revisão

The Broad Homeostatic Role of Natriuretic Peptides

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ABSTRACT

A brief non-inclusive review on natriuretic peptides (NP), their receptors, and their main functional properties is presented. The three main NP, atrial (ANP), brain (BNP) and C-type (CNP) are considered. Guanylyl cyclase receptors modulate all the known systemic effects of NP. Clearance receptors determine the metabolic disposal of NP and in this manner regulate their plasma levels and/or local tissue concentrations. Structure-function properties, and homeostatic properties of NP receptors are presented. ANP, which plays a major role in pressure-volume homeostasis, is discussed in relationship to its effects on renal hemodynamic and excretory functions, inhibition of the renin-angiotensin-aldosterone system, vasorelaxant, and third-spacing action. For BNP special attention is directed to its role as a negative modulator of ventricular remodeling, in view of its anti-hypertrophic, anti-fibrotic and anti-inflammatory effects in the heart. The major effect of CNP in promoting vertebral and longitudinal bone growth is briefly addressed. Finally, emphasis is placed on the recent discovery that ANP affects fat metabolism in humans due to its powerful lipolytic action. **(Arq Bras Endocrinol Metab 2006;50/2:198-207)**

Keywords: Natriuretic peptides; ANP, BNP and CNP; Guanylyl cyclase and clearance receptors; Blood pressure-volume homeostasis; Growth, hypertrophy and remodeling; Lipolysis

RESUMO

O Amplo Papel Homeostático dos Peptídeos Natriuréticos.
Este trabalho apresenta uma breve revisão parcial sobre os peptídeos natriuréticos (NP), seus receptores e suas principais propriedades funcionais. Serão discutidos os três principais NP: atrial (ANP), cerebral (BNP) e tipo-C (CNP). Os receptores guanilil-ciclase modulam todos os efeitos sistêmicos conhecidos dos NP. Receptores de clareamento determinam o catabolismo dos NP e, desta maneira, regulam seus níveis plasmáticos e/ou sua concentração tecidual. As propriedades do tipo estrutura-função e homeostáticas dos receptores de NP são apresentadas. O ANP, que tem um importante papel na homeostase pressão-volume, é discutido em relação aos seus efeitos sobre a hemodinâmica renal e funções de excreção, inibição do sistema renina-angiotensina-aldosterona, vaso-relaxamento e ação no terceiro espaço. Quanto ao BNP, especial atenção é focada no seu papel como um modulador negativo da remodelação ventricular, em vista de seus efeitos anti-hipertróficos, anti-fibróticos e anti-inflamatórios no coração. O principal efeito do CNP em promover crescimento ósseo vertebral e longitudinal é discutido brevemente. Finalmente, enfatiza-se a recente descoberta de que o ANP afeta o metabolismo de gorduras em humanos, devido à sua poderosa ação lipolítica. **(Arq Bras Endocrinol Metab 2006;50/2:198-207)**

Descritores: Peptídeos natriuréticos; ANP, BNP e CNP; Guanilil-ciclase e receptores de clareamento; Homeostase pressão arterial-volume; Crescimento, hipertrofia e remodelação; Lipólise

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A SURPRISING SET of diversified functional properties of natriuretic peptides is being unveiled since their discovery and chemical characterization in the early 80s. For an inclusive review of the early literature see reference (1). Atrial natriuretic peptide (ANP), the most intensely studied member of the natriuretic peptide family, was initially thought to be exclusively a hormone that directly regulates salt excretion, induces vasorelaxation, and consequently participates in blood pressure-volume homeostasis. However, early studies already demonstrated that ANP, in addition to its natriuretic, vasoactive and blood pressure lowering effects, is a powerful inhibitor of the renin-angiotensin-aldosterone system (2-4). Moreover, ANP was also shown to have a broad spectrum of vasoactive effects, antagonizing all known hormonal and non-hormonal vasoconstrictors, with a small agonist (vasoconstrictive) action on its own (1,5). These early studies led us to postulate that ANP act as a physiological antagonist of the prevalent salt retaining homeostatic mechanisms resulting from evolutionary selection when mammals migrated from the salt-rich environment of the seas to the salt-poor environment of the land (6).

Studies in the past decades further advanced the knowledge of the broad scope of the homeostatic role of natriuretic peptides. Thus, natriuretic peptides were shown not only to inhibit the renin-angiotensin-aldosterone system but also to affect many other actions of hormones, paracrines/ autocrines, cytokines and growth factors. Moreover, natriuretic peptides counteract fibrotic, and inflammatory stimuli that are responsible for hypertrophic and remodeling responses in the heart and possibly in the vasculature (7,8). Natriuretic peptides, particularly CNP, also promote vertebral and longitudinal bone growth and play a role in the development of the reproductive and central nervous systems (7). Finally, of particular interest to the readers of this issue is the recent finding that administered ANP has a potent lipolytic effect in humans (9).

Several effects of natriuretic peptides were shown to result in the negative modulation of normal physiological responses, e.g., increases in blood pressure, blood volume, heart remodeling, and fat tissue mass, to impede that these responses reach pathological levels. Thus, natriuretic peptides function as controllers of normal homeostasis, and are likely to play a major protective role against prevalent risk factors for end-organ damage such as hypertension, remodeling of the heart, and obesity. In this article I will briefly review the biology of natriuretic peptides and their receptors, focusing mainly on basic properties that help in the understanding of the complex homeostatic role of these novel endocrines.

THE MAIN NATRIURETIC PEPTIDES

Shortly after the discovery of atrial natriuretic factor by Adolpho de Bold and colleagues in 1981, we and others determined the chemical nature and the fundamental cardiorenal effects of the factor, which is nowadays known as atrial natriuretic peptide (ANP). ANP is a 28 amino acids polypeptide with a conserved central core between disulfide linked cysteines (1,10). ANP is mostly found in cardiac atria where it is stored in secretory granules as a 136 amino acids pro-hormone (1). Upon its secretion, induced by increases in atrial pressure and stretch, the pro-hormone is processed by a serine protease, named corin, to the active 28 amino acids peptide. Very recent evidence shows that mice with experimental deletion of the corin gene lack circulating ANP, and consequently become hypertensive (11). The chemical structure of ANP is exceedingly well preserved across mammalian species. Soon after the characterization of ANP, several laboratories purified and chemically characterized other related peptides, including brain natriuretic peptide (BNP), urodilatin (URO) and C-type natriuretic peptide (CNP). They all share a conserved core structure with ANP but have different N and C terminal extensions (1).

Although BNP was initially purified from brain — hence its name — it is present at the highest concentration in the heart, and within the heart in ventricular myocytes (12). Contrary to ANP and CNP, there is considerable species specificity for BNP. BNP shares the same receptors and have the same effects as ANP when administered to experimental animals (13). In normal conditions the concentration of BNP in plasma and cardiac atrial tissue is far lower than that of ANP, making it difficult to envision a significant role of this hormone in blood pressure-volume homeostasis in physiological conditions. However, in pathological conditions, such as in ventricular hypertrophy, the synthesis of BNP in myocytes increases markedly leading to plasma levels that are similar or even higher than those of ANP (12,14). In cardiac ventricles, BNP is not stored in granules but is constitutively released by myocytes suggesting that it has an important paracrine effect in the regulation of ventricular mass (see below). There is now convincing evidence to indicate that plasma levels of BNP may serve as an important biochemical marker to detect ventricular hypertrophy and heart failure in humans (14).

C-type natriuretic peptide (CNP) lacks a C-terminal extension and is widely distributed in the mammalian organism, being present mostly in brain, bone, and in vascular endothelial cells (7,15). CNP is not secreted to blood but has important local paracrine and/or autacoid actions in endochondral ossification, in the vasculature, and in the central nervous system (7). URO (ANP32) has a four amino acids extended N-terminal, and has been detected in urine and distal tubular cells (16). URO is not secreted into blood. When administered to mammals, URO's effects are undistinguishable from those of ANP. It has been postulated that URO acts from the luminal side of distal nephron sites to decrease sodium reabsorption, but firm evidence in this regard is still lacking (15).

NATRIURETIC PEPTIDE RECEPTORS

There are two main classes of natriuretic peptide receptors (NPR). Guanylyl cyclase (GC) receptors mediate all the known cardiovascular, renal, osteogenic and lipolytic effects of natriuretic peptides. Clearance receptors (NPRC) — as the name implies — have an important role in the removal of natriuretic peptides from the circulation, and/or a modulatory role in the regulation of their local tissue concentration. For more inclusive reviews of natriuretic peptide receptors see references (1,7,17-19).

Guanylyl cyclase receptors of natriuretic peptides

There are two forms of guanylyl cyclase receptors for natriuretic peptides, named type A (GC-A or NPRA) and type B (GC-B or NPRB). Upon ligand binding, GC receptors generate cGMP, the main second messenger of the actions of all natriuretic peptides (7,17,19). Genetic deletion of the GC-A receptor in mice confirmed that this receptor mediate the effects of natriuretic peptides on blood pressure-volume homeostasis (20). GC receptors differ markedly from other classes of receptors (e.g., G-coupled receptors) as they contain in a single molecule an extracellular binding sequence, a single transmembrane domain, and in the cytoplasmic domain, the enzyme guanylyl cyclase separated from the transmembrane domain by an intervening tyrosine-kinase like sequence that controls the guanylyl cyclase activity (7,17,19). Thus, in a single molecule GC receptors contain the three integral domains (acceptor, effector and modulator) that relay the actions of natriuretic peptides. In G-coupled receptors, the most abundant class of receptors in mammals, these domains are present in three separate molecules, i.e., the receptor molecule proper (e.g., adrenergic receptor), the effector enzyme (e.g., adenylyl cyclase), and the modulatory protein(s) (G-proteins).

The characteristic structure of GC receptors summarized above confers unique activation and inactivation properties that are important for the understanding of the homeostatic role of natriuretic peptides in physiological and pathophysiological conditions. In the absence of ligand (ANP, CNP or BNP), the tyrosine kinase-like domain (TKL) inhibits the activity of the guanylyl cyclase, and therefore the generation of cGMP. In this manner basal levels of the second messenger are kept to a minimum. Upon ligand binding, there is a conformational change in the cytoplasmic domain that favors the binding of ATP (and possibly other yet unknown cytoplasmic factors) to the TKL domain resulting in an allosteric desinhibition of the guanylyl cyclase activity (17-19,21,22). Thus, activation of GC receptors (generation of cGMP) by natriuretic peptides is rapidly achieved by desinhibition of the guanylyl cyclase activity. As soon as ATP (and possibly other cytoplasmic factors) bind to the TKL domain there is also an allosteric change in GC that markedly decreases, by yet unknown mechanisms, the affinity of the receptor, resulting in a rapid dissociation of natriuretic peptides from the GC receptor, and hence a prompt termination of the response (6,22-24).

It is noteworthy that the GC receptor, contrary to NPRC and most other receptor classes, is an integral membrane protein that does not undergo internalization upon ligand binding, and consequently does not down-regulate or mediate lysosomal hydrolysis of ligand (22,23). This finding was somewhat controversial in the past but it has recently been fully confirmed (24). Lack of down-regulation does not, however, imply that there is no receptor desensitization. Long-term desensitization of GC receptors upon exposure to high concentration of natriuretic peptides or heterologous desensitization by activation of protein kinase C is a well-defined phenomenon, which is due to receptor dephosphorylation (19). From a pathophysiological point of view it is likely that receptor desensitization plays an important, albeit not sole, role in the characteristic resistance to ANP's natriuretic effect when plasma levels of ANP are chronically elevated such as, e.g., in congestive heart failure (1,6).

The structure-function properties for GC receptors described above have important physiological consequences. As we have previously described, GC receptors act in a staccato mode with rapid on-off receptor occupancy resulting in rapid responses as

plasma or local levels of natriuretic peptides increase and immediate termination of the response when levels return to normal (6,23). This particular kinetics, together with the lack of down regulation of GC receptors, assures that there are always unoccupied GC receptors ready to respond to stimuli that increase ANP or other natriuretic peptides. The rapid off-rate also permits a rapid termination of the response when the stimuli for the increase in ANP are removed. Once natriuretic peptides are dissociated from the GC receptors, they are removed from the circulation by the clearance receptors (see below).

Clearance receptors of natriuretic peptides

Clearance receptors of natriuretic peptides (NPCR) are by far the most abundant class of natriuretic peptide receptors, comprising more than 95% of the total population of these receptors in several organs, including the kidney and the vasculature (17). Curiously in fat tissues of non-primates, NPRC is also the most abundant receptor for NP but in primates the predominant receptor is GC-A (9). This explains why ANP does not have a lipolytic action in non-primates while it has a major lipolytic effect in humans (see below). NPRC has a single membrane spanning domain, a large extracellular domain that binds with high affinity all members of the family of natriuretic peptides, and a very short cytoplasmic domain (37 amino acids) (1,17,18). The extracellular domain has significant homology with the extracellular domain of the GC receptor. However, NPRC, but not GC receptors, are able to bind synthetic truncated forms of natriuretic peptides (see below). The short cytoplasmic domain is a characteristic common to all known clearance and/or transport receptors, such as, e.g., low-density lipoprotein, and asialoglycoprotein receptors (1,17,18).

Contrary to GC receptors that have very strict stereochemical requirements for binding of natriuretic peptides, NPRC is quite promiscuous in this regard, accepting peptides containing as few as five conserved amino acids (Arg-Ile-Asp-Arg-Ile) of the ring structure of the natriuretic peptide sequence (1,17,18). The clearance function of NPRC was discovered in my laboratory in 1987, using a shortened analog of ANP (C-ANP_{4-23}), and an isolated perfused rat kidney preparation (25). We also found that administration of C-ANP_{4-23} to intact rats led to a major increase in the plasma levels of ANP by blocking its binding to NPRC, and in this manner decreasing ANP metabolism and removal from the circulation. Soon thereafter the clearance function of NPRC was further established by showing that administration of C-ANP_{4-23} or C-ANP_{11-15} decreases the metabolic clearance of endogenous or administered ANP (26,27). Other investigators presented evidence that in some tissues and cells, NPRC activation may lead to a decrease in cell cAMP and stimulation of phosphatyl inositol pathway. They postulated that NPRC might mediate physiological effects of natriuretic peptides (7,19). To date, however, there is no evidence that NPRC mediate any of the known systemic effects of natriuretic peptides. More recently, studies in mice with genetic deletion of NPRC fully confirmed the clearance function of these receptors (28). In these studies no evidence was found that NPRC directly mediates functional effects of natriuretic peptides.

The clearance function of NPRC is due to receptor-mediated endocytosis, with subsequent lysosomal hydrolysis of ANP, and rapid and efficient recycling of internalized NPRC to the cell surface (29). In vitro, ANP and other natriuretic peptides are also metabolic substrates for neutral endopeptidase (NEP). Under normal conditions, this enzyme probably plays a relatively minor role in the metabolic clearance of ANP in vivo (17,27). However, when a large fraction of NPRC is occupied by high plasma levels of endogenous ANP, such as occurs, e.g., in congestive heart failure, or after the administration of synthetic ANP or artificial NPRC ligands, NEP may play a more substantial role in the metabolic clearance of natriuretic peptides (27).

The balance between ANP's rate of secretion by the atria, and its metabolic clearance rate at peripheral tissues determines plasma levels of the hormone. ANP dissociates very slowly from surface C receptors, resulting in sufficient resident time for the endocytosis of receptor-ligand complexes. Thus, clearance receptors continuously deliver bound ANP to lysosomes, and returns to the cell surface to mediate additional cycles of removal of ANP from the circulation (17,18,29). In this manner, clearance receptors act as a hormonal buffer system to impede large and inappropriate plasma fluctuations of ANP and BNP, to rapidly bring plasma levels of ANP and BNP to basal levels once the stimulus for its secretion ceases, and to dispose of ANP, BNP and CNP molecules dissociated from GC receptors. Consequently, clearance receptors also regulate the concentration of natriuretic peptides at the tissue and cell levels. The combination of the staccato and the continuous modes of guanylyl cyclase and clearance receptors allows for a precise modulation of the broad homeostatic role of natriuretic peptides (6). The clearance function of NPRC may be of therapeutic interest in the future since it raises the possibility of

increasing plasma and tissue levels of endogenous natriuretic peptides by blocking their cellular metabolism. Unfortunately, orally active ligands of NPRC were not yet developed.

THE MAIN EFFECTS OF NATRIURETIC PEPTIDES

ANP is the main circulating natriuretic peptide involved in the regulation of renal function and plasma volume in physiological conditions. Increases in plasma volume, and arterial or pulmonary blood pressure lead to significantly elevated plasma levels of ANP. On the other hand, plasma levels of BNP are more often related to ventricular overload conditions. The direct stimulus for the secretion of ANP is atrial stretch or pressure such as occurs in volume expansion and hypertension (1). Once secreted ANP will act on the kidney to increase glomerular filtration rate, and salt and fluid excretion. On the vasculature, ANP antagonizes vasoconstriction, and on systemic capillaries it increases hydraulic permeability facilitating the shift of fluid from the intravascular to the interstitial compartment (third spacing effect). ANP inhibits the renin-angiotensin-aldosterone system, antagonizes sympathetic effects, and inhibits the synthesis and/or the effects of other hormones or paracrines/autocrines involved in volume pressure homeostasis, including endothelin, and vasopressin (1,4,6,30). As can be surmised, all of these effects are geared to maintain blood pressure and volume within normal limits.

Null mice (gene knockout) for ANP or corin (which converts pro-ANP to ANP), or for the GC-A receptor have increased blood pressure, inability to excrete salt under salt loading conditions, and inability to regulate plasma volume appropriately (11,31-34). These genetic studies confirm the major role of ANP and GC-A receptors in blood pressure-volume homeostasis.

It is unlikely that BNP or CNP are involved in the physiological regulation of blood pressure and volume either as circulating hormones or as paracrines/autocrines. BNP is constitutively secreted by ventricular myocytes, the rate of secretion increasing with ventricular overload (14). Under normal conditions, plasma levels of BNP are much lower than those of ANP, and as mentioned above, BNP interact with the same receptors as ANP with similar affinity. Nevertheless, when BNP is administered intravenously in humans it has the same overall effects as described above for ANP (14). BNP is the natriuretic peptide that entered the pharmaceutical armamentarium for the treatment of acute heart failure as at high plasma levels, similarly to ANP it decreases both pre-load and after-load. Physiologically, the most likely function of BNP is to counteract hypertrophic and remodeling stimuli in the heart (see below).

Circulating levels of CNP are practically undetectable, and even when administered in vivo CNP has little natriuretic or blood pressure lowering effect compared to ANP and BNP (7). The possible reason for this is that CNP acts through GC-B rather than GC-A receptors, and GC-B expression in kidney and in the intact vasculature is relatively small. CNP has been postulated to be a local vasodilator but null mice for CNP do not develop hypertension (7). Recent findings suggest that CNP may also have a vasoprotective role inhibiting vascular proliferation and vascular inflammatory responses (7). To date, however, the most striking paracrine/autocrine effect of CNP is its effect on vertebral an longitudinal bone growth (see below).

THE ROLE OF NATRIURETIC PEPTIDES IN BLOOD PRESSURE-VOLUME HOMEOSTASIS

Renal actions of natriuretic peptides

In the kidney ANP increases GFR, an effect that is mainly due to an increase in glomerular capillary hydrostatic pressure that results from efferent arteriolar constriction and afferent arteriolar dilation (6). In normal conditions, ANP does not alter or even slightly decreases renal blood flow (RBF). ANP is the only known endogenous substance that may increase GFR in face of a decrease in blood pressure, and an unchanged or even decreased RBF (2). However, when kidneys are vasoconstricted such as in experimental acute renal failure, ANP dilates the renal vasculature and increases renal blood flow (1,2).

The natriuretic effect of ANP results from an increase in sodium load to the base of the inner medullary collecting duct, an effect that is essential for a robust natriuretic response, and subsequently disruption of load-reabsorption balance in this nephron segment (1,6). The reasons for the increase in sodium load to the IMDC are multiple, and include: increase in GFR, decrease in inner medullary hypertonicity, and direct tubular effects that decrease sodium reabsorption in nephron segments proximal to the inner medullary collecting duct (1,6). Since ANP is a powerful inhibitor of the renin-angiotensin-aldosterone system it will indirectly act on tubular sites that are targets of this system, including proximal tubular sites

(angiotensin) and distal nephron sites (angiotensin, and aldosterone). Finally, inhibition of sodium reabsorption by the inner medullary collecting duct also contributes to ANP (and possibly URO)-induced natriuresis (30) As pointed out above, BNP and CNP are unlikely to modulate sodium excretion under physiological conditions.

When the renal hemodynamic effects of ANP are precluded, ANP fails to elicit an important increase in sodium excretion (1,35). Abnormal renal hemodynamics, together with GC-A receptor desensitization (see above), explains the resistance of the kidney to the natriuretic effect of endogenous or administered ANP in some pathological cases of volume retention such as congestive heart failure or the nephrotic syndrome (1,6).

ANP as a functional antagonist of the renin-angiotensin-aldosterone system

ANP inhibits renin secretion by the kidney and aldosterone synthesis by the adrenal gland, resulting in a decrease in plasma levels of these antinatriuretic hormones (2-4). ANP decrease in renin secretion is due to the increase in sodium load to the macula densa brought about by an increase in GFR and possibly a decrease in proximal tubule reabsorption of sodium (1-3). ANP inhibits aldosterone synthesis by a direct action on adrenal zona glomerulosa cells, and indirectly by decreasing plasma renin (1,2,4). ANP also antagonizes all of the known effects of angiotensin II, including its peripheral vasoconstriction, growth promoting activity in vascular smooth muscle cells, stimulation of proximal sodium fluid reabsorption, and central dipsogenic effect (1,4,6). The inhibition of the renin-angiotensin-aldosterone system by administered ANP in humans is striking. At infusion rates that barely increased plasma levels of the hormone and only slightly increased fluid and electrolyte excretion by the kidney, ANP markedly decreased plasma aldosterone levels and plasma renin in humans (1,4).

Cardiovascular actions of natriuretic peptides

The effects of ANP on cardio-circulatory hemodynamics are complex and depend on the baseline status of the cardiovascular system. In normotensive experimental animals and humans, ANP decreases blood pressure slightly but consistently (1,36). In several models of hypertension in experimental animals, ANP decreases blood pressure markedly (1,37). The main mechanism of the blood pressure lowering effect of ANP also depends on the baseline status of the cardiovascular system. In normotension, ANP decreases blood pressure by decreasing cardiac output, while calculated total peripheral resistance remains unchanged (36). ANP decreases cardiac output by lowering plasma volume, and consequently central venous pressure (36). In turn, the decrease in plasma volume is the result of a shift of fluid from the intravascular to the interstitial compartment by an increase in capillary hydraulic permeability (38).

In experimental models of volume-dependent hypertension (e.g., DOC-salt hypertension in rats), the ANP-induced decrease in blood pressure is due mainly to a decrease in cardiac output (37). However, in renin-dependent models of hypertension (e.g., 2K-1C, Goldblatt hypertension in rats), ANP decreases blood pressure by decreasing total peripheral resistance, and cardiac output may be even slightly increased due to the relieve in afterload (37). These apparently contradictory mechanisms are entirely consistent with our previous observations that natriuretic peptides are not vasorelaxant per se but are functional antagonists of vasoconstriction (1,5,6). The results are also consistent with our observation that ANP has a primary role in shifting fluid from the intravascular to the interstitial compartment, a phenomenon that, together with its natriuretic effect, makes ANP a central player in the homeostatic mechanisms to maintain plasma volume within normal limits (1,6,38). It is noteworthy that in the lung, ANP may have a protective effect against edema, not only because of its effect of decreasing central venous pressure but also because the increase in hydraulic permeability in the low pressure lung capillaries will favor absorption rather than filtration.

The early findings of the central role of ANP in plasma volume homeostasis were recently confirmed by specifically deleting the gene for the GC-A receptors in the vascular endothelium of mice (EC GC-A KO) (34). In the EK GC-A KO mice blood volume increased even under normal dietary sodium intake. As the direct vasorelaxant and natriuretic effects of infused ANP was preserved in these experiments it was concluded that the increase in plasma volume was due to a decrease in capillary permeability to fluid (34).

Studies with a non-peptidic antagonist of guanylyl cyclase (GC) receptors of natriuretic peptides (HS 142-1) revealed that, as indicated in the earlier studies, ANP plays an important role in blood pressure-volume homeostasis (6). Still more compelling are the recent results of genetic experiments in which genes for ANP or GC-A were deleted (6,20,28,39). These studies, together with the evidence summarized

above, show that ANP is not simply an emergency hormone involved in the response to acute volume overload, as has been assumed by some investigators, but that it has a significant role in the adaptation to chronic volume expansion.

ANTI-HYPERTROPHYC, ANTI-FIBROTIC AND ANTI-INFLAMATORY EFFECTS OF NATRIURETIC PEPTIDES IN THE HEART

One of the surprising findings of experiments with genetic deletion of GC-A receptors or ANP in mice was the major increase in cardiac mass, which was independent of the increase in blood pressure (8,31,40). Specific deletion of GC-A in the heart, rather than systemic deletion of this receptor in the entire organism, has a hypotensive instead of a hypertensive effect. This hypotension is due to an increase synthesis/release of ANP (40). In GC-A null mice, cardiac mass as well as fibrosis, and the concentration of collagen increases markedly. These responses are exaggerated with an hypertrophic stimulus such as aortic banding, leading to a major decrease in cardiac function (8,40). It is likely that the major effect of natriuretic peptides on cardiac hypertrophy and remodeling is mediated by an increase in the constitutive secretion of BNP by the cardiac myocytes since it has been shown that plasma levels of BNP are strongly correlated to the level of cardiac hypertrophy and cardiac failure (14).

Experiments in vitro also demonstrated that BNP antagonizes the effects of TGF-β (a major profibrotic and pro-inflammatory growth factor in the heart) on cell growth, production of collagen I and fibronectin, as well as inhibition of the expression of several pro-inflammatory, pro-fibrotic and pro-transformation genes in cultured cardiac myofibroblasts (41). This study also found that BNP by itself only affects the expression of a few genes while altering the effects of TGF-β in literally hundred of genes (41). This extends to the growth/remodeling effects of natriuretic peptides, the previous extensive evidence from physiological experiments that as far as blood pressure-volume homeostasis is concerned natriuretic peptides act primarily to counteract the effects of several vasoconstrictive and salt retaining stimuli (6).

The studies briefly described above demonstrate that natriuretic peptides negatively impact on cardiac growth and remodeling elicited by hypertrophic stimuli. If CNP has a similar effect in the vasculature as has been suggested (7), then natriuretic peptides may also be considered major players in counteracting end-organ damage in the entire cardiovascular system. In this manner, these recent findings have a potential to open new avenues for the management of end-organ damage in the heart and vasculature.

THE ROLE OF CNP: EFFECTS ON BONE, REPRODUCTIVE ORGANS, CENTRAL NERVOUS SYSTEM, AND VASCULATURE

CNP, the third member of the natriuretic peptide family, is the only known physiological ligand of GC-B receptors. For a recent review on CNP and GC-B receptors see reference (7). Here we will only briefly mention the main effects of CNP. The most dramatic phenotype resulting from experimental genetic deletion of CNP or the GC-B receptor in mice is lack of growth of longitudinal bones and vertebra (impaired endochondral ossification, dwarfism) and lack of development of the female reproductive tract (7,15,42). Null mice for CNP have a shortened life-span, which is apparently due to respiratory impairment caused by the abnormal ossification of the skull and vertebra (15,42). Null mice for ANP or the GC-A receptor do not show ossification abnormalities, whereas genetic deletion or mutation of NPRC in mice leads to the opposite phenotype, i.e., overgrowth of longitudinal bones and vertebra resulting in mice with a hunch-back (kyphosis) and elongated limbs (7,28). The reason of this phenotype is that the local concentration of natriuretic peptides, particularly CNP secreted by condrocytes, is increased in the absence of NPRC due to the lack of receptor-mediated metabolism of this peptide (7,28).

Several other effects of CNP have been postulated in basis of in vitro experiments or infusion in experimental animals. As CNP is not secreted to blood, these effects must, if present in physiological conditions, result from paracrine or autocrine effects of this peptide. These effects include regulation of fibroblast growth and deposition of extracellular matrix; functional antagonism of the effects of growth factors and cytokines; anti-growth, anti-remodeling and anti-inflammatory effects in the systemic vasculature; relaxation of smooth muscle in respiratory tract, intestine and oviduct; inhibition of nerve growth factor-stimulated proliferation of olfactory neuron precursors; and neuro-endocrine control of reproduction (7). The physiological and pathophysiological role of these and other proposed effects of CNP remain to be elucidated.

EFFECT OF NATRIURETIC PEPTIDES ON LIPID METABOLISM IN ADIPOCYTES

The recent reports on acute effects of natriuretic peptides on lipid metabolism add another possible layer of functions to their already broad range biological properties. Since 1986 it was known that infusion of ANP in humans increases plasma free fatty acids (9,43). However, the mechanisms and possible functional significance of this finding was only investigated in more detail in the past 5 years. For a recent inclusive review of the field the reader is directed to reference (9).

Addition of ANP or BNP to isolated human adipocytes or infusion of these peptides into humans markedly increases hydrolysis of triacylglycerol leading to the production of non-esterified free fatty acids (NEFA) and glycerol. The effect of natriuretic peptides on lipolysis is similar to that produced by β receptor agonists (9). Acute control of lipolysis has been known to be under the aegis of the autonomic nervous system and insulin. The net effect of the autonomic nervous system depends on a balance between activation of adrenergic β receptors, enhancing lipolysis, and α_2 receptors, inhibiting lipolysis. Binding of agonists to β adrenergic receptors increases cAMP and PKA activity, which in turn phosphorylates perilipin and hormone-sensitive lipase (HSL), enhancing the translocation of lipid droplet to the cell surface of adipocytes, and ultimately facilitating the efflux of NEFA and glycerol from human fat cells.

The pathway for the lipolytic action of natriuretic peptides is binding to GC-A receptors, increases in cGMP, and activation of PKG, which ultimately converges to the same end point, i.e., activation of perilipin and HSL (44). Indeed, the action of permeable analogs of cGMP mimics that of ANP in isolated adipocytes (9). Since the effects of catecholamines and natriuretic peptides in adipocytes converge to the same end-point by different pathways, the lipolytic action of natriuretic peptides is not eliminated by β-receptor blockade, a finding which may be of importance in patients using beta blockers (see below) (9).

Insulin is a major regulator of lipid metabolism, increasing the resynthesis of tryacylglycerol and NEFA and in this manner increasing fat storage. The pathway goes through a decrease in cAMP levels (by increasing phosphodiasterase activity), and consequently inhibition of PKA, leading to a lower activity of HSL and decrease in lipolysis. Thus, catecholamines and insulin act in opposite direction on the cAMP-PKA pathway, and consequently interact for the overall final effect on lipolysis. On the other hand the lipolytic action of natriuretic peptides is independent of the anti-lipolytic effect of insulin. Consequently, while insulin inhibits the effect of isoproterenol (a β adrenergic receptor agonist) it has no effect on the lipolytic action of natriuretic peptides (9). In addition to the effect on lipolysis, ANP also inhibits leptin release in human adipocytes from obese individuals, an effect that is secondary to its lipolytic action (43).

An interesting finding of the studies on the effects of natriuretic peptides on lipolysis is that they are detectable only in primates, including humans, and not in other species, including rodents, rabbit and dogs. It was demonstrated that the reason for this specificity is that only primates have a high density of GC-A as well and NPRC receptors, whereas in other species the predominant receptor is NPRC with very low expression of GC-A receptors. Thus, clearance receptors are not directly involved in the mediation of the lipolytic action of natriuretic peptides. However, epidemiological studies have suggested that clearance receptors may play a role in modulating abdominal adiposity since it was found that in individuals carrying an allele variant in the promoter region of NPRC that is associated with lower expression of NPRC in adipocytes have a lower prevalence of overweight, obesity and abdominal adiposity (45). A possible explanation for this epidemiological finding is that reduced density of NPRC in human adipocytes will increase local concentrations of natriuretic peptides and in this manner favors fat mobilization. However, further studies are necessary to establish the role of natriuretic peptides and of their clearance receptors in the modulation of local levels of natriuretic peptides in human adipocytes. One of the difficulties in this regard is that only primates have both a significant density of GC-A and NPRC, making it difficult to establish adequate animal models.

The potency of natriuretic peptide-induced lipolysis is the greatest for ANP, followed by BNP. CNP has very little lipolytic action, indicating that GC-A, not GC-B receptors mediate the effects of natriuretic peptides on lipid metabolism. ANP infused into humans at doses that raise plasma concentrations to levels similar to those found, e.g., in advanced congestive heart failure, has a potent lipolytic effect as measured both by microdialysis techniques in subcutaneous adipose tissue and by plasma levels of NEFA and glycerol (9,46). The levels of ANP attained in these experiments were still way above normal plasma levels raising the question, not yet fully answered, if the observed effect of natriuretic peptides on fat metabolism is physiological. Under normal resting

conditions it is difficult to envision a homeostatic loop between the heart and fat tissue to regulate lipid metabolism. However, it is known that strenuous exercise is a potent stimulus for fat mobilization as well as for release of ANP and BNP from the heart into the circulation. Fatty acids may serve as important source of energy in strenuous exercise, and therefore it is possible that the homeostatic loop heart–natriuretic peptides–fat tissue, acting in conjunction with the autonomic nervous system, is adapted to respond to energy requirements during stress conditions. In this sense it is interesting that in patients with beta blockade there is a magnification of the secretion of natriuretic peptides to the circulation after strenuous exercise. This would tend to maintain high levels of lipolysis even in when the lipolytic action of catecholamines is blocked (9).

It has been postulated that increase in fat mass may lead to an overexpression of NPRC leading to a decrease in plasma levels of ANP (45). As described above, lower plasma levels of ANP will lead to sodium retention, and an increase in plasma volume, resulting in an increase blood pressure. The increase expression of NPRC in fat cells will lower the local concentration of ANP, and in this manner inhibit lipolysis (and stimulate leptin secretion) favoring fat storage. This would contribute to a vicious cycle of the relationship between obesity, decrease plasma concentration of ANP and hypertension (45). Further work is needed to establish this postulate on firm experimental grounds.

Studies on the relationship between natriuretic peptides and lipid metabolism in physiological and pathophysiological conditions are still in their infancy. Future research is bound to significantly advance our understanding of the cellular and molecular mechanisms, the physiological control processes, and possible avenues of therapeutic intervention in this new aspect of the field of natriuretic peptides.

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