

STAYING CURRENT

The renal and cardiovascular effects of natriuretic peptides

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Wong PC, Guo J, Zhang A. The renal and cardiovascular effects of natriuretic peptides. *Adv Physiol Educ* 41: 179–185, 2017; doi: 10.1152/advan.00177.2016.—The landmark report by de Bold et al. in 1981 signified the heart as one of the endocrine organs involved in fluid and salt balance (de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. *Life Sci* 28: 89–94, 1981). Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are secreted from cardiomyocytes in response to cardiac stretch as in the case of heart failure, whereas C-type natriuretic peptide (CNP) is secreted from endothelial and renal cells in response to cytokines and endothelium-dependent agonists, such as acetylcholine. Binding ANP or BNP to natriuretic peptide receptor A induces cyclic guanylyl monophosphate as second messenger in the target cells to mediate the following: natriuresis; water diuresis; increasing glomerular filtration rate; decreasing systemic sympathetic activities; plasma volume; cardiac output and blood pressure; and curbing mitoses of heart fibroblasts and hypertrophy of cardiovascular muscle cells. ANP, BNP, and CNP are cleared from the bloodstream by natriuretic peptide receptor C and degraded by an ectoenzyme called neprilysin (NEP). The plasma levels of BNP are typically >100 pg/ml in patients with congestive heart failure. Sacubitril/valsartan is an angiotensin receptor NEP inhibitor that prevents the clinical progression of surviving patients with heart failure more effectively than enalapril, an angiotensin-converting enzyme inhibitor. A thorough understanding of the renal and cardiovascular effects of natriuretic peptides is of major importance for first-year medical students to gain insight into the significance of plasma levels of BNP in patients with heart failure.

atrial natriuretic peptide; ANP; BNP; CNP; natriuresis; natriuretic peptide receptors

KISCH (23) revealed dense granules in the atrial myocytes of guinea pigs in 1956. Twenty years later, Marie et al. (30) showed a progressive decrease in the atrial-specific granules in rats subjected to different types of water and sodium load. In 1981, de Bold et al. (9) reported that intravenous injection of atrial extract induced a rapid and potent natriuretic response in rats, signifying the heart as one of the endocrine organs involved in fluid and salt balance (36). Subsequently, a 28-amino acid peptide with diuretic and natriuretic activity was purified from rat and human atrial muscle named as atrial natriuretic factor and α -human atrial natriuretic polypeptide, respectively (14, 21). Atrial natriuretic peptide (ANP) is synthesized and stored in the atrial granules as a prohormone with 126 amino acids, proANP, which is split by corin, a trypsin-like serine protease on cell surface, into an amino terminal portion, NT-proANP1–98, and an active hormone, ANP99–126, or ANP1–28, which is secreted in response to increased

tension of the atrial wall (Fig. 1) (26, 29, 41, 44, 57). Secretion of ANP can also be affected by hypoxia, age, sex, renal function, change of heart rate, and stimuli of glucocorticoids, vasopressin (anti-diuretic hormone), endothelin A, angiotensin II (ANG II), and catecholamines (5, 26, 40, 44).

With a pharmacological spectrum similar to that of ANP, brain natriuretic peptide (BNP) was first isolated in the porcine brain in 1988 and in the human atrium in 1990 (20, 54). Although low levels of BNP are stored with ANP in atrial granules, the cardiac ventricle rather than the atrium is the main source of plasma BNP in normal subjects and patients with congestive heart failure (CHF), whereas the reverse is true in the case of left ventricular hypertrophy without systolic dysfunction (34, 35). BNP is deglycosylated from a 108-amino acid prohormone, proBNP, and further processed by furin, an intracellular pro-protein convertase, and corin into an amino terminal fragment, NT-proBNP1–76, and a biologically active peptide, BNP77–108, or BNP1–32 (Fig. 1). BNP is also produced by the cardiac fibroblast on which it exerts its anti-fibrotic action (26, 29, 41, 44, 57).

C-type natriuretic peptide (CNP) was initially identified in porcine brain in 1990, and CNP immunoreactivity was shown to be positive in human endothelial cells, renal cells, and plasma (29, 32, 52, 56). Cleavage of a prohormone with 103 amino acids, proCNP, by proteolytic enzymes, such as furin, produces an amino terminal fragment of NT-proCNP1–81 (50) and one of the two biologically active peptides, CNP1–22 and CNP1–53 (Fig. 1) (26, 29, 41, 44). Transforming growth factor- β markedly stimulates expression of CNP mRNA and increases CNP secretion in cultured bovine endothelial cells (56).

A version of ANP, urodilatin, is produced in renal distal tubules with a local function in sodium and volume regulation in the kidney (59).

ANP, BNP, and CNP are phylogenetically, functionally, and structurally related peptides for the regulation of fluid homeostasis in vertebrates, and each of them has a 17-amino acid ring formed by a disulfide bond (Fig. 1) (59).

Natriuretic Peptide Receptors

Both natriuretic peptide receptor A (NPR-A) and natriuretic peptide receptor B (NPR-B) contain an extracellular ligand-binding domain, a single transmembrane spanning region, an intracellular domain consisting of a protein kinase-like homology domain, and a particulate guanylyl cyclase (GC) catalytic domain. Binding ANP or BNP to NPR-A induces the intracellular portion of particulate GC to generate cGMP from guanosine triphosphate. As an intracellular second messenger, cGMP then activates protein kinase G (PKG) and phosphodiesterase for the target cells to exert effects (Fig. 1) (12, 26, 41, 57–59).

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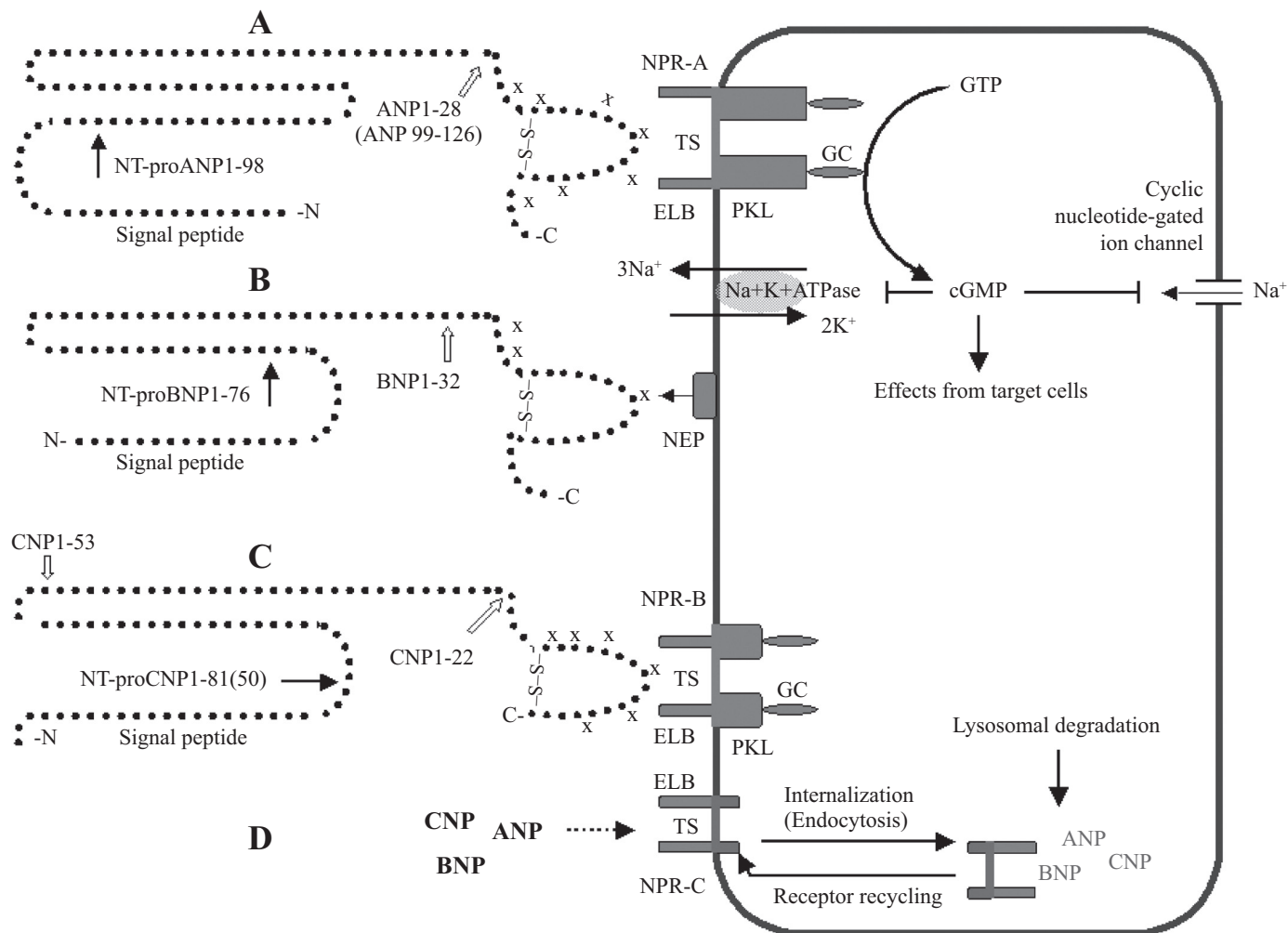


Fig. 1. Schematic structures of natriuretic peptides and their receptors. *A*: proANP1–126 with its signal peptide is cleaved to NT-proANP1–98 and the active hormone ANP1–28 by corin, a trypsin-like serine protease on cell surface (open arrow). Binding ANP to the extracellular ligand-binding (ELB) domain of natriuretic peptide receptor A (NPR-A) induces the particulate guanylyl cyclase (GC) catalytic domain to generate cGMP from guanosine triphosphate (GTP) as second messenger to inhibit cyclic nucleotide-gated ion channels and Na⁺-K⁺-adenosine triphosphatase (Na⁺-K⁺-ATPase), as well as activate protein kinase G and phosphodiesterase for target cells to exert effects. *B*: proBNP1–108 with its signal peptide is cleaved to NT-proBNP1–76 and the active hormone BNP1–32 by furin, an intracellular pro-protein convertase, and corin (open arrow). *C*: proCNP1–103 with its signal peptide is cleaved to NT-proCNP1–81 (50) and the active hormones CNP1–22 and CNP1–53 by proteolytic enzymes such as furin (open arrows). x, Site of neprilysin (NEP) action (29, 57, 58). *D*: binding ANP, BNP, or CNP to natriuretic receptor C (NPR-C) induces internalization (endocytosis), resulting in lysosomal degradation of ANP, BNP, and CNP, and reappearance of the NPR-C in the cell membrane (43, 58). NPR-B, natriuretic peptide receptor B; PKL, protein kinase-like homology domain; TS, transmembrane spanning region.

NPR-A and NPR-B are found in the kidney, vascular smooth muscle, adrenal gland, the heart, and the brain. NPR-A has greater affinity for ANP than BNP and CNP, whereas NPR-B prefers CNP to ANP and BNP (Fig. 1) (26, 58).

Natriuretic peptide receptor C as a clearance receptor. In 1987, Maack et al. (28) reported that they injected C-ANP (4–23), a ring-depleted analog of ANP, into isolated perfused rat kidney and detected no renal effects, although C-ANP (4–23) binds to 99% of the receptors there. When they injected C-ANP (4–23) into the intact anesthetized rats, the rats responded with markedly increased plasma levels of endogenous immunoreactive ANP and increased sodium excretion. These suggest that such a C-ANP (4–23)-binding receptor, natriuretic peptide receptor C (NPR-C), might work uniquely as a clearance receptor to which ANP binds, without inducing any detectable effect, until C-ANP (4–23) is occupying most of the

NPR-Cs and thus allowing more ANP in the bloodstream to induce natriuresis (28).

Subsequent studies reveal that NPR-C is a disulfide-linked homodimer homologous to the extracellular domains of NPR-A and NPR-B, with only 37 intracellular amino acids for potential signaling functions. Constituting ~94% of the total ANP binding sites in endothelial cells, NPR-C is evident in the adrenal, brain, heart, kidney, mesentery, and vascular smooth muscle tissue. NPR-C binds ANP, BNP, and CNP to clear them from the extracellular environment through receptor-mediated internalization; lysosomal degradation of ANP, BNP, and CNP; and recycling of NPR-C (Fig. 1) (26, 42, 43, 57, 58).

Proteolysis of Natriuretic Peptides

ANP, BNP, and CNP can be rapidly degraded by neprilysin [neutral endopeptidase (NEP)], a zinc-containing and mem-

brane-bound ectoenzyme that cleaves substrates on the amino side of hydrophobic residues. Seven ANP cleavage sites have been identified, mainly in the ring structure (Fig. 1) (29, 43). NEP catalyzes the degradation of ANG II, bradykinin, and endothelin A as well (22).

ANP is also cleaved by insulin-degrading enzyme, a zinc metalloprotease in cell membrane and cytoplasmic compartment (62).

Renal Effects of ANP

ANP boosts natriuresis and water diuresis. Reabsorption of Na^+ in the inner medullary collecting duct depends mainly on the apical amiloride-sensitive Na^+ channel (cyclic nucleotide-gated ion channel) that allows passive Na^+ entry from the renal tubular lumen and basolateral Na^+ - K^+ -adenosine triphosphatase (Na^+ - K^+ -ATPase) that helps Na^+ - K^+ - 2Cl^- cotransporter actively pump Na^+ out of the epithelial cell into the peritubular space and, eventually, the bloodstream. ANP leads to inhibition of apical Na^+ channel function and basolateral Na^+ - K^+ -ATPase activity, resulting in decreased reabsorption of Na^+ from the inner medullary collecting duct and increased urinary excretion of Na^+ known as natriuresis (Fig. 1) (57). ANP induces cGMP to inhibit the Na^+ channel by a phosphorylation-independent mechanism, as well as by a mechanism of activating cGMP-dependent protein kinase (27). ANP inhibits basolateral Na^+ - K^+ -ATPase through PKG-induced phosphorylation in a cGMP-dependent manner (57).

ANP also boosts natriuresis by directly inhibiting renin release from juxtaglomerular (granular) cells through a cGMP-mediated process without changes in intracellular Ca^{2+} . It decreases the synthesis and release of aldosterone, as well as the anti-natriuretic effects, thus boosting natriuresis (5, 24).

There are conflicting results of studies on the role of ANP in direct inhibition of vasopressin release from the posterior pituitary (17). However, Matsukawa and Miyamoto (31) show that circulating ANP inhibits the ANG II-induced release of vasopressin from the posterior pituitary where the blood-brain barrier does not exist, thus boosting water diuresis by curbing the effects of vasopressin on water reabsorption in the distal tubule.

It has been demonstrated that ANP causes diuresis and natriuresis, at least in part by inhibiting the V2 receptor-mediated action of vasopressin in the collecting ducts (18).

ANP increases glomerular filtration rate. ANP increases glomerular filtration rate (GFR) through its direct vasodilation effects on the afferent arterioles, which allows more blood to enter the glomerulus for ultrafiltration. ANP also reverses afferent arteriolar vasoconstriction induced by norepinephrine (37, 60). The spasmolytic effects of ANP on vascular smooth muscle may involve one or more of the following mechanisms: inhibition of Ca^{2+} release from the sarcoplasmic reticulum, inhibition of Ca^{2+} influx, and enhancement of active Ca^{2+} extrusion (5).

Several studies suggest that ANP directly increases the glomerular capillary ultrafiltration coefficient K_f by inducing relaxation of the contractile intraglomerular mesangial cells in the space between capillary endothelium and podocytes, resulting in expansion of capillary surface area available for filtration. ANP also relaxes the mesangial cells precontracted with

ANG II, and these mesangial cells have specific, high-affinity ANP receptors that induce cGMP in response to ANP (5).

There are controversies on the vasoconstriction effects of ANP on the efferent arteriole: Veldkamp et al. (60) observed no changes of the diameter of efferent arteriole after tropical use of ANP, whereas others report dilatation of the afferent arteriole and constriction of the efferent arteriole in ANP bath (25).

Hemodynamic Effects of ANP

ANP induces hypovolemia and decreases blood pressure. de Bold et al. (9) report a reduced blood pressure and increased hematocrit in rats after infusion of atrial extract. In bilaterally nephrectomized rats, ANP infusion significantly lowers the blood pressure, with an average of 14% decrease in plasma volume and 7% increase in hematocrit, suggesting increased permeability of capillaries and efflux of fluid from the circulatory system. ANP boosts the rate of clearance of radioiodinated albumin from the circulatory system in mice (2, 7, 9). Infusion of ANP at a rate of $2.5 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 90 min induces a significant fall in plasma volume and an increase in transcapillary efflux of intravascular albumin in humans as well. A decrease in plasma volume contributes to a decrease in both cardiac output and blood pressure (2, 63).

ANP leads to activation of the cGMP-dependent PKG that stimulates the Ca^{2+} /calmodulin-dependent endothelial nitric oxide (NO) synthase to help produce more NO for relaxation of the vascular smooth muscle cells, resulting in a decrease in systemic blood pressure (12). It is interesting that ANP may also induce Ca^{2+} /calmodulin-dependent endothelial NO synthase in the aorta artery, ventricle, and kidney by binding to either NPR-A with cGMP as second messenger, or 67-kDa NPR-C coupled with inhibitory guanine nucleotide-regulatory protein to inhibit adenylyl cyclase (8, 57).

ANP inhibits sympathetic activities. ANP decreases sympathetic outflows by modulating ganglionic neurotransmission rather than increasing discharge from cardiac mechanoreceptors with inhibitory vagal afferents (13). A decrease in sympathetic activities contributes to a decrease in systemic vasoconstriction and thus a decrease in systemic blood pressure.

The Role of Natriuretic Peptides in CHF

Resulting from any structural or functional impairment of ventricular filling or ejection of blood, heart failure is a clinical syndrome associated with arterial underfilling, tissue hypoperfusion, and central venous congestion. Arterial underfilling decreases the inhibitory signals from the baroreceptors of the carotid sinus and aortic arch to the sympathetic nervous system, leading to increased sympathetic activities that increase plasma norepinephrine and vasopressin, as well as renin secretion that activates renin-angiotensin-aldosterone system (RAAS), including ANG II and aldosterone. Sympathetic nervous system and RAAS augment cardiac output by increasing heart rate, contractility, preload, and number of contractile elements at the expense of increased cardiac workload and oxygen consumption. ANG II and norepinephrine induce hypertrophy of cardiomyocytes. ANG II also boosts proliferation of cardiac fibroblasts in CHF (11, 15, 64).

Anti-hypertrophic and anti-fibrotic effects of ANP. In the cell culture of aortic smooth muscle from rats, ANP acts to inhibit

cell proliferation stimulated by platelet-derived growth factor and suppresses ANG II-induced RNA and protein syntheses by 30–40% with a concomitant reduction of cell sizes (1). ANG II and platelet-derived growth factor stimulate the extracellular-regulated kinase-2 and stress-activated protein kinase (p38 MAPK) activities and their protein levels by two- to fourfold in cultured human vascular smooth muscle cells. The studies of Sharma et al. (50) suggest that ANP inhibits such activities and protein expression by 65–75% in human vascular smooth muscle cells transiently transfected with NPR-A.

ANG II or endothelin A stimulates the DNA synthesis in the cell culture of cardiac fibroblasts from rat pups, which is inhibited by ANP (16). ANP may curb the aldosterone-induced cardiomyocyte growth and fibroblast migration and proliferation by inhibiting the synthesis and release of aldosterone (51). ANP also acts to attenuate the growth of cardiomyocytes and fibroblasts, most likely as a result of inhibiting norepinephrine-stimulated Ca^{2+} influx (6).

All of ANP, BNP, and CNP have cardiorenal protective properties, although CNP has the most anti-fibrotic and least renal effects (29, 41, 44, 59).

Plasma levels of ANP, BNP, and NT-proBNP rise in CHF. Since ANP and BNP are secreted from the cardiomyocytes in response to stretch, it is not surprising that the plasma levels of ANP and BNP typically rise up in excess of 100 pmol/l and 100 pg/ml, respectively, in patients with CHF. With a half-life of 22 min, BNP has been shown to have greater stability in vitro and better diagnostic performance than ANP, whose half-life is 2 min (5, 44, 49).

In 2002, McCullough et al. (33) reported that, at a blood level of 100 pg/ml, BNP had a sensitivity of 90% and specificity of 73% in determining the presence of CHF. Indeed, more than a dozen studies show that elevated BNP levels in patients with CHF are associated with increased risk of death or cardiovascular events (10).

The cleavage of a proBNP produces an active segment of BNP and an inactive one of NT-proBNP: both increase with age and in CHF, both are cleared by the kidney, but BNP is also cleared by NEP and NPR-C and, therefore, has a shorter half-life than NT-proBNP, 22 and 70 min, respectively (26). In 2005, Januzzi Jr. et al. (19) reported that NT-proBNP at a cutoff point of 450 pg/ml was highly sensitive and specific for the diagnosis of acute CHF for patients under 50 yr of age, and the same was true for a cutoff point of 900 pg/ml for patients 50 yr of age and beyond. An NT-proBNP level <300 pg/ml was optimal for ruling out acute CHF, with a negative predictive value of 99% (19). Currently, it is recommended that an NT-proBNP level >1,800 pg/ml is indicative of CHF in patients >75 yr of age. In patients with chronic kidney disease, an NT-proBNP level >1,200 pg/ml suggests CHF for those <50 yr of age and >4,502 pg/ml for those between 50 and 75 yr old (53).

There is significant risk for death or hospitalization for patients with CHF, if the BNP or NT-proBNP value does not fall after aggressive CHF care. However, the use of NEP inhibitor that prevents NEP from degrading BNP may lower the levels of NT-proBNP but not BNP in a successful treatment of CHF. Other causes for elevated levels of BNP and NT-proBNP include anemia, pulmonary hypertension, sepsis, severe burns, and cancer chemotherapy. Some patients with

advanced CHF may have normal BNP or NT-proBNP levels or have falsely low BNP levels because of obesity (53, 59, 64).

Therapeutic Potentials of Natriuretic Peptides

The clinical status of the patients with acute CHF improves after intravenous administration of ANP or its human recombinant form, carperitide, or nesiritide, a recombinant form of human BNP, but the short half-life of these agents limits their routine use. With a 12-amino acid extension to the COOH-terminal of the native ANP, mutant ANP is more resistant to NEP and insulin-degrading enzyme degradation. Similarly, fusing native human CNP with a COOH-terminal sequence of dendroaspis natriuretic peptide found in snake venom, cenderitide natriuretic peptide is less susceptible to NEP degradation and elicits potent natriuretic and diuretic effects, increases GFR, inhibits renin, and induces less hypotension than BNP. It also shows anti-fibrotic actions in rats with experimental cardiac fibrosis. Both cenderitide natriuretic peptide and mutant ANP are currently under clinical development programs for further testing (62).

One way to increase the levels of endogenous ANP, BNP, and CNP is to decrease the rate of degradation by inhibiting NEP. However, a pure NEP inhibitor, such as candoxatril, increases not only the levels of ANP, but also those of ANG II that nullify the effects of the former. With the ability to inhibit both NEP and angiotensin-converting enzyme (ACE), omapatrilat shows a better profile than candoxatril in terms of decrements in blood pressure and vascular resistance. As bradykinin is degraded by both NEP and ACE, a simultaneous inhibition of them by omapatrilat increases the levels of bradykinin that favor the development of angioedema. This and the lack of substantial benefit of omapatrilat comparing to enalapril in patients with CHF preclude its clinical use (22). Preserving the ACE mechanism for the degradation of bradykinin, sacubitril/valsartan is an angiotensin receptor NEP inhibitor that contains the NEP inhibitor prodrug sacubitril and the ANG II receptor antagonist valsartan in a molar ratio of 1:1. A recent study, PARADIGM-HF, concludes that such an agent prevents the clinical progression of surviving patients with heart failure more effectively than the ACE inhibitor enalapril (22, 39).

Physiological vs. Pharmacological Effects of ANP

Since most of the renal and cardiovascular effects of ANP are observed after the use of large doses of ANP, it is difficult to pinpoint which effect is physiological and which one is not. To tackle the problem, Anderson et al. (3) infused human α -ANP in 30 ml of 5% glucose solution at a rate of 1.2 pmol·kg⁻¹·min⁻¹ for 3 h in seven water-loaded normal subjects, achieving plasma ANP concentrations within the upper part of the physiological range: 20.9 ± 1.9 pmol/l. The infusion of ANP causes a significant increase in natriuresis and water diuresis, as well as a decrease in plasma renin activity, without significant changes in pulse rate and blood pressure (3). All of these results are confirmed in a similar study by Richards et al. (46), who infused ANP at a rate of 2 pmol·kg⁻¹·min⁻¹ for 2 h, with peak levels of plasma ANP at 100 pg/ml, in six normal men without significant changes of GFR.

RAAS may be suppressed by natriuretic peptide-independent mechanisms. Eight seated subjects on standard sodium intake receive isotonic saline intravenously at a rate of ~660 ml/h (11 ml/min) for 4 h without significant changes in mean arterial blood pressure, GFR, urinary excretion rates of cGMP and NO metabolites, and blood levels of oxytocin and ANP. Natriuresis occurs within 1 h of infusion with a sixfold increase in the last hour, whereas plasma renin activity, ANG II, and aldosterone begin to decrease progressively 1 h after the start of infusion (45). The results of this study suggest that a small and continuous fluid load induces natriuresis by suppression of RAAS rather than stimulation of ANP release from the cardiac atrium.

In another study, eight normal subjects are infused with 2 liters of isotonic saline in 1 h, which results in increased urinary excretion of sodium and elevated plasma levels of ANP and cGMP initially. The latter two peak at 15 min after the end of saline infusion, sustain for another 45 min, and begin to decline gradually and concomitantly with the levels of cGMP in urine. The urinary sodium excretion remains increased up to 5 h after the end of saline infusion, during which plasma renin activity and aldosterone are markedly reduced (48). This is consistent with another study in which the endogenous plasma concentrations of immunoreactive atrial natriuretic factor rise rapidly 5 min after 33% blood volume expansion with whole blood in 15 min in the anesthetized, open chest rats with aortic snare to maintain constant blood pressure, and natriuresis peaks at 15–30 min after the transfusion (4). Given a short half-life of 2 min for ANP, it is possible that acute volume expansion induces ANP for the initial natriuresis, and the subsequent natriuresis results from the prolonged suppression of RAAS by natriuretic peptide-independent mechanisms.

The results of these studies suggest that natriuresis and water diuresis are most likely physiological effects induced by endogenous ANP in response to volume overload.

Inferences from studies of gene knockout mice. Mice lacking a functional *Npr1* gene encoding NPR-A exhibit hypertension and marked cardiac hypertrophy with interstitial fibrosis, in association with enhanced activation of proinflammatory cytokines, probably via nuclear factor- κ B-mediated signaling pathway (38, 61). Selective deletion of *GC-A* gene for NPR-A in smooth muscle of knockout (KO) mice abolishes the direct vasodilatation effects of ANP, but the KO mice maintain normal arterial blood pressure. In contrast, selective deletion of *GC-A* genes for NPR-A in endothelium of KO mice results in significant arterial hypertension and cardiac hypertrophy with an 11–13% increase in total plasma volume. Intravenous infusion of ANP increases the rate of clearance of radio-iodinated albumin from the circulatory system in the control, but not in the KO, mice with selective deletion of *GC-A* gene for NPR-A in endothelium, indicating a critical role of NPR-A-mediated increases in endothelial permeability for the hypovolemic, hypotensive effects of ANP (47).

The results of these studies suggest that enhancement of endothelial permeability as well as hypovolemic, hypotensive, anti-hypertrophic, and anti-fibrotic effects may be physiologically induced by endogenous ANP.

Summary

Secretion of ANP and BNP from cardiac chambers signifies the heart as one of the endocrine organs involved in the fluid

and salt balance by interacting with RAAS, endothelin A, vasopressin, NO, sympathetic nervous system, the kidney, etc. ANP also curbs mitoses of heart fibroblasts and hypertrophy of cardiovascular muscle cells in CHF.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

P.C.Y.W. conceived and designed research; P.C.Y.W. and J.G. analyzed data; P.C.Y.W. and J.G. prepared figure; P.C.Y.W. and J.G. drafted manuscript; P.C.Y.W., J.G., and A.Z. edited and revised manuscript; A.Z. approved final version of manuscript.

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