar to that of Michel et al., who recently reported that ACE inhibition in a rat model of heart failure normalized plasma ANF levels. The reduction of preload and afterload and thus of cardiac hypertrophy probably culminated in decreased ANF synthesis and release by ventricles in A-C shunt rats since both total content and concentration were diminished after drug treatment. On the other hand, a reduction of atrial filling pressure may have caused a decreased secretion of ANF by the atria. Diminished ANF release by the atria and synthesis by the ventricles may, in turn, have contributed to lower plasma COOH-terminal ANF levels. However, plasma NH_2-terminal ANF concentration did not show a parallel reduction. This is probably a reflection of their distinct catabolic pathways (28), which could be affected differently during CHF. No data are available on this subject.

Despite high plasma ANF levels, A-C shunt rats had lower hematocrit with a tendency towards lower urinary sodium excretion and urinary volume than sham-operated controls, indicating sodium retention and an increased plasma volume. These attenuated renal responses to ANF may be secondary to renal ANF receptor downregulation (29) and/or RAS activation, as well as renal hemodynamic abnormalities. A-C shunt rats given captopril or losartan presented an increase of urinary volume from the 1st and 2nd day of treatment, respectively. Furthermore, urinary volume in both treated A-C shunt groups was higher than in A-C shunt or sham-operated controls from the 3rd day of treatment. This result was similar to the data of Xie et al., (32), who recently demonstrated that losartan induced substantial diuresis, natriuresis and chloruresis in Munich-Wistar rats. Increased urinary volume in captopril-treated rats could also be secondary to their augmented water intake. In our

experiment, urinary sodium excretion was not increased in losartan-treated rats (data not shown), which may have been due to significantly decreased food intake implying reduced sodium consumption. Diminished food intake could have been secondary to a gastrointestinal response to the drug or to taste loss. An enhanced renal response may result from upregulation of functional ANF receptors in the kidneys, which may be secondary to decreased circulating ANF levels, or to systemic and/or local renal RAS inhibition.

In our experiments, urinary cGMP excretion in A-C shunt rats receiving the AT_1 receptor antagonist was decreased from the 3rd day of drug administration. Furthermore, A-C shunt rats treated with the ACE inhibitor also had a tendency towards lower urinary cGMP excretion although this decrease was not significant. Thus, reduced urinary cGMP excretion is consistent with lower plasma ANF levels in treated rats. It has been demonstrated that ACE recovery in serum after ACE inhibition is faster than in the heart and kidney (9), suggesting that tissue ACE suppression could be responsible for the longterm effect of captopril.

From our results, we conclude that the beneficial effects of captopril or losartan administration are related to the inhibition of systemic and/or tissue ANG II production or to the antagonistic action on ANG II receptors. However, increased bradykinin (3), reduced sympathetic adrenergic nerve terminal catecholamine release (17) or elevated vasodilator prostaglandin levels (26) may also contribute to the action of ACE inhibition.

In summary, the present studies demonstrate that chronic treatment with ACE inhibitors or ANG II receptor antagonists has beneficial effects in rats with moderate high-output heart failure. Under inhibition of systemic and/or tissue RAS, plasma ANF might exert its actions more effectively. Thus, both treated A-C shunt groups present improved hemodynamics, diminished water retention and obvious regression of cardiac hypertrophy. Therefore, neurohormonal vasoconstrictor and ANF systems tend to reach

a new balance at more "normal" levels, reversing or slowing the development of heart failure. Our results suggest that losartan is an effective agent in the treatment of CHF.

TABLE 3.1

EFFECT OF CAPTOPRIL OR LOSARTAN TREATMENT ON MEAN ARTERIAL, RIGHT ATRIAL, AND LEFT VENTRICULAR END-DIASTOLIC PRESSURE, HEART RATE AND FIRST DERIVATIVE OF LEFT VENTRICULAR PRESSURE IN AORTOCAVAL SHUNT RATS

Group	MAP mmHg	RAP mmHg	LVEDP mmHg	dP/dt max mmHg/sec	Heart rate beats/min
Sham (n=11)	104.18 ± 2.78	1.98 ± 0.44	0.28 ± 0.87	4392.82 ± 270.99	377.82 ± 10.54
Aortocaval shunt (n=9)	84.96 ± 3.17†	5.01 ± 0.73†	5.33 ± 2.08†	3798.61 ± 306.85	352.44 ± 7.84
Aortocaval shunt losartan (n=10)	75.63 ± 2.16†Δ	4.50 ± 0.45†	2.85 ± 0.61	3689.30 ± 231.32	367.30 ± 9.55
Aortocaval shunt captopril (n=10)	74.80 ± 3.49†Δ	4.33 ± 0.59†	1.81 ± 0.75Δ	3694.20 ± 184.96	368.00 ± 5.64

Values are means ± SEM; MAP, mean arterial pressure; RAP, right atrial pressure; LVEDP, left ventricular end diastolic pressure; dP/dt max, 1st derivative of left ventricular pressure

† $p<0.01$ vs sham

Δ $p<0.05$ vs aortocaval shunt

TABLE 3.2

EFFECT OF CAPTOPRIL OR LOSARTAN TREATMENT ON TISSUE WEIGHTS AND CARDIAC HYPERTROPHY

Group	Sham (n=11)	A-C shunt (n=9)	A-C shunt losartan (n=10)	A-C shunt captopril (n=10)
BW (g)	408.64 ± 8.60	399.56 ± 5.00	385.60 ± 6.34	399.10 ± 6.37
HW (mg)	1225.73 ± 26.73	1974.11 ± 76.89†	1665.70 ± 69.24††ΔΔ	1720.30 ± 32.41††ΔΔ
HW (mg.100g^{-1} BW)	300.63 ± 6.55	493.59 ± 16.56††	432.00 ± 16.36††ΔΔ	432.47 ± 12.81††ΔΔ
RVW (mg)	192.55 ± 6.30	324.11 ± 18.42††	282.70 ± 15.60††Δ	300.56 ± 7.63††
RVW (mg.100g^{-1} BW)	47.28 ± 1.73	80.88 ± 3.83††	73.26 ± 3.69††	78.51 ± 3.49††
LVW (mg)	832.00 ± 13.32	1236.56 ± 36.49††	1088.70 ± 49.53††ΔΔ	1128.10 ± 21.28††Δ
LVW (mg.100g^{-1} BW)	204.23 ± 4.26	309.14 ± 6.35††	281.99 ± 10.64††Δ	283.77 ± 8.91††Δ
RAW (mg)	73.55 ± 2.48	167.44 ± 8.95††	139.11 ± 9.15††Δ	140.50 ± 8.68††Δ
RAW (mg.100g^{-1} BW)	18.04 ± 0.61	41.87 ± 2.07††	36.08 ± 2.17††Δ	35.33 ± 2.34††Δ
LAW (mg)	25.73 ± 0.87	56.22 ± 3.92††	47.50 ± 2.54††Δ	46.70 ± 2.23††Δ
LAW (mg.100g^{-1} BW)	6.32 ± 0.24	14.07 ± 0.96††	12.28 ± 0.53††Δ	11.73 ± 0.60††Δ
RVW/LVW ratio	0.23 ± 0.01	0.26 ± 0.01††	0.26 ± 0.01††	0.26 ± 0.01††
RAW/LAW ratio	2.88 ± 0.10	3.02 ± 0.11	2.92 ± 0.10	3.05 ± 0.20
LW (mg)	1455.82 ± 67.87	1790.33 ± 80.54††	1601.80 ± 71.25	1677.33 ± 69.63†
LW (mg.100g^{-1} BW)	355.52 ± 13.05	447.78 ± 18.52††	414.28 ± 12.96†	430.78 ± 19.57††

Values are means ± SEM

BW = body weight; HW = heart weight; LW = lung weight; RVW = right ventricular free wall weight; LVW = left ventricular free wall + septum weight; LAW = left atrial weight; RAW = right atrial weight; † $p<0.005$ vs sham; †† $p<0.01$ vs sham; Δ $p<0.05$ vs A-C shunt; ΔΔ $p<0.01$ vs A-C shunt

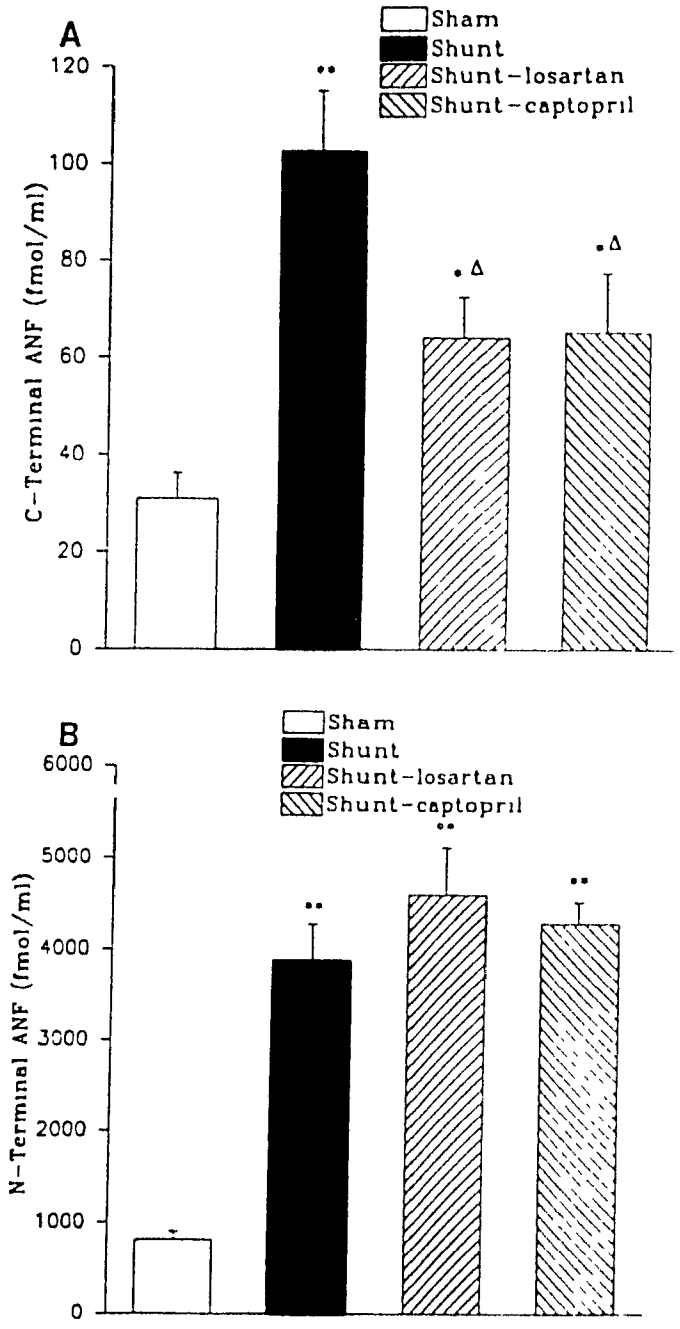

Fig. 3.1: Effect of captopril or losartan treatment on plasma (A) COOH- and (B) NH_2-terminal ANF in aortocaval shunt rats.

* $p<0.05$ vs sham; ** $p<0.01$ vs sham; Δ $p<0.05$ vs shunt

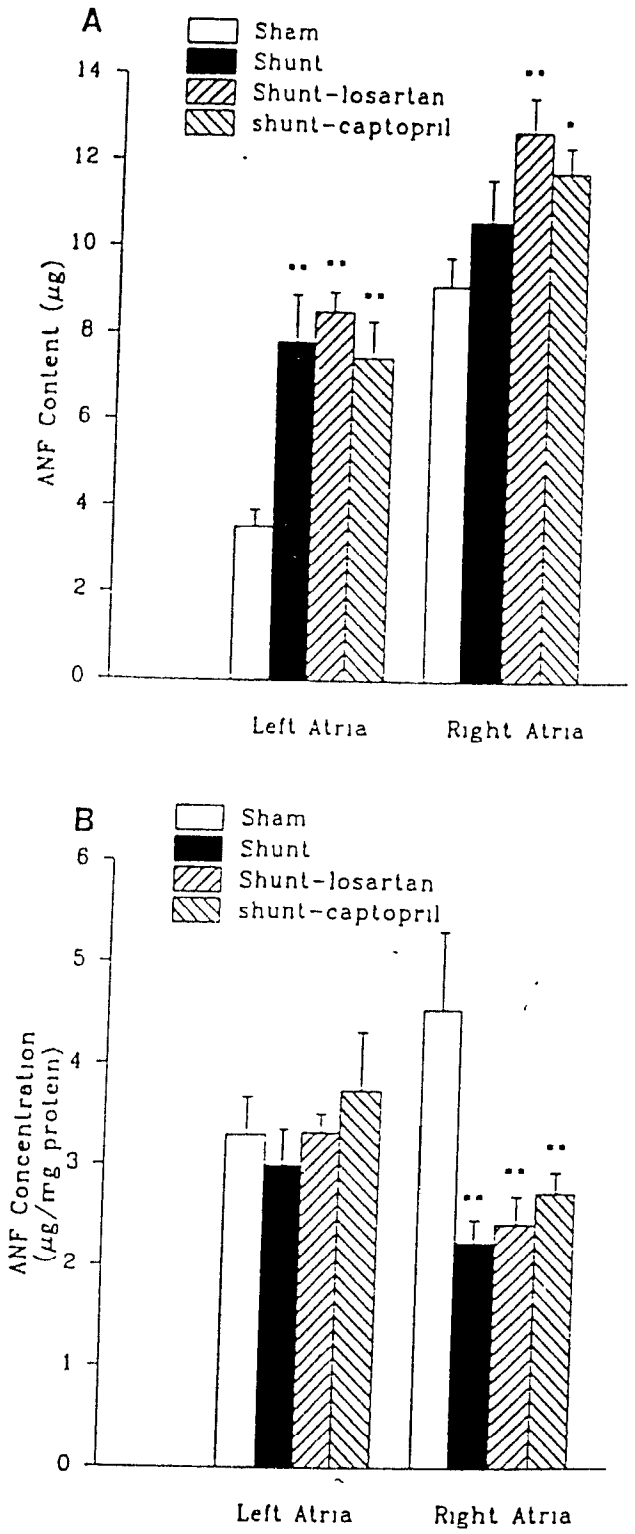

Fig. 3.2: Effect of captopril or losartan treatment on immunoreactive ANF levels in atria of aortocaval shunt rats.

* $p<0.05$ vs sham; ** $p<0.01$ vs sham

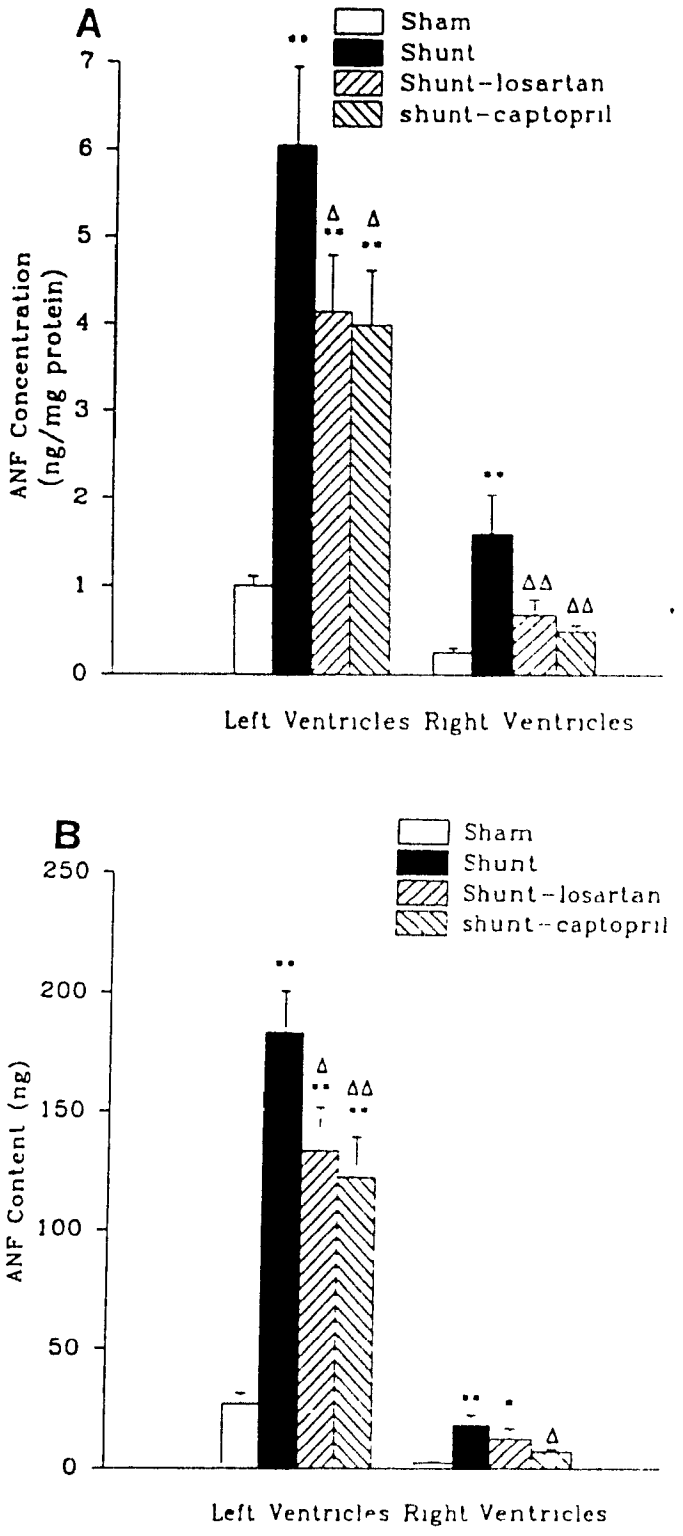

Fig. 3.3: Effect of captopril or losartan treatment on immunoreactive ANF levels in ventricles of aortocaval shunt rats.

* $p<0.05$ vs sham; ** $p<0.01$ vs sham; Δ $p<0.05$ vs shunt; ΔΔ $p<0.01$ vs shunt

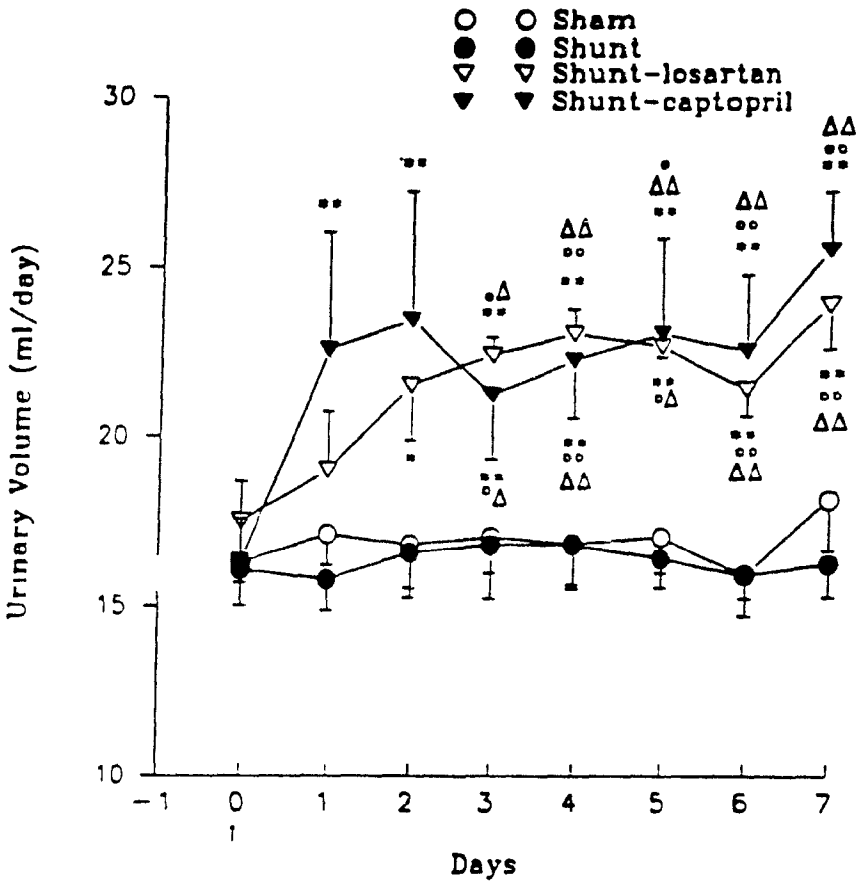

Fig. 3.4: Effect of captopril or losartan treatment on urinary volume in aortocaval shunt rats. Arrow indicates treatment initiation.

* $p<0.05$ vs day 0; ** $p<0.01$ vs day 0; Δ $p<0.05$ vs shunt; ΔΔ $p<0.01$ vs shunt; o $p<0.05$ vs sham; o o $p<0.01$ vs sham

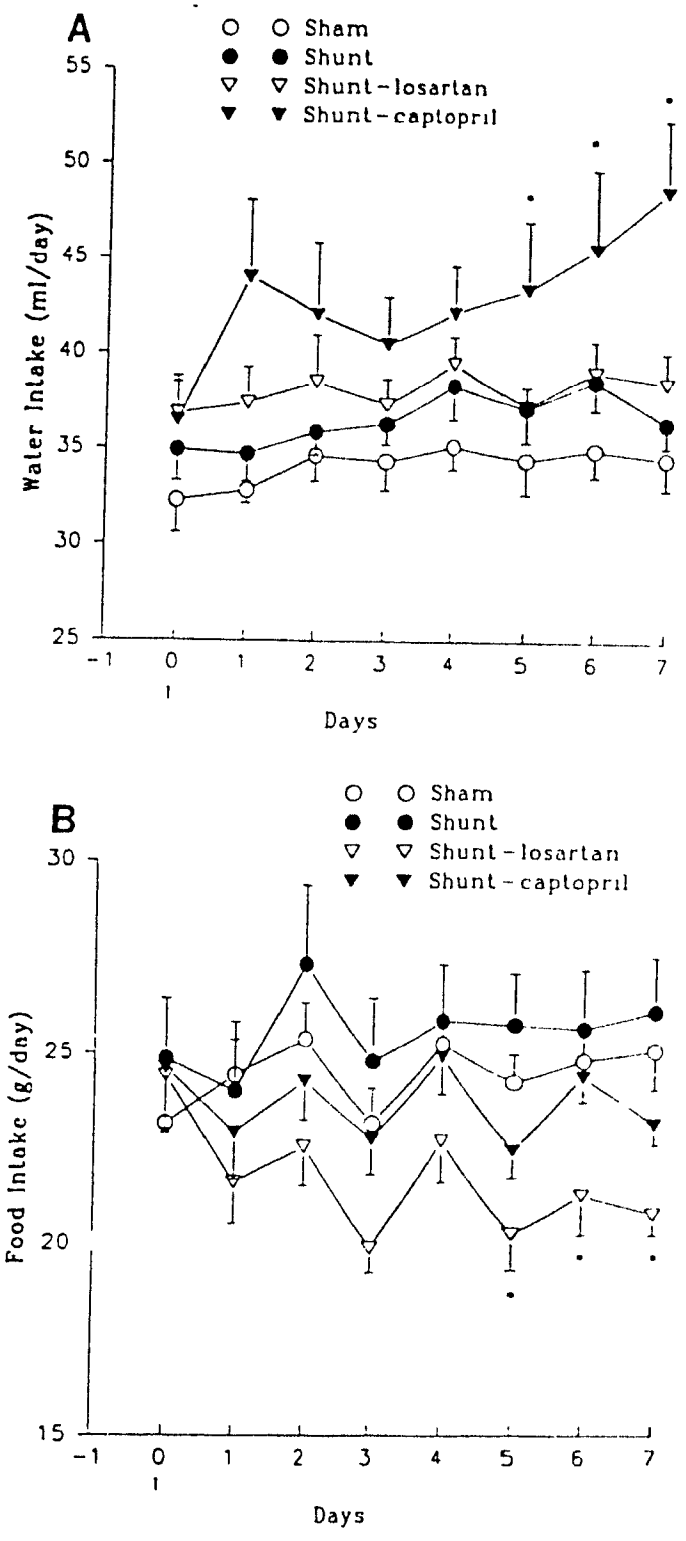

Fig. 3.5: Effect of captopril or losartan treatment on (A) water and (B) food intakes in aortocaval shunt rats. Arrow indicates treatment initiation.

* $p < 0.05$ vs any other groups

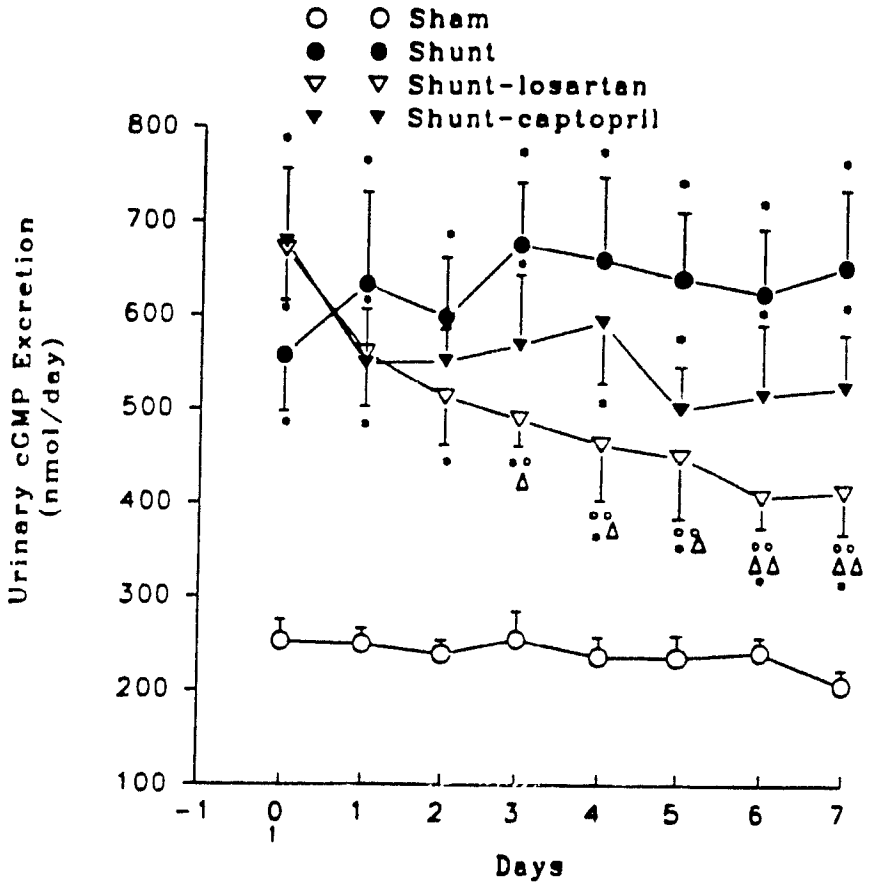

Fig. 3.6: Effect of captopril or losartan treatment on urinary cGMP excretion in aortocaval shunt rats. Arrow indicates treatment initiation.

o $p<0.05$ vs day 0; o o $p<0.01$ vs day 0; Δ $p<0.05$ vs shunt; ΔΔ $p<0.01$ vs shunt; * $p<0.01$ vs sham

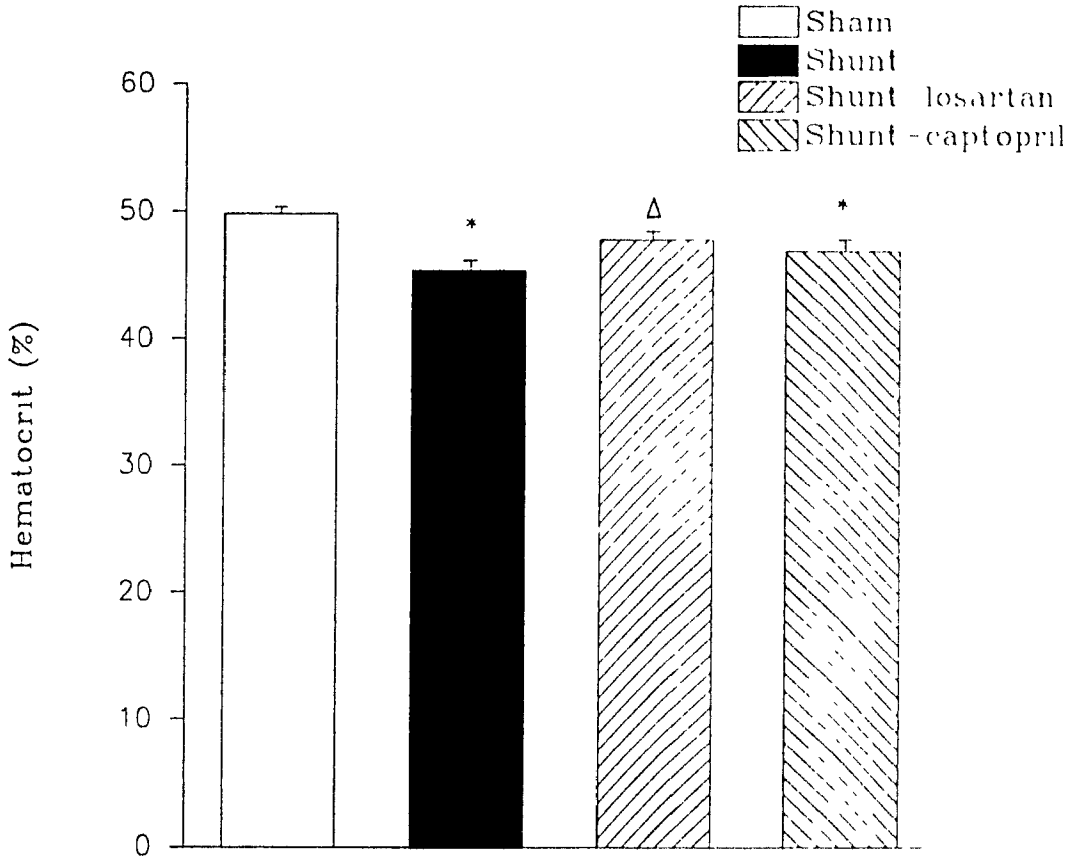

Fig. 3.7: Effect of captopril or losartan treatment on hematocrit in aortocaval shunt rats.

* $p < 0.01$ vs sham; Δ $p < 0.05$ vs shun

3.6 ACKNOWLEDGMENTS

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3.7 REFERENCES

1. Bloch, K.D., J.G. Seidman, J.D. Naftilan, J.T. Fallon, and C.E. Seidman. Neonatal atria and ventricles secrete atrial natriuretic factor via tissue-specific secretory pathways. *Cell* 47: 695-702, 1986.
2. Brogden, R.N., P.A. Todd, and E.M. Sorkin. Captopril: an update of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. *Drug* 36: 540-600, 1988
3. Carretero, O.A., S. Miyazaki, and A.G. Scicli. Role of kinins in the acute antihypertensive effect of the converting enzyme inhibitor, captopril. *Hypertension* 3: 18-22, 1981.
4. Chiu, A.T., D.E. McCall, W.A. Price, P.C. Wong, D.J. Carini, J.V. Duncia, R.R. Wexler, S.E. Yoo, A.L. Johnson, and P.B M.W M Timmermans Nonpeptide angiotensin II receptor antagonist. VII Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.* 252: 711-718, 1990.
5. Consensus Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure. *New Engl. J. Med.* 316: 1429-1435, 1987.
6. Drexler, H., K. Lindpaintner, W. Lu, B Schaeffer, and D Ganten. Transient increase in the expression of cardiac angiotensinogen in rat model of myocardial infarction and failure (abstract). *Circulation* 80(Suppl II) II-459, 1989
7. Dzau V.J, and J.R Ingelfinger. Molecular biology and pathophysiology of the intrarenal renin-angiotensin system *J Hypertension* 7(Suppl 7) S3-S8, 1989
8. Dzau, V.J. Significance of the vascular renin-angiotensin pathway. *Hypertension* 8: 553-559, 1986.
9. Fabris, B., B. Jackson, R. Cubela, F.A.O Mendelsohn, and C I. Johnston: Angiotensin converting enzyme in the rat heart: studies of its inhibition in vitro and ex vivo. *Clin. Exp. Pharmacol. Physiol.* 16. 309-313, 1989

10. Francis, G.S. Neurohormonal mechanisms involved in congestive heart failure. *Am. J Cardiol.* 55: 15A-21A, 1985.

11. Garcia R., D. Lachance, and G. Thibault. Atrial natriuretic factor release and natriuresis in rats with high-output heart failure. *Am. J. Physiol.* 259· H1374-H1379, 1990.

12. Garcia, R. and S. Diebold. A simple, rapid and effective method of producing aortocaval shunts in the rat. *Cardiovasc. Res.* 24: 430-432, 1990.

13. Gutkowska, J., J. Genest, G. Thibault, R. Garcia, P. Larochelle, J.R. Cusson, O. Kuchel, P. Hamet, A. De Léan, and M. Cantin. Circulating forms and radioimmunoassay of atrial natriuretic factor. In: Rosenblatt, M., Jacobs, J. W. (eds): Endocrinology and Metabolism Clinics of North America, Atrial Natriuretic Factor, Philadelphia, W.B. Saunders Co., 1987, p. 183.

14. Hirsch, A T., C.E. Talsness, H. Schunkert, P. Martin, and V.J. Dzau. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. Circ. Res. 69: 475-482, 1991.

15. Ichikawa, I., J.M. Pfeffer, M.A. Pfeffer, T.H. Hostetter, and B.M. Brenner. Role of angiotensin II in the altered renal function of congestive heart failure. *Circ. Res.* 55: 669-675, 1984.

16. Katoh, Y., I. Komuro, Y. Shibasaki, H. Yamaguchi, and Y. Yazaki. Angiotensin II induces hypertrophy and oncogene expression in cultured rat heart myocytes (abstract). *Circulation* 80(suppl II): II-450, 1989.

17. Khairallah, P.A. Action of angiotensin on adrenergic nerve ending: inhibition of norepinephrine uptake. *Fed. Proc.* 31: 1351-1357, 1972.

18. Kon, V., A. Yared, and I. Ichikawa. Role of the renal sympathetic nerves in mediating hypoperfusion of renal cortical microcirculation in experimental congestive heart failure and acute extracellular fluid volume depletion. *J. Clin. Invest.* 76: 1913-1920, 1985.

19. Lindpaintner, K., M.J. Wilhelm, M. Jin, T. Unger, R.E. Lang, B.A. Schoelkens, and D. Ganten. Tissue renin-angiotensin systems: focus on the heart. *J. Hypertension* 5 (Suppl): 2: 33-38, 1987.

20. Massie, B.M., J.F. Tubau, J. Szlachcic, and B.F. O'Kelly. Hypertensive heart disease: The critical role of left ventricular hypertrophy. J. Cardiovasc. Pharmacol. 13(Suppl. 1): S18-S24, 1989.

21. Michel, J.P., A.L. Lattion, J.I. Salzmann, M.L. Cerol, M. Philippe, J.P. Camilleri, and P. Corvol. Hormonal and cardiac effects of converting enzyme inhibition in rat myocardial infarction. *Circ. Res* 62: 641-650, 1988.

22. Pfeffer, J., M. Pfeffer, and E. Braunwald. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. *Circ Res*. 57: 84-95, 1985

23. Riegger, A.J.G. The role of the atrial natriuretic factor in congestive heart failure. Progress in Pharmacology and Clinical Pharmacolog 713: 101-115, 1990.

24. Rogers, T.B., S.T. Gaa, and I.S. Allen. Identification and characterization of functional angiotensin II receptor on cultured heart myocytes *J. Pharmacol. Exp. Ther.* 236: 438-442, 1986.

25. Spector, T. Refinement of a Coomassie blue method of protein quantification. *Anal. Biochem.* 86: 142-146, 1978.

26. Swartz, S.L., G.H. Williams, N.K. Hollenberg, L. Levine, R.G Dluhy, and T.J. Moore. Captopril-induced changes in prostaglandin production. Relationship to vascular responses in normal man. J. Clin. Invest 65:1257-1264, 1980.

27. Thibault, G., K.K. Murthy, J. Gutkowska, N.G. Seidah, C Lazure, M Chrétien, and M. Cantin. NH_2-terminal fragment of rat pro-atrial natriuretic factor in the circulation: Identification, radioimmunoassay and half-life *Peptides* 9: 47-53, 1988.

28. Thibault, G., M. Nemer, J. Drouin, J.P. Lavigne, J. Ding, C. Charbonneau, R. Garcia, J. Genest, G. Jasmin, M. Sole, and M. Cantin. Ventricles are a major site of atrial natriuretic factor synthesis and release in cardiomyopathic hamster with heart failure. *Circ. Res.* 65: 71-82, 1989.

29. Tsunoda, K., F.A.O. Mendelsohn, P.M. Sexton, S.Y. Chai, G.P. Hodsman, and C.I. Johnston. Decreased atrial natriuretic peptide binding in renal medulla in rats with chronic heart failure. Circ. Res. 62: 155-161, 1988.

30. Tsutamoto, T., K. Bito, and M. Kinoshita. Plasma atrial natriuretic polypeptide as an index of left ventricular end-diastolic pressure in patients with chronic left-side heart failure. *Am. Heart J.* 117: 599-606, 1989.

31. Wong, P.C., W.A. Price, A.T. Chiu, J.V. Duncia, D.J. Carini, R.R. Wexler, A.L. Johnson, and P.B.M.W.M. Timmermans. Nonpeptide angiotensin II receptor antagonist. IX. Antihypertensive activity in rats of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.* 252. 726 732, 1990.

32. Xie M.-H., F.-Y. Liu, P C. Wong, P.B M.W.M. Timmermans, and M.G. Cogan. Proximal nephron and renal effects of DuP 753, a nonpeptide angiotensin II receptor antagonist. *Kidney Int.* 38: 473-479, 1990.

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSION

The present work describes the role of ANF in rats with chronic high-output heart failure by (a) characterizing plasma and tissue ANF levels, hemodynamics and renal function at different stages of moderate chronic high-output heart failure in rats; (b) assessing the contribution of the atria and ventricles to plasma ANF levels; and (c) investigating the role of ANF and RAS as well as the effects of losartan in rats with A-C shunts. The results obtained in this study provide deeper insight into the role of ANF in rats with high-output heart failure.

4.1 ANF synthesis and secretion in A-C shunt rats

In order to study the contribution of the atria and ventricles to plasma ANF levels in rats with high-output heart failure, this study has characterized changes in plasma and cardiac tissue ANF levels during the development of heart failure and after the treatment of ACE inhibitor or ANG II receptor antagonist.

Atrial stretch, induced by an increase in preload or afterload, is known to be a potent stimulus of ANF release (60, 69) As reported previously in both human and experimental CHF (15, 37), plasma COOH- and NH_2-termianal ANF levels were greatly elevated at different stages of heart failure in the A-C shunt groups (Chapter II and III). A positive correlation between plasma ANF and RAP or LVEDP was observed in A-C shunt rats but not in sham-operated animals (Chapter II). Treatment with captopril or losartan normalized LVEDP and tended to decrease RAP, consequently reducing plasma COOH-terminal ANF levels (Chapter III). These results provide further evidence that chronically increased cardiac filling pressure, induced by augmented cardiac preload, is a sustained stimulus for ANF release. Moreover, decreased atrial ANF concentrations but not content (which was even increased), and increased ANF concentrations and content in either ventricles of A-C shunt rats indicate that elevated cardiac filling pressure stimulates not only ANF release but also ANF

synthesis in each cardiac chamber. This in turn contributes to elevated plasma ANF levels in rats with moderate high-output heart failure (Chapter II and III).

Despite greatly elevated plasma ANF levels in A-C shunt rats (Chapter II and III), it may be possible that the degree of increased plasma ANF is not appropriate for the magnitude of the stimulus, which consequently results in decreased $U_{Na}V$ and UV, and increased blood volume (Chapter II and III) in A-C shunt rats. The results of this study have shown that plasma ANF levels tend to decrease during the development of heart failure (Chapter II). However, we have not observed a deficiency of ANF in either atria or ventricles. The results of this study also indicate that LVEDP is significantly higher in A-C shunt rats at early stages but not at later stages of the development of CHF than in their respective sham-operated controls, suggesting that a decrease in cardiac filling pressure may have occurred during later stages. Furthermore, reduced atrial receptor responses may occur after long-term stretch (40, 134). Thus, the present study indicates that an inappropriate secretion of ANF or the gradual decrease of plasma ANF levels during the development of CHF may be due to either reduced sensitivity to atrial stretch or to declining cardiac filling pressure but not exhausted ANF storage.

4.2 The role of elevated plasma ANF levels in rats with high-output heart failure

Present study indicates that plasma ANF levels and urinary cGMP are increased in A-C shunt rats at all time periods. However, A-C shunt animals presented lower hematocrit, and lower or tendency to lower $U_{Na}V$ and UV at all observed times (Chapter II and III). Increased plasma ANF levels and urinary cGMP excretion is inconsistent with attenuated renal function, suggesting that factors other than circulating ANF cause a marked reduction in ANF's biological action in CHF. Although many factors may involve attenuated ANF effects in CHF, RAS appears to play a key role in this regard because of its multiple biological activities and its presence in tissue.

Therefore, this study comparatively investigated the chronic hemodynamic, hormonal, and renal effects of ACE inhibition with captopril, ANG II receptor antagonism with losartan, and interactions of RAS and ANF in rats with a moderate degree of high output heart failure. This is the first study where the chronic effects of losartan have been investigated in rats with moderate high-output heart failure. The treatment with captopril or losartan decreased MAP and returned LVEDP to the sham-operated control values. Both treatments also presented a tendency to be decreased in RAP. These results suggest a fall in both pre- and afterload in treated A-C shunt rats (Chapter III). A-C shunt rats treated with losartan or captopril had an increased urinary volume when compared with untreated A-C shunt or sham-operated controls. This enhanced renal response may have resulted from the upregulation of functional ANF receptors in the kidneys, which may be secondary to decreased circulating ANF levels, or to systemic and/or local renal RAS inhibition (Chapter III). Both treatments increased hematocrit in A-C shunt rats (although the hematocrit was not significantly changed in A-C shunt rats treated with captopril), suggesting a decrease in blood volume. A clear regression of cardiac hypertrophy, mediated by inhibiting local cardiac ANG II production or antagonizing the action of ANG II at its receptors, or by decreasing cardiac pre- and afterload, was evident in both treated A-C shunt rats (Chapter III). Diminished ANF release by the atria and synthesis by the ventricles may have contributed to lower plasma ANF levels. Thus, both treated A-C shunt rats present improved hemodynamics, diminished water retention, and an obvious regression of cardiac hypertrophy. Decreased plasma ANF and urinary cGMP after being treated with losartan or captopril suggest that ANF reaches a new and more "normal" steady state after the RAS block.

Taken together, the results of this study indicate that elevated plasma ANF levels may play an important role in maintaining relative "normal" hemodynamics and body fluid homeostasis by opposing the RAS in rats with moderate high output heart failure. However, a relative deficiency of plasma ANF and/or an attenuated response to

endogenous ANF, mediated by activated the neurohormonal vasoconstrictor systems, may contribute to the pathophysiology and development of CHF at different stages.

4.3 Conclusions

The following can concluded from the present work:

A. Chronic increased cardiac filling pressure stimulated not only ANF release but also ANF synthesis in each cardiac chamber, which in turn contributed to elevate plasma ANF in A-C shunt rats.

B. Increased plasma ANF levels may play an important role in the maintenance of sodium and body fluid homeostasis by opposing the neurohormonal vasoconstrictor systems in A-C shunt rats.

C. An attenuated renal response to ANF and sodium and water retention is present in A-C shunt rats.

D. The activation of neurohormonal vasoconstrictor systems and the gradual decrease of plasma ANF levels may contribute to sodium and water retention at different stages of this experimental model of heart failure.

E. Under inhibition of systemic and/or tissue RAS, plasma ANF may exert its actions more effectively.

F. Chronic treatment with ACE inhibitors or ANG II receptor antagonists has beneficial effects in rats with moderate high-output heart failure

G. Both treated A-C shunt groups present improved hemodynamics, diminished water retention, and obvious regression of cardiac hypertrophy.

H. Neurohormonal vasoconstrictor and ANF systems in both treated A-C shunt animals tend to reach a new balance at more "normal" levels, reversing or slowing the development of heart failure.

The mechanisms of attenuated ANF's actions in CHF is an area that deserves further investigation. Further research in this area may provide some insight into the pathophysiological role of ANF and the therapy of CHF.

4.4 Claims to originality

The present work describes for the first time:

A) Hemodynamic parameters, and tissue and plasma ANF during the development of CHF induced by A-C shunt.

B) Chronic treatment with losartan, a nonpeptide ANG II antagonist, present improved hemodynamics, diminished water retention and an obvious regression of cardiac hypertrophy in rats with moderate high-output heart failure.

C) Losartan, like ACE inhibition, is an effective agent in the treatment of CHF.

REFERENCES

1. Aalkjaer, C., Mulvany, M.J., and Nyborg, N.C.B. Atrial natriuretic factor causes specific relaxation of rat renal arcuate arteries. Br. J. Pharmacol. 86:447-453, 1985.
2. Abassi, Z., Burnett, JC Jr., Grushka, Hoffman, A., Haramati A. Winaver, J. Atrial natriuretic peptide and renal cGMP in rats with experimental heart failure. Am. J. Physiol. 261: R858-R864, 1991.
3. Anand-Srivastava, M.B., A.K. Srivastava, and M. Cantin. Pertussi toxin attenuates atrial natriuretic factor-mediated inhibition of adenylate cyclase. J. Bio. Chem. 262:4931-4934, 1987.
4. Arai H, Nakao K, Saito H, Morii N, Sugawara S, Yamada T Itoh H, Shiono S, Mukoyama M, Ohkubo H, Nakanishi S, Imura H. Augmented expression of atrial natriuretic polypeptide (ANP) gene in ventricles of spontaneously hpertensive rats (SHR) and SHR-stroke prone. Circ. Res. 62:926-930, 1988.
5. Atarashii, K., Mulrow, P J., Franco-Saenz, R., Snajdar, R., amd Rapp, J. Inhibition of aldosterone production by an atrial extract. Science (Washington, DC), 224: 992-994, 1984.
6. Awazu, M., T. Imada, V. Kon, T. Inagami, and I. Ichikawa. Role of endogenous atrial natriuretic peptide in congestive heart failure. Am. J. Physiol. 257: R641-R646, 1989.
7. Ballermann, B.J., Hoover, R.L., Karnovsky, M.J. and Brenner, B.M. Physiological regulation of atrial peptide receptors in rat renal glomeruli. J. Clin. Invest., 76:2049-2056, 1985.
8. Bie, P., Wang, B.C., Leadley, R.J., Jr., and Goetz, K.L. Hemodynamic and renal effects of low-dose infusions of atrial peptide in awake dogs. Am. J. Physiol. 254:R161-R169, 1988.

9. Bloch, K.D., J.A. Scott, J.B. Zisfein, J.T. Fallon, M.N. Margolies, C.E. Seidman, G.R. Matsveda, C.J. Homcy, R.M. Graham, and J.G. Seidman. Biosynthesis and secretion of proatrial natriuretic factor by cultured rat cardiocytes. Science Wash. DC 230: 1168-1171, 1985.

10. Bodak, A., Cluzeaud, F., Gastineau, P. and Hatt, P.Y. Degree of granulation of atrial cardiocytes: its decrease after aorto-caval fistula in the rats. Basic Res. Cardiol. 74:509-517, 1979.

11. Burnett, J.C., Jr., Granger, J.P., and Openorth, T.J. Effects of synthetic atrial natriuretic factor on renal function and renin release. 247: F863-F866, 1984

12. Chang, M.S., Lowe, D.G., Lewis, M., Hellmiss, R , Chen, E., and Goeddel, D.V. Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclase. Nature 341.68-72, 1989.

13. Chartier, L, Schiffrin, E.L., Thibault, G, and Garcia, R. Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH, and potassium *in-vitro* and angiotensin II-induced steroidogenesis *in-vivo*. Endrocrinology 115:2026-2028, 1984.

14. Chinkers, M., Garbers, D.L., Chang, M.S., Lowe, D.G., Chin, H.M., Goeddel, D.V., and Schulz, S. A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. Nature (London) 338·78-83, 1989.

15. Cody, R.J., Atlas, S.A., Laragh, J.H., Kubo, S.H. Covit, A.B., Ryman, K.S., Shaknovich, A., Pondolfino, K., Clark, M., Camargo, M.J.F , Scarborough, R.M., and Lewicki, J.A. Atrial natriuretic factor in normal subjects and heart failure patients. Plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. J. Clin. Invest. 78·1362-1374, 1986

16. Cogan, M.G. Atrial natriuretic factor can increase renal solute excretion primarily by raising glomerular filtration. Am. J. Physiol. 250: F710-F714, 1986.

17. Crozier, I.G., Ikram, H., Gomez, H.J., Nicholls, M.G., Espiner, E. A., and Warner, N.J. Hemodynamic effects of atrial peptide infusion in heart failure. Lancet 2: 1242-1245, 1986.

18. Davis, J.O. and Freeman, R.H. Mechanisms regulating renin release. Physiol. Rev. 56:1-56, 1976.

19. Davis, C.I., and J.P. Briggs. Effect of atrial natriuretic peptides on renal medullary solute gradients. Am. J. Physiol. 253: F679-F684, 1987.

20. de Bold, A.J. Heart atria granularity effects changes in water-electrolyte balance. Proc. Soc. Exp. Bio. Med. 161:508-511, 1979.

21. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci. 28:89-94, 1981.

22. de Wardener H.E., Mills I.H., Clapham W.F., and Hayter C.J. Studies on the efferent mechanism of sodium diuresis which follows the administration of intravenous saline in the dog. Clin. Sci. 21:249-258, 1961.

23. Dietz, J.R. Release of natriuretic factor from rat heart-lung preparation by atrial distension. Am. J. Physiol. 247: R1093-R1096, 1984.

24. Dillingham MA, Anderson RJ. Inhibition of vasopressin action by atrial natriuretic factor. Science 231:1572-1573, 1986.

25. Drexler, H., Hirth, C., Stasch, H.-P., Lu, W., Neuser, D., and Just, H. Vasodilatory action of endogenous atrial natriuretic factor in a rat model of chronic h art failure as determined by monoclonal ANF antibody. Circ. Res. 66: 1371-1380, 1990.

26. Dunn, B.R., I. Ichikawa, J.M. Pfeffer, J.L. Trop, and B.M. Brenner. Renal and systemic hemodynamic effects of synthetic atrial natriuretic peptide in the anesthetized rat. Circ. Res. 59: 237-246, 1986.

27. Elliot, M.E. and Goodfriend, T.L. Inhibition of aldosterone synthesis by atrial natriuretic factor. Fed. Proc. Fed. Am. Soc. Exp. Biol. 45: 2376-2381, 1986.

28. Epstein, M., R.D. Loutzenhiser, E. Friedland, R.M. Aceto, M.J.F. Camargo, and S.A. Atlas. Relationship of increased plasma atrial natriuretic factor and renal sodium handling during immersion-induced central hypervolemia in normal humans. J. Clin. Invest. 79:738-745, 1987.

29. Feller, S.M., M. Gagelmann and W. G. Forssmann. Urodilatin: a newly described member of the ANP family. Trends Pharmacol. Sci. 10:93-94, 1989.

30. Flückiger, J.R., B. Waeber, G. Matsueda, B. Delaloye, J. Nussberger and H.R. Brunner. Effect of atriopeptin III on hematocrit and volemia of nephrectomized rats. Am. J. Physiol 251: H880-H883, 1986.

31. France HA, Dixon RAF, Blaine EH, Siegel PKS. Ventricular atrial natriuretic factor in the cardiomyopathic hamster model of congestive heart failure. Circ. Res. 62:31-36, 1988.

32. Fuller, F., Porter, J.G., Arfsten, A.E., Miller, J., Schilling, J W., Scarborough. R.M., Lewicki, J.A., and Schenk, D.B Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clone. J. Biol. Chem. 263: 9395-9401, 1988.

33. Gagelmann, M., D. Hock, and W. G. Forssmann. Urodilatin (CDD-ANP-95-126) is not biologically inactivated by a peptidase from dog kidney cortex membranes in contrast to atrial natriuretic peptide/cardiodilatin (α-hANP/CDD-99-126). FEBS Lett. 233:249-254, 1988.

34. Garcia R, Thibault G, Cantin M, and Genest J. Effect of a purified atrial natriuretic factor on rat and rabbit vascular strips and vascular bed. Am. J. Physiol. 247:R34-R39, 1984.

35. Garcia, R. G. Thibault, J. Gutkowska, P. Hamet, M. Cantin, and J. Genest. Effect of chronic infusion of synthetic atrial natriuretic factor (ANF 8-33) in conscious two-kidney, one-clip hypertensive rats. Proc. Soc. Exp. Biol. Med. 178: 155-159, 1985.

36. Garcia R and S. Diebold. Simple, rapid, and effective method of producing aortocaval shunts in the rat. Cardiovascular Research 24:430-432, 1990.

37. Garcia, R., D. Lachance, and G. Thibault. Atrial natriuretic factor release and natriuresis in rats with high-output heart failure. Am. J. Physiol. 259:H1374-H1379, 1990.

38. Garvin, J.L. Inhibition of JV by ANF in rat proximal straight tubules requires angiotensin. Am. J. Physiol. 257:F907-F911, 1989.

39. Glembotski, C.C., and T.R. Gibson. Molecular forms of immunoreactive atrial natriuretic peptide released from cultured rat atrial myocytes. Biochem. Biophys. Res. Commun. 132:1008-1017, 1985.

40. Greenberg TT, Richmond WH, Stocking RA, Gupta PD, Meehan JP, Henry JP. Impaired atrial receptor responses in dogs with heart failure due to tricuspid insufficiency and pulmonary artery stenosis. Circ Res 32:424-433, 1973.

41. Gunning, M., Ballermann, B.J., Silva, P., Brenner, B.M. and Zeidel, M.L. Brain natriuretic peptide: interaction with renal ANP system. Am. J. Physiol. 258:F467-F472, 1990.

42. Hamet, P., J. Tremblay, S.C. Pang, R. Garcia, G. Thibault, J. Gutkowska, M. Cantin, and J. Genest. Effect of native and synthetic atrial natriuretic factor on cyclic GMP. Biochem. Biophys. Res. Commun. 123:515-527, 1984.

43. Hamet P, Tremblay J, Pang SC, Skuherska R, Schiffrin EL, Garcia R, Cantin M, Genest J, Palmour R, Ervin FR, Martin S, and Goldwater R. Cyclic GMP as mediator and biological marker of atrial natriuretic factor. J. Hypertens. 4 (suppl 2):S49-S56; 1986.

44. Harris, P.J., Thomas, D., and Morgan, T. O. Atrial natriuretic peptide inhibits angiotensin-stimulated proximal tubular sodium and water reabsorption. Nature (London), 326: 697-698, 1987.

45. Hatt, P.Y. The juxtaglomerular apparatus. In: Ultrastructure of the kidney. pp101-141, Eds: A.J. Dalton, F. Haguenau (New York 1967).

46. Heim, J.M., S. Kiefersauer, H.J. Fulle, and R. Gerzer. Urodilatin and B-ANF: binding properties and activation of particulate guanylate cyclase. Biochem. Biophys. Res. Commun. 163:37-41, 1989.

47. Henrich, W.L., Needleman, P., and Campbell, W.B. Effect of atriopeptin III on renin release *in vitro*. Life Sci. 39:993-1001, 1986

48. Henry, J.P., Gauer, O.H., and Reeves, J.L. Evidence of the atrial location of receptors influencing urine flow. Circ. Res. 4:85-90, 1956.

49. Hirata, Y., Shichiri, M., Emori, T., Marumo, F. Kangawa, K., and Matsuo, H. Brain natriuretic peptide interacts with atrial natriuretic peptide receptor in cultured rat vascular smooth muscle cells FEBS Lett 238 425-418, 1988.

50. Hodsman, G.P., K. Tsunoda, K. Ogawa, and C.I. Johnston. Effects of posture on circulating atrial natriuretic peptide. Lancet 2. 1427-1429, 1985

51. Huang, C.K., J. Lewicki, L.K. Johnson, and M G. Cogan Renal mechanism of action of rat atrial natriuretic factor. J. Clin Invest. 75: 769-773, 1985.

52. Imada, T., R. Takayanagi, and T Inagami. Identification of a peptidase which processes atrial natriuretic factor precursor to its active form with 28 amino acid residues in particulate fraction of rat atrial homogenate. Biochem. Biophys. Res. Commun. 143: 587-592, 1987.

53. Jamieson J.D. and Palade G.E. Specific granules in atrial muscle cells. J. Cell Biol 23:151-172, 1964.

54. Keeton, T.K. and Campbell, W.B. The pharmacologic alteration of renin release. Pharmacol. Rev. 31:81-227, 1981.

55. Kisch B. Electron microscopy of the atrium of the heart. I. Guinea pig. Exp Med Surg 14:99-112, 1956.

56. Kojima M, Minamino N, Kangawa K, Matsuo H. Cloning and sequence analysis of a cDNA encoding a precursor for rat C-type natriuretic peptide (CNP). FEBS Lett 276:209-213, 1990.

57. Koller KJ, Lowe DG Bennett GL, Minamino N, Kangawa K, Matsuo H. Goeddei DV. Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide. Science 252:120-123, 1991.

58. Kudo T, Baird A. Inhibition of aldosterone production in the adrenal glomerulosa by atrial natriuretic factor. Nature 312:756-757, 1985.

59. Kurtz, A., Bruna, R.D., Pfeilschifter, J., Taugner, R., and Bauer, C. Atrial natriuretic peptide inhibits renin release from juxtaglomerular cells by a cGMP-mediated process. Proc. Natl. Acad. Sci. U.S.A. 83:4769-4774, 1986.

60. Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger TH. Atrial natriuretic factor - a circulating hormone stimulated by volume loading. Nature 314:264-266, 1985.

61. Lee RT, Bloch KD, Pfeffer JM, Pfeffer MA, Neer EJ, Seidman CE. Atrial natriuretic factor gene expression in ventricles rats with spontaneous biventricular hypertrophy. J. Clin. Invest 81: 431-434, 1988.

62. Lee, M.E., Miller, W.L., Edwards, B.S., and Burnett, J.C. Jr. Role of endogenous atrial natriuretic factor in acute congestive Heart Failure. J. Clin. Invest. 84: 1962-1966, 1989.

63. Leitman, D.C., J.W. Andresen, T. Kuno, Y. Kamisaki, J.K. Chang, and F. Murad. Identification of multiple binding sites for atrial natriuretic factor by affinity cross-linking in cultured endothelial cells. J. Biol. Chem. 261: 11650-11655, 1986.

64. Leitman, D.C., Andresen, J.W., Catalana, R.M., Walaman, S.A., Tuan, J.J., and Murad, F. Atrial natriuretic peptide binding, cross-linking, and stimulation of cyclic GMP accumulation and particulate cyclase activity in cultured cells. J. Biol. Chem. 263:3720-3728, 1988.

65. Light, D.B., E.M. Schwiebert, K.H. Karlson, and B.A. Stanton. Atrial natriuretic peptide inhibits a cation channel in renal innermedullary collecting duct cells. Sciences 243: 383-385, 1989.

66. Linden, R.J., and N. Sreeharan. Humoral nature of the urine response to stimulation of atrial receptors. Quart. J. Exp. Physiol. 66:431-438, 1981.

67. Maack T., Suzuki, M., Almeida, F.A., Nussenzveig, D., Scarborough, R.M., McEnroe, G.A., and Lewicki, J.A. Physiological role of silent receptors of atrial natriuretic factor. Science (Washington, DC), 238: 675-678, 1987.

68. Maki, M., R. Takayanagi, K.S. Misono, K.N. Pandey, C. Tibbetts and T. Inagami. Structure of atrial natriuretic factor precursor deduced from cDNA sequence. Nature Lond. 309:722-724, 1984.

69. Manning, P.T., D. Schwartz, N.C. Katsube, S.W. Holmberg, and P. Needleman. Vasopression-stimulated release of atriopeptide: endocrine antagonists in fluid homeostasis. Science 229:395-397, 1985.

70. Margulies KB, Heublein DM, Perrlla MA, Burnett JC Jr. ANF-mediated renal cGMP generation in congestive heart failure. Am. J. Physiol. 260:F562-F568, 1991

71. Martinez-Palomo, A., S.A. Bencosme. Electron microscopic observations on myocardial specific granules and residual bodies in vertebrates. Anat. Res. 154:473, 1966.

72. Martinez-Palomo A, Bencosme SE. Changes in the content of myocardial specific granules in adrenal regeneration hypertension. An electron microscopic study. Fed. Am. Soc. Exp. 25:476, 1966.

73. Mercadier, J.-J., J.-L Samuel, J.-B. Michel, M.-A. Zongazo, D. Delabastie, A.-M. Lompre, C. Wisnewsky, L. Rappaport, B. Levy, and K. Schwartz. Atrial natriuretic factor gene expression in rat ventricle during experimental hypertension. Am. J. Physiol. 257: H979-H987, 1989.

74. Mercadier JJ, Zongaro MA, Urbain R, Allen PD, Komajda M, Carayon A. Schwartz K: Myocardial levels of atrial natriuretic factor and of its mRNA are highly variable in end-stage heart failure. (abstract) J. Mol. Cell Cardiol 22(suppl IV):S40, 1990.

75. Minamino N, Aburaya M, Ueda S, Kangawa K, Matsuo H. The presence of brain natriuretic peptide of 12,000 daltons in porcine heart. Biochem. Biophys. Res. Commun. 155:740-746, 1988.

76. Minamino, N., Kangawa, K., and Matsuo, H. N-terminally extended form of C-type natriuretic peptide (CNP-53) identified in porcine brain. Biochem. Biophys. Res. Commun., 170: 973-979, 1990.

77. Minamino N, Makino Y, Tateyama H, Kangawa K, Matsuo H. Characterization of immunoreactive human C-type natriuretic peptide in brain and heart. Biochem. Biophys. Res. Commun. 179:535-542, 1991.

78. Misono, K., H. Fukumi, R.T. Grammer, and T. Inagami. Rat atrial natriuretic factor: complete amino acid sequence and disulfide linkage essential for biological activity. Biochem. Biophys. Res. Commun. 119:524-529, 1984.

79. Misono, K.S., R.T. Grammer, J.W. Rigby, and T. Inagami. Photoaffinity labeling of atrial natriuretic factor receptor in bovine and rat adrenocortical membranes. Biochem. Biophys. Res. Commun. 130:994-999, 1985.

80. Moe GW, Canepa-Anson R and Armstrong. Atrial natriuretic factor: Pharmacokinetics and cyclic GMP responses in relation to biologic effects in severe heart failure. J. Cardiovasc Pharmacol. 19:691-700, 1992.

81. Mukoyama, M., Nakao, K., Saito, Y., Ogawa, Y., Hosoda, K., Suga, S.I., Shirakami, G., Jougasaki, M., Imura, H. Increased human brain natriuretic peptide in congestive heart failure. New Engl. J. Med. 323:757-758, 1990.

82. Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, Shirakami G, Jougasaki M, Obata K, Yasue H. Brain natriuretic peptide as a novel cardiac hormone in humans: Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J. Clin. Invest. 87:1402-1412, 1991.

83. Murad, F. Cyclic guanosine monophosphate as a mediator of vasodilation. J. Clin. Invest. 78:1-5, 1986.

84. Naruse, M., Obana, K., Naruse, K., Sugino, N., Demura, H., Shizume, K., and Inagami, T. Antisera to atrial natriuretic factor reduces urinary sodium excretion and increases plasma renin activity in rats. Biochem. Biophys. Res. Commun. 132:954-960, 1985.

85. Nemer, M., M. Chamberland, D. Sirois, S. Argentin, J. Drouin, R.A. Dixon, R.A. Zivin, and J.H. Condra. Gene structure of human cardiac hormone precursor, pronatriodilatin. Nature Lond 312:654-656, 1984.

86. Nishida, Y., A. Miyata, H. Morita, N. Uemura. K. Kangawa, H. Matsuo, and H. Hosomi. Lack of neural control of atrial natriuretic peptide release in conscious dogs. Am. J. Physiol. 53:F1164-F1170, 1987.

87. Obana, K., Naruse, M., Naruse, K., Sakurai, H. Demura, H., Inagami, T., and Shizume, K. Synthetic rat atrial natriuretic factor inhibits *in vitro* and *in vivo* renin secretion in rats. Endocrinology 117:1282-1284, 1985.

88. Oikawa, S., M. Imai, A. Ueno, S. Tanaka, T. Noguchi, H. Nakazato, K. Kangawa, A. Fukuda, and H. Matsuo. Cloning and sequence analysis of cDNA encoding a precursor of human atrial natriuretic polypeptide. Nature Lond. 309:724-726, 1984.

89. Oikawa, S., M. Imai, C. Inuzuka, Y., Y. Tawaragi, H. Nakazato, and H. Matsuo. Structure of dog and rabbit precursors of atrial natriuretic polypeptide deduced from nucleotide sequence of cloned cDNA. Biochem. Biophys. Res. Commun. 132: 892-899, 1985.

90. Okamoto H. An electron microscopic study of the specific granules in the atrial muscle cells upon administration of agents affecting autonomic nerves. Arch. Histol. Jpn. 30:467-478, 1969.

91. Osol, G., Halpern, W., Tesfamariam, B., Nakayama, K., and Weinberg, D. Synthetic atrial natriuretic factor does not dilate resistance-sized arteries. Hypertension 8:606-610, 1986.

92. Palade, G. E. Secretory granules in the atrial myocardium (Abstr.). Anat. Res. 139:262, 1961.

93. Raine, A.E.G., Erne, P., Bürgisser, E., Müller, F.B., Bolli, P., Burkart, F., Bühler, F.R. Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. N. Engl. J. Med. 315: 533-537, 1986.

94. Rankin, A.J., Wilson, N., and Lesome, J.R., Effects of autonomic stimulation on plasma immunoreactive atrial natriuretic peptide in the anesthetized rabbit. Can. J. Physiol. Pharmacol. 65:532-537, 1986.

95. Riegger, G.A.J., Kromer, E.P., and Kochsiek, K. Human Atrial natriuretic peptide: plasma levels, hemodynamic, hormonal, and renal effects in patients with severe congestive heart failure. J. Cardiovasc. Pharmacol. 8:1107-1112, 1986.

96. Riegger, GAJ. The role of the atrial natriuretic factor in congestive heart failure. Progress in Pharmacology and Clinical Pharmacology 713: 101-115, 1990.

97. Riegger, G.A.J., D. Elsner, W.G. Forssmann, and E.P. Kromer. Effects of ANP-(95-126) in dogs before and after induction of heart failure. Am. J. Physiol. 259 (Heart Circ. Physiol. 28): H1643-H1648, 1990.

98. Roy, D.R. Effect of synthetic ANP on renal and loop of Henle functions in young rat. Am. J. Physiol. 251:F200-F225, 1986.

99. Sagnella, G.A., Markandu, N.D., Shorf, A.C., and MacGregor, G.A. Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. Lancet 2:1208-1211, 1985.

100. Saito, Y., K. Nakao, K. Nishimura, A. Sugawara, K. Okumara, K. Obata, R. Sonoda, T. Ban, H. Yasue, and H. Imura. Clinical application of atrial natriuretic polypeptide in patients with congestive heart failure: beneficial effects on left ventricular function. Circulation 76:115-124, 1987.

101. Saito Y, Nakao K, Arai H, Nishimura K, Okumura K, Obata K, Takemura G, Fujiwara H, Sugawara A, Yamada T, Ito H, Mukoyama M, Hosoda K, Kawai C, Ban T, Yasue H, Imura H. Augmented expression of atrial natriuretic polypeptide gene in ventricle of human failing heart. J. Clin. Invest. 83:298-305, 1988.

102. Saito Y, Nakao K, Ito H, Yamada T, Mukoyama M, Arai H, Hosoda K, Shirakami G, Suga S, Minamino N, Kangawa K, Matsuo H, Imura H. Brain natriuretic peptide is a novel cardiac hormone. Biochem. Biophys. Res. Commun. 158:360-368, 1989.

103. Samson, W.K. Atrial natriuretic factor inhibits dehydration and hemorrhage-induced vasopressin release. Neuroendocrinology 40: 277-279, 1985.

104. Saxenhofer, H., A. Paselli, P. Weidmann, W. G. Forssmann, A. Bub, P. Ferrari, and S. G. Shaw. Urodilatin, a natriuretic factor from kidneys, can modify renal and cardiovascular function in men. Am. J. Physiol. 259 (Renal Fluid Electrolyte Physiol. 28): F832-F838, 1990.

105. Scarborough, R. M., Schenk, D.B., McEnroe, G.A., Arfsten, A. Kang, L.L., Schwartz, K. and Lewicki, J.A. Truncated atrial natriuretic peptide analogs. Comparsion between receptor binding and stimulation of cyclic GMP accumulation in cultured vascular smooth muscle cells. J. Biol. Chem. 261:12960-12964, 1986.

106. Schultz-Knappe, P., K. Forssmann, F. Herbst, D. Hock, R. Pipkorn, and W.G. Forssmann. Isolation and structural analysis of "urodilation," a new peptide of the cardiodilatin-(ANP)-family, extracted from human urine. Klin. Wochenschr. 66:752-759, 1988.

107. Schulz, S., Singh, S., Bellet, R.A., Singh, G. Tubb, D.J., Chin, H., and Garbers, D.L. The primary structure of a plasma membrane guanylate cyclase demonstrates diversity within this new receptor family. Cell 58: 1155-1162, 1989.

108. Schwab TR, Edwards BS, Heublein DM, and Burnett J.C. Jr.. The role of atrial natriuretic peptide in volume expansion natriuresis. Am. J. Physiol. 251:R310-R313, 1986.

109. Scriven, T.A. and Burnett, J.C., Jr. Effects of synthetic atrial natriuretic peptide on the renal function and renin release in acute experimental heart failure. Circulation 72:892-897, 1985.

110. Seidman, C.E., A.D. Duby, E. Choi, R.M. Graham, E. Haver, E., C. Homcy, J.A. Smith, and J.G. Seidman. The structure of rat preproatrial natriuretic factor as defined by a complimentary DNA clone. Science Wash. DC 225:324-326, 1984.

111. Seihamer JJ, Arfsten A, Miller JA, Lundquist P, Scarborough RM, Lewicki JA, Porter JG. Human and canine gene homologs of porcine natriuretic peptide. Biochem. Biophys. Res. Commun. 165:650-658, 1989.

112. Singhal, P.C., S. Decandido, J. A. Satriano, D. Schlondorff, and R. M. Hays. Atrial natriuretic peptide and nitroprusside cause relaxation of rat mesangial cells. Am. J. Physiol. 257: C86-C93, 1989.

113. Smith, J.B., and Lincoln, T.M. Angiotensin decreases cyclic GMP accumulation produced by atrial natriuretic factor. Am. J. Physiol. 253: C147-C150, 1987.

114. Song, D.L., Kohse, K.P., and Murad, F. Brain natriuretic factor: Augmentation of cellular cyclic GMP, activation of particular guanylate cyclase and receptor binding. FEBS Lett. 232:125-129, 1988.

115. Sudoh T, Kangawa K, Minamino N, and Matsuo H. A new natriuretic peptide in porcine brain. Nature 332:78-81, 1988.

116. Sudoh T, Minamino N, Kangawa K, and Matsuo H. C type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. Biochem. Biophys Res Commun 168:863-870, 1990.

117. Suzuki, M., Almeida, F.A., Nussenzveig, D.R., Sawyer, D., and Maack, T. Binding and functional effects of atrial natriuretic factor in isolated rats kidney. Am. J. Physiol. 253: F917-F928, 1987.

118. Takahashi T, Allen P.D., and Izumo S. Expression of A-, B-, and C-type natriuretic peptide genes in failing and developing human ventricles. Correlation with expression of the Ca^{2+}-ATPase gene. Circ. Res. 71.9-17, 1992.

119 Takayanagi, R., I. Tanaka, M. Maki and T. Inagami. Effects of changes in water-sodium balance on levels of atrial natriuretic factor messenger RNA and peptide in rats. Life Sci. 36: 1843-1848, 1985.

120. Takemura G, Fujiwara H, Mukoyama M, Saito Y, Nakao K, Kawamura A, Ishida M, Kida M, Kida M, Uegaito T, Tanaka M. Expression and distribution of atrial natriuretic peptide in human hypertrophic ventricle of hypertensive hearts and hearts with hypertrophic cardiomyopathy. Circulation 83:181-190, 1991.

121. Tawaragi Y. Fuchimura K, Tanaka S, Minamino N, Kangawa K, Matsuo H. Gene and precursor structure of human C-type natriuretic peptide. Biochem. Biophys. Res. Commun. 175:645-651, 1991.

122. Thibault, G., M. Nemer, J. Drouin, J.P. Lavigne, J. Ding, C. Charbonneau, R. Garcia, J. Genest, G. Jasmin, M. Sole, and M. Cantin. Ventricles as a major site of atrial natriuretic factor synthesis and release in cardiomyopathic hamsters with heart failure. Circ. Res. 65:71-82, 1989.

123. Tikkanen, I., Fyhrquist, R., Metsärinne, K., Leidenius, R. Plasma atrial natriuretic peptide in cardiac disease and during infusion in healthy volunteers. Lancet II:66-69, 1985.

124. Trippodo, N.C., A. Januszewicz, F.E. Cole, A.A. Macphee, B.L. Pegram, and M.B. Kardon. Conversion factors of high to low molecular weight forms of atrial natriuretic factor. Federation Proc. 45: 2091-2095, 1986.

125. Trippodo, N.C., Kardon, M.B., Pegram, B.L., Cole, F.E., and MacPhee, A.A. Acute haemodynamic effects of the atrial natriuretic hormone in rats. J. Hypertens., 4 (Suppl. 2): S35-S40, 1986.

126. Trippodo, N.C., Cole, F.E., MacPhee, A.A., and Pegram, B.L. Biologic mechanisms of atrial natriuretic factor. J. Lab. Clin. Med. 109:112-119, 1987.

127. Tsuchimochi H, Kurimoto F, Ieqki K, Koyama H, Takaku F, Kawana M, Kimata S, Yazaki Y: Atrial natriuretic peptide distribution in fetal and failed adult human hearts. Circulation 78:920-927, 1988.

128. Tsunoda, K., F.A.O. Mendelsohn, P.M. Sexton , S.Y. Chai, G.P. Hodsman, and C.I. Johnston. Decreased atrial natriuretic peptide binding in renal medulla in rats with chronic heart failure. Circ. Res. 62: 155-161, 1988.

129. Villarreal, D., R.H. Freeman, and R. A. Johnson. Renal effects of ANF (95-126), a new atrial peptide analogue, in dogs with experimental heart failure. Am. J. Hypertens. 4:501-515, 1991.

130. Vuolteenaho O., O. Arjamaa, and N. Ling. Atrial natriuretic polypeptides (ANP): rat atria store high molecular weight precursor but secrete processed peptides of 25-35 amino acids. Biochem. Biophys. Res. Commun. 129:82-88, 1985.

131. Wilkins, M.R., Settle, S.L., and Needleman, P. Augmentation of the natriuretic activity of exogenous and endogenous atriopeptin in rats by inhibition of guanosine 3', 5'-cyclic monophosphate degradation. J. Clin. Invest. 85: 1274-1297, 1990.

132. Yosimura M, Yasue H, Morita E, Sakaino N, Jougasaki M, Kurose M, Mukoyama M, Saito Y, Nakao K, Imura H. Hemodynamic, renal, and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure. Circ. Res. 84:1581-1588, 1991.

133. Zeidel, M.L., P. Silva, B.M. Brenner, and J.L. Seifter. Cyclic GMP mediates effects of atrial peptides on medullary collecting duct cells. Am. J. Physiol. 252: F551-F559, 1987.

134. Zucker I, Earle AM, Gilmore JP. The mechanism of adaptation of left atrial stretch receptors in dogs with chronic congestive heart failure. J. Clin. Invest. 60:323-331, 1977.