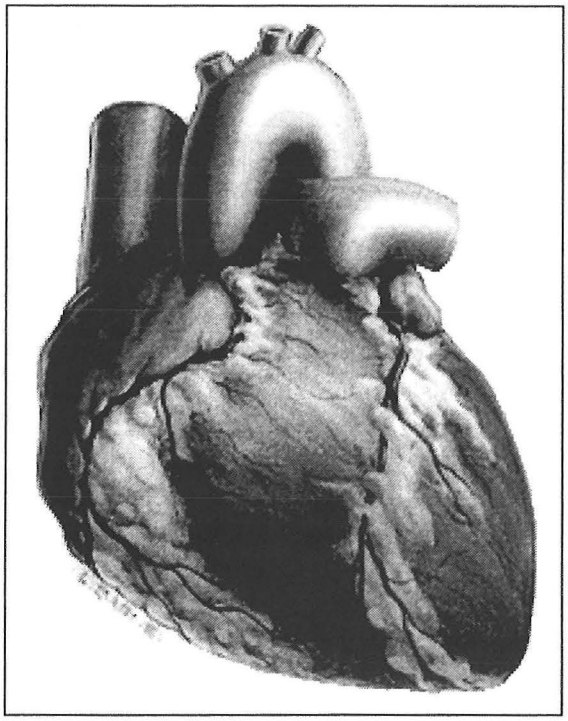
## The Endocrine Heart:

### Reconciling the Hemodynamic and Neurohormonal "Paradigms" of Heart Failure Progression



This is to acknowledge that Daniel L. Dries, M.D., M.P.H., has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Dries will not be discussing off-label uses in his presentation.

**Daniel L. Dries, MD, MPH**
**Assistant Professor of Medicine**
**Heart Failure Research Group**
**Division of Cardiology**
**Interests: mechanisms of congestive heart failure progression, cardiorenal interaction in heart failure, genetic epidemiology related to susceptibility to LVH and CHF.**

## I. Historical Background

Medical historians attribute the discovery of the endocrine heart and the cardiac hormonal system (CHS), also referred to as the natriuretic peptide system, to 1956 when simultaneously there was made a physiologic and pathological discovery. Bruno Kisch reported the electron microscope appearance of atrial secretory granules and Drs. Henry and Gauer described what has come to be known as the Henry-Gauer reflex: diuresis induced by atrial distention[1]. However, supporting the contention that "there is little new under the sun", one can argue that the first historical description of the endocrine function of the heart was made approximately 2000 years ago by the Roman historian Flavius. He referred to the workers that dived underwater during the construction of the maritime harbor of Caesarea as *urinatores.* The diuresis caused by the frequent changes in intrathoracic pressure forced them to urinate frequently. Our understanding of the CHS advanced futher in the early 1980's when Adolfo deBold, Harold Sonnenberg, and colleagues demonstrated that the infusion of an extract obtained from rat atrial appendages resulted in natriuresis and diuresis.

## II. Physiology of the Cardiac Hormonal System

The myocardium responds to acute increases in transmural distending pressure ('stretch") by releasing natriuretic peptides into the circulation[2]. The primary natriuretic peptides synthesized and released by the heart are atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). ANP is primarily synthesized by the atria, in particular the left-atrial appendage and stored in secretory granules for rapid release. BNP, so-called because of its initial discovery in the porcine brain, is primarily produced by the ventricles in a constitutive manner and *not* stored in granules. The natriuretic peptides ANP and BNP exert many beneficial *compensatory* actions within their target tissues: vasodilation, natriuresis, and a direct suppression of the sympathetic nervous system and the renin-angiotenin-aldosterone system. A third natriuretic peptide, CNP, is produced by endothelial cells as the natriuretic peptide of a local endothelial autocine/paracrine natriuretic peptide system that regulates vascular tone and exerts anti-proliferative actions on vascular smooth muscle cells[3, 4]. Appreciable levels of CNP are not found in the circulation and it binds to a specific receptor distinct from that of ANP or BNP. Additional undiscovered natriuretic peptides may exist. For example, recently, a novel new potent natriuretic peptide has been identified in the venom of the green mamba snake[5-7].

### *The afferent limb of the CHS.*

The basic stimulus for the release of ANP and BNP from myocardial cells into the circulation is an increase in transmural cardiac distending pressure, not increases in atrial or ventricular pressure per se. To illustrate this point, Burnett and colleagues [8] demonstrated that circulating levels of ANP do not increase in a canine model of cardiac tamponade. The explanation is that in cardiac tamponade a *balanced* increase in intracardiac and pericardial pressures occurs and this does not result in increased transmural distending pressure.

The release of ANP from the secretory granules is triggered by increases in intracellular calcium concentrations. Chronic and sustained increases in atrial stretch will deplete ANP granules within 24 hours. Consequently, sustained increases in ANP release into the circulation require increased transcription of the ANP gene which takes takes longer than 24 hours. In

contrast, increases in BNP gene transcription are seen as early as 4 hours after sustained increases in ventricular loading conditions[9 10].

Both ANP and BNP are synthesized as precursor peptide molecules (ANP precursor is 156 amino acids and BNP is 145 amino acids ) that are subsequently processed into active hormones. The atrial secretory granules store pro-ANP, so processing occurs subsequent to calcium-mediated exocytosis from the storage granules. Final processing cleaves the amino-terminal amino acid chain yielding short cytoplasmic terrminal peptide fragments that are the biologically active forms of ANP and BNP (figure 1). The lopped structure formed by the disulfide bond between cysteine residues is required for biological activity.

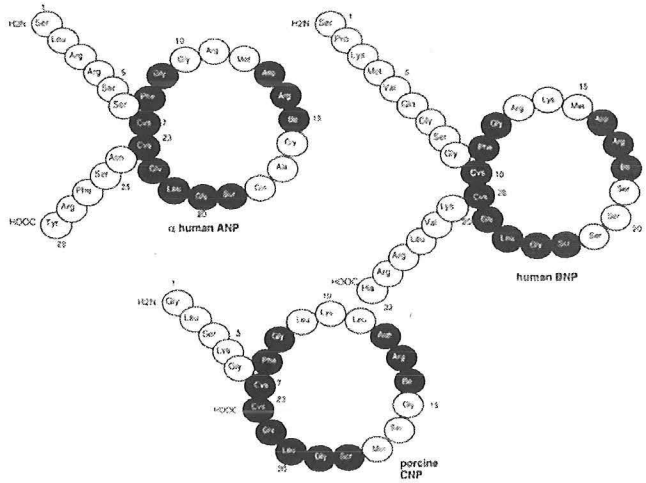



Figure 1

The enzyme responsible for the processing of pro-ANP and pro-BNP has recently been identified[11, 12]. Corin is a type II transmembrane serine protease. The active catalytic portion of this enzyme resides on the extracellular membrane of cardiomyocytes. In vitro experiments in cultured atrial cell lines has demonstrated that corin processes both pro-ANP and pro-BNP. The processing of pro-peptide hormones is thought to occur during the process for acute release into the circulation. The pro-hormones undergo active exocytosis into the extracellular space where the catalytic region of corin cleaves the N-terminal region at a specific amino acid sequence as the pro-hormones exit the cell[13]. Some in vitro data suggests that a portion of this processing may occur within the myocytes prior to release (Wu, personal communication). Thus, corin conceptually can be thought of as the "cardiac hormonal converting enzyme". It is a critical component of the afferent limb of the endogenous CHS.

***The efferent limb.***

ANP and BNP mediate their physiologic effects within target tissues by interacting with a specific receptor called the type-A natriuretic peptide receptor (NPR-A)[14]. The NPR-A receptor is a homodimer that consists of 4 distinct domains: an extracellular ligand binding domain, a transmembrane domain, a kinase homology domain, and a guanylate cyclase domain that is capable of converting GTP into cGMP (figure 2). CNP binds to the type-B natruretic peptide receptor located on fibroblasts and endothelial cells. The structure of the type B receptor (NPR-B) is similar to NPR-A. The natriuretic peptide clearance receptor, NPR-C, has an extracellular ligand binding similar to NPR-A, however, it lacks the intracellular guanylate cyclase domain.

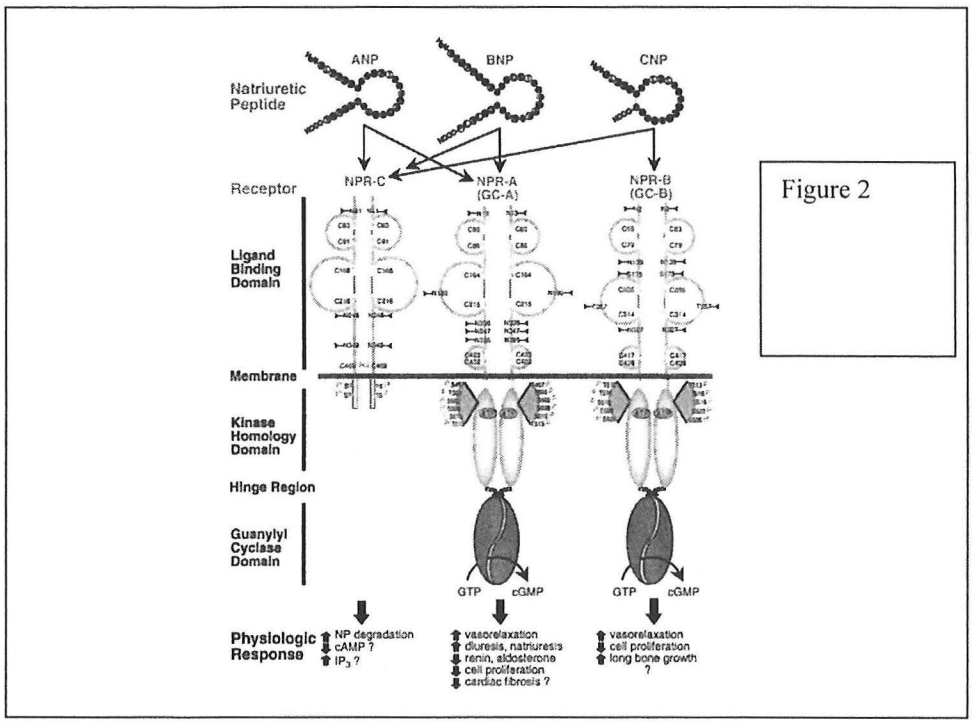



Figure 2

The binding of ANP or BNP to the NPR-A receptor causes a conformational change in the receptor that activates the *particulate* guanylate cyclase domain leading to the conversion of GTP to c-GMP within the cell[15, 16]. The production of c-GMP is the essential second messenger that initiates the intracellular signaling cascades that culminate in the physiologic effects of ANP and BNP in target tissue. The guanylate cyclase domain that is part of the NPR-A receptor is termed *particulate* guanylate cyclase and it is distinct from *soluble* guanylate cyclase that generates cGMP in response to nitric oxide. Unlike particulate guanylate cyclase, soluble guanylate cyclase circulates freely in the cytoplasm without association with a surface receptor.[17] Perhaps because of the localization of particulate GC close to the cell membrane, a portion of the cGMP produced by the natriuretic peptides egresses from the cell. Changes in plasma or urinary cGMP are reflective of the actions of ANP and BNP in target tissue[18, 19] and are used to indirectly measure the in vivo physiologic activity of ANP and BNP[20, 21].

Local control of the physiologic effects of ANP or BNP in target tissue is the result of the balance between binding of hormone to NPR-A, clearance by binding to the natriuretic peptide clearance receptor (NPR-C) or degradation by neutral endopeptidase (figure 3). Both the clearance receptor and neutral endopeptidase are believed to equally contribute to cardiac hormone degradation in heart failure[22]. Upon binding of ANP or BNP to the clearance receptor, the entire complex is internalized, the peptide hormone is degraded in lysosomes, and the receptor complex is then recycled to the cellular membrane. There is some evidence that the cytoplasmic portion of the NPR-C receptor may have a signaling function. For example, several experiments have suggested that the cytoplasmic portion of the NPR-C receptor can inhibit activation of adenylate cyclase activity[23]. Neutral endopeptidase is a zinc-dependent enzyme abundantly expressed in most of ANP and BNP's target tissues. This enzyme directly degrades ANP and BNP by cleavage of the disulfide link in ANP and BNP. There exists enthusiasm for inhibitors of neutral endopeptidase to augment the bioactivity of ANP and BNP and several agents are being tested in ongoing clinical trials[24-26].

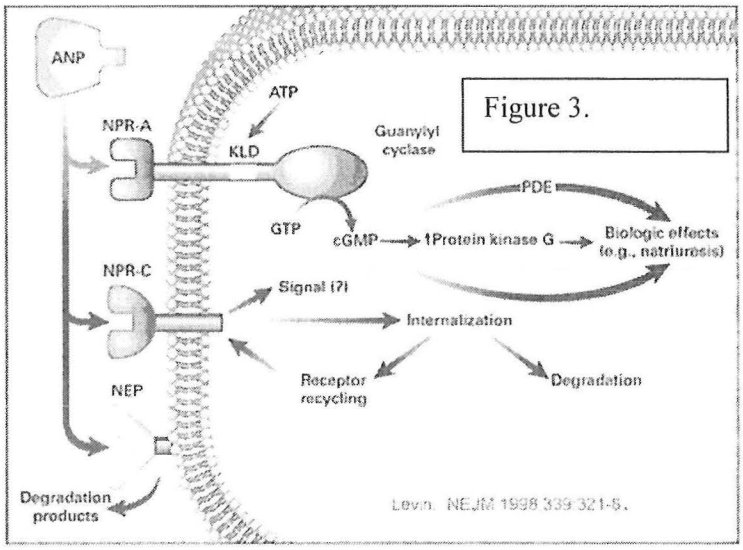



Figure 3.

The ability of the natriuretic clearance receptor (NPR-C) to modulate the local physiologic effects of ANP and BNP was demonstrated in a murine model in which the gene for the NPR-C receptor was disrupted[27]. The double NPR-C gene knockout mice (no NPR-C receptor's produced) demonstrated a phenotype characterized by significantly prolonged circulating ANP half-life, hypotension, and increased natriuresis compared to wild-type mice. Interestingly, a human polymorphism of the NPR-C gene's promoter that increases transcription of the NPR-C gene is associated with lower ANP levels and increased blood pressure in obese individuals[28].

***cGMP-dependent protein kinase G mediates many of the actions of ANP and BNP***

The cGMP produced by the NPR-A receptor upon ANP or BNP binding activates cGMP-dependent protein kinase termed protein kinase-G (PKG).[29-32]. This kinase exists as a homodimer and there are two forms: PKG-I and PKG-II[33]. The kinase exists within the myocardium itself in which the predominant form is PKG-I[34]. The kinase activity of PKG-I and PKG-II activates a variety of molecular signaling pathways that influence ion channel permeability, gene activation and protein-protein interactions within target tissues[30, 31, 35].

*Natriuresis.*

The natriuretic effect of the natriuretic peptides is mediated by PKG-dependent inhibition of sodium reabsorption in the inner medullary collecting duct. This is the result of a coordinated inhibition of apical sodium channels and basolateral Na/K ATPase[36]. Both effects appear to be PKG-dependent because the effects of the natriuretic effects are mimicked by 8-bromo-cGMP, the membrane permeable analogue of cGMP and blocked by a specific inhibitor of PKG.

*Smooth muscle relaxation.*

The smooth muscle relaxation effects of the natriuretic peptides are also PKG-dependent[29, 37]. In smooth muscle cells, an increase in intracellular calcium causes smooth muscle cell contraction by activation of the calcium/calmodulin dependent myosin light chain kinase, which phosphorylates myosin light chain and activates the contractile myosin adenosine triphosphatase (ATPase). The net phosphorylation state of myosin light chain is determined by

the relative activity of myosin light chain phosphatase (MLCP) and a myosin light chain kinase (MLCK). Myosin light chain phosphatases is coupled to PKG-I in smooth muscle cells. When ANP or BNP bind to NPR-A receptors in smooth muscle, the subsequent activation of PKG results in phosphorylation of MLCP. This decreases the phosphatase activity of MLCP and shifts the balance between the activity of MLCP and MLCK. The result is net dephosphorylation of myosin light chain, decreased contractile apparatus interactions and smooth muscle cell relaxation[38].

*Neurohormonal suppression.*

The direct suppressive actions of ANP and BNP on renin-angiotensin-aldosterone (RAAS) activation are PKG-mediated as well. For example, overexpressed cGMP-dependent protein kinases inhibits cAMP-dependent renin release from the kidney[39]. Likewise, knockout of the NPR-A gene in mice increases the renal production of renin messenger RNA [40]. In mice with knockout of the PKG-II gene, there is increased renin synthesis, as determined by increases in rennin messenger RNA, under stimulatory (low salt diet plus ramipril) and inhibitory conditions (high-salt diet)[41]. ANP and BNP also directly suppress endothelin and aldosterone synthesis and release. In a cultured zona-glomerulosa cell line, activation of the NPR-A receptors directly suppresses the production of aldosterone[42]. In the porcine aorta, the inhibition of angiotenisn-II mediated endothelin release by ANP and BNP is c-GMP dependent[43].

**III. CHS in Asymptomatic Left-Ventricular Dysfunction**.

The classic symptoms of congestive heart failure, dyspnea on exertion, orthopnea and paroxysmal nocturnal dyspnea are invariably the result of abnormal increases in left-ventricular end-diastolic pressure. Therefore, the existence of "asymptomatic left-ventricular dysfunction" (ALVD) necessarily implies retained ability to regulate intravascular volume and prevent abnormal increases in intracardiac filling pressures. This early stage of ventricular systolic dysfunction has been reproduced in animal models using a rapid ventricular pacing. The results of these studies demonstrate that the cardiac hormonal system (CHS) is of central importance to the maintenance of the asymptomatic stage of left-ventricular systolic dysfunction.

For example, in a low-ANP model of acute congestive heart failure produced by inferior vena cava constriction, characterized by decreased cardiac output without increases in atrial pressures or ANP, marked sodium retention, vasoconstriction, and activation of the renin–angiotenin-aldosterone (RAAS) system result. However, these findings were not observed in a *high-ANP* model produced by rapid ventricular pacing, despite similar reductions in cardiac output[44]. When exogenous ANP was infused into the circulation of the vena-caval constricted dogs, sodium retention, vasoconstriction and activation of the RAAS system was prevented[44]. Redfield and associates demonstrated that in a pacing-induced canine model of ALVD there was activation of the cardiac hormonal system as evidenced by an elevation in ANP, no activation of the RAAS, and normal cardiorenal response to a fluid challenge [45]
(Table 1).

**Table 1. Canine Model of ALVD**

| | Normals | | ALVD | |
|---|---|---|---|---|
| | Baseline | VE | Baseline | VE |
| Uvol (ml/min) | 0.40 | 5.59 | 0.62 | 5.04 |
| UNa (meq/min) | 44 | 693 | 34 | 630 |
| FELi (%) | 13 | 36 | 12 | 49 |

VE=volume expansion with saline bolus

Table 1. In response to volume expansion (VE) caused by rapid infusion of isotonic saline, there were no significant differences in ALVD dogs compared to controls with regards to increases in fractional sodium excretion, total sodium excretion or urine volume.

There are only limited data on the pathophysiology that underlies ALVD in humans. The only major clinical trial to examine patients with ALVD was the Studies of Left Ventricular Dysfunction (SOLVD) Prevention Trial[46] published in 1992. In the SOLVD Prevention trial, participants were enrolled if they had no symptoms of CHF (NYHA Class I) and a left ventricular ejection fraction $\leq 0.35$. Baseline neurohormones were measured in a subset of the participants. Interestingly The neurohormonal profile (Table 2) is similar to that seen in animal models of ALVD; namely, activation of the endogenous cardiac hormonal system (CHS) *precedes* activation of the RAAS[47] as demonstrated by the significant increase in ANP in patients with ALVD compared to controls, but no significant increase in plasma renin activity (PRA).

**Table 2. Neurohormal Activation in ALVD vs. CHF**

| | Control | SOLVD Prevention | SOLVD Treatment |
|---|---|---|---|
| PNE | 316.5 | 422 | 507 |
| ANP | 48.3 | 102.8 | 148 |
| PRA | 0.6 | 0.7 | 1.7 |
| AVP | 1.8 | 2.3 | 3.0 |

Francis et al. Circulation 1990; 82: 1724.

These data suggest that early activation of the cardiac hormonal system maintains cardiorenal homeostasis and suppresses activation of the RAAS. This is consistent with experimental data that demonstrate a direct suppressive effect of the cardiac hormones on renin and aldosterone synthesis and release[39-41]. The precise pathophysiology that underlies the transition from ALVD to symptomatic heart failure is not known. However, the critical event may be an "escape" of the RAAS from the suppressive actions of the natriuretic peptides.

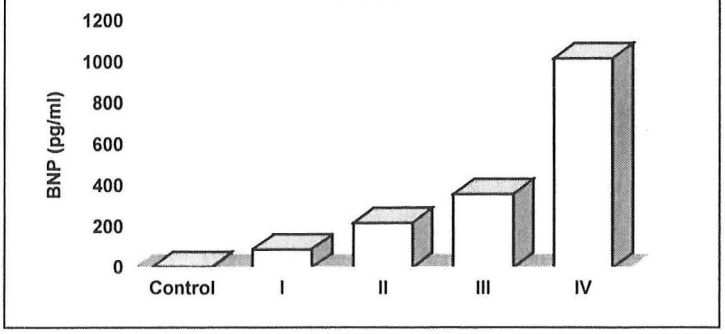
The SOLVD Prevention trial published in 1992 is an old clinical trial, yet it remains the only randomized, controlled clinical trial that enrolled patients with ALVD. In that trial, enalapril reduced the risk for the composite endpoint of death or hospitalization for heart failure, but did not significantly reduce the risk for all-cause

mortality. Based on the SOLVD Prevention trial it is recommended that all patients with ALVD should receive ACE-inhibition therapy. It is not known if beta-blockers reduce progression of ALVD to heart failure. Although clearly effective in all symptomatic stages of heart failure[48-51], there are no randomized clinical trials that have tested the ability of beta-blocker therapy to reduce the risk for progresion of ALVD to heart failure. Additional novel therapeutic approaches based upon an understanding of the unique pathophysiology of ALVD and the role of the cardiac hormonal system may improve our ability to reduce the risk for progression of ALVD to heart failure. For example, given the role of the cardiac hormonal system in suppressing RAAS activation and maintaining the cardiorenal response to volume challenges, an intriguing strategy would be to augment the actions of ANP and BNP pharmacologically with the use of neutral endopeptidase (NEP) inhibitors, either as monotherapy or combined with ACE-inhibitors (e.g. omapatrilat)[25, 52-57].

**IV. CHS in Advanced Heart Failure.**

Despite the recognized importance of the cardiac hormonal system (CHS) in ALVD and mild heart failure, it was not clear that it continued to act as an important compensatory system once heart failure became severe. The CHS is progressively activated as the severity of CHF increases (figure 4). Are the increased levels of the natriuretic peptides exerting any important function once symptoms of heart failure develop or are the physiologic effects of ANP and BNP easily over-powered by the adverse neurohormonal mileu that characterizes advanced CHF?

Figure 4.




In an effort to address these questions experimentally, Wada and colleagues[58]created a canine model of severe heart failure using rapid and sustained ventricular pacing. The neurohormonal profile mimicks that seen in advanced stages of human heart failure. The investigators then administered HS-121 to a subset of the dogs. HS-121 is a specific antagonist for NPR-A so it blocks the biological actions of ANP and BNP in all target tissues expressing NPR-A. The investigators noted rapid declines in renal function and activation of adverse neurohormonal systems upon blockade of the biological actions of ANP and BNP by HS-121 (Table 3).

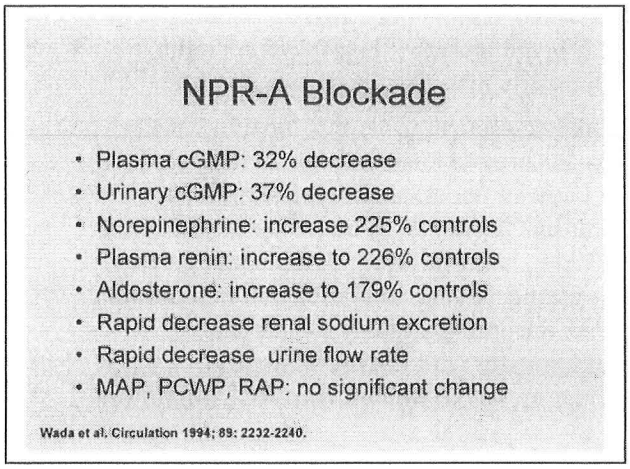



Table 3. Administration of HS-121, a competitive blocker of NPR-A, resulted in rapid increases in sympathetic and RAAS activation as well as decreased natriuresis. The acute decline in plasma and urinary cGMP suggests a decline in cardiac hormonal bioactivity in target tissues.

These data to demonstrate that the CHS continues to exert a tonic compensatory role in all stages of heart failure, in particular the ability of ANP and BNP to suppress excessive activation of the RAAS and sympathetic systems and to promote renal homeostasis. Additional support for the hypothesis that the CHS is an important *compensatory system* in advanced heart failure is provided by the data demonstrating the benefit of synthetic BNP infusions (nesiritide) in advanced heart failure. When administered to patients with decompensated moderate to severe systolic heart failure, nesiritide effectively reduced pulmonary capillary wedge pressure, but also significantly reduced the circulating levels of norepinephrine, aldosterone and endothelin[59-61]. The extent of neurohormonal suppression was greater than that seen with intravenous nitroglycerin despite comparable changes in hemodynamic parameters that can drive neurohormonal activation. Anecdotally, in our experience, the use of BNP has been associated with enhanced diuresis without the problem of increase in serum creatinine during aggressive diuresis.

Perhaps arguing against the importance of the cardiac hormonal system as a stabilizing compensatory system in moderate to severe heart failure are the results of the OVERTURE (Omapatrilat Versus Enalapril Randomized Trial of Reducing Events) heart failure trial recently reported (unpublished data) at the ACC 51rst Scientific Session 2002. Omapatrilat is a combined ACE and NEP-inhibitor. A total of 5770 patients with NYHA Class II-IV CHF and EF < 30% were randomized to enalapril (10 mg/day) or omapatrilat (40 mg/d). There was no significant difference between enalapril or omapatrilat with regards to the composite primary end point of all-cause mortality/CHF hospitalizations. There may be several potential explanations for the negative results. One interpretation is that the incremental increase in circulating ANP and BNP that occurs with omapatrilat offered no advantage over enalapril because the cardiac hormones are not critical to the pathophysiology of progression once heart failure becomes advanced. There may be other explanations for these negative results. As we will discuss subsequently, despite the increased levels of BNP and ANP in advanced CHF, there is a blunting of their target tissue biological actions. On average, vasopeptidase inhibition will raise circulating levels of ANP and BNP ~ 4-fold and this degree of increase may not be sufficient to overcome the mechanisms that contribute to target tissue resistance to BNP. In fact further sustained increases may contribute to further resistance over time. With intravenous infusions with nesiritide, acute physiologic effects are seen in patients with advanced heart failure. However, intravenous infusions of nesiriitde can increase

circulating BNP-levels ~10-fold (in the 5000 pg/ml range) and this may be sufficient to overcome the resistance mechanisms that we will discuss later.

## V. BNP as a Biomarker in Patients with Heart Failure

A great deal of attention has recently been focused on the utility of BNP as a diagnostic and prognostic biomarker[62-65]. An elevated BNP has been consistently corrrelated with an increased risk for morbidity and mortality in heart failure[66-71], as recently reviewed by Maisel [72] and during Dr. James de Lemos' medical grand rounds here at UT Southwestern. In order to understand *why* BNP is such a robust clinical biomarker for adverse events, despite its known *beneficial and compensatory* physiologic effects, a brief review of some of the major studies of BNP as a biomarker in heart failure will provide the conceptual framework.

In a recent clinical cohort study under the direction of Maeda and associates[70], a cohort of patients with decompensated moderate to severe systolic heart failure underwent standard therapy (not protocol directed) including more aggressive diuresis and addition of standard medications. At baseline and at 3-months after initial randomization a neurohormonal profile was measured in each subject. By 3-months patients had on average improved clinically (Table 4) as evidenced by improved NYHA class, and significant decreases in norepinephrine, endothelin, interleukin-6 and ANP and BNP (Table 4).

Table 4. BNP Guided CHF Therapy

| | Baseline | 3-months |
|---|---|---|
| NYHA | 3.4 ± 0.05 | 2.2 ± 0.05 |
| LVEF | 23 ± 0.09 | 33 ± 1.3 |
| ANP (pg/ml) | 208 ± 19 | 80 ± 7.2 |
| BNP (pg/ml) | 917 ± 96 | 285 ± 37 |
| PNE pg/ml) | 968 ± 84 | 431 ± 32 |
| ET-1 (pg/ml) | 4.58 ± 0.23 | 2.69 ± 0.1 |
| IL-6 (pg/ml) | 15.2 ± 2.5 | 5.0 ± 0.7 |

The 3-month value of IL-6 and BNP were independently associated with all-cause mortality and the composite endpoint of death or CHF hospitalization. When stratified by the median 3-month BNP or IL-6 levels, the patients with BNP levels above the median value of 170 pg/ml had a significant increase in mortality (figure 5).

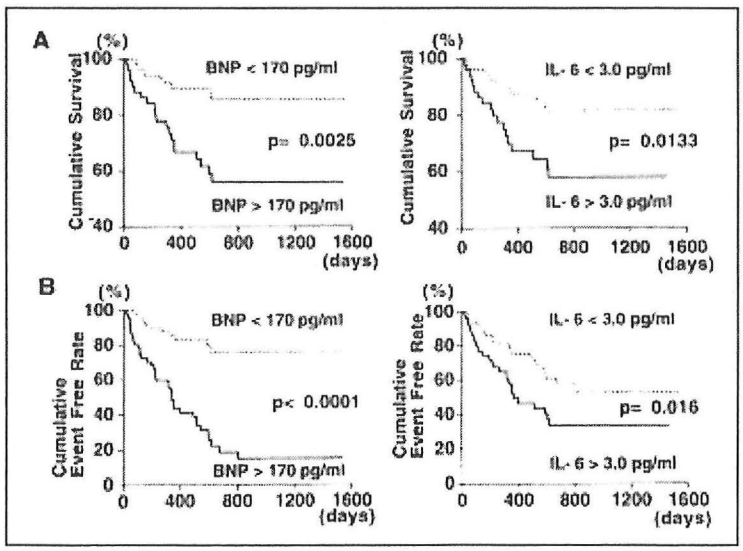



Figure 5. When divided into cohorts according to the mean values of BNP (170 pg/ml), patients above the median value demonstrated significant increases in all-cause mortality and risk for the combined endpoint of death or CHF hospitalization. Similar trends were demonstrated for IL-6, a marker of generalized cytokine activation. Note that stratification was based on median 3-month BNP and IL-6 values.

## VI. Understanding BNP as Biomarker

***What is tailored hemodynamic therapy for advanced refractory heart failure?***

Tailored hemodynamic therapy [73-75] is a general approach to advanced decompensated heart failure that targets intracardiac filling pressures rather than cardiac output per se. In brief, a pulmonary artery catheter is used to guide therapy after obvious volume reservoir (anasarca or ascites) is removed. Initially a combination of intravenous vasodilators, typically nitroprusside, and diuretics is used during the initial 24 hours and to approach as possible the following hemodynamic goals: a mean pulmonary capillary wedge pressure $\leq$ 16mmHg, mean right atrial pressure of $\leq$ 8 mm Hg, systemic vascular resistance $\leq$ 1200 and maintaining a systolic blood pressure $\geq$ 80 mmHg. Once obtained the next 48 hours are used to transition the patient to an oral regimen that maintains these hemodynamics. The ultimate goal is to redesign the outpatient oral regimen. Note that cardiac output is not the primary target, but rather normalization of ventricular loading conditions. Obtaining these hemodynamic targets consistently improves cardiac index secondarily by improving forward stroke-volume via a reduction in mitral regurgitation, and the extent of improvement in cardiac index is as dramatic if not better than that obtained with intravenous inotropic agents[76]. It has been demonstrated that the improvements in hemodynamics achieved after transition to an oral medical regimen are maintained long-term when these patients are followed in a dedicated heart failure clinic and become familiar with the proper use of a flexible outpatient diuretic regimen[77].

In general, many of our patients with advanced heart failure have elevated cardiac filling pressures despite any symptoms of congestion and despite no evidence of edema or crackles on auscultation of the lungs. Drazner and colleagues reported that one of the most sensitive indicators of elevated left-sided filling pressures in patients with advanced heart failure is right atrial pressure (figure 6) as demonstrated by the tight correlation between left-sided and right-sided cardiac pressures in 000 patients with advanced heart failure[78]. In the population of patients with advanced heart failure, the most common "mistake" is not to recognize volume-overload. This is because the usual clinical signs are absent. Although quite specific when present, rales and peripheral edema are evident in less than 25% of patients with abnormally high left-sided pressures. The most sensitive signs of increased left-sided filling pressures is elevation of the jugular venous pressure (~80% sensitivity). A "square wave" response upon performing the Valsalva maneuver is also a reliable sign of increased left-sided filling pressures[79].

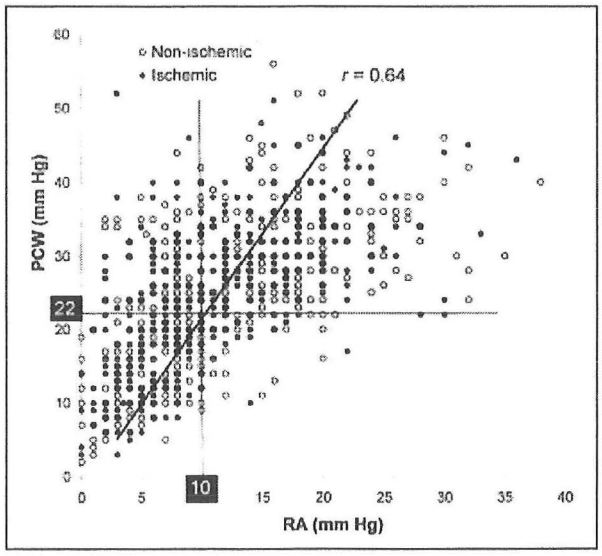



Fig 6. In 1000 patients with advanced ischemic and non-ischemic cardiomyopathy there is a linear correlation between RA and PCWP. In general a RA pressure of 10 correlated with a PCWP of 22 mm Hg.

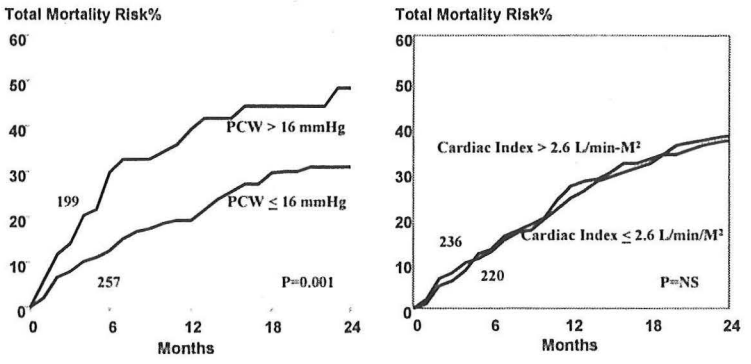
In our experience using this approach on the CHF service, many of our patients are rendered asymptomatic in terms of symptoms of congestion (a dry cough being one of the frequent symptoms of elevated pressures) when their baseline PCWP, not infrequently as high as 30-35 mmHg, is reduced below a threshold value of 25 mmHg. Tailored therapy, therefore, *targets filling pressures-below the usual threshold for development of symptoms of congestion.* There is debate Is there any Cab *normalization* of cardiac filing pressures beyond relief of symptoms of congestion important to reduce the rate of heart failure progression?

***Post-therapy intracardiac filling pressures correlate with prognosis in advanced CHF.***

Supporters of the neurohormonal paradigm of heart failure advocate that hemodynamics are not important in the neurohormonal paradigm of heart failure other than their relation to symptoms of congestion. This continues to be a lively debate in the heart failure community. Opponents of the aggressive approach of tailored hemodynamic therapy point to numerous studies that found no association of hemodynamics with prognosis in heart failure. However, as pointed out by Stevenson [73-75] these studies used *baseline* hemodynamics in their analyses and in general baseline hemodynamics for patients with decompensated heart failure are similar and do not provide the ability to risk-stratify patients over time.

*Post-therapy* hemodynamics are associated with prognosis[73, 75, 80]. For example, Fonarow and associates at UCLA demonstrated that when patients with refractory end-stage heart failure underwent tailored therapy, the patients could be risk-stratified when divided into subsets based on the ability to achieve a normal pulmonary-capillary wedge pressure (PCWP $\leq$ 16 mmHg). The all-cause mortality was significantly greater for the patients unable to lower PCWP to a value $\leq$ 16 mmHg. The two sub-groups based on post-therapy PCWP were indistinguishable when baseline hemodynamics were compared.

**Figure 7 . Early Response of PCW but not CI Predicts Subsequent Mortality in Advanced Heart Failure**



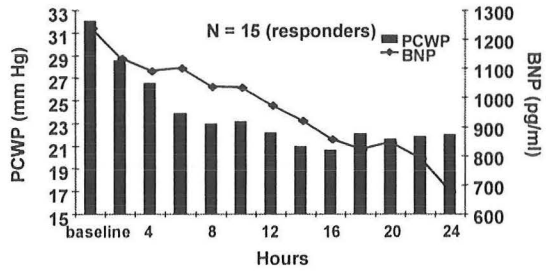

**Final hemodynamic measurement in 456 advanced HF patients after tailored vasodilator therapy. Fonarow Circulation 1994;90:I-488**

These data suggest that when using an aggressive treatment strategy such as tailored hemodynamic therapy (to be discussed subsequently) the ability to approximate hemodynamic goals or near-normal filling pressure provides an ability to risk-stratify patients. They do not

suggest that a further reduction in cardiac filling pressures beyond relief of symptoms of congestion may be beneficial in reducing heart failure progression. The approach termed "tailored therapy" has never prospectively been demonstrated to improve mortality in advanced heart failure. This approach is undergoing prospective evaluation in the NIH-sponsored ESCAPE trial. In this study, patients with refractory NYHA IIIB or IV heart failure are being randomized to management using clinical assessment alone, aimed at normalizing filling pressures based on clinical exam findings (e.g. JVP less than 8 cm) or treatment guided by a PA-catheter.

Recently, Maisel and colleagues demonstrated a tight correlation between changes in PCWP and BNP during treatment of decompensated CHF (figure 8).

**Figure 8. Correlation of Change in PCWP with BNP**




Maisel, A. et al. J Cardiac Failure, Vol. 7, No. 1, 2001

Integrating these data, one intriguing potential explanation based on these data fro BNP's prognostic import in CHF is its correlation with post-therapy ventricular loading conditions. In other words, patients with increased post-therapy BNP levels are at increases risk for heart failure progression because they have persistently increased cardiac filling pressures. This can be the case despite symptomatic improvement. For example, based om Maisel's data, it is likely that on average, the patients in Fonarow's study (figure 7) with a PCWP > 16mmHg had higher levels of BNP than those with the ability to obtain a PCWP $\leq$ 16 mmHg. Of course, the acceptance of this hypothesis implies that hemodynamics, specifically ventricular loading conditions, contribute to progression of heart failure. Are there data to support this concept?

***Ventricular loading conditions may cause heart failure progression.***

Ventricular loading conditions may directly influence progression and prognosis in heart failure. For example, chronically increased filling pressures increase ventricular wall stress and promote myocyte slippage and the process of progressive ventricular dilatation[81]. This is consistent with the notion that "dilatation begets dilatation". Decreasing ventricular end-diastolic pressure in theory will reduce wall tension for a given ventricular diameter and may reduce the rate of dilatation. Left-ventricular size, specifically the end-diastolic dimension, is independently associated with increased mortality in advanced heart failure[82].

A recent study demonstrated that BNP elevation was independently associated with the risk for sudden cardiac death in moderate to severe CHF[83]. If BNP is related to cardiac filling pressures, how are ventricular filling pressures in turn related to arrhythmic risk? It is known that

increased ventricular loading conditions can lead to changes in repolarization[84]. Loading conditions are often heterogenous in the ventricle so these changes in repolarization can cause heterogeneity in refractoriness and promote re-entry arrhythmias[85]. In addition, increased myocyte stretch and increased ventricular loading conditions can increase calcium overload, predispose to early after-depolarizations and triggered automaticity leading to arrhythmias[86].

It is also increasingly apparent that loading conditions in the ventricle alter myocardial gene expression[87-89]. This has been confirmed in patients supported with left-ventricular assist devices (LVAD's). If working properly, LVAD's normalize left-ventricular loading conditions. Several investigators have demonstrated that after LVAD implantation, myocardial expression of pro-inflammatory cytokines and the cardiac hormones ANP and BNP are reduced[90, 91]. Increased ventricular loading conditions enhance activation and gene-expression of the metalloproteinases that degrade collagen, fibronectin and other components of the extracellular matrix[92]. Therefore, metalloproteinase activation plays a critical role in the adverse ventricular remode4ling that occurs as heart failure progresses. In addition, persistent cytokine activation is induced by increased ventricular loading conditions. Cytokine activation is independently associated with heart failure mortality[70] and cytokine activation, such as TNF-alpha, contributes to further activation of the metalloproteinase proteolytic family[93]. Thus, the gene expression profile activated by increased ventricular loading conditions might contribute directly to the adverse remodeling process that defines progression of heart failure.

In addition, Johnson and colleagues recently reported the hemodynamic and neurohormonal responses to tailored hemodynamic therapy[94]. Neurohormonal measurements were made before the initiation of nitroprusside, with each patient maintaining his or her oral medical regimen until the morning of the catheterization, after optimal hemodynamics were approximated on intravenous medication (time point A) but patients were holding all oral medications, including ACE-inhibitors, and after conversion to an oral regimen (time point C) [Table 5A]. Oral medications were withheld from the morning of catheterization to time point A. Therefore, the neurohormonal changes reflect the consequences of lowered intracardiac filling pressures in the absence of oral medications such as ACE-inhibitors. On average, hemodynamics improved substantially (Table 5B).

Table 5A. Neurohormonal Response to Tailored Therapy

| N=34 pts | Baseline | A | B |
|---|---|---|---|
| ANP (pg/ml) | 201 ± 30 | 96 ± 14 | 74 ± 12 |
| BNP (pg/ml) | 175 ± 22 | 126 ± 20 | 82 ± 13 |
| PNE (pg/ml) | 858 ± 96 | 817 ± 97 | 608 ± 47 |
| Renin (ng/ml/hr) | 15.4 ± 2.2 | 30.4 ± 1.8 | 32.0 ± 1.4 |
| Aldo (ng/dl) | 16.5 ± 3.4 | 28.8 ± 5.9 | 16.3 ± 3.2 |
| Endothelin (pg/ml) | 7.7 ± 0.6 | 5.5 ± 0.5 | 5.2 ± 0.3 |

A=mean 1.4 days; IV nitroprusside (all orals held)

B=mean 3.4 days; after transition to roal regimen

Johnson and colleagues; J Am Coll Cardiol 2002; 39:1623-9.

Table 5B. Hemodynamic response to tailored therapy

| N=34 pts | Baseline | B |
|---|---|---|
| RA | 15 ± 1 | 8 ± 1 |
| PCWP | 31 ± 1 | 18 ± 1 |
| CI | 1.70 ± 0.08 | 2.58 ± 0.09 |
| SVR | 1780 ± 94 | 1109 ± 50 |
| SBP | 84 ± 2 | 77 ± 2 |

A=mean 1.4 days; IV nitroprusside (ACE, orals held)

B=mean 3.4 days; after transition to oral regimen

Johnson and colleagues; J Am Coll Cardiol 2002; 39:1623-9.

These data demonstrate that cardiac hemodynamics and neurohormonal activation, including activation of the CHS, are closely related. Note that the decline in ANP and BNP precedes the decline in plasma norepinephrine. It is also correlated with activation of the RAAS system as evidenced by increases in renin and aldosterone. Might this reflect a release from the suppressive actions of ANP and BNP on the RAAS? In some ways the changes in renin and aldosterone are similar to the changes seen when Wada and associated gave HS-121 to dogs with severe heart failure (Table 3), except that when the NPR-A receptor antagonist was administered plasma norepinephrine rose sharply. The slow decline in norepinephrine in the case of tailored therapy may reflect the balance between two opposing mechanisms: 1. ) tendency for norepinephrine (PNE) to increase as ANP and BNP fall and 2.) endency for PNE to fall as cardiac output improves and the increased stroke volume decreases central sympathetic outflow via baroreflexes[95]. As we shall see later, the indisputable link that Johnson and colleague's data demonstrates between hemodynamics, neurohormones and the cardiac hormonal system provides a conceptual framework within which to better understand the complex cardiorenal interactions that likely underlie the phenomenon of aggravated renal dysfunction during intensive therapy for decompensated heart failure.

***Attenuation of the physiologic effects of the cardiac hormones in advanced CHF.***

A less recognized fact in understanding BNP's role in heart failure is the recognition that as heart failure progresses, the physiologic effects of the cardiac hormones are markedly attenuated[21]. Tsutomota and colleagues examined the physiology of the cardiac hormonal system across the pulmonary vascular bed in patients with various degrees of heart failure severity ranging from NYHA Class II-IV[96]. All patients underwent a right heart catheterization to position a pulmonary-artery catheter. Samples were drawn from the pulmonary artery and pulmonary capillary wedge region to determine levels of ANP, BNP and cGMP. The patients were divided into two groups. Group I consisted of patients defined as mild heart failure O(NYHA Class II) and group 2 was defined as severe heart failure (NYHA Class III/IV). The values of ANP, BNP and cGMP were plotted for the each individual in each group. These data are shown below.

Figure 1. Correlation between plasma atrial natriuretic peptide (ANP) and cyclic guanosine monophosphate (cGMP) concentrations in the main pulmonary artery in group I (functional class II)

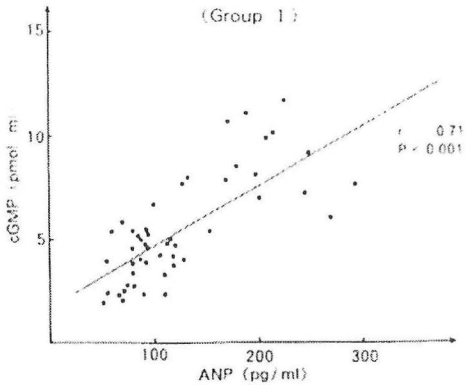
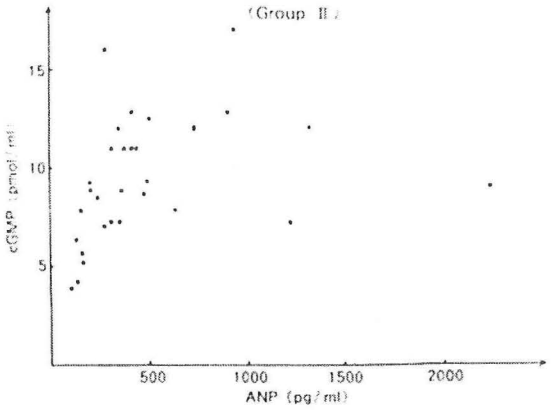
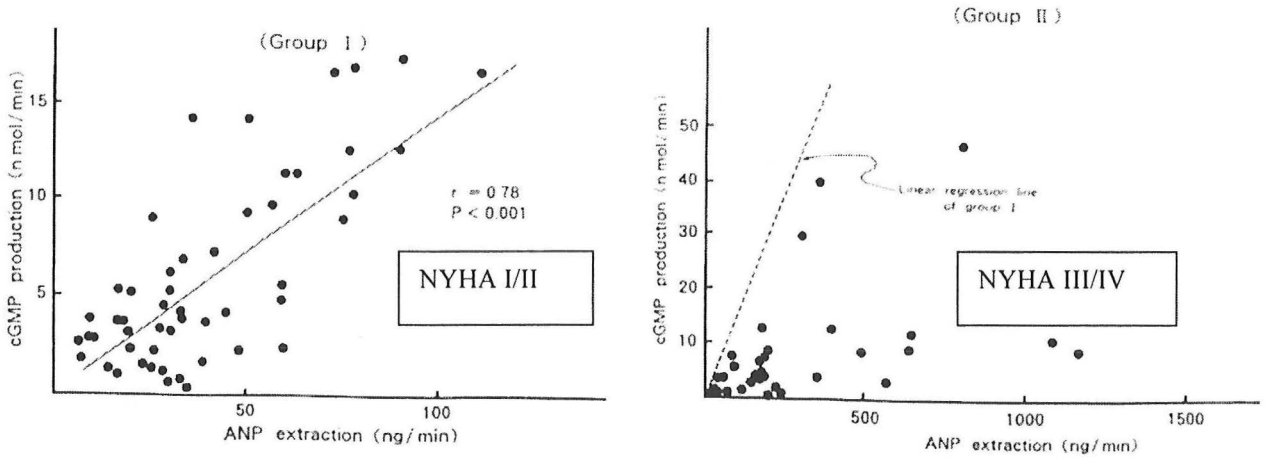



Figure 2. Comparison between plasma atrial natriuretic peptide (ANP) and cyclic guanosine monophosphate (cGMP) concentrations in the main pulmonary artery in group II (functional class III or IV).




It is demonstrated that in the group with mild CHF, there existed a positive linear correlation between the level of ANP and cGMP. The same was seen for the BNP versus cGMP relationships in the two groups (data not shown). In the group with advanced heart failure,

however, there was no correlation between the pulmonary-artery region concentration of ANP and cGMP. In fact, the average cGMP value was similar to that of the group with mild heart failure, despite a nearly five-fold elevation in ANP values, and appeared to plateau. The investigators also measured the molar extraction of ANP across the pulmonary vascular bed relative to pulmonary vascular production of cGMP. This was accomplished by measuring these values in the pulmonary artery and the pulmonary vein. As demonstrated below, patients with advanced heart failure demonstrated uncoupling of cardiac hormone extraction from cGMP production across the pulmonary vascular circuit as shown below.




***The prognostic impact of desensitization of the cardiac hormonal system's efferent limb.***

There is only one study that indirectly addressed the question as to the prognostic impact of the degree of blunting of the physiologic actions of ANP or BNP in heart failure (attenuation of the efferent limb of the CHS). Maeda and colleagues[97] followed a cohort of patients with moderate systolic heart failure over an average of 2 years. At baseline each patient had a measurement of plasma BNP and cGMP. The investigators compared the slopes of the BNP versus cGMP relationships in survivors compared to non-survivors. A reduced slope would indicate attenuated physiologic activity for the cohort because for any level of BNP the corresponding level of cGMP on average was lower. The results demonstrated a reduced slope in these relationships in non-survivors (figure 9). This suggests that patients with more severe attenuation of the efferent limb of the CHS are at increased risk for mortality. It may be the case that *in an individual patient*, the degree to which the physiologic effects of BNP are blunted may continue independent prognostic information. For example, in two otherwise similar CHF patients with identical BNP values, these data would suggest the patient with lower cGMP levels in the plasma, an indirect measure of the target tissue actions of BNP, would have a higher mortality risk.

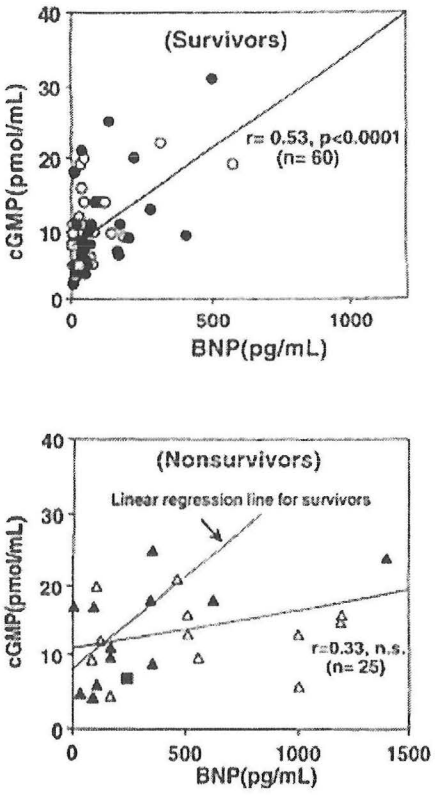



Figure 9. The baseline slope of the plasma BNP versus cGMP relationships were plotted and compared for the survivors and non-survivors. It appears that despite increased BNP values on average in the non-survivors, the average cGMP values reached a plateua at a value similar to the survivors. Moreover, the slope of the BNP versus cGMP relationship was significantly lower in the non-survivors, implying greater attenuation of the biological actions of BNP in these subjects.

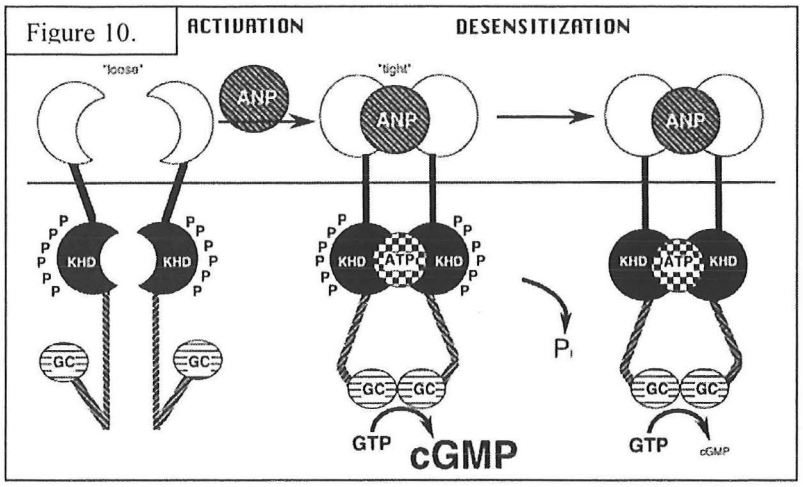
***Causes of blunted target tissue bioactivity of cardiac hormones in advanced CHF.***

There are several mechanisms leading to the reduced physiologic effects of the cardiac hormones in advanced heart failure. The degradation of the natriuretic peptides is increased in advanced heart failure due to upregulation of the clearance receptor (NPR-C) [98]and increased neutral endopeptidase activity [99]. This decreases the amount of ANP or BNP available to bind to the NPR-A receptor at the target tissue level thereby reducing the effects of the peptide hormones in target tissue. Clearly, these two mechanisms alone would explain the data from Tsutomota and colleagues [96] that demonstrated that ANP and BNP were "cleared" across the vascular bed despite no corresponding increase in cGMP production in the patients with more advanced heart failure. In addition, there is evidence for upregulation of the phosphodiesterase isoforms that degrade cGMP in patients with more advanced heart failure [100-102].

In addition, the phenomenon of *homologous desensitization* of the NPR-A receptor explains a substantial proportion of the reduced bioactivity of ANP and BNP in advanced heart failure[14, 103]. Persistent stimulation of the NPR-A receptor results in a loss of guanylate cyclase activity. The mechanisms for this receptor desensitization process are complex. Although the cardiac peptide hormones can suppress transcription of the NPR-A receptor, [104], receptor down-regulation does not appear to be the primary mechanism for receptor desensitization. The primary mechanism is *dephosphorylation* of the kinase homology domain (figure 2). Phosphorylation of the serine and threonine residues in the kinase homology domain permits ligand binding to exert a conformational change across the receptor that is activates the guanylate cyclase domain [105-107]. The conformational change that ANP and BNP cause by binding to the receptor to activate the guanylate cyclase region and convert GTP to cGMP also "exposes" the kinase homology domain's serine and threonine residues to an unidentified phosphatase, possibly protein

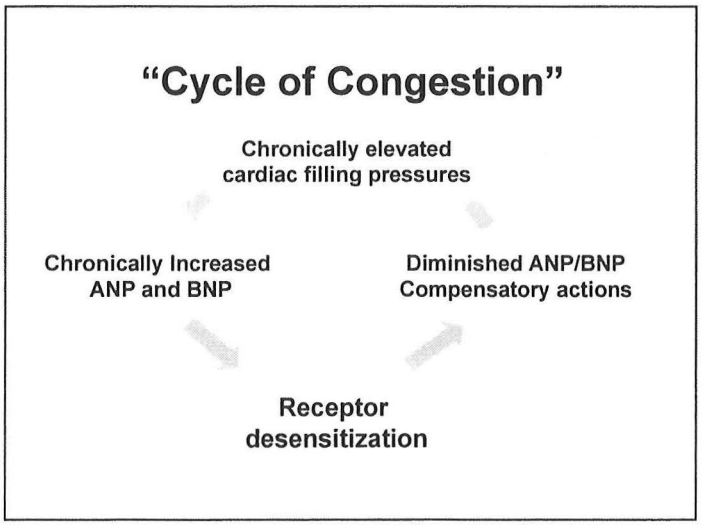
phosphatase 5[108]. This results in dephosphorylation of this domain and receptor desensitization (figure 10). Interestingly, site-directed mutagenesis studies in which glutamate was substituted for the 6 phosphorylation sites of NPR-A's kinase homology domain produced a "constitutively phosphorylated," receptor with a 10-fold increase in cGMP production upon ANP stimulation and resistance to homologous desensitization[103]. The process of de-phosphorylation as the mechanism of receptor desensitization is unique to the guanylate cyclase receptor family and the exact opposite of that seen for G-protein coupled receptors, such as the beta-adrenergic receptor.

Figure 10.




***Integrating the hemodynamic and neurohormonal paradigms of CHF progression.***

Chronic elevations in cardiac filling pressure may contribute to homologous desensitization and in doing so contribute to heart failure progression. . For example, chronic elevations in ventricular loading conditions lead to chronic increases in circulating ANP and BNP in patients with heart failure. The resultant chronic exposure of the NPR-A receptor to the levels of ANP and BNP that characterize advanced heart failure would be expected to result in homologous desensitization of the receptor. If we could prevent sustained elevations in ANP and BNP in CHF by a strategy aimed at targeting near-normal filling pressures perhaps we could reverse or prevent homologous desensitization. This is speculative at the present time, but the ongoing ESCAPE trial will provide an opportunity to evaluate the effect of reductions in cardiac filling pressure on the efferent limb of the cardiac hormonal system.



*Aggravated renal dysfunction during treatment of decompensated CHF.*

It is known that the treatment of decompensated heart failure can result in rapid and dramatic declines in ANP and BNP. It is intriguing to speculate that sustained reductions in cardiac filling pressure over time may reduce circulating ANP and BNP levels and "reverse" the desensitization of the CHS. This might impact in a beneficial way the ability to maintain fluid homeostasis. However, the abrupt declines in ANP and BNP that occur during reductions in chronically elevated cardiac filling pressures may lead to a transient period of cardiorenal instability.

The phenomenon of aggravated renal dysfunction (ARD) during the acute treatment phase of decompensated heart failure, defined as a 25% or greater increase in serum creatinine during the treatment of decompensated heart failure, may occur in 20-25% of patients. It significantly prolongs hospital length-of-stay and increases costs[109]. The clinical response to ARD, often based on incomplete knowledge of the underlying pathophysiology, may not be appropriate. For example, ARD is often attributed to "overdiuresis", and the clinical response often is to decrease or hold diuretics or even administer fluid. Another explanation is "over-vasodilation" and this may limit attempts to uptitrate ACE-inhibitors to target doses.

Recent data suggests that ARD is not explained by "overdiuresis" or abnormal hemodynamics. For example, Weinfeld and colleagues [110] examined the renal responses to tailored hemodynamic therapy in 30 patients with decompensated heart failure admitted to the cardiomyopathy service at Brigham and Women's hospital. Patients were stratified into those that did (N=5) and did not (N=25) develop ARD, defined as a 25% or greater increase in creatinine during treatment for decompensated heart failure. The patients that experienced ARD had improvements in cardiac output, SVR, and filling left and right-sided cardiac filling pressures that were indistinguishable from those that maintained renal function (Table 6). Multivariate logisitic regression analysis identified age, pre-treatment GFR and atrial fibrillation to be independently associated with the risk for ARD.

**Table 6. Aggravated Renal Dysfunction**

| | ARD (N=5) | | No ARD (N=25) | |
|---|---|---|---|---|
| | Baseline | Post | Baseline | Post |
| CVP | 11 ± 3 | 10 ± 6 | 13 ± 5 | 9 ± 4 |
| PCWP | 24 ± 4 | 20 ± 4 | 28 ± 7 | 22 ± 6 |
| CO | 4.2 ± 0.5 | 4.2 ± 5 | 3.9 ± 1.0 | 4.5 ± 1.0 |
| SVR | 1480 ± 290 | 1180 ± 230 | 1400 ± 520 | 1080 ± 240 |
| RPP | 66 ± 8 | 64 ± 10 | 64 ± 11 | 61 ± 10 |

Several lines of evidence support the hypothesis that abrupt changes in natriuretic peptide levels might directly and indirectly contribute to the development of ARD. Johnson and associates data Table 5A) as well as Maisel's data (figure 8) clearly demonstrate that tailored

hemodynamic therapy results in rapid declines in ANP and BNP within 24 hours and that the decline in ANP and BNP *precedes* the decline in norepinephrine and is associated with activation of the RAAS[94]. Interstingly, the increase in renin activity despite no adverse changes in hemodynamic parameters is similar to the increase in renin that occurred when Wada and associated administered HS-121, the NPR-A antagonist, to dogs with severe pacing-induced heart failure[58]. The final piece of supportive evidence comes from a report from the surgical literature[111]. In this study, the investigators evaluated a total of 61 patients undergoing cardiac surgery for valvular or coronary artery disease or both to test the hypothesis that the magnitude of reduction in chronically elevated left atrial pressure would be related to the decrease in post-operative renal sodium excretion. They found that the immediate post-operative decline in left-atrial pressure (from 16.7 ±1.o to 9.4 ± 0.4 mmHg) was inversely correlated with post-operative urine output ($r=0.69$; $p<0.001$) and sodium excretion ($r=0.51$; $p<0.005$). Moreover, a significant postoperative decline in ANP (from 150 ±22 to 65 ± 14 pg/ml; $p<0.01$) and increase in renin activity (from 2.5 ± 0.6 to 8.7 ± 3.2; $P<0.05$) occurred in those patients with a 7 mmHg or greater decline in left-atrial pressure.

***Other potential mechanisms explaining BNP's prognostic import in CHF.***

The association of increases with BNP with adverse prognosis in heart failure might also relate to the correlation of BNP gene transcription with activation of other pathways that influence disease progression. For example, cytokine activation, in particular interleukin-6 and TNF-alpha, can directly activate BNP gene transcription[112-114]. Cytokines have been independently associated with mortality in patients with heart failure[70, 115-118].

A highly speculative hypothesis is that BNP is associated with prognosis in heart failure as a marker for calcineurin activation. As demonstrated first by Molkentin, Olson and colleagues[119], selective overexpression of calcineurin signaling pathway is sufficient to cause cardiac hypertrophy and increase transcription of the BNP gene. There exists controversy as to whether or not calcineurin activity is markedly increased in advanced systolic heart failure[120]. Some investigators have suggested that calcineurin activity is increased and potentially harmful by contributing to abnormal function of the sarcoplasmic endoplasmic reticulumm ATPase, a critical regulator of intracellular calcium handling[121]. Phospholamban interacts and controls the activity of SERCA. When phospholamban is dehosphorylated it decreases the activity of SERCA contributing to diastolic calcium overload and diminished calcium release during systole[122]. Calcineurin when activated is a phosphatase that might contribute to dephosphorylation of phospholamban. BNP might be correlated with calcineurin activation in CHF. This remains an area of active research.

***Summary: Potential explanations for the prognostic import of elevated BNP in CHF:***

1. association with intracardiac filling pressures
2. association with degree of attenuation of efferent bioactivity
3. association with degree of calcineurin activation?
4. marker for persistent cytokine activation?
5. combination of above or other factors?

## VII. Future Research Directions

The UT Southwestern heart failure research group in cooperation with the McDermott Center's Program for Genomic Applications (PGA) and the Reynolds Cardiovascular Research Center remains very interested in a better understanding of the causes for the apparent increased risk for mortality, largely accounted for by an increased risk for CHF progression, in African-Americans with CHF, despite adjustment for a variety of potential confounders[123]. There was some speculation that this might reflect differences in the response to therapy. Recent data for beta-blockers [124]and a recent report from the SOLVD database exploring racial response to ACE-inhibitors suggest that this is not the case. It is apparent from the demographics of patients enrolled in the major heart failure clinical trials that the epidemiology of heart failure is quite different in African-Americans compared to Caucasians and that these differences might account for differences in the natural history of the disease in African-Americans with heart failure[125-128]. Our laboratory is focusing on the cardiac hormonal system and other compensatory systems to identify allelic variants within these genes that might increase susceptibility to left-ventricular hypertrophy, the transition from LVH to systolic heart failure, and progression of established systolic heart failure.

## References


1. Forssmann WG, Richter R, Meyer M. The endocrine heart and natriuretic peptides: histochemistry, cell biology, and functional aspects of the renal urodilatin system. Histochem Cell Biol 1998; 110:335-57.
2. Thibault G, Amiri F, Garcia R. Regulation of natriuretic peptide secretion by the heart. Annu Rev Physiol 1999; 61:193-217.
3. Chen HH, Burnett JC, Jr. C-type natriuretic peptide: the endothelial component of the natriuretic peptide system. J Cardiovasc Pharmacol 1998; 32:S22-8.
4. Matsuo H. Discovery of a natriuretic peptide family and their clinical application. Can J Physiol Pharmacol 2001; 79:736-40.
5. Lisy O, Jougasaki M, Heublein DM, et al. Renal actions of synthetic dendroaspis natriuretic peptide. Kidney Int 1999; 56:502-8.
6. Schirger JA, Heublein DM, Chen HH, et al. Presence of Dendroaspis natriuretic peptide-like immunoreactivity in human plasma and its increase during human heart failure. Mayo Clin Proc 1999; 74:126-30.
7. Lainchbury JG, Lisy O, Burnett JC, Jr., Meyer DM, Redfield MM. Actions of a novel synthetic natriuretic peptide on hemodynamics and ventricular function in the dog. Am J Physiol Regul Integr Comp Physiol 2002; 282:R993-8.
8. Edwards BS, Zimmerman RS, Schwab TR, Heublein DM, Burnett JC, Jr. Atrial stretch, not pressure, is the principal determinant controlling the acute release of atrial natriuretic factor. Circ Res 1988; 62:191-5.
9. de Bold AJ, Ma KK, Zhang Y, de Bold ML, Bensimon M, Khoshbaten A. The physiological and pathophysiological modulation of the endocrine function of the heart. Can J Physiol Pharmacol 2001; 79:705-14.
10. Langenickel T, Pagel I, Hohnel K, Dietz R, Willenbrock R. Differential regulation of cardiac ANP and BNP mRNA in different stages of experimental heart failure. Am J Physiol Heart Circ Physiol 2000; 278:H1500-6.
11. Yan W, Sheng N, Seto M, Morser J, Wu Q. Corin, a mosaic transmembrane serine protease encoded by a novel cDNA from human heart. J Biol Chem 1999; 274:14926-35.
12. Hooper JD, Scarman AL, Clarke BE, Normyle JF, Antalis TM. Localization of the mosaic transmembrane serine protease corin to heart myocytes. Eur J Biochem 2000; 267:6931-7.
13. Wu F, Yan W, Pan J, Morser J, Wu Q. Processing of pro-atrial natriuretic peptide by corin in cardiac myocytes. J Biol Chem 2002; 277:16900-5.
14. Potter LR, Hunter T. Guanylyl cyclase-linked natriuretic peptide receptors: structure and regulation. J Biol Chem 2001; 276:6057-60.
15. Sharma RK, Yadav P, Duda T. Allosteric regulatory step and configuration of the ATP-binding pocket in atrial natriuretic factor receptor guanylate cyclase transduction mechanism. Can J Physiol Pharmacol 2001; 79:682-91.
16. Duda T, Yadav P, Jankowska A, Venkataraman V, Sharma RK. Three dimensional atomic model and experimental validation for the ATP- Regulated Module (ARM) of the atrial natriuretic factor receptor guanylate cyclase. Mol Cell Biochem 2001; 217:165-72.
17. Hofmann F, Ammendola A, Schlossmann J. Rising behind NO: cGMP-dependent protein kinases. J Cell Sci 2000; 113:1671-6.
18. Wong KR, Xie MH, Shi LB, et al. Urinary cGMP as biological marker of the renal activity of atrial natriuretic factor. Am J Physiol 1988; 255:F1220-4.
19. Gerzer R, Witzgall H, Tremblay J, Gutkowska J, Hamet P. Rapid increase in plasma and urinary cyclic GMP after bolus injection of atrial natriuretic factor in man. J Clin Endocrinol Metab 1985; 61:1217-9.

20. Tsutamoto T, Kinoshita M, Hisanaga T, et al. Comparison of hemodynamic effects and plasma cyclic guanosine monophosphate of nicorandil and nitroglycerin in patients with congestive heart failure. Am J Cardiol 1995; 75:1162-5.
21. Tsutamoto T, Kanamori T, Wada A, Miyauchi N, Murata T, Kinoshita M. Limitations of compensation by endogenous atrial natriuretic peptide in heart failure. Jpn Circ J 1992; 56:489-93.
22. Rademaker MT, Charles CJ, Kosoglou T, et al. Clearance receptors and endopeptidase: equal role in natriuretic peptide metabolism in heart failure. Am J Physiol 1997; 273:H2372-9.
23. Pagano M, Anand-Srivastava MB. Cytoplasmic domain of natriuretic peptide receptor C constitutes Gi activator sequences that inhibit adenylyl cyclase activity. J Biol Chem 2001; 276:22064-70.
24. Lapointe N, Rouleau JL. Cardioprotective effects of vasopeptidase inhibitors. Can J Cardiol 2002; 18:415-20.
25. Floras JS. Vasopeptidase inhibition: a novel approach to cardiovascular therapy. Can J Cardiol 2002; 18:177-82.
26. Nawarskas JJ, Anderson JR. Omapatrilat: a unique new agent for the treatment of cardiovascular disease. Heart Dis 2000; 2:266-74.
27. Matsukawa N, Grzesik WJ, Takahashi N, et al. The natriuretic peptide clearance receptor locally modulates the physiological effects of the natriuretic peptide system. Proc Natl Acad Sci U S A 1999; 96:7403-8.
28. Sarzani R, Dessi-Fulgheri P, Salvi F, et al. A novel promoter variant of the natriuretic peptide clearance receptor gene is associated with lower atrial natriuretic peptide and higher blood pressure in obese hypertensives. J Hypertens 1999; 17:1301-5.
29. Lincoln TM, Dey N, Sellak H. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. J Appl Physiol 2001; 91:1421-30.
30. Silberbach M, Roberts CT, Jr. Natriuretic peptide signalling: molecular and cellular pathways to growth regulation. Cell Signal 2001; 13:221-31.
31. Francis SH, Corbin JD. Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. Crit Rev Clin Lab Sci 1999; 36:275-328.
32. Smolenski A, Burkhardt AM, Eigenthaler M, et al. Functional analysis of cGMP-dependent protein kinases I and II as mediators of NO/cGMP effects. Naunyn Schmiedebergs Arch Pharmacol 1998; 358:134-9.
33. Francis SH, Poteet-Smith C, Busch JL, Richie-Jannetta R, Corbin JD. Mechanisms of autoinhibition in cyclic nucleotide-dependent protein kinases. Front Biosci 2002; 7:d580-92.
34. Lohmann SM, Vaandrager AB, Smolenski A, Walter U, De Jonge HR. Distinct and specific functions of cGMP-dependent protein kinases. Trends Biochem Sci 1997; 22:307-12.
35. White RE. Cyclic GMP and ion channel regulation. Adv Second Messenger Phosphoprotein Res 1999; 33:251-77.
36. Beltowski J, Wojcicka G. Regulation of renal tubular sodium transport by cardiac natriuretic peptides: two decades of research. Med Sci Monit 2002; 8:RA39-52.
37. Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP. Molecular mechanism of cGMP-mediated smooth muscle relaxation. J Cell Physiol 2000; 184:409-20.
38. Surks HK, Mochizuki N, Kasai Y, et al. Regulation of myosin phosphatase by a specific interaction with cGMP- dependent protein kinase Ialpha. Science 1999; 286:1583-7.

39. Gambaryan S, Wagner C, Smolenski A, et al. Endogenous or overexpressed cGMP-dependent protein kinases inhibit cAMP- dependent renin release from rat isolated perfused kidney, microdissected glomeruli, and isolated juxtaglomerular cells. Proc Natl Acad Sci U S A 1998; 95:9003-8.
40. Shi SJ, Nguyen HT, Sharma GD, Navar LG, Pandey KN. Genetic disruption of atrial natriuretic peptide receptor-A alters renin and angiotensin II levels. Am J Physiol Renal Physiol 2001; 281:F665-73.
41. Wagner C, Pfeifer A, Ruth P, Hofmann F, Kurtz A. Role of cGMP-kinase II in the control of renin secretion and renin expression. J Clin Invest 1998; 102:1576-82.
42. Olson LJ, Lowe DG, Drewett JG. Novel natriuretic peptide receptor/guanylyl cyclase A-selective agonist inhibits angiotensin II- and forskolin-evoked aldosterone synthesis in a human zona glomerulosa cell line. Mol Pharmacol 1996; 50:430-5.
43. Kohno M, Yokokawa K, Horio T, Yasunari K, Murakawa K, Takeda T. Atrial and brain natriuretic peptides inhibit the endothelin-1 secretory response to angiotensin II in porcine aorta. Circ Res 1992; 70:241-7.
44. Lee ME, Miller WL, Edwards BS, Burnett JC, Jr. Role of endogenous atrial natriuretic factor in acute congestive heart failure. J Clin Invest 1989; 84:1962-6.
45. Redfield MM, Aarhus LL, Wright RS, Burnett JC, Jr. Cardiorenal and neurohumoral function in a canine model of early left ventricular dysfunction. Circulation 1993; 87:2016-22.
46. Studies of left ventricular dysfunction (SOLVD)--rationale, design and methods: two trials that evaluate the effect of enalapril in patients with reduced ejection fraction. Am J Cardiol 1990; 66:315-22.
47. Francis GS, Benedict C, Johnstone DE, et al. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). Circulation 1990; 82:1724-9.
48. Foody JM, Farrell MH, Krumholz HM. beta-Blocker therapy in heart failure: scientific review. Jama 2002; 287:883-9.
49. Bandyopadhyay S, O'Mahony MS. Beta-blockers in left ventricular systolic dysfunction--from evidence to practice. Age Ageing 2002; 31:23-8.
50. Sleiman OI, Murin J, Ghanem WM. Benefits of beta-blockers in heart failure. Bratisl Lek Listy 2001; 102:365-9.
51. Sleiman OI, Murin J, Ghanem WM. Beta-blockers in heart failure. Bratisl Lek Listy 2001; 102:332-7.
52. Corti R, Burnett JC, Jr., Rouleau JL, Ruschitzka F, Luscher TF. Vasopeptidase inhibitors: a new therapeutic concept in cardiovascular disease? Circulation 2001; 104:1856-62.
53. Weber MA. Vasopeptidase inhibitors. Lancet 2001; 358:1525-32.
54. Kubota E, Dean RG, Hubner RA, Balding LC, Johnston CI, Burrell LM. Evidence for cardioprotective, renoprotective, and vasculoprotective effects of vasopeptidase inhibitors in disease. Curr Hypertens Rep 2001; 3 Suppl 2:S31-3.
55. Chen HH, Cataliotti A, Burnett JC, Jr. Role of the natriuretic peptides in the cardiorenal and humoral actions of omapatrilat: insights from experimental heart failure. Curr Hypertens Rep 2001; 3 Suppl 2:S15-21.
56. Nawarskas J, Rajan V, Frishman WH. Vasopeptidase inhibitors, neutral endopeptidase inhibitors, and dual inhibitors of angiotensin-converting enzyme and neutral endopeptidase. Heart Dis 2001; 3:378-85.
57. Nathisuwan S, Talbert RL. A review of vasopeptidase inhibitors: a new modality in the treatment of hypertension and chronic heart failure. Pharmacotherapy 2002; 22:27-42.

58. Wada A, Tsutamoto T, Matsuda Y, Kinoshita M. Cardiorenal and neurohumoral effects of endogenous atrial natriuretic peptide in dogs with severe congestive heart failure using a specific antagonist for guanylate cyclase-coupled receptors. Circulation 1994; 89:2232-40.
59. Hobbs RE, Mills RM, Young JB. An update on nesiritide for treatment of decompensated heart failure. Expert Opin Investig Drugs 2001; 10:935-42.
60. Colucci WS. Nesiritide for the treatment of decompensated heart failure. J Card Fail 2001; 7:92-100.
61. Colucci WS, Elkayam U, Horton DP, et al. Intravenous nesiritide, a natriuretic peptide, in the treatment of decompensated congestive heart failure. Nesiritide Study Group. N Engl J Med 2000; 343:246-53.
62. Maisel A. B-type natriuretic peptide levels: a potential novel "white count" for congestive heart failure. J Card Fail 2001; 7:183-93.
63. Morrison LK, Harrison A, Krishnaswamy P, Kazanegra R, Clopton P, Maisel A. Utility of a rapid B-natriuretic peptide assay in differentiating congestive heart failure from lung disease in patients presenting with dyspnea. J Am Coll Cardiol 2002; 39:202-9.
64. Maisel AS, Koon J, Krishnaswamy P, et al. Utility of B-natriuretic peptide as a rapid, point-of-care test for screening patients undergoing echocardiography to determine left ventricular dysfunction. Am Heart J 2001; 141:367-74.
65. Dao Q, Krishnaswamy P, Kazanegra R, et al. Utility of B-type natriuretic peptide in the diagnosis of congestive heart failure in an urgent-care setting. J Am Coll Cardiol 2001; 37:379-85.
66. Harrison A, Morrison LK, Krishnaswamy P, et al. B-type natriuretic peptide predicts future cardiac events in patients presenting to the emergency department with dyspnea. Ann Emerg Med 2002; 39:131-8. t&artType=abs&id=a121483&target=.
67. Burger MR, Burger AJ. BNP in decompensated heart failure: diagnostic, prognostic and therapeutic potential. Curr Opin Investig Drugs 2001; 2:929-35.
68. Wu AH. B-type natriuretic peptide and its clinical utility in patients with heart failure. MLO Med Lab Obs 2001; 33:10-4; quiz 16, 19.
69. Tamura K, Takahashi N, Nakatani Y, Onishi S, Iwasaka T. Prognostic impact of plasma brain natriuretic peptide for cardiac events in elderly patients with congestive heart failure. Gerontology 2001; 47:46-51.
70. Maeda K, Tsutamoto T, Wada A, et al. High levels of plasma brain natriuretic peptide and interleukin-6 after optimized treatment for heart failure are independent risk factors for morbidity and mortality in patients with congestive heart failure. J Am Coll Cardiol 2000; 36:1587-93.
71. Yu CM, Sanderson JE. Plasma brain natriuretic peptide--an independent predictor of cardiovascular mortality in acute heart failure. Eur J Heart Fail 1999; 1:59-65.
72. Maisel A. B-type natriuretic peptide levels: diagnostic and prognostic in congestive heart failure: what's next? Circulation 2002; 105:2328-31.
73. Stevenson LW, Tillisch JH, Hamilton M, et al. Importance of hemodynamic response to therapy in predicting survival with ejection fraction less than or equal to 20% secondary to ischemic or nonischemic dilated cardiomyopathy. Am J Cardiol 1990; 66:1348-54.
74. Stevenson LW. Tailored therapy before transplantation for treatment of advanced heart failure: effective use of vasodilators and diuretics. J Heart Lung Transplant 1991; 10:468-76.
75. Stevenson LW. Tailored therapy to hemodynamic goals for advanced heart failure. Eur J Heart Fail 1999; 1:251-7.

76. Stevenson LW, Bellil D, Grover-McKay M, et al. Effects of afterload reduction (diuretics and vasodilators) on left ventricular volume and mitral regurgitation in severe congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 1987; 60:654-8.
77. Steimle AE, Stevenson LW, Chelimsky-Fallick C, et al. Sustained hemodynamic efficacy of therapy tailored to reduce filling pressures in survivors with advanced heart failure. Circulation 1997; 96:1165-72.
78. Drazner MH, Hamilton MA, Fonarow G, Creaser J, Flavell C, Stevenson LW. Relationship between right and left-sided filling pressures in 1000 patients with advanced heart failure. J Heart Lung Transplant 1999; 18:1126-32.
79. Drazner MH, Rame JE, Stevenson LW, Dries DL. Prognostic importance of elevated jugular venous pressure and a third heart sound in patients with heart failure. N Engl J Med 2001; 345:574-81.
80. Stevenson LW, Massie BM, Francis GS. Optimizing therapy for complex or refractory heart failure: a management algorithm. Am Heart J 1998; 135:S293-309.
81. Gerdes AM, Capasso JM. Structural remodeling and mechanical dysfunction of cardiac myocytes in heart failure. J Mol Cell Cardiol 1995; 27:849-56.
82. Lee TH, Hamilton MA, Stevenson LW, et al. Impact of left ventricular cavity size on survival in advanced heart failure. Am J Cardiol 1993; 72:672-6.
83. Berger R, Huelsman M, Strecker K, et al. B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. Circulation 2002; 105:2392-7.
84. Eckardt L, Kirchhof P, Breithardt G, Haverkamp W. Load-induced changes in repolarization: evidence from experimental and clinical data. Basic Res Cardiol 2001; 96:369-80.
85. Pye MP, Cobbe SM. Arrhythmogenesis in experimental models of heart failure: the role of increased load. Cardiovasc Res 1996; 32:248-57.
86. Fozzard HA. Afterdepolarizations and triggered activity. Basic Res Cardiol 1992; 87:105-13.
87. Depre C, Davies PJ, Taegtmeyer H. Transcriptional adaptation of the heart to mechanical unloading. Am J Cardiol 1999; 83:58H-63H.
88. Margulies KB. Ventricular unloading and myocyte recovery: insight gained into the pathophysiology of congestive heart failure. Curr Cardiol Rep 2000; 2:181-8.
89. Heerdt PM, Holmes JW, Cai B, et al. Chronic unloading by left ventricular assist device reverses contractile dysfunction and alters gene expression in end-stage heart failure. Circulation 2000; 102:2713-9.
90. Uray IP, Connelly JH, Thomazy V, et al. Left ventricular unloading alters receptor tyrosine kinase expression in the failing human heart. J Heart Lung Transplant 2002; 21:771-82.
91. Uray IP, Connelly JH, Frazier O, Taegtmeyer H, Davies PJ. Altered expression of tyrosine kinase receptors Her2/neu and GP130 following left ventricular assist device (LVAD) placement in patients with heart failure. J Heart Lung Transplant 2001; 20:210.
92. Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. Circ Res 2002; 90:520-30.
93. Bradham WS, Bozkurt B, Gunasinghe H, Mann D, Spinale FG. Tumor necrosis factor-alpha and myocardial remodeling in progression of heart failure: a current perspective. Cardiovasc Res 2002; 53:822-30.
94. Johnson W, Omland T, Hall C, et al. Neurohormonal activation rapidly decreases after intravenous therapy with diuretics and vasodilators for class IV heart failure. J Am Coll Cardiol 2002; 39:1623-9.
95. Floras JS. Arterial baroreceptor and cardiopulmonary reflex control of sympathetic outflow in human heart failure. Ann N Y Acad Sci 2001; 940:500-13.

96. Tsutamoto T, Kanamori T, Wada A, Kinoshita M. Uncoupling of atrial natriuretic peptide extraction and cyclic guanosine monophosphate production in the pulmonary circulation in patients with severe heart failure. J Am Coll Cardiol 1992; 20:541-6.
97. Tsutamoto T, Wada A, Maeda K, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. Circulation 1997; 96:509-16.
98. Andreassi MG, Del Ry S, Palmieri C, Clerico A, Biagini A, Giannessi D. Up-regulation of 'clearance' receptors in patients with chronic heart failure: a possible explanation for the resistance to biological effects of cardiac natriuretic hormones. Eur J Heart Fail 2001; 3:407-14.
99. Perrella MA, Margulies KB, Wei CM, Aarhus LL, Heublein DM, Burnett JC, Jr. Pulmonary and urinary clearance of atrial natriuretic factor in acute congestive heart failure in dogs. J Clin Invest 1991; 87:1649-55.
100. Walter M, Unwin R, Nortier J, Deschodt-Lanckman M. Enhancing endogenous effects of natriuretic peptides: inhibitors of neutral endopeptidase (EC.3.4.24.11) and phosphodiesterase. Curr Opin Nephrol Hypertens 1997; 6:468-73.
101. Margulies KB, Burnett JC, Jr. Inhibition of cyclic GMP phosphodiesterases augments renal responses to atrial natriuretic factor in congestive heart failure. J Card Fail 1994; 1:71-80.
102. Supaporn T, Sandberg SM, Borgeson DD, et al. Blunted cGMP response to agonists and enhanced glomerular cyclic 3',5'- nucleotide phosphodiesterase activities in experimental congestive heart failure. Kidney Int 1996; 50:1718-25.
103. Potter LR, Hunter T. A constitutively "phosphorylated" guanylyl cyclase-linked atrial natriuretic peptide receptor mutant is resistant to desensitization. Mol Biol Cell 1999; 10:1811-20.
104. Cao L, Wu J, Gardner DG. Atrial natriuretic peptide suppresses the transcription of its guanylyl cyclase-linked receptor. J Biol Chem 1995; 270:24891-7.
105. Wedel BJ, Foster DC, Miller DE, Garbers DL. A mutation of the atrial natriuretic peptide (guanylyl cyclase-A) receptor results in a constitutively hyperactive enzyme. Proc Natl Acad Sci U S A 1997; 94:459-62.
106. Foster DC, Wedel BJ, Robinson SW, Garbers DL. Mechanisms of regulation and functions of guanylyl cyclases. Rev Physiol Biochem Pharmacol 1999; 135:1-39.
107. Foster DC, Garbers DL. Dual role for adenine nucleotides in the regulation of the atrial natriuretic peptide receptor, guanylyl cyclase-A. J Biol Chem 1998; 273:16311-8.
108. Chinkers M. Protein phosphatase 5 in signal transduction. Trends Endocrinol Metab 2001; 12:28-32.
109. Krumholz HM, Chen YT, Vaccarino V, et al. Correlates and impact on outcomes of worsening renal function in patients > or =65 years of age with heart failure. Am J Cardiol 2000; 85:1110-3.
110. Weinfeld MS, Chertow GM, Stevenson LW. Aggravated renal dysfunction during intensive therapy for advanced chronic heart failure [see comments]. Am Heart J 1999; 138:285-90.
111. Shannon RP, Libby E, Elahi D, et al. Impact of acute reduction in chronically elevated left atrial pressure on sodium and water excretion. Ann Thorac Surg 1988; 46:430-7.
112. He Q, LaPointe MC. Interleukin-1beta regulation of the human brain natriuretic peptide promoter involves Ras-, Rac-, and p38 kinase-dependent pathways in cardiac myocytes. Hypertension 1999; 33:283-9.
113. He Q, LaPointe MC. Interleukin-1beta regulates the human brain natriuretic peptide promoter via Ca(2+)-dependent protein kinase pathways. Hypertension 2000; 35:292-6.

114. Harada E, Nakagawa O, Yoshimura M, et al. Effect of interleukin-1 beta on cardiac hypertrophy and production of natriuretic peptides in rat cardiocyte culture. J Mol Cell Cardiol 1999; 31:1997-2006.
115. Ferrari R. Interleukin-6: a neurohumoral predictor of prognosis in patients with heart failure: light and shadow. Eur Heart J 2002; 23:9-10.
116. Kell R, Haunstetter A, Dengler TJ, Zugck C, Kubler W, Haass M. Do cytokines enable risk stratification to be improved in NYHA functional class III patients? Comparison with other potential predictors of prognosis. Eur Heart J 2002; 23:70-8.
117. Tabet JY, Lopes ME, Champagne S, Su JB, Merlet P, Hittinger L. Inflammation, cytokines and anti-inflammatory therapies in heart failure. Arch Mal Coeur Vaiss 2002; 95:204-12.
118. Orus J, Roig E, Perez-Villa F, et al. Prognostic value of serum cytokines in patients with congestive heart failure. J Heart Lung Transplant 2000; 19:419-25.
119. Molkentin JD, Lu JR, Antos CL, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 1998; 93:215-28.
120. Lim HW, Molkentin JD. Calcineurin and human heart failure. Nat Med 1999; 5:246-7.
121. Munch G, Bolck B, Karczewski P, Schwinger RH. Evidence for calcineurin-mediated regulation of SERCA 2a activity in human myocardium. J Mol Cell Cardiol 2002; 34:321-34.
122. Munch G, Bolck B, Brixius K, et al. SERCA2a activity correlates with the force-frequency relationship in human myocardium. Am J Physiol Heart Circ Physiol 2000; 278:H1924-32.
123. Dries DL, Exner DV, Gersh BJ, Cooper HA, Carson PE, Domanski MJ. Racial differences in the outcome of left ventricular dysfunction. N Engl J Med 1999; 340:609-16.
124. Yancy CW, Fowler MB, Colucci WS, et al. Race and the response to adrenergic blockade with carvedilol in patients with chronic heart failure. N Engl J Med 2001; 344:1358-65.
125. Yancy CW. Heart failure in African Americans: a cardiovascular engima. J Card Fail 2000; 6:183-6.
126. Yancy CW. The role of race in heart failure therapy. Curr Cardiol Rep 2002; 4:218-25.
127. Yancy CW. Heart failure in blacks: etiologic and epidemiologic differences. Curr Cardiol Rep 2001; 3:191-7.
128. Yancy CW. Treatment of heart failure in African Americans: clinical update. Ethn Dis 2002; 12:S1-19-26.