afterload reducers. It is therefore, not possible to conclude that Natrecor has an adverse effect on renal function. It is quite possible that any afterload reducer has detrimental effects on renal function. As per Dr. Throckmorton's review most elevations of creatinine re-approached pre-treatment values with the longer duration of follow up.

Table Global-23: Creatinine Increases of Specified Amounts or Specified Percent.

| | Placebo and Standard Treatment (n=173) | Natrecor 0.015 ug/Kg/min (n=169) | Natrecor 0.03 ug/kg/min (n=167) | Natrecor 0.06 ug/kg/min (n=26) | p-Value |
|---|---|---|---|---|---|
| > 1.0 mg/dl increase | 5 (3%) | 9 (5%) | 11 (7%) | 0 | 0.24 |
| > 0.5 mg/dl Increase | 13 (8%) | 25 (15%) | 32 (19%) | 4 (15%) | 0.02 |
| | | | | | |
| >100% Increase | 4 (2%) | 5 (3%) | 6 (4%) | 0 | 0.68 |
| > 50% Increase | 5 (3%) | 17 (10%) | 25 (15%) | 3 (12%) | 0.003 |
| > 25% Increase | 27 (16%) | 43 (25%) | 46 (28%) | 7 (27%) | 0.04 |

Hypotension:

The adverse event profile, particularly for symptomatic hypotension does not appear to be consistent with a half-life of Nesiritide of approximately 20 minutes. The sponsor supplies some data in the Advisory Committee Briefing Document dated 11 January 1999 (Table 6-13, reproduced as Global Table 24). There were 14/169 (8.3%) patients in the 0.015 infusion regimen cohort and 23/167 (13.8%) in 0.030 ug/kg/min cohort who developed symptomatic hypotension. The median time till the onset of hypotension was approximately 6 hours. Seventeen of the 37 subjects had symptomatic hypotension that lasted more than 2 hours. Twenty-two of those with symptomatic hypotension had the infusion discontinued, but the timing of the discontinuation relative to the onset of hypotension is unclear.

Table Global - 24: Profile of Symptomatic Hypotension for Active Treatments (Adapted from Sponsor's Briefing Table 6-13)

| | Natrecor 0.015 ug/kg/min (n=169) | Natrecor 0.03 ug/kg/min (n=167) |
|---|---|---|
| Number with Hypotension | 14 ( 8.3%) | 23 (13.8%) |
| Time of Onset | | |
| < 1 Hour | 0 | 1 |
| 1 to <3 Hours | 4 | 3 |
| 3 to < 6 Hours | 3 | 7 |
| 6 to 24 Hours | 7 | 11 |
| Unknown | 0 | 1 |
| Severity | | |
| Mild | 5 | 4 |
| Moderate | 9 | 12 |
| Severe | 0 | 7 |
| Duration | | |
| ≤ 30 min | 5 | 5 |
| 31 to 60 minutes | 2 | 5 |
| 61 to 120 minutes | 2 | 2 |
| >2 to 7 Hours | 4 | 8 |
| > 7 Hours | 1 | 3 |
| Dose Not Changed | 3 | 1 |
| Dose Decreased | 3 | 8 |
| Discontinued | 8 | 14 |
| Inotrope added (Dobutamine/Dopamine) | 0 | 3 |

In summary, the onset of hypotension was slow, the duration for which hypotension persisted was long and the intensity of the hypotension was often moderate-severe. Higher infusion rates lead to excessive blood pressure decreases.

Need for Swan Ganz- Intrarterial monitoring:

Lastly, is invasive hemodynamic monitoring necessary to assure safety of Natrecor? There is not a very strong data base from which to make an empirical decision. Perhaps the only data base that would be useful would be a comparison of the safety from study #704.325 with that of study #704.326. There was absolute requirement for a Swan-Ganz in study #704.325 and an optional requirement in #704.326. Those who received a Swan-Ganz in study #704.326 did not necessarily have them inserted at the time of infusion, but may have been instrumented during the course of hospitalization.

Cardiovascular adverse events were greater in study # 704.326. Both hypotension and symptomatic hypotension was increased in study #704.326 (Table Global-23).

One argument for inserting a Swan-Ganz catheter is that there is a pressing need to balance cardiac benefit (decrease in wedge pressure) versus the drop of blood pressure before deciding on further upward titration. Since the proposed dosing instructions would not recommend upward titration, the need for a Swan-Ganz catheter appears less pressing.

Table Global –25 Selected Adverse Event Profiles Study #704.325 and #704.326 Through Day 14.

| | Study #704.325 | | | | Study #704.326 | | | |
|---|---|---|---|---|---|---|---|---|
| | PBO/ Standard Care (n=42) | Nesiritide 0.015 (n=43) | Nesiritide 0.030 (n=42) | All Subjects (n=127) | Standard Care (n=102) | Nesiritide 0.015 (n=103) | Nesiritide 0.030 (n=100) | All Subjects (n=305) |
| Any Cardiovascular | 12 (29%) | 27 (63%) | 15 (36%) | 54 (43%) | 54 (53%) | 67 (65%) | 67 (67%) | 186 (62%) |
| Hypotension | 2 (5%) | 9 (21%) | 8 (19%) | 19 (15%) | 18 (18%) | 83 (80%) | 79 (79%) | 248 (82%) |
| Symptomatic Hypotension | 0 (0%) | 2 (21%) | 5 (19%) | 7 (6%) | 7 (7%) | 11 (11%) | 24 (24%) | 42 (14%) |
| Ventricular Tachycardia | 5 (12%) | 9 (21%) | 3 (1%) | 17 (13%) | 22 (22%) | 23 (22%) | 23 (23%) | 68 (22%) |

In summary, if the approved regimen is a single infusion rate with no upward titration, no Swan-Ganz catheter appears to be necessary.

Kinetics (see Dr Sadrieh's review for more details)

The kinetic behavior of Natrecor was studied in single bolus (#704.305 and #704.312), multiple bolus (#704.309, #704.310) and intravenous infusion studies (#704.306, #704.307, #704.311 and #704.325). All studies were carried out in patients with CHF, with the exception of #704.312, which enrolled patients who were post-CABG. The kinetic profile of hBNP, measured during the intravenous bolus studies, was generally fit to a two-compartment model. Nesiritide concentrations rapidly decayed, with an initial half-life of approximately 1-2 minutes. This phase of decay accounted for approximately 30% of the AUC. The secondary half-life was approximately 20 minutes and this accounted for approximately 70% of the AUC.

No information was collected during the development of hBNP on the resultant concentrations of ANP. Since hBNP and ANP interact both at the kinetic and dynamic levels and since ANP appears more potent than hBNP (at least in increasing plasma cGMP), it is possible that some of the time course and effects of hBNP are due to alteration of ANP concentrations in the CHF population.

Pharmacodynamics:

The relationship between the hemodynamic effect and the concentration of hBNP are likely to be complex. Any effects of hBNP are likely to result not only from its own actions but also through any interaction with ANP. In addition, binding of hBNP to guanylyl cyclase sets in motion a cascade of

intracellular events (including increases in cGMP, protein kinase C and the various phosphorylated proteins). The time course of the offset of the intracellular cascade is not addressed in this submission.

The terminal kinetic half-life of hBNP, after single or multiple individual boluses, is approximately 20 minutes. The onset of hBNP's effect on PCWP in studies #704.311 (Figure Global-2, derived from p. 26 of Dr. Throckmorton's DRAFT review) and study #704.325 (Figure Global-3, derived from p. 48 of Dr. Throckmorton's DRAFT review), as well as the offset of effect after discontinuation of the infusion in study #704.311 (Figure Global- 4, derived from p. 27 of Dr. Throckmorton's DRAFT review) appears to define the dynamic half-life of hBNP as far longer than the 20 minute kinetic half-life.

Considering the lower dose groups, the drug effect at approximately 1-1.5 hours (3-5 half-lives) of the infusion, when steady state should have occurred, represents less than 50% of the 6 hour effect occurs. The lack of steady-state dynamic effect is all the more remarkable since these subjects received a bolus of drug before the start of the infusion. The bolus should be contributing to the hemodynamic effects, particularly at the early time points.

The offset kinetics of Natrecor also appear to have a greater than a 20-minute half-life. Two-hours (approximately 6 kinetic half-lives) after discontinuing treatment in study #704.311, fifty percent of the drop in the PCWP is still present (Figure Global-5).

The results of study #704.307 also suggest that the kinetic-dynamic relationship of Natrecor is complex. In this study, a total of 20 subjects received, in a crossover design, either placebo or infusions of Natrecor at increasing doses of 0.003, 0.01, 0.03, 0.1 ug/kg/min. Each of the infusion rates was maintained for 1.5 hours, except the last highest infusion rate, which was maintained for 3 hours. Midway during the study, the highest infusion rate (0.1 ug/kg/min) was discontinued and the duration of the next highest dose (now highest dose) was increased to 3 hours. Blood for hBNP measurements was collected after 60 minutes of each of the infusion rates and also one-hour after discontinuing the infusion. Invasive hemodynamics was also measured after 60 minutes of each of the infusion regimens (and also at approximately 3 hours during the highest infusion) and 1-hour after the end of the infusion.

Figure Global-3 PCWP Onset Study #704.3311

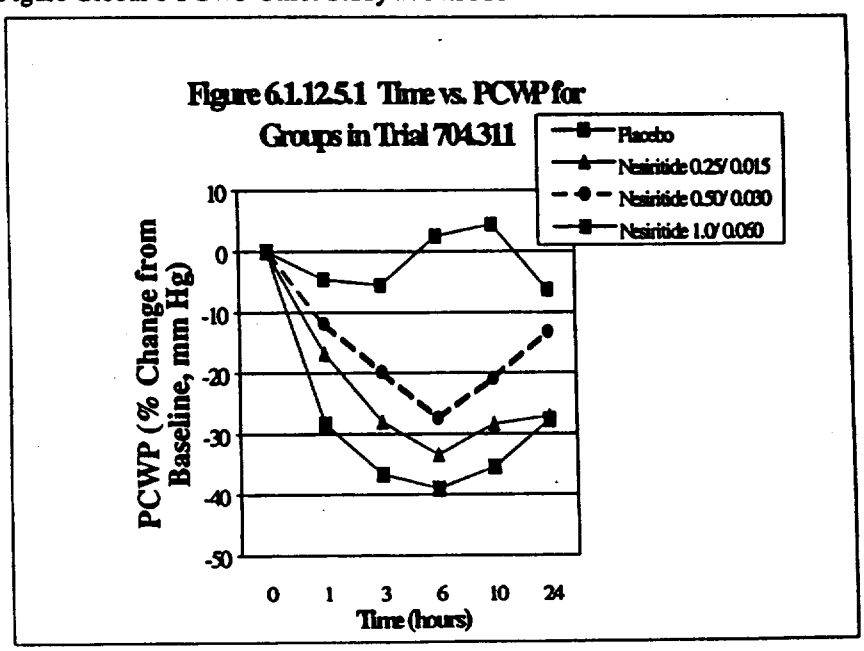


Figure Global-4 Onset Data Study #704.325

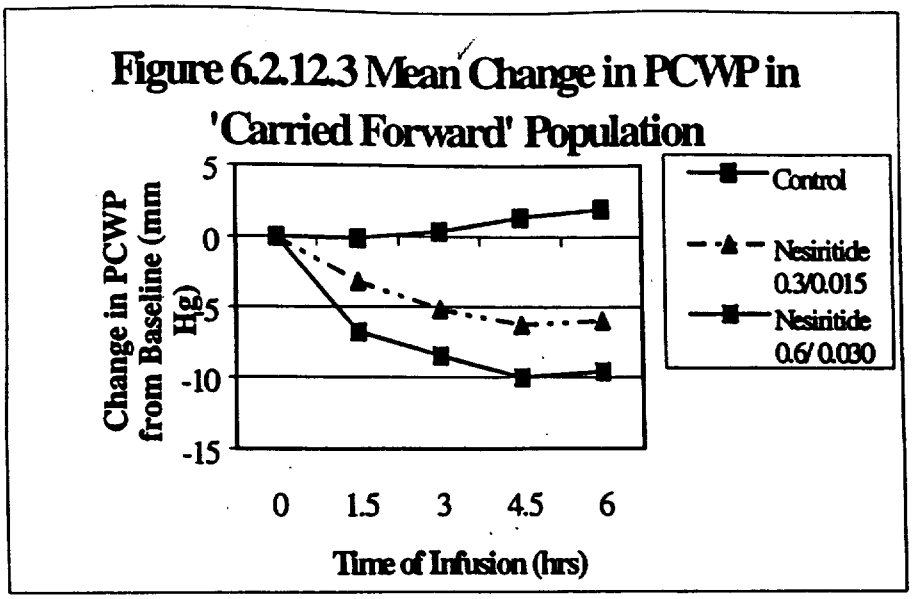
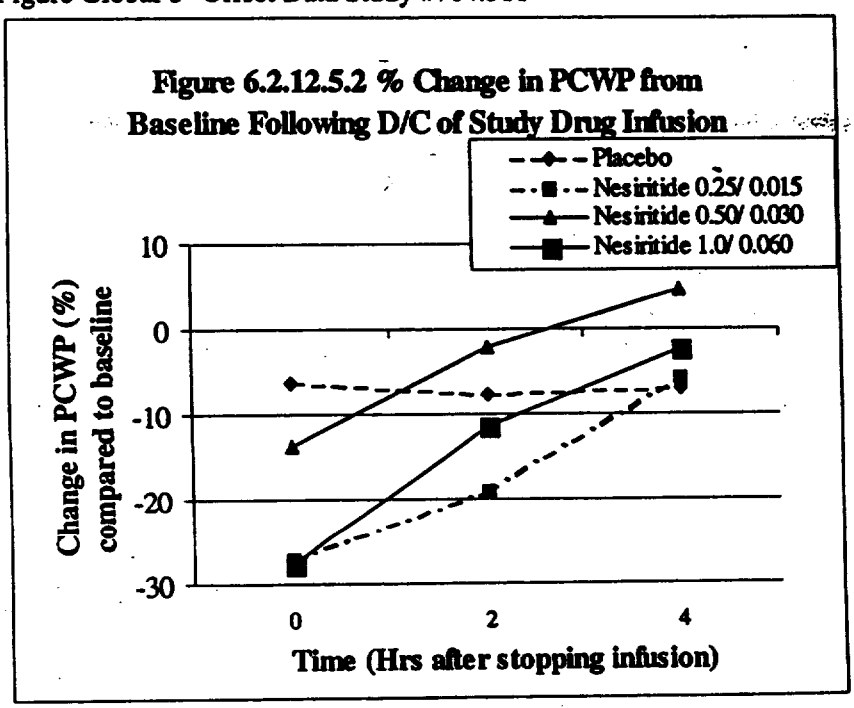



Figure Global-5- Offset Data Study #704.311




Plasma concentrations of hBNP, measured 1-1.5 hours into each infusion rate, were approximately linear with the infusion rate. The residual hBNP concentration one-hour after discontinuation of the infusion was equivalent to an infusion rate of 0.005 ug/kg/min. As the results (Table Global-26) show, the residual effect 1-hour after discontinuation of the infusion reflects effects similar to those during the 0.03 ug/kg/min

infusion rate, a factor of 6 greater than predicted. The results of this study are also consistent with a marked hysteresis effect of hBNP-concentrations and its hemodynamic effects.

Table Global- 26 Study #704.307 Hemodynamic Effect (Placebo Subtracted )at 60-90 minutes during the infusion phase and 1-hour after discontinuation of the infusion

| Parameter | Baseline Value | Natrecor Infusion Rates in ug/kg/min | | | | |
|---|---|---|---|---|---|---|
| | | 0.003 | 0.01 | 0.03 | 0.1 | 1-hour Post infusion |
| PCWP (mm Hg) | 1.7 | -3.7 | -6.8 | -10.3 | -15.5 | -12.2 |
| MRAP (mm Hg) | 0.5 | -2.1 | -2.6 | -4.7 | -5.4 | -5.0 |
| SVR (dynes*sec*cm-5) | -117 | 10 | -92 | -224 | -392 | -151 |
| CI (l/min/M2) | -0.1 | -0.1 | +0.1 | +0.3 | +0.6 | +0.1 |
| DBP (mm (Hg) | 1.5 | -4.2 | -4.1 | -7.9 | -8.6 | -9.8 |
| SBP (mm Hg) | 1.8 | -4.7 | -5.0 | -9.0 | -10.4 | -12.7 |
| Heart Rate (BPM) | -1.5 | -2.6 | -3.9 | 0.4 | 7.1 | 4. 3 |

With respect to other hemodynamic parameters, the time course for MRAP, SVR, CI, SBP and HR for study #704.311 and #704.325 are displayed in Global Figure 6 (derived from Figures 5-4 and 5-5 of the sponsor's briefing document of 11 January 1999). MRAP and SBP seem to have a time course pattern with a slow onset of effect. The time-course of these effects also appears inconsistent with the 20 minute Natrecor half-life. SVR and CI, on the other hand, appear to peak at or before the 1 to 1.5 hour measurement.

Pharmacokinetic-Pharmacodynamic Analysis:

Dr. Miller of the Agency re-analyzed the steady state portion of this study, by modeling the data to a sigmoidal E-max model [7] using NONMEM. This analysis, however, did not include the information from the single post-infusion, offset kinetic and dynamic measurements. This model predicted an EC50 of approximately 2.4-3.1 ug/ml. Concentrations in this range are generated by steady state infusions of Natrecor of 0.015 and 0.030 ug/kg/min (see p. 62 of Dr. Sadrieh's review on the concentrations generated during study #704.325). The corresponding maximum hemodynamic effects are shown in Table Global-27. There were a small number of subjects (n=6) who had hemodynamic measurements during study #704 both at 1-1.5 hours and 2.5 to 3 hours during the infusion. There did not appear to be an increase in measurable dynamic effects in going from the 1-1.5 and 2.5 –3 hours. Limiting the data to the steady state measurements, no "effect-compartment" was necessary to fit the PCWP data.

Table Global-27 Dynamic Parameters of Study 704.307 (95% Confidence Interval) "Steady State" Values

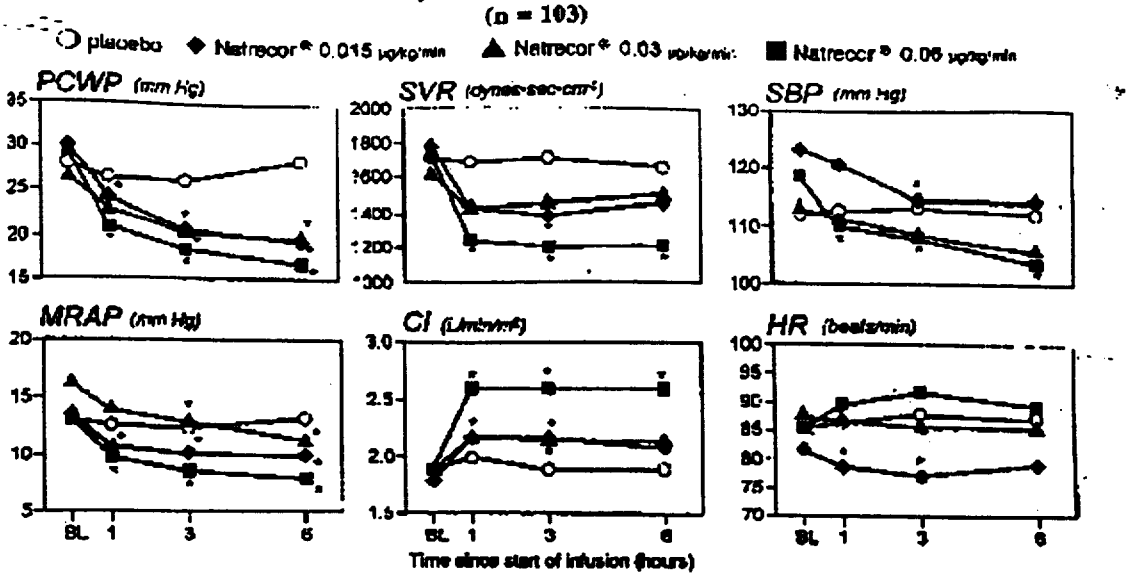
| Parameter | Baseline | Emax | EC50 pg/ml | Comments |
|---|---|---|---|---|
| PCWP (mm Hg) | 24.6 (22.4-26.7) | 16.2 (13.6-18.8) | 2400 (1500-3300) | Data reflects class II and III patients the two class IV patients had lower responses and were not included in this data |
| CI (L/min/$M^2$) | 2.02 (1.90-2.15) | 0.68 (0.27-1.08) | 3100 (700-5500) | There appeared to be a significant negative relationship between weight and CI. |
| SVR (dynes.sec.$cm^{-5}$ | 1500 (1.347-1.653) | -450 (-18 to –750) | 2400 (500-4300) | There appeared to be a significant negative effect of weight |

In summary, the onset and offset effects of Natrecor on PCWP for study #704.311, the onset of effect from study #704.325, as well as the single offset time point for study #704.307 suggest that the kinetic-dynamic relationship of Natrecor and PCWP is complex. The dynamic function half-life is

[7] The sigmoidal factor was 1, so that the equation degenerated into a standard Emax model.

Figure 5-4

**Studies 704.311**
**Hemodynamic Effects of Natrecor®**
**(n = 103)**



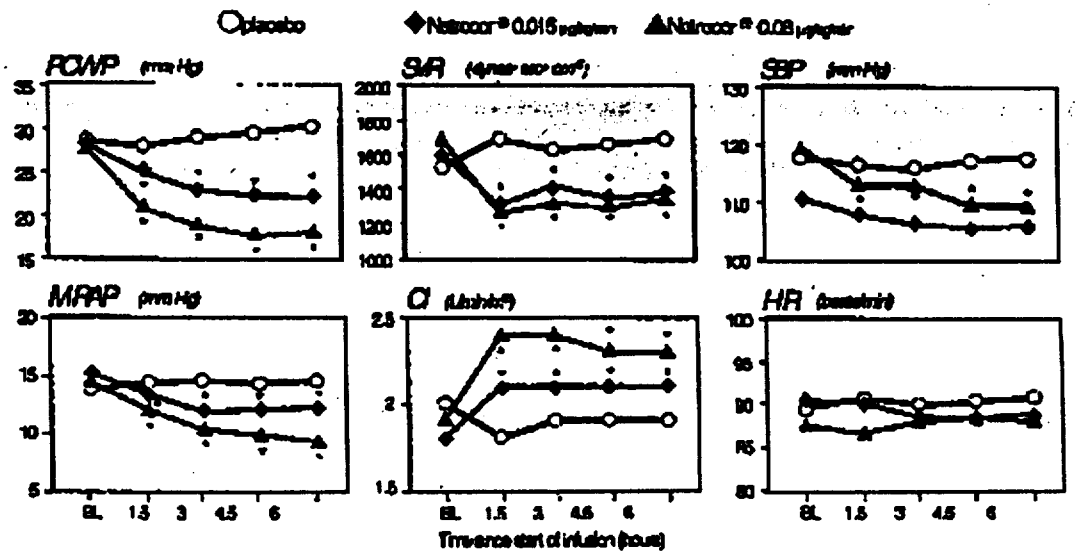

Plotted values represent treatment group means.

* $p < 0.05$ vs. placebo, by pairwise contrast within carry forward ANOVA of change from baseline



Figure 5-5

**Studies 704.325**
**Hemodynamic Effects of Natrecor®**
**(n = 127)**




Plotted values represent treatment group means.

* $p < 0.05$ vs. placebo, by pairwise contrast within carry forward ANOVA of change from baseline

Figure Global-6 Time Course of Other Hemodynamic Parameters

:

apparently much longer than the kinetic half-life. The NONMEM analysis performed by Dr. Miller is the only information that suggests an Emax relationship between kinetics and PCWP, that is dynamics and

kinetics are tightly linked. These results, however, were derived from steady-state measurements with data derived form a small number of subjects

The kinetic-dynamic relationship for other measured hemodynamics such as MRAP and SBP also appear to have a prolonged half-life till onset of effect. Still other hemodynamic parameters, most notably CI and SVR appear to peak rapidly, with maximal effects observed at the first measured time point. The time courses for the offset of these hemodynamic effects have not been analyzed.

Consequences of the Kinetic –Dynamics Effects of Natrecor:

The relationship between the kinetics and dynamics of hBNP is not optimal for a drug meant to acutely alter hemodynamics. During the course of an infusion, any modification of the infusion rate of Natrecor is not immediately, or even rapidly, translated into the corresponding hemodynamic effects. Overzealous up-titration of hBNP, in order to rapidly optimize cardiac hemodynamics, would in all likelihood overshoot, leading to profound and long lasting hypotension (the most frequently reported adverse event). The duration of time to wait prior to altering the infusion rate is left unclear by the database.

There is little or no information as to how to adjust Natrecor dosing in subjects on stable doses of concurrent vasoactive medications that may be used for the treatment of decompensated CHF. During all clinical studies these medications were discontinued from between 1 to 6 hours, before instituting treatment with Natrecor. Since the effects of Natrecor are not easily titrated, addition of this drug to ongoing treatments may overshoot and again lead to excessive hypotension.

Dosing, Dose Response and Use with Concurrent medications:

I would recommend approval of only a single regimen, consisting of a bolus of 0.3 ug/kg followed by a constant infusion of 0.015 ug/kg/min. Although hemodynamics appears to monotonically change with increases in infusion rate, signs and symptom of CHF are not additionally improved at doses greater than the 0.015 infusion regimen. Adverse events, particularly hypotension, are more frequent, more severe and more prolonged among those treated with the 0.03 ug/kg/min infusion regimen than those treated with the 0.015 regimen. A smaller proportion of patients tolerated the 0.03 than the 0.015ug/kg/min infusion regimens.

Attempting to construct a set of dosing instructions for this drug point out the weakness of this submission. Few patients in the database were titrated to higher doses to achieve a set of desired hemodynamic effects. Consequently, there is no empiric database that demonstrates any titration scheme to be safe and effective. Kinetic/dynamic models are poorly developed and poorly supported by this NDA. Consequently, basing a titration scheme, which defines the need for a bolus, the frequency at which infusion rates should be changed and the particular hemodynamic parameter upon which to base such infusion rate changes, cannot be supported either by empirical or theoretical considerations. Lower infusion rates[8] as well as higher infusion[9] rates as part of a titration scheme, would likely expand the utility of Natrecor for the

[8] With respect to the low dose-range of Natrecor, 0.003 ug/kg/min was the lowest dose that was infused (without a bolus; study #704.307). All 20 subjects who were enrolled into this study received the low (0.003 ug/kg/min) dose, which demonstrated a modest effect all hemodynamic parameters with the exception of SVR (Table Global-24). The next lowest dose (0.01 ug/kg/min) appears to decrease wedge pressure, MRAP and also SVR. Doses, possibly as low as 0.003 ug/kg/min, therefore, might be useful, particularly if infused long enough to achieve steady state.

[9] The highest dose that was infused was 0.1 ug/kg/min (study # 704.307). The sponsor discontinued this dose after nine patients were infused and two patients discontinued because of a hypotension, one that required inotropic support. During the pivotal clinical studies, patients with stable CHF received a maximal infusion rate of 0.06 ug/kg/min (n=26) and those with decompensated CHF received a maximal dose of 0.03 ug/kg/min. Of those treated with either 0.015 or 0.030 ug/kg/min infusions, the percentage of patients with hypotension that led to discontinuation was substantially more frequent and of the hypotension was of greater severity of this hypotension on the 003 than 0.015 infusion rate.

treatment of CHF. Absent the empiric database or a reliable kinetic/dynamic model, I have no way of recommending how to implement use of these doses.

Normally, I would not recommend for approval a drug with such a minimal database. However, I find it difficult to ignore the flawed but strongly suggestive benefit that Natrecor had in ameliorating the signs/symptoms of CHF. I am reasonably comfortable that the 0.015 ug/kg/min infusion regimen is sufficiently safe on its own for approval.

17 pages redacted from this section of the approval package consisted of draft labeling

# APPENDIX B
# (Reference)

*Mechanisms of Disease*

FRANKLIN H. EPSTEIN, M.D., *Editor*

# NATRIURETIC PEPTIDES

ELLIS R. LEVIN, M.D., DAVID G. GARDNER, M.D., AND WILLIS K. SAMSON, PH.D.

IN 1981, de Bold and his colleagues made the seminal observation that infusion of extracts of atrial tissue into rats caused a copious natriuresis.[1] This then led to the isolation and cloning of atrial natriuretic peptide, the first member of a family of peptides with potent natriuretic, diuretic, and vasorelaxant activity.[2] Subsequent contributions from many investigators have expanded our understanding of the family of natriuretic peptides, their receptors, and their cellular actions that regulate physiologic functions. Studies using drugs to inhibit the function of some natriuretic peptide receptors or to prevent the degradation of natriuretic peptides have confirmed the importance of these peptides. These investigations in animals and humans have established that the natriuretic peptides have a role in the body's defense against hypertension and plasma volume expansion. This review will highlight recent developments in the physiologic and pathophysiologic functions of the natriuretic peptides and the implications for their use in treating patients with cardiovascular diseases.

## BIOCHEMISTRY AND MOLECULAR BIOLOGY

The natriuretic peptide family consists of three peptides: atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide (Fig. 1). The precursor prohormone for each is encoded by a separate gene. The tissue-specific distribution and regulation of each peptide are unique.

Atrial natriuretic peptide is produced primarily in the cardiac atria. Several hormones and neurotransmitters, such as endothelin, arginine vasopressin, and catecholamines, directly stimulate the secretion of atrial natriuretic peptide. Increased atrial-wall tension, reflecting increased intravascular volume, is the dominant stimulus for its release. The messenger RNA transcript for atrial natriuretic peptide is approximately 1 kb in size and encodes a precursor protein (pro–atrial natriuretic peptide) of 126 amino acids. Cleavage of human pro–atrial natriuretic peptide releases a 98-amino-acid amino-terminal fragment, as well as a 28-amino-acid carboxy-terminal fragment that is mature atrial natriuretic peptide. Both fragments circulate in the plasma, and their concentrations are increased in patients with increased intravascular volume, such as patients with congestive heart failure. Fragments of the amino-terminal molecule also are present in plasma, and some data suggest they have biologic actions similar to those of atrial natriuretic peptide (see below).[3] Little atrial natriuretic peptide is produced by ventricular tissue in normal adults, but it is present in the ventricular tissue of fetuses and neonates and in hypertrophied ventricles.[4,5]

The atrial natriuretic peptide gene is also expressed in the kidney, in which alternative processing of the precursor generates a 32-amino-acid peptide called urodilatin.[6] Urodilatin may be important for the local regulation of sodium and water handling in the kidney.

Brain natriuretic peptide was originally identified in extracts of porcine brain. It is present in human brain, but there is considerably more in the cardiac ventricles. Human pro–brain natriuretic peptide contains 108 amino acids; processing releases a mature 32-amino-acid molecule and an amino-terminal fragment. Both circulate in the plasma, and the concentrations are high in patients with ventricular hypertrophy or congestive heart failure.

C-type natriuretic peptide is the third member of the family. Two C-type natriuretic peptide molecules, 22 and 53 amino acids in length, have been identified in vivo. Each is derived from the single pro–C-type natriuretic peptide precursor through different processes, and the 22-amino-acid form is contained within the carboxy-terminal portion of the 53-amino-acid form. The 22-amino-acid peptide predominates in the central nervous system, anterior pituitary, kidney, vascular endothelial cells, and plasma and is more potent than the 53-amino-acid form. The plasma concentration of C-type natriuretic peptide is very low.

Other related peptides include guanylin and uroguanylin. These are 15- and 16-amino-acid peptides, respectively, that are produced primarily in the gastrointestinal mucosa, in which they activate guanylyl cyclase to generate cyclic guanosine monophosphate (cGMP). These peptides may regulate salt and water transport across the intestinal mucosa, and they may also coordinate intestinal absorption with subsequent renal excretion of sodium.[7]

From the Departments of Medicine and Pharmacology, University of California, Irvine, and the Veterans Affairs Medical Center, Long Beach, Calif. (E.R.L.); the Department of Medicine and the Metabolic Research Unit, University of California, San Francisco (D.G.G.); and the Department of Physiology, University of North Dakota School of Medicine, Grand Forks (W.K.S.). Address reprint requests to Dr. Levin at the Long Beach Veterans Hospital, Medical Service (111-I), 5901 E. 7th St., Long Beach, CA 90822.

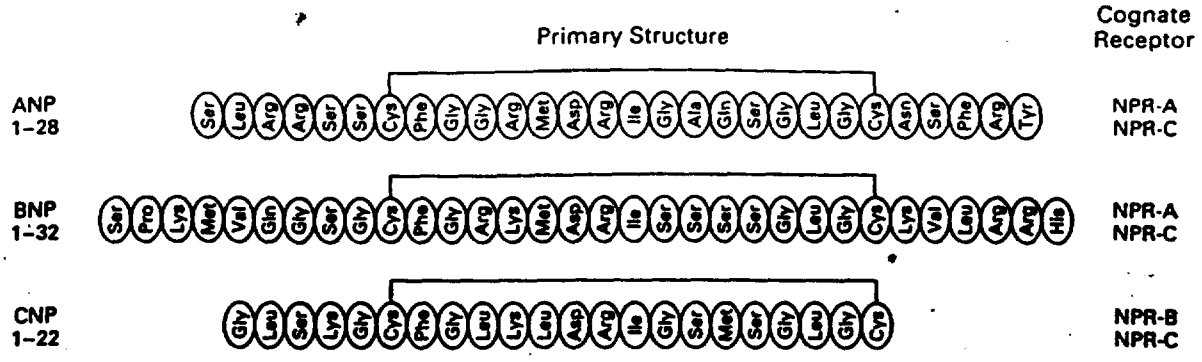



**Figure 1. Amino Acid Sequences of the Three Human Natriuretic Peptides.**

The bracket shows the location of the cystine bridges present in each peptide. The major natriuretic peptide receptors (NPRs) to which each peptide binds are listed on the right. ANP denotes atrial natriuretic peptide, BNP brain natriuretic peptide, and CNP C-type natriuretic peptide.

## NATRIURETIC PEPTIDE RECEPTORS

### Guanylyl Cyclase Receptors

The natriuretic peptides exert their effects through interaction with high-affinity receptors on the surface of target cells (Fig. 2). Three natriuretic peptide receptors (A, B, and C) have been identified in mammalian tissues. Natriuretic peptide receptors A and B are linked to the cGMP-dependent signaling cascade and mediate many of the cardiovascular and renal effects of the natriuretic peptides. Natriuretic peptide receptors A and B are structurally similar, with approximately 44 percent homology in the ligand-binding extracellular domain.[8] The A receptor binds both atrial and brain natriuretic peptides, with preference for atrial natriuretic peptide. C-type natriuretic peptide is the natural ligand for the B receptor. The A receptor is the most abundant type in large blood vessels, but there are also some B receptors. The B receptors predominate in the brain. Both receptors are present in the adrenal glands and the kidney. In both A and B receptors, the extracellular portion is linked to the intracellular portion by a single membrane-spanning segment. The intracellular portion contains a kinase-like domain, followed by the guanylyl cyclase catalytic domain. Binding of the natriuretic peptides to their receptors activates guanylyl cyclase, leading to an elevation in intracellular cGMP.

### Natriuretic Peptide–Clearance Receptor

Natriuretic peptide receptor C is involved in clearance of the peptides.[9] The natriuretic peptides bind to it and are internalized and enzymatically degraded, after which the C receptor returns to the cell surface. It is a homodimer protein in which each monomer has a single membrane-spanning segment. All three natriuretic peptides bind to this receptor with equal affinity. Circulating natriuretic peptides also are inactivated by cleavage by neutral endopeptidases present within renal tubular cells and vascular cells. Each system accounts for approximately half of natriuretic peptide turnover in sheep,[10] but their relative contributions in humans are not known.

## ACTIONS OF NATRIURETIC PEPTIDES

### Cardiovascular Actions

In animals, sustained low-dose infusions of atrial natriuretic peptide reduce peripheral vascular resistance and lower blood pressure,[11] but high doses increase peripheral vascular resistance despite the decrease in blood pressure,[12] suggesting counterregulatory activation of baroreceptors.

The atrial natriuretic peptide–dependent decrease in blood pressure results in part from a reduction in cardiac preload caused by shifting of intravascular fluid into the extravascular compartment (Fig. 3).[13] This reflects increased permeability of the vascular endothelium and perhaps increased hydraulic pressure in the capillary bed. However, extravasation of fluid into the extravascular compartment is not the sole mechanism for the reduction in preload. Atrial natriuretic peptide increases venous capacitance and promotes a natriuresis that reduces extracellular-fluid volume. The latter results from the direct effects of atrial natriuretic peptide on the kidney (see below) and from suppression of the renin–angiotensin–aldosterone axis.[14]

Atrial natriuretic peptide reduces sympathetic tone in the peripheral vasculature. This reduction is probably caused by dampening of baroreceptors, by suppression of the release of catecholamines from autonomic nerve endings, and especially by suppression of sympathetic outflow from the central nervous system.[15,16] Atrial natriuretic peptide lowers the activation threshold of vagal afferents, thereby suppressing the reflex tachycardia and vasoconstriction that accompany the reduction in preload and ensuring a sustained decrease in mean arterial pressure.

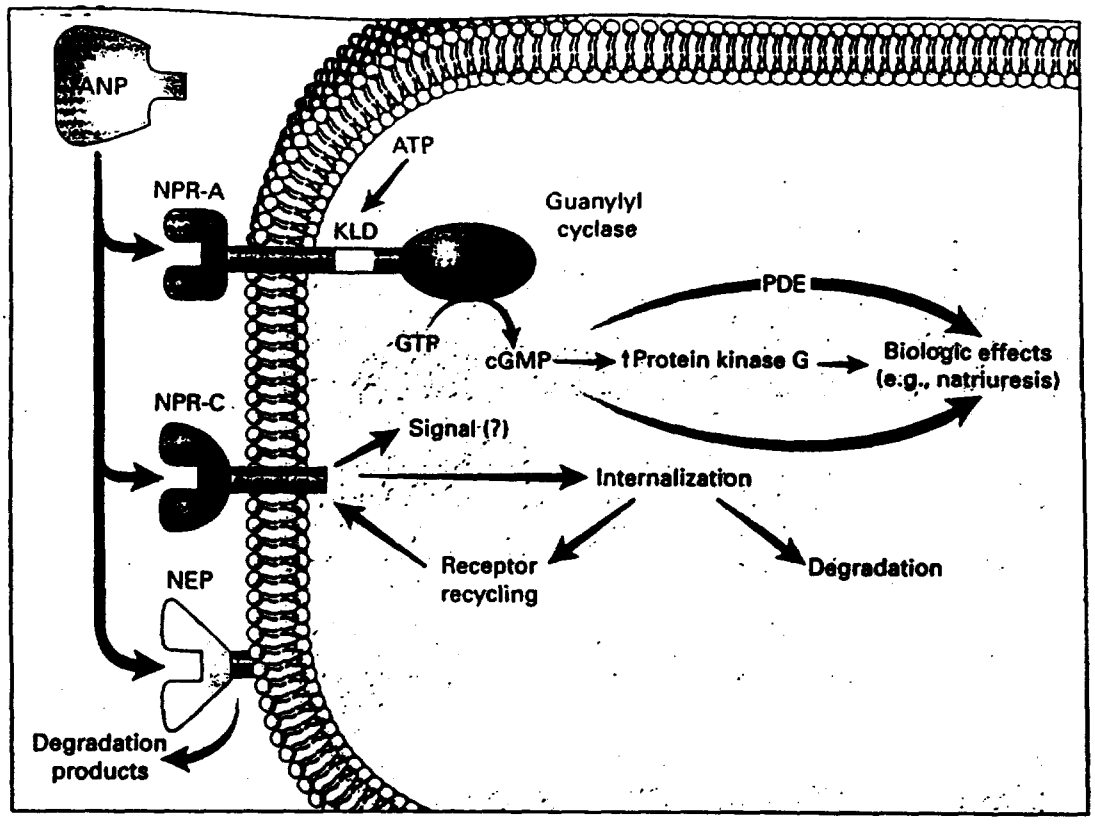



**Figure 2. Action of Atrial Natriuretic Peptide at Target Cells.**

**Atrial natriuretic peptide (ANP) binds to natriuretic peptide receptor A (NPR-A) and, in an ATP-dependent fashion, stimulates the intrinsic guanylyl cyclase activity of the receptor. Cyclic guanosine monophosphate (cGMP) exerts its biologic effects indirectly through cGMP-dependent protein kinase G or one or more phosphodiesterases (PDEs), or by direct action on effectors such as amiloride-sensitive sodium channels in the kidney. ATP dependence requires the kinase-like domain (KLD) of the receptor. Atrial natriuretic peptide also binds to natriuretic peptide receptor C (NPR-C), after which it is internalized and degraded. The C receptor may also have independent signaling functions. Finally, atrial natriuretic peptide may be degraded by the extracellular neutral endopeptidases (NEPs) in the kidney and vasculature. GTP denotes guanosine triphosphate.**

Brain natriuretic peptide has cardiovascular effects very similar to those of atrial natriuretic peptide. C-type natriuretic peptide is a more potent dilator of veins than the other two peptides.

Each natriuretic peptide has antimitogenic activity in both the cardiovascular system and other organ systems. Atrial natriuretic peptide and C-type natriuretic peptide inhibit mitogenesis in cultured vascular cells and in balloon-injured carotid arteries in rats,[17,18] mainly through a cGMP-dependent mechanism. This implies that the natriuretic peptides may modulate growth within the vascular wall in disorders such as atherosclerosis, hypertension, and postangioplasty restenosis.

### Renal Actions

The natriuretic and diuretic actions of natriuretic peptides are due to both renal hemodynamic and direct tubular actions (Fig. 3). The increase in renal blood flow caused by atrial natriuretic peptide does not last as long as the natriuretic action, suggesting two separate effects. Atrial natriuretic peptide stimulates dilatation of afferent renal arterioles and constriction of efferent arterioles, leading to increased pressure within the glomerular capillaries.[19] This increased pressure causes increased glomerular filtration. The peptide also increases the accumulation of cGMP in mesangial cells, which relaxes these cells and thereby increases the effective surface area for filtration.[20,21]

However, plasma concentrations of atrial natriuretic peptide that do not increase the glomerular filtration rate cause natriuresis, indicating that the peptide has direct tubular actions. The latter could involve locally produced natriuretic peptides (e.g., urodilatin) acting by a paracrine mechanism[6] or systemic atrial natriuretic peptide. Atrial natriuretic peptide can inhibit angiotensin II–stimulated sodium and water transport in proximal convoluted tubules.[22] In cortical col-

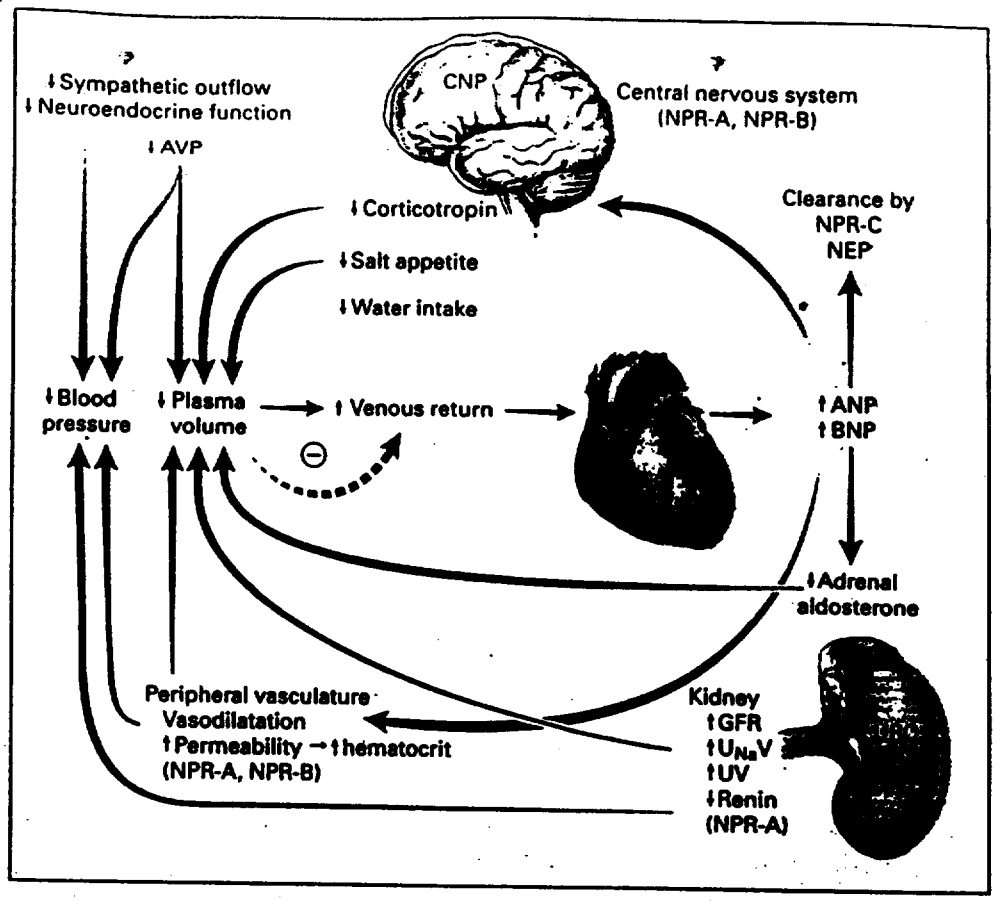



**Figure 3. Physiologic Effects of Natriuretic Peptides Released from the Heart When Venous Return Is Increased.**

Increased secretion of the natriuretic peptides reduces blood pressure and plasma volume through coordinated actions in the brain, adrenal gland, kidney, and vasculature. The minus sign indicates that a decrease in plasma volume leads to a decrease in venous return, which in turn decreases the secretion of the natriuretic peptides. URO denotes urodilatin; NEP neutral endopeptidase; CNP C-type natriuretic peptide; NPR-A, NPR-B, and NPR-C natriuretic peptide receptors A, B, and C, respectively; AVP arginine vasopressin; ANP and BNP atrial and brain natriuretic peptides, respectively; GFR glomerular filtration rate; $U_{Na}V$ urinary sodium excretion; UV urinary volume; and BP blood pressure. The receptors that mediate the functions of the natriuretic peptides are indicated in parentheses.

lecting ducts, it inhibits tubular water transport by antagonizing the action of vasopressin.[23] In the inner medullary collecting duct, it stimulates cGMP production and blocks sodium absorption.[24-27]

In humans, infusions of atrial or brain natriuretic peptide at doses that raise their plasma concentrations slightly above normal result in diuresis and natriuresis, unrelated to changes in blood pressure. These infusions reduce plasma renin and aldosterone concentrations and inhibit angiotensin II–stimulated aldosterone secretion.[13] C-type natriuretic peptide also inhibits aldosterone secretion, but it has little effect on arterial pressure or salt and water excretion.[28,29] Urodilatin, the unique renal atrial natriuretic peptide, stimulates diuresis and natriuresis at doses lower than the doses of atrial natriuretic peptide required to produce diuresis and natriuresis.[30] It appears to be more resistant to endopeptidase inactivation, which perhaps explains its relative potency and suggests an advantage over atrial natriuretic peptide as a therapeutic agent.

Studies using HS-142-1, a competitive antagonist of natriuretic peptide in binding to receptor A or B, provide additional support for the importance of these peptides in renal function.[31] In normal animals or in animals with experimentally induced heart failure, this drug blocks natriuretic peptide–induced natriuresis and diuresis, increases renal vascular resistance, and increases plasma renin, aldosterone, and catecholamine concentrations.[32-35] Similarly, the drug reduces renal plasma flow and glomerular filtration in diabetic or cirrhotic rats with ascites.[36,37] These results

imply that natriuretic peptides may have a role in the pathogenesis of renal dysfunction in these disorders.

#### Actions on the Central Nervous System

Although plasma atrial natriuretic peptide and brain natriuretic peptide do not cross the blood–brain barrier, they reach sites in the central nervous system outside this barrier (e.g., the subfornical organ, hypothalamic median eminence, and area postrema). All three natriuretic peptides, particularly C-type natriuretic peptide, are produced in the brain. Pressor hormones or amines such as endothelin,[38] vasopressin,[39] and norepinephrine,[40] but not angiotensin II, stimulate the release of atrial natriuretic peptide from cultured hypothalamic neurons. The actions of the natriuretic peptides in brain reinforce those in the periphery (Fig. 3). For example, the peripheral natriuretic effects are amplified by the central inhibition of salt appetite and water drinking,[41,42] which complements the renal diuretic effects of the peptide. Furthermore, atrial natriuretic peptide inhibits the secretion of vasopressin and, in some studies, corticotropin through effects on the brain and pituitary.[43] Each of these effects implies coordinated central and peripheral actions in controlling fluid and electrolyte homeostasis.

Natriuretic peptides act in the brain stem to decrease sympathetic tone.[15,16,44] In rats with genetic forms of hypertension, inhibiting the actions of endogenous atrial natriuretic peptide in the nucleus tractus solitarii further elevates blood pressure, suggesting that the peptide has a role in the tonic regulation of cardiovascular baroreceptor signal to this region of the brain.[16]

The mechanism of action of atrial and C-type natriuretic peptides in brain may best be explained by the distribution of receptor subtypes. The natriuretic peptide C receptor is found throughout the central nervous system, perhaps reflecting its antigrowth effects in glia.[45] The A receptor predominates in areas adjacent to the third ventricle that are not separated from the blood by the blood–brain barrier, a position that allows binding of circulating atrial natriuretic peptide as well as of centrally produced peptide.[46] This receptor appears to mediate the effects of atrial natriuretic peptide on salt appetite and water drinking. Natriuretic peptide B receptors predominate in the hypothalamus and other rostral brain regions, where the peptides inhibit secretion of arginine vasopressin and paradoxically stimulate sympathetic tone.

## PATHOPHYSIOLOGY

#### Cardiovascular Disease

The natriuretic peptides clearly defend against excess salt and water retention. Rats immunized against their own atrial natriuretic peptide cannot excrete a water load normally.[47] The roles of these peptides are perhaps best defined in patients with congestive heart failure. The cardiac hypertrophy that accompanies myocardial failure leads to increased ventricular production of atrial natriuretic peptide and brain natriuretic peptide. Their release into plasma is further stimulated by stretching of the failing atrial and ventricular myocardium and by elevated plasma concentrations of angiotensin II and endothelin-1.

In animals with congestive heart failure, the secretion of atrial natriuretic peptide inhibits the production of catecholamines, angiotensin II, aldosterone, and endothelin-1, and infusion of antagonists of natriuretic peptide A or B receptor (e.g., HS-142-1) results in marked increases in the plasma concentrations of these hormones.[35] The volume-contracting and vasodilative properties of atrial natriuretic peptide reduce systemic vascular resistance, decrease intracardiac filling pressure, and improve myocardial performance. As shown in vitro, atrial natriuretic peptide inhibits the growth of cardiac fibroblasts,[48] potentially limiting the proliferative remodeling of the heart by retarding collagen deposition. Atrial natriuretic peptide can also induce cardiac myocyte apoptosis.[49] Thus, through both direct actions and indirect actions (i.e., afterload reduction), the natriuretic peptides potentially limit the myocardial proliferative or hypertrophic response to injury or ischemia.

Patients with congestive heart failure have high plasma concentrations of atrial and brain natriuretic peptides. The concentrations are correlated with the extent of ventricular dysfunction, rising by as much as a factor of 30 in patients with advanced heart disease (New York Heart Association class IV).[50] Increasing plasma natriuretic peptide concentrations are correlated with the development of cardiac arrhythmias and the degree of hemodynamic compromise, and high concentrations predict poor long-term survival.[51] Plasma brain natriuretic peptide concentrations may correlate with outcome more closely.[52] The role of C-type natriuretic peptide, if any, in heart failure is not known.

In early left ventricular dysfunction, activation of the renin–angiotensin–aldosterone system and renal sympathetic nervous system is inhibited by atrial natriuretic peptide. Blocking this action of the peptides results in accelerated progression to overt heart failure,[53] further indicating the importance of atrial natriuretic peptide in maintaining renal perfusion and urine flow. However, renal responsiveness to natriuretic peptides decreases as heart failure worsens, even as the plasma concentrations of the peptides rise. This probably reflects changes in renal hemodynamics and a combination of receptor down-regulation and increased cGMP phosphodiesterase activity.[54] This decreased responsiveness leads to enhanced local actions of angiotensin II and the sympathetic nervous system in the kidney, resulting in salt retention and further deterioration of cardiac function.[55]

Similarly, relative unresponsiveness to endogenous atrial natriuretic peptide may also contribute to the volume overload associated with acute renal failure.

### Hypertension

Studies in rodents have defined the role of the natriuretic peptides in preventing the development of hypertension. Transgenic mice overexpressing the genes for atrial natriuretic peptide[56] or brain natriuretic peptide[57] have plasma natriuretic peptide concentrations that are at least 10 times higher than those in normal littermates, and their systolic blood pressure is 20 to 30 mm Hg lower. Transgenic mice overexpressing atrial natriuretic peptide do not develop pulmonary hypertension when exposed to chronic hypoxia, a finding that implicates this peptide in the defense against this disorder.

Findings in mice with inactivation of the gene for atrial natriuretic peptide are also evidence that this peptide has a role in the defense against elevated blood pressure. Animals with homozygous inactivation of the atrial natriuretic peptide gene that are fed a low-salt diet have slightly elevated basal blood pressure, and it rises markedly when they are fed more salt.[58] Heterozygotes have normal basal blood pressure, but it rises when they are fed a very high salt diet.

Therefore, even partial deficiency of atrial natriuretic peptide impairs the ability to maintain normal blood pressure. The renal and electrolyte response to salt loading is not greatly impaired in either homozygotes or heterozygotes, however, indicating compensation for the loss of these atrial natriuretic peptide actions in the kidney.[59] The ability of atrial natriuretic peptide to defend against salt-induced hypertension probably reflects several actions, including natriuresis, vasodilatation, and inhibition of sympathetic tone.[15]

Cardiac enlargement is routinely found in homozygous atrial natriuretic peptide knockout mice. Thus, deficiency of atrial natriuretic peptide may amplify the humoral or local cardiac-growth–stimulating effects of hypertension in these animals.

Disruption of the natriuretic peptide A receptor in mice also leads to hypertension, but the phenotype differs from that of atrial natriuretic peptide knockout mice. Mice with inactivation of the A receptor gene have elevated basal blood pressure, but do not respond to salt loading with additional increases in blood pressure.[60] Additional studies in these animals suggest that atrial natriuretic peptide acts through the A receptor in the kidney to excrete sodium and water after volume expansion.[61]

The differences in blood-pressure response to salt loading in the two types of mice suggest that in mice lacking atrial natriuretic peptide, another natriuretic peptide (brain natriuretic peptide?), presumably acting through the A receptor, prevents hypertension under low-salt conditions. This compensation would be missing in natriuretic peptide A receptor knockout mice. In the latter mice, high blood pressure in the basal state may activate regulatory mechanisms that prevent further elevations after salt loading. Whether mutations of the natriuretic peptides or their receptors contribute to the development of hypertension in humans is not known.

Natriuretic peptides clearly have a role in the response to increased sodium retention caused by an excess of mineralocorticoids. When aldosterone is hypersecreted or exogenous mineralocorticoid is administered, sodium is retained for only a few days, after which there is escape from the sodium-retaining action of the mineralocorticoid. The concentration of plasma atrial natriuretic peptide, but not of brain natriuretic peptide, rises coincident with escape.[62] Administration of HS-142-1 to rats significantly impairs urinary salt and water excretion and amplifies the increased blood-pressure response after the administration of exogenous mineralocorticoid.[34,62] Collectively, these findings identify an important role of atrial natriuretic peptide in defending against mineralocorticoid-induced and salt-induced hypertension.

## THERAPEUTIC USES

Several studies in humans have examined the efficacy of atrial natriuretic peptide in the treatment of disorders as divergent as hypertension, renal insufficiency, and congestive heart failure. Administration of atrial natriuretic peptide reduces blood pressure and promotes sodium excretion in patients with essential hypertension.[63] It also lowers blood pressure and improves central hemodynamics (including the cardiac index) in patients with chronic heart failure.[64]

Atrial natriuretic peptide, in the form of anaritide (amino acid fragment 102 to 126), has been investigated as therapy in patients with acute renal failure. In a multicenter, randomized, placebo-controlled trial involving 504 critically ill patients with acute renal failure, the patients with oliguric renal failure had improved dialysis-free survival 21 days after treatment.[65] However, for the group as a whole, atrial natriuretic peptide did not improve dialysis-free survival, and in patients with nonoliguric renal failure it may have been detrimental.

A number of neutral endopeptidase inhibitors capable of inhibiting the degradation of atrial natriuretic peptide have been developed, including several that are active when taken orally. Treatment of humans with these inhibitors leads to the expected increase in plasma atrial natriuretic peptide concentrations and sodium excretion. In patients with chronic congestive heart failure, administration of the neutral endopeptidase inhibitor candoxatrilat significantly increased sodium excretion.[66] The magnitude of the effect was closely related to base-line cardiac output, implying that the maintenance of renal perfusion is important for drug efficacy. There was also a sus-

tained drop in left and right atrial pressures mediated, at least in part, by the inhibition of neurohormonal activity. Paradoxically, one group found that higher doses of candoxatrilat induced systemic vasoconstriction rather than vasodilatation, with an increase in systemic vascular resistance and a decrease in the cardiac index.[67] Because neutral endopeptidase inhibitors impair the degradation of angiotensin II, inhibitors of angiotensin-converting enzyme or angiotensin II receptor may augment the beneficial effects of neutral endopeptidase inhibitors when given in combination with them. The overall evidence to date suggests that these drugs are likely to be beneficial in selected patients with congestive heart failure.

## CONCLUSIONS

The natriuretic peptides defend against excess salt and water retention, inhibit the production and action of vasoconstrictor peptides, promote vascular relaxation, and inhibit sympathetic outflow. These actions lead to a reduction in blood pressure that is most apparent in states of volume excess. These peptides may also restrain cardiac growth or the development of compensatory cardiac hypertrophy. By binding to all three classes of receptors, the natriuretic peptides act in concert to regulate cardiovascular function. Administration of natriuretic peptides and maneuvers that enhance their cellular actions or prevent their degradation continue to be evaluated for therapeutic efficacy in patients with heart failure and may be useful in other states of excessive intravascular volume. If successful, manipulating the natriuretic peptide environment may form the basis for new strategies to control the manifestations of cardiovascular disease.

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# Review of Pharmacokinetic/Pharmacodynamic Non-pivotal Studies.

Abraham Karkowsky, M.D., Ph.D. /S/

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HFD 110 CSO /AKarkowsky/ DThrockmorton/
NBadrich/ L. Ceci.

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Summary

This review contains the results of six small Natrecor studies (# 704.305, #704.306, # 704.307, # 704.309, # 704.310, # 704.312). None of the conclusions that are drawn from these studies can be stated with anything resembling strong conviction. The consequent conclusions, however, do not strongly differ from larger and better-controlled studies, which were reviewed by Dr. Throckmorton. Below are summarized some of these lukewarm conclusions derived from the above 6 studies.

- Plasma concentrations of Natrecor, when this drug is administered as an intravenous bolus, decays biphasically. The duration of kinetic sampling was too short and the concentrations of Natrecor at later time-points approach those of endogenous hBNP. It is, therefore, not possible to exclude higher order decay processes or measure them with any degree of accuracy.
- The half-life of the first decay process is rapid (~1minutes). The half-life of the second decay process is approximately 20 minutes. The AUC of the second decay process represents approximately 70% of the total AUC.
- When Natrecor is administered either as a bolus or a constant infusion to patients with CHF and systolic dysfunction, PCWP, MRAP and SVR decrease. Cardiac output increases slightly.
- When Natrecor is administered either as a bolus or as a constant infusion to patients with CHF or patients who are post-surgery for CABG, both systolic and diastolic blood pressures decrease and heart rate increases.
- When dynamic effects such as PCWP, Cardiac Index and SVR are modeled to measurements, which were collected during the ascending phase of a dose escalation study, the data are well fit to a sigmoidal $E_{max}$ model.
- $EC_{50}$ values for PCWP, CI and SVR were approximately 2.4-3.1 ng/ml. $E_{max}$ for PCWP, CI and SVR are 16.2 mm Hg, 0.68 L/min/M2 and –450 dynes*sec*$cm^{-5}$, respectively.
- There is, however, an apparent dissociation between serum concentrations and hemodynamic effect when considering the single post-infusion time point of this ascending infusion study. Despite substantial drops in plasma hBNP concentrations there appeared to be little recovery of PCWP, SVR, blood pressures and heart rate.
- Following single intravenous boluses of Natrecor, peak effects on PCWP, CO (or CI) and SVR appear to peak at 30-60 minutes, well after peak concentrations of Natrecor have substantially decayed.
- No patients died during the infusion period. There were three patients who died between 6 and 30 days post infusion; two, placebo subjects and one Natrecor Bolus patient.
- Adverse events, which occurred in those treated with drug, included hypotension. Among those treated post CABG in an uncontrolled data base oliguria occurred in 4 patients among those treated with boluses of Natrecor.

In five of these six studies which are reviewed in this document, Natrecor was administered to patients with CHF, NYHA Class II-IV. The sixth study was a dose-exploring, open-label study with patients who were hypertensive immediately post-coronary artery bypass surgery (#704.312).