

*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

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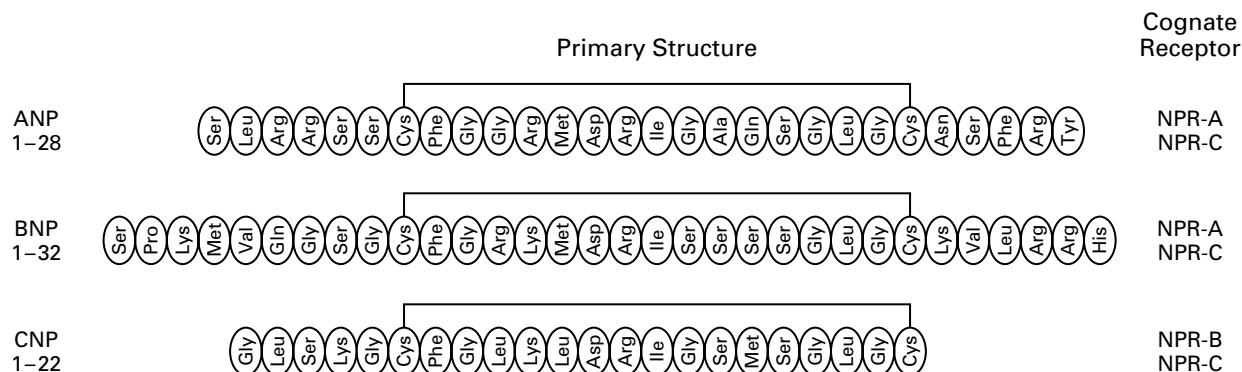


Figure 1. Amino Acid Sequences of the Three Human Natriuretic Peptides.

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.

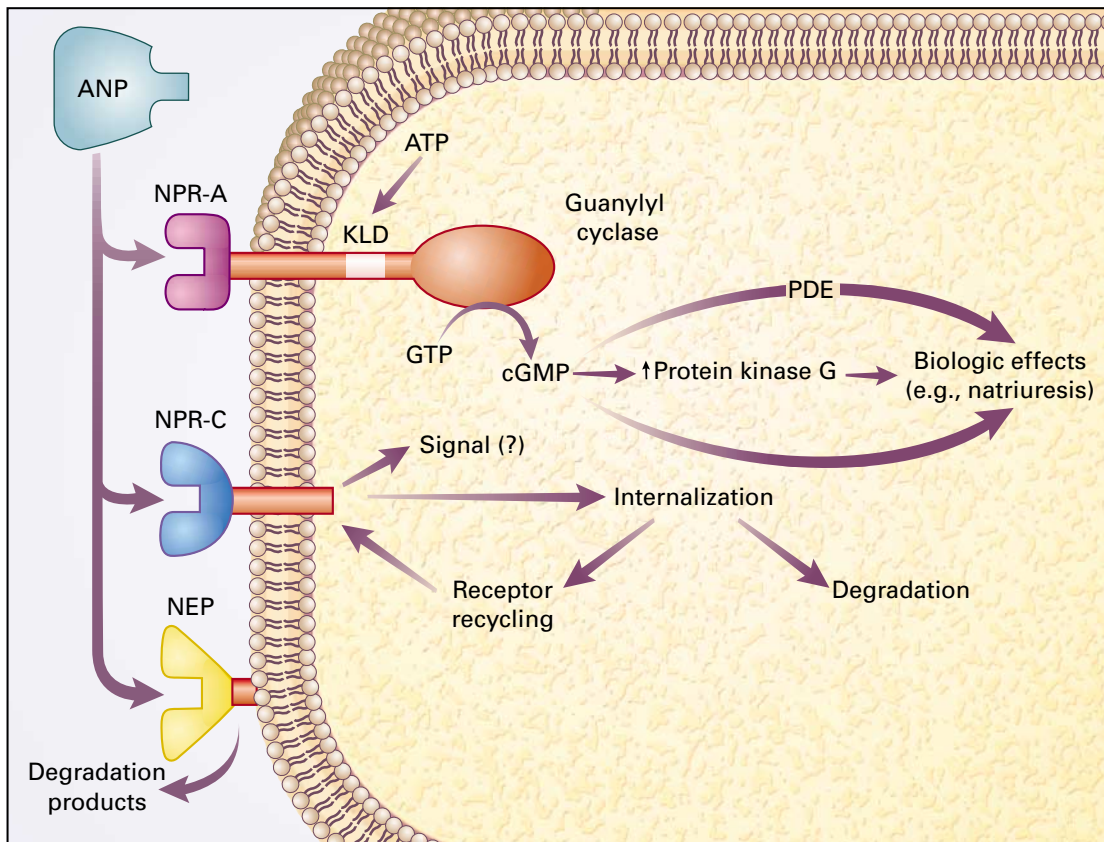


Figure 2. Action of Atrial Natriuretic Peptide at Target Cells.

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 post-angioplasty 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 mechanism⁶ 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-

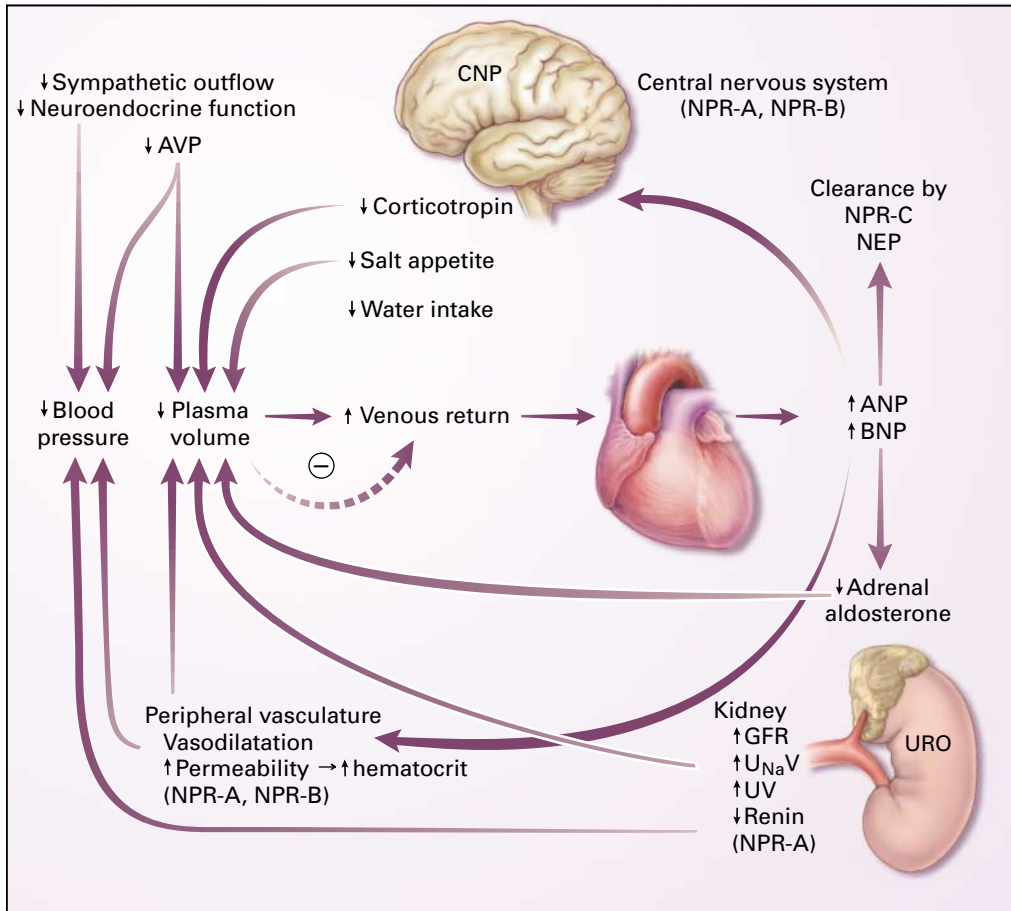


Figure 3. Physiologic Effects of Natriuretic Peptides Released from the Heart When Venous Return Is Increased. Increased secretion of the natriuretic peptides reduces blood pressure and plasma volume through coordinated actions in the brain, adrenal gland, kidney, and vasculature. The minus sign indicates 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_{NaV} 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 pep-

tide 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 solitarius 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 peptide⁵⁶ or brain natriuretic peptide⁵⁷ 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 knock-

out 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.

Supported by grants from the Medical Research Service of the Department of Veterans Affairs, the National Institutes of Health, and the National Science Foundation.

We are indebted to Dr. Eric Espiner for his comments.

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