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Natriuretic Peptides, Their Receptors, and Cyclic Guanosine Monophosphate-Dependent Signaling Functions

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Natriuretic peptides are a family of structurally related but genetically distinct hormones/paracrine factors that regulate blood volume, blood pressure, ventricular hypertrophy, pulmonary hypertension, fat metabolism, and long bone growth. The mammalian members are atrial natriuretic peptide, B-type natriuretic peptide, C-type natriuretic peptide, and possibly osteocrin/musclin. Three single membrane-spanning natriuretic peptide receptors (NPRs) have been identified. Two, NPR-A/GC-A/NPR1 and NPR-B/GC-B/NPR2, are transmembrane guanylyl cyclases, enzymes that catalyze the synthesis of cGMP. One, NPR-C/NPR3, lacks intrinsic enzymatic activity and controls the local concentrations of natriuretic peptides through constitutive receptor-mediated internalization and degradation. Single allele-inactivating mutations in the promoter of human NPR-A are associated with hypertension and heart failure, whereas homozygous inactivating mutations in human NPR-B cause a form of short-limbed dwarfism known as acromesomelic dysplasia type Maroteaux. The physiological effects of natriuretic peptides are elicited through three classes of cGMP binding proteins: cGMP-dependent protein kinases, cGMP-regulated phosphodiesterases, and cyclic nucleotide-gated ion channels. In this comprehensive review, the structure, function, regulation, and biological consequences of natriuretic peptides and their associated signaling proteins are described. (*Endocrine Reviews* 27: 47–72, 2006)

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Abbreviations: ANP, Atrial natriuretic peptide; AVP, arginine-vasopressin; BNP, B-type natriuretic peptide; CNG, cyclic nucleotide-gated; CNP, C-type natriuretic peptide; FGF3, fibroblast growth factor-3; GC-A, guanylyl cyclase A; HSP, heat shock protein; IP_3, inositol 1,4,5-trisphosphate; MMP, matrix metalloproteinase; NPR, natriuretic peptide receptor; NPR-C, natriuretic peptide clearance receptor; PDE, phosphodiesterase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PMA, phorbol 12-myristate 13-acetate.

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I. Introduction and Historical Background

ALTHOUGH PHYSIOLOGICAL EXPERIMENTS had long predicted a humoral link between the heart and kidneys (1), De Bold *et al*. (2) reported the first direct evidence for such a substance in 1981. They found that the iv injection of atrial, but not ventricular, homogenates into rats elicited a rapid decrease in blood pressure that was accompanied by increased renal sodium and water excretion. After this seminal observation, several groups purified peptides of varying sizes from atrial tissue that possess both natriuretic and smooth muscle-relaxing activity (3–6). These peptides were given a number of different names such as atrial natriuretic factor, cardionatrin, cardiodilatin, atriopeptin, and atrial natriuretic peptide (ANP); the latter description is most often used today. B-type natriuretic peptide (BNP), which was

originally called brain natriuretic peptide (7), and C-type natriuretic peptide (CNP) (8) were subsequently purified from porcine brain extracts based on their ability to relax smooth muscle. All three members contain the conserved sequence CFGXXXDRIXXXXGLGC (Fig. 1) where X is any amino acid. The flanking cysteines form a 17-amino-acid disulfide-linked ring that is required for biological activity (3).

In 1984, ANP was shown to elevate cGMP concentrations in rat tissues, primary cell cultures, and urine (9). During the same year, ANP was reported to activate particulate, but not soluble, guanylyl cyclase activity in various rat tissue homogenates (10, 11) in a manner that correlated with vascular smooth muscle relaxation (11).

Initial photoaffinity labeling and/or chemical cross-linking of ^{125}I-labeled ANP to whole cells or membranes revealed proteins of 60 and 120–140 kDa as estimated by reducing SDS-PAGE (12–15). Purification of the smaller protein (16) and subsequent cloning of its cDNA (17) predicted a disulfide-linked homodimeric receptor with a large extracellular binding domain, a single membrane-spanning region, and only 37 intracellular amino acids. This receptor is generally referred to as the natriuretic peptide clearance receptor, NPR-C or NPR3. Purification of the higher molecular weight protein revealed that the ANP binding activity cofractionated with guanylyl cyclase activity (18–21). Cloning of the cDNA for this receptor, known as natriuretic peptide receptor-A (NPR-A), guanylyl cyclase A (GC-A) or natriuretic

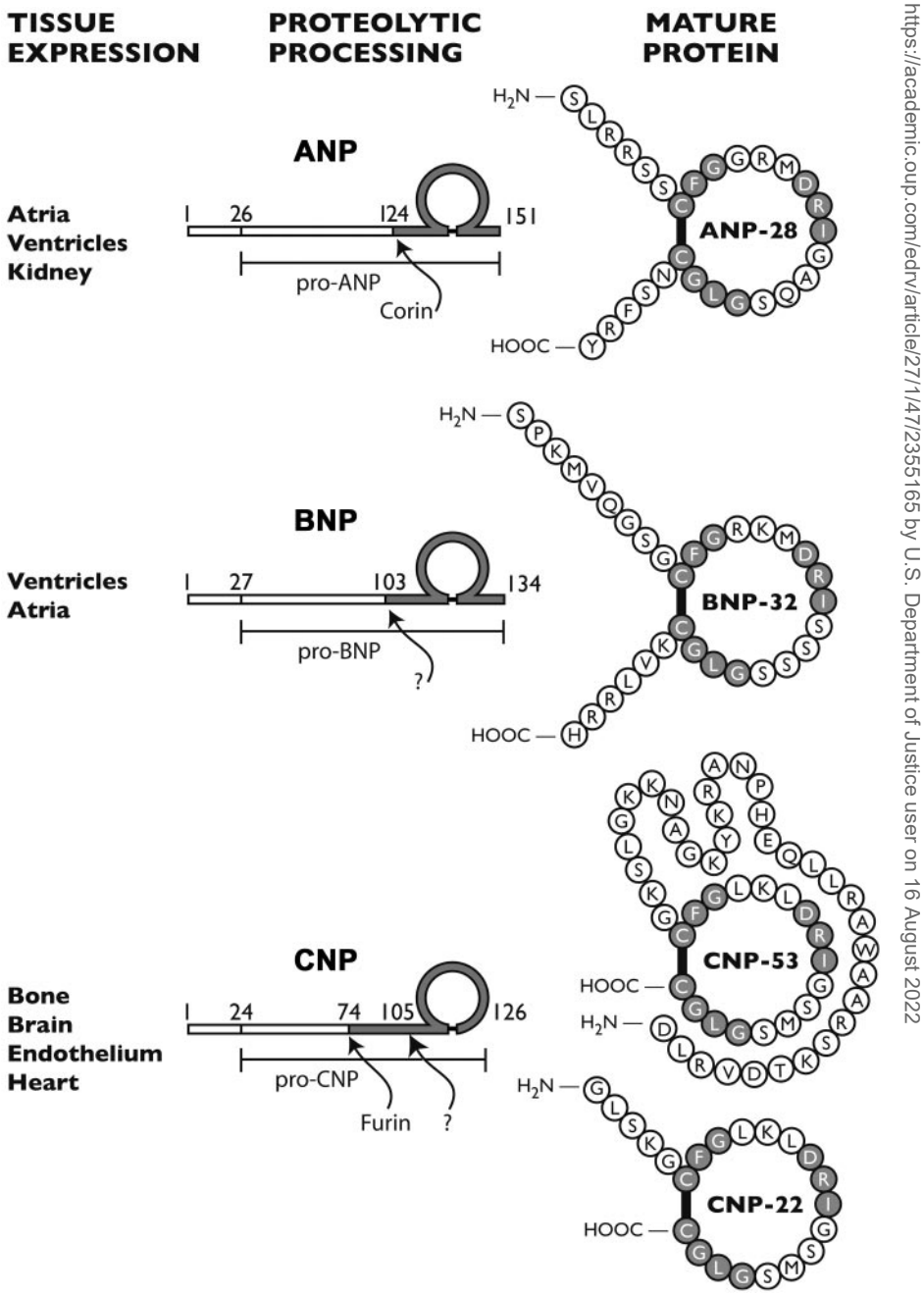

FIG. 1. Natriuretic peptide expression, processing, and structure. ANP, BNP, and CNP are expressed in the indicated tissues as pre-pro-hormones. The signal sequences are cleaved to form pro-ANP, -BNP, and -CNP. The peptides are further proteolytically processed to form mature peptides. ANP is cleaved by corin. The enzyme responsible for BNP cleavage has not been definitively identified. Cleavage of pro-CNP by furin *in vitro* results in a 53-amino-acid peptide. An unknown enzyme further processes CNP to a 22-amino-acid form as well. All three mature peptides contain a conserved 17-residue disulfide-linked ring structure that is required for activity. The disulfide bond is shown in *black*, and invariant residues within the ring are *shaded*.

peptide receptor 1 (NPR1) was obtained by probing a rat brain cDNA library with a sea urchin receptor guanylyl cyclase homolog (22, 23). Surprisingly, sequence analysis suggested that the hormone binding and guanylyl cyclase domains resided within the same polypeptide. This was confirmed when cells transfected with the NPR-A cDNA, but not with empty vector, displayed marked ^{125}I-ANP binding and ANP-dependent cGMP elevations. As a result of the same library screen, a second guanylyl cyclase-linked natriuretic peptide receptor was identified and was called guanylyl cyclase B (GC-B), natriuretic peptide receptor-B (NPR-B), or natriuretic peptide receptor 2 (NPR 2) (24, 25). The specificity of the ligand-guanylyl cyclase receptor interaction was determined in transfected cells. ANP and BNP stimulate NPR-A, whereas CNP stimulates NPR-B (26, 27) (Fig. 2).

II. Natriuretic Peptides

In mammals, there are generally three natriuretic peptides: ANP, BNP, and CNP, although CNP does not stimulate "natriuresis" at physiological concentrations. Teleosts have a novel family member called ventricular natriuretic peptide instead of BNP, whereas only CNP is expressed in sharks (28). Evolutionary analysis indicates that ANP and BNP evolved from CNP gene duplication events (29). Hence, CNP is the most ancient family member.

A. Atrial natriuretic peptide

All natriuretic peptides are synthesized as preprohormones (Fig. 1). Human preproANP is 151 amino acids in length. Cleavage of the amino terminal signal sequence results in the 126-amino-acid proANP, which is the predominant form stored in atrial granules. ProANP is rapidly cleaved upon secretion by the transmembrane cardiac serine protease called corin to form the biologically active carboxyl-terminal 28-amino-acid peptide (30). Mice lacking corin have undetectable levels of the mature form of ANP in heart tissue and are hypertensive (31). Alternative processing of proANP by an unknown protease in the kidney generates a 32-residue peptide called urodilatin, which may be important in regulating renal sodium and water excretion (32).

ANP is primarily expressed and stored in granules in the atria, although it is present at lower concentrations in other tissues such as the ventricles and kidney (Fig. 1). The primary stimulant for ANP release is atrial wall stretch resulting from increased intravascular volume (33, 34). Once secreted, ANP perfuses into the coronary sinus, which facilitates distribution to its various target organs in a true endocrine manner. In addition, hormones such as endothelin (35), angiotensin (36), and arginine-vasopressin (AVP) (37) stimulate ANP release (38), as do water immersion (39) and head down tilt (40). Plasma levels of ANP in normal patients are approximately 10 fmol/ml and are elevated 10- to 30-fold in patients with congestive heart failure (41, 42) (Table 1).

The human ANP gene is found on chromosome 1p36.2 (Table 1). The mouse gene is located on chromosome 4 (43). The ANP gene, like the BNP and CNP genes, contains three exons. Disruption of the murine ANP gene (Nppa) results in marked hypertension, which was initially suggested to be salt-sensitive (Table 2) (44). However, a subsequent report from the same laboratory found that blood pressures in ANP-deficient animals are not influenced by dietary salt intake (45).

B. B-type natriuretic peptide

BNP was initially purified from porcine brain extracts and given the name brain natriuretic peptide (7). However, it was subsequently found in much higher concentrations in cardiac ventricles from patients or animals undergoing cardiac stress such as congestive heart failure or myocardial infarction (42).

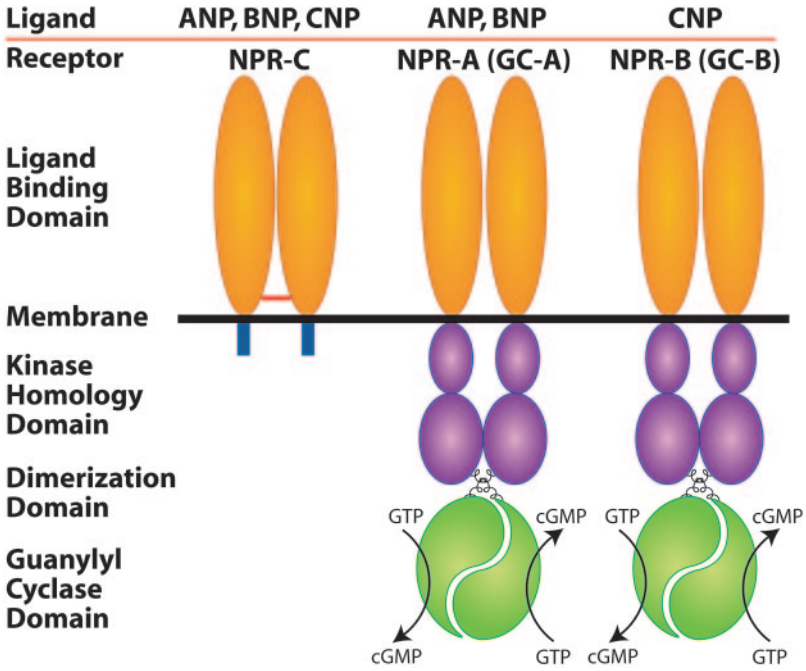

FIG. 2. Natriuretic peptide receptor topology and ligand preferences. Natriuretic peptides bind three proteins, NPR-A, NPR-B, and NPR-C. NPR-A and NPR-B are membrane-bound guanylyl cyclases consisting of an extracellular ligand binding domain, a single hydrophobic transmembrane region, and intracellular kinase homology, dimerization, and carboxyl-terminal guanylyl cyclase domains. The catalytic domain is hypothesized to form a dimer in a head-to-tail arrangement that contains two active sites. NPR-C is approximately 30% identical to NPR-A and NPR-B in the extracellular ligand-binding domain but contains only 37 intracellular amino acids. *Red horizontal line* indicates an intermolecular disulfide bond.

TABLE 1. Human natriuretic peptide gene locus, plasma concentration, and half-life

	ANP	BNP	CNP
Gene name	NPPA	NPPB	NPPC
Gene locus	1p36.2	1p36.2	2q24-qter
Plasma half life (min)	~2 (1.7 –3.1) [328, 329]	~20 (19.5 –22.6) [330]	2.6 [331]
Plasma concentration (pmol/liter)			
Normal	6.4 ± 0.9 [42] (1.1 –13.7) [42, 332–334]	0.9 ± 0.007 [42] (0.9 –6) [42, 332–335]	1.4 ± 0.6 [332] (1.4 –1.9) [332, 336, 337]
In congestive heart failure	87 ± 12 [321] (26 –164) [42, 321, 332]	87 ± 11 [321] (3.9 –267) [42, 321, 332, 335]	1.4 ± 0.2 [332] (1.4 –1.85) [332, 338]
In myocardial infarction	33.4 ± 6.1 [321] (33.4 –55.3) [321, 337, 339]	60 ± 9.4 [287] (26.6 –62.2) [321, 337, 339]	N.D.
In pulmonary arterial hypertension	14.0 [260] (8.8 –20.5) [260]	15.3 [260] (9.2 –49.4) [260]	N.D.
In chronic renal failure	43 ± 11 [332] (43 –48) [321, 332]	130 ± 37.4 [332] (28 –130) [321, 332, 335, 340]	3.0 ± 0.4 [332]
In subarachnoid hemorrhage	5.9 ± 1.0 [341] (5.46 –10.5) [341, 342]	15.1 ± 3.8 [341] (0.64 –23.2) [341, 342]	2.0 –2.6 [343] (0.91 –9.1) [342, 343]
In cirrhosis	27.98 ± 3.71 [333]	16.0 ± 1.91 [333] (1.2 –43.1) [333, 335]	1.36 ± 0.18 [336]

The gene loci for each human natriuretic peptide are shown. The approximate plasma half-life and plasma concentration of each natriuretic peptide in normal and various disease states also are shown. Values represent the mean patient value from a representative study. Values in *parentheses* represent the range of mean values from several studies. Reference numbers are in *brackets*. N.D., Not determined.

For this reason, it is currently referred to as BNP or "B-type natriuretic peptide," but not "brain natriuretic peptide." Human BNP is synthesized as a preprohormone of 134 residues containing a signal sequence that is cleaved to yield a 108-amino-acid prohormone (Fig. 1). Additional cleavage by an unknown protease results in an inactive 76-residue amino-terminal (nt) fragment and a 32-residue carboxyl-terminal biologically active peptide. Fully processed BNP length varies between species. Human, pig, and dog BNP is 32 amino acids (46, 47), whereas rat and mouse BNP is 45 amino acids (48, 49).

Although BNP is stored with ANP in atrial granules, BNP is not stored in granules in the ventricles. Instead, ventricular BNP production is transcriptionally regulated by cardiac wall stretch resulting from volume overload. The nuclear transcription factor, GATA 4, plays a dominant role in regulating this process (50, 51). Healthy individuals have plasma BNP concentrations of approximately 1 fmol/ml (3.5 pg/ml) or about one tenth that of ANP (Table 1). In contrast, plasma BNP concentrations of patients with congestive heart failure are elevated between 200- and 300-fold. The enormous range of plasma BNP concentrations between normal and sick individuals makes it an ideal indicator of cardiac stress (42). Several studies indicate that elevated BNP levels correlate with poor prognoses (see *Section XI*).

The human BNP gene is only 8 kb upstream of the ANP gene on chromosome 1p36.2 (Table 1). The mouse gene is located on chromosome 4. Disruption of both alleles of the murine BNP gene (Nppb) yields normotensive animals that develop pressure-sensitive ventricular fibrosis (Table 2) (52). Hence, at least in mice, BNP is not an endocrine regulator of blood pressure but rather a paracrine regulator of the heart.

TABLE 2. Phenotypes of mice and humans with inactivation mutations in genes that code for natriuretic peptides and their receptors

Gene	Mouse knockout phenotype	Human diseases
ANP	Hypertension Cardiac hypertrophy	
BNP	Ventricular fibrosis	
CNP	Dwarfism	
NPR-A	Hypertension Ventricular fibrosis Cardiac hypertrophy	Hypertension Ventricular fibrosis
NPR-B	Dwarfism, seizures Female sterility Decreased adiposity	Dwarfism (AMDM)
NPR-C	Giantism Hypotension	

Knockout mice for each of the natriuretic peptides and receptors have been generated. The characteristic phenotype of each knockout animal is listed. Human genetic mutations have been identified for two of the receptors as shown. AMDM, Acromesomelic dysplasia, type Maroteaux.

C. C-type natriuretic peptide

CNP is the most highly expressed natriuretic peptide in the brain and is found in high concentrations in chondrocytes (53, 54) and cytokine-exposed endothelial cells (55). It is not stored in granules. In cultured endothelial cells, its secretion is up-regulated by TNF-α (56), TGF-β (55), IL-I (56), and sheer stress (57) and suppressed by insulin (58). CNP is the most conserved natriuretic peptide. For instance, both 22- and 53-amino-acid versions of CNP are identical in humans, pigs, and rats. Human proCNP contains 103 residues, and the intracellular endoprotease furin has been shown to process proCNP to the mature 53-amino-acid peptide *in vitro* (Fig. 1) (59). In some tissues, CNP-53 is cleaved to CNP-22 by an unknown extracellular enzyme. Although CNP-22 and CNP-53 elicit similar if not identical functions (60, 61), their tissue expression differs. CNP-53 is the major form in the brain (62), endothelial cells (63), and heart (64), whereas CNP-22 predominates in human plasma (63) and cerebral spinal fluid (65). Normal plasma CNP concentrations (both forms) are in the low femtomole per milliliter range (63) and are minimally (66), if at all (67), elevated in patients with congestive heart failure.

The human CNP gene is located between 2q24 and the 2q terminus (Table 1) (68, 69). The mouse gene is located on chromosome 1 (69). Disruption of the murine CNP gene (Nppc) results in normotensive mice that display severe dwarfism and undergo early death as a result of impaired endochondral ossification (Table 2) (see Fig. 9) (70).

D. Osteocrin/musclin

Using signal-trap strategies, two different groups identified a peptide with limited similarity to natriuretic peptides. Interestingly, one group found it primarily in bone and named it osteocrin (71), whereas another group found it primarily in muscle and named it musclin (72). In a paper presented at the American Society of Bone and Mineral Research Conference in 2004 (73), the osteocrin group reported that osteocrin binds with high affinity to NPR-C, but not to NPR-A or NPR-B, in a manner that is competitive with ANP. When NPR-A and NPR-C were expressed in the same cells, osteocrin increased ANP-dependent cGMP elevations, presumably by blocking NPR-C mediated ANP degradation. Transgenic expression of osteocrin under the bone specific collagen type I promoter resulted in mice with elongated bones and marked kyphosis, which is similar to the phenotype of mice transgenically overexpressing BNP (74) or CNP (75) or lacking NPR-C (76, 77). These data suggest that osteocrin increases local CNP levels in the growth plate by blocking binding to NPR-C (see *Section X.L*).

III. Natriuretic Peptide Receptors

There are three known natriuretic peptide binding proteins in mammals: NPR-A, NPR-B, and NPR-C (Fig. 2). They are also known as GC-A, GC-B, and the clearance receptor, or as NPR1, NPR2, and NPR3, respectively. NPR-A and NPR-B represent two of the five transmembrane guanylyl cyclases found in humans (78). The other members of the family are GC-C, the receptor for the intestinal peptides guanylin and uroguanylin, and Ret-GC-1 and Ret-GC-2, retinal enzymes that regulate the photoreceptor dark cycle. The third natriuretic peptide receptor, NPR-C, does not possess any known intrinsic enzymatic activity.

A. Natriuretic peptide receptor A

Human and rat NPR-A mRNA are highly expressed in kidney, adrenal, terminal ileum, adipose, aortic, and lung tissues (Table 3) (23, 25, 79). *In situ* hybridization analysis of rhesus monkey tissues indicated that NPR-A mRNA is prevalent in the kidney, adrenal glomerulosa, adrenal medulla, pituitary, cerebellum, and endocardial endothelial cells (80). In the brain, NPR-A mRNA was observed in the mitral cell layer of the olfactory bulb, medial habenula, subfornical organ, and area postrema (81, 82). It was also observed in forebrain white matter tracts, suggesting synthesis in glial cells. Western blot analysis detected high NPR-A protein levels in rodent lung, kidney, adrenal, testis, and liver tissue (Table 3) (83, 84). NPR-A was purified to apparent homogeneity from rat lung (18) and bovine adrenal cortex (19, 85). In cultured cells, NPR-A is readily found in primary vascular smooth muscle and kidney mesangial cells. Its expression decreases dramatically with continued propagation (86). In fact, we are not aware of any immortalized cell line that expresses high levels of this receptor, although low expression is observed in some human embryonic kidney 293 (87) and rat PC-12 pheochromocytoma (88) cell lines (Table 3).

TABLE 3. Natriuretic peptide receptor tissue and cell line expression

Receptor	Tissue expression	Cell line expression
NPR-A	Adrenal, brain, VSM, lung, kidney, adipose, heart	Primary VSMC Primary renal mesangial PC-12 Some 293 cell lines
NPR-B	Chondrocytes, brain, lung, VSM, uterus	Primary VSMC Smooth muscle (A10, A7R5) Fibroblast (NIH3T3, Balb3T3) Chondrocyte (ATDC5)
NPR-C	Most tissues	A10 VSMC 3T3 fibroblast

The tissue expression pattern of each natriuretic peptide receptor is shown. Natriuretic peptide receptor expression in commonly studied cell lines also is listed. VSM, Vascular smooth muscle; VSMC, VSM cell.

The guanylyl cyclase-linked natriuretic peptide receptors have a growth factor receptor-like topology consisting of an extracellular ligand-binding domain of approximately 450 amino acids, a 20- to 25-residue single hydrophobic membrane-spanning region, and an intracellular domain of approximately 570 amino acids (Fig. 2). The latter is made of a 250-amino-acid kinase homology domain, a roughly 40-residue coiled-coil dimerization domain, and approximately 250-amino-acid carboxyl-terminal guanylyl cyclase catalytic domain (78). The rank order of NPR-A activation by natriuretic peptides is ANP $\geq$ BNP $\gg$ CNP (26, 27).

The extracellular domain of rat NPR-A contains three intramolecular disulfide bonds between Cys-60/Cys-86, Cys-164/Cys-215, and Cys-423/Cys-432 (see Ref. 89 for graphic depiction of disulfide bonds), but no intermolecular disulfide bonds (90). When fractionated by SDS-PAGE, NPR-A exhibits considerable size heterogeneity, which is primarily due to differential N-linked glycosylation. Sequencing of the amino termini of human natriuretic peptide receptor-IgG fusion proteins purified from Chinese hamster ovary cells indicated that Asn-2 and Asn-13 of NPR-A are glycosylated (91). A soluble extracellular domain of rat NPR-A purified from Cos cells is glycosylated on Asn-13, Asn-180, Asn-306, Asn-347, and Asn-395 (92). The role of glycosylation in the regulation of NPR-A is controversial. Some investigators found that terminal glycosylation affects ligand binding (93, 94), whereas others did not (92, 95, 96).

Under basal conditions, NPR-A is phosphorylated on four serines (Ser-497, Ser-502, Ser-506, and Ser-510) and two threonines (Thr-500 and Thr-513) within a stretch of 17 amino acids at the amino-terminal portion of its kinase homology domain. Conversion of any phosphorylated residue to alanine decreases receptor-associated phosphate, changes tryptic phosphopeptide mapping patterns, and reduces hormone-dependent guanylyl cyclase activity. The mutation of four or more phosphorylation sites to alanine yields a hormonally unresponsive receptor, indicating that phosphorylation of

NPR-A is absolutely required for hormonal activation (87). Whether NPR-A contains additional phosphorylation sites is currently unknown but remains a possibility because sea urchin homologs have stoichiometries of 15–17 moles of phosphate per mole of receptor (97, 98).

The crystal structure of the glycosylated, unliganded, dimerized extracellular domain of rat NPR-A was solved at 2.0 Å resolution (99). The monomer contains a type I periplasmic binding protein fold and consists of two interconnected subdomains with each containing a central β-sheet flanked by α-helices. An apparent chloride ion is buried within the amino portion of each monomer. Chloride was reported to be absolutely required for ANP binding to NPR-A (100); however, this observation has not been confirmed. Although originally proposed to adopt a tail-to-tail V-shaped dimer with the apex being closest to the membrane, the crystal structure of the extracellular domain bound to a truncated form of ANP revealed that the receptor forms a head-to-head, A-like dimer with a stoichiometry of one molecule of ANP to two molecules of receptor (101). Data from studies where the proposed dimerization interfaces were mutated are consistent with an A-shaped, not V-shaped, model (102, 103). Because ANP has no internal symmetry, binding of ANP to NPR-A is asymmetric.

NPR-A has been shown to associate with a limited number of partners. NPR-A expressed in 293 cells interacts with heat shock proteins 70 and 90 (HSP70 and HSP90), molecular chaperones required for proper protein folding and/or trafficking (104). HSP90 is hypothesized to bind within the NPR-A kinase homology domain because deletion of the intracellular or the kinase homology domains disrupts the interaction. Inhibition of HSP90 activity decreases ANP-stimulated cGMP production, presumably due to decreased processing and/or folding of NPR-A (104). Additionally, the kinase homology domain of NPR-A was shown to associate with protein phosphatase 5 (105) and cGMP-dependent protein kinase Iα (106) in two-hybrid screens. To date, neither interaction has been reported in mammalian cells. cGMP-dependent protein kinase Iα was suggested to phosphorylate and activate NPR-A in a feed forward mechanism (106). However, we find that neither overexpression nor lack of expression (null animals) of cGMP-dependent protein kinase Iα has any effect on the phosphorylation status or guanylyl cyclase activity of NPR-A (344).

The human NPR-A gene is approximately 16 kb, contains 22 exons and 21 introns, and is located on chromosome 1q21–22 (23, 107). The rat NPR-A gene (Npr1) spans about 17.5 kb and also contains 22 exons and 21 introns (108). It lacks a definitive TATA box but contains three putative Sp1 binding sites. The murine NPR-A gene has been disrupted by two separate laboratories (109, 110). The null animals have high blood pressure, cardiac hypertrophy, and ventricular fibrosis (Table 2) (109, 110). One group also found that male mice lacking NPR-A died at 6 months of age due to a catastrophic cardiovascular event (110), but this was later attributed to the genetic background of the mice (111). In humans, a single allele mutation was identified in the promoter of the NPR-A gene that decreases receptor expression by about 70% (112). Interestingly, of the eight Japanese patients identified with this mutation, seven had hypertension and one had congestive heart failure. Hence, every time a loss of function mutation was identified in the NPR-A gene, it was associated with disease. In contrast, a separate study involving 498 New Zealand patients failed to observe this mutation, suggesting that it may be rare outside of Japan (113).

B. Natriuretic peptide receptor B

NPR-B mRNA was found in lung, brain, adrenal, kidney, uterus, and ovary tissue (Table 3) (25, 79, 114). *In situ* hybridization studies found detectable NPR-B mRNA in the adrenal medulla, pituitary, cerebellum, and skin (80). NPR-B is the predominant natriuretic peptide receptor in the brain. NPR-B mRNA was detected throughout the neuroaxis, being abundantly expressed in the limbic cortex, neocortex, olfactory bulb, hippocampus, and amygdala (81). Intense staining was found in preoptic-hypothalamic neuroendocrine circuits and in motor nuclei of cranial nerves. In a separate study, high levels of NPR-B mRNA were found throughout the hypothalamus and the neural lobe of the pituitary (82). NPR-B protein has been found at relatively high concentrations in fibroblasts (Table 3) (115–117).

NPR-B has the same overall topology as NPR-A (Fig. 2). The disulfide-bonding pattern of NPR-B has not been chemically determined, but mutagenesis-based studies are consistent with intramolecular disulfide bonds between Cys-53 and Cys-79, Cys-205 and Cys-314, as well as Cys-417 and Cys-426 (118). Similarly, the glycosylation sites of NPR-B have not been chemically determined, but mutagenesis studies suggest that five of the seven extracellular asparagines are glycosylated (119, 120). The mutation of Asn-24 to Asp resulted in a 90% loss in CNP binding, which is probably due to improper folding or cellular targeting of the receptor (120). NPR-B is phosphorylated on Thr-513, Thr-516, Ser-518, Ser-523, and Ser-526 (121). Similarly to NPR-A, mutating any of these residues to alanine reduces hormone-dependent guanylyl cyclase activity. Whether additional unidentified phosphorylation sites exist is unknown. No crystal structure has been reported for any domain of NPR-B. Multiple splice variants of NPR-B have been identified, including a species lacking enzymatic activity that can function in a dominant-negative manner (122, 123). Whether these truncated variants participate in CNP signaling is unknown. The rank order of activation of NPR-B by natriuretic peptides is CNP $\gg$ ANP $\geq$ BNP. To date, studies on purified NPR-B have not been reported.

Two loss of function mouse models exist for NPR-B (Table 2). Targeted deletion of exons 3 through 7, which encode the carboxyl-terminal half of the extracellular domain and transmembrane segment of NPR-B, by homologous recombination results in dwarfism and female sterility (124). The heterozygous animals were significantly shorter than the wild-type animals as well. A spontaneous mutation resulting from a T to G transversion, which causes the substitution of a highly conserved leucine with an arginine in the guanylyl cyclase domain of NPR-B, also results in dwarfism in mice containing two defective alleles (*cn/cn*) (125). Female infertility was not noted in this mouse model. Interestingly, the targeted deletion of CNP or NPR-B resulted in mice that had

significantly higher mortality rates than the single mutation-containing *cn/cn* mice. One possible explanation for these seemingly disparate results is that CNP binding to NPR-B signals through another mechanism in addition to cGMP synthesis.

The human NPR-B gene spans about 16.5 kb, contains 22 exons, and is located on chromosome 9p21–12 (126). Similar to NPR-A, the NPR-B promoter lacks a defined TATA box but contains multiple putative Sp1 binding sites. The mouse gene, Npr2, is found on chromosome 4. Homozygous loss of function mutations in human NPR-B have been identified in patients with a rare form of short-limbed dwarfism called acromesomelic dysplasia, type Maroteaux (Table 2) (127). Sterility was not noted in these patients. Similarly to the "knockout" mice, patients with a single mutated NPR-B allele were statistically shorter than the average person from their respective populations (127).

C. Natriuretic peptide clearance receptor

NPR-C mRNA is found in atrial, mesentery, placenta, lung, kidney, and venous tissue (79, 128) and in aortic smooth muscle and aortic endothelial cells (Table 3) (17). *In situ* hybridization studies found detectable NPR-C mRNA in kidney, adrenal, heart, cerebral cortex, and cerebellum tissue (80). NPR-C protein was purified to apparent homogeneity from bovine lung (129) and vascular smooth muscle cells (16).

The extracellular domain of NPR-C is about 30% identical to NPR-A and NPR-B (130). However, unlike the cyclase-linked receptors, it contains only 37 intracellular amino acids and no guanylyl cyclase activity (17) (Fig. 2). The extracellular domain of human NPR-C is glycosylated on Asn-41, Asn-248, and Asn-349 and contains two sets of intramolecular disulfide bonds between Cys63-Cys91 and Cys168-Cys216 that are conserved in NPR-A and NPR-B (131). One intermolecular bond was identified in bovine NPR-C at Cys469 (132), whereas in human NPR-C two intermolecular disulfide bonds were found at Cys-428 and Cys-431 (131). Hence, unlike NPR-A and NPR-B, NPR-C is a disulfide-linked homodimer (Fig. 2). NPR-C is phosphorylated on serine residues when overexpressed in hamster cells (133).

The crystal structures of the unbound and CNP-bound versions of the NPR-C extracellular domain indicate a ligand to receptor stoichiometry of 1:2 with a membrane distal dimerization interface or A-shaped dimer (134). Hormone binding was found to induce a 20-Å closure of the membrane proximal domains of the dimer.

The affinity of NPR-C for natriuretic peptides is ANP $\geq$ CNP $>$ BNP in both humans and rats (27, 91). Dissociation constants range from 10 to 140 pM (27). The differential affinity of NPR-C for the cardiac family members may contribute to the longer serum half-life of BNP compared with ANP (Table 1). NPR-C, but not NPR-A or NPR-B, also binds the synthetic ANP analog, c-ANF (ANP 4–23), which is missing the carboxyl-terminal tail and a portion of the disulfide ring structure (135). Hence, functions that are stimulated by c-ANF, but not ANP, have been suggested to result from NPR-C-dependent signaling. However, caution is advised when interpreting these experiments because c-ANF indirectly increases NPR-A-dependent responses by blocking NPR-C-dependent ANP degradation (135).

Loss of function mutations in mice indicate that the major function of NPR-C is to clear natriuretic peptides from the circulation or extracellular milieu through receptor-mediated internalization and degradation (76, 77). Like many nutrient type transmembrane receptors, such as the transferrin or low-density lipoprotein receptors, the internalization of NPR-C is constitutive. In other words, it is independent of ligand binding (136). NPR-C internalization is abolished by hypertonic sucrose treatment, which causes clathrin disassembly, suggesting that the endocytosis is mediated by clathrin-coated pits (137). ^{125}I-ANP hydrolysis also is disrupted by cellular treatment with NH_4Cl or chloroquine, suggesting that NPR-C-bound ligand undergoes lysosomal hydrolysis followed by receptor recycling to the cell surface (136, 138).

In contrast, a number of laboratories have reported signaling functions for NPR-C (139). The NPR-C selective agonist c-ANF (ANP 4–23) reduces adenylyl cyclase activity in membranes or cAMP concentrations in whole cells (140). This effect is inhibited by pertussis toxin treatment, which is consistent with a requirement for Gi- or Go-protein activation (141). The inhibition was blocked with an antibody specific for the intracellular domain of NPR-C (142), whereas small peptide fragments of the NPR-C intracellular domain mimic the inhibition (143). Similarly, the ability of CNP to inhibit catecholamine efflux from pheochromocytoma cells is dependent on NPR-C protein levels and is inhibited by an antibody against the intracellular portion of NPR-C (144, 145). NPR-C has been shown to stimulate phospholipase C in a G protein-dependent manner as well (146–148).

The human NPR-C gene is located on chromosome 5p14-p13, spans more than 65 kb, and contains eight exons and seven introns (149). The mouse NPR-C gene, Npr3, is located on chromosome 15. Multiple loss of function mouse models exist for NPR-C (Table 2). Targeted inactivation of both alleles of the NPR-C gene by homologous recombination results in animals that have a reduced ability to clear ^{125}I-ANP from their circulation (two thirds longer half-life), reduced ability to concentrate urine, and long bone overgrowth (77). However, circulating levels of ANP and BNP in the knockout animals are similar to those in wild-type animals, suggesting the existence of a feedback mechanism for ANP and BNP synthesis. In addition to the targeted deletion model, three different strains have been identified that contain recessive loss of function mutations in the gene for NPR-C (76). Longjohn mice contain a 36-bp in-frame deletion between positions 195 and 232 that results in a 12-amino acid deletion. Longjohn2 animals contain a C to T transition at position 283 that results in a premature stop codon. The *strigosus* strain, which is Latin for long and emaciated, has a C to A transversion at position 502 that results in an Asp to His substitution. All mutations are found in the extracellular ligand-binding domain and presumably disrupt ligand binding, although this has not been formally demonstrated. Like the animals with the targeted deletion, these animals exhibited marked skeletal overgrowth. A lack of body fat deposits also was noted upon necropsy. Interestingly, none of the animals with homozygous loss of function NPR-C mutations were

impaired in any known natriuretic peptide response. On the contrary, these animals display phenotypes associated with exaggerated NPR-A and NPR-B actions, for example, hypotension and gigantism, respectively. These data suggest that NPR-A or NPR-B mediates the known effects of natriuretic peptides that have been identified to date, at least in mice. However, it is possible that NPR-C mediates some yet to be discovered natriuretic peptide function. In our opinion, the demonstration of natriuretic peptide functions that are intact in NPR-A- and NPR-B-expressing animals, but absent in animals lacking functional NPR-C, is essential to support a signaling role for NPR-C in mice.

IV. Activation of NPR-A

In the basal state, NPR-A is a higher-ordered oligomer, and its guanylyl cyclase activity is tightly repressed (Fig. 3). Evidence for dimers, trimers, and quatramers exists (150–152) (Fig. 3). Unlike growth factor receptors, ligand binding does not lead to further oligomerization (150, 152). Analysis of the crystal structure of NPR-A indicates that one molecule of ANP binds per two molecules of NPR-A and causes a Ferris wheel-like translocation of the two juxtamembrane domains with little change in interdomain distance (101). However, these data are not consistent with a report showing that a version of NPR-A containing a mutant unpaired juxtamembrane cysteine forms a disulfide dimer upon hormone binding, suggesting that ANP binding decreases the distance between the juxtamembrane regions of the monomers (153). Through an unknown mechanism, this activation signal is transmitted across the plasma membrane, which initiates a series of subsequent events. First, the normal repression exerted by the kinase homology domain is relieved. The kinase homology domain is thought to repress NPR-A because receptors lacking this domain are constitutively active (154, 155). At this point, it is hypothesized that the guanylyl cyclase domains come together in a head to tail arrangement to form two active sites per dimer. This idea is based on the crystal structure of adenylyl cyclase, not guanylyl cyclase, because the latter does not exist. However, a similar structure is likely between the two cyclases because a surprisingly few number of amino acid changes were shown to convert a guanylyl cyclase to an adenylyl cyclase (156) and vice versa (157). Second, the affinity of the hormone-binding domain for ligand decreases, which increases the dissociation rate (158, 159). Finally, the regulatory phosphorylation sites on the kinase homology domain are dephosphorylated, which desensitizes the receptor (Fig. 3) (160). Quantitation of thiophosphate incorporation into active and desensitized forms of NPR-A suggests that the dephosphorylation is primarily the result of reduced receptor phosphorylation, with only slight increases in receptor dephosphorylation (161). Currently, the identities of these regulatory enzymes are unknown. However, NPR-A is dephosphorylated by two separate phosphatase activities in crude membranes. One is inhibited by microcystin and does not require a metal cofactor for activity. The other requires magnesium or manganese for activity but is not inhibited by microcystin (162).

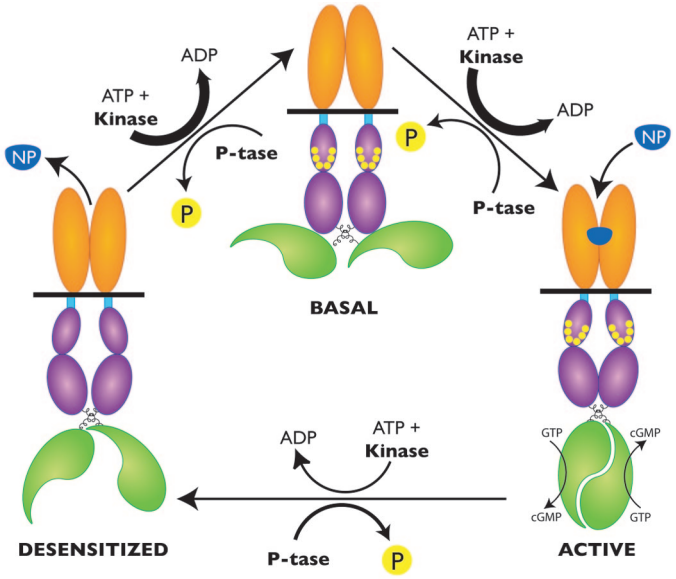

FIG. 3. Hypothetical model for NPR-A and NPR-B activation and desensitization. Three states of receptor activation are labeled "basal," "active," and "desensitized." In the basal state, NPR-A and NPR-B are higher ordered oligomers (shown here as dimers for simplicity). In the basal state, they are phosphorylated on five (NPR-B) or six (NPR-A) known sites within the kinase homology domain (*purple*). Phosphates are indicated by the *small yellow spheres*. It is hypothesized that phosphorylation "licenses" the receptor for hormonal activation. The rate of phosphorylation or dephosphorylation is indicated by the thickness of the respective *arrows*. Natriuretic peptide (NP; *blue*) binding to the highly phosphorylated, inactive basal receptor induces a conformational change that brings the juxtamembranes regions of the extracellular domain together. This activation signal is transduced across the membrane, which is hypothesized to relieve the repression of the kinase homology domain on the guanylyl cyclase domain (*green*). This allows the cyclase domains to dimerize. Each dimer is envisioned to contain two active sites. Prolonged ligand exposure stimulates receptor dephosphorylation, which results in reduced activity via a process called desensitization. The dephosphorylation primarily results from inhibition of the phosphorylation process. Release of ligand and rephosphorylation returns the enzyme to its basal state.

ANP was originally shown to increase guanylyl cyclase activity in crude membranes in the absence of ATP (10). However, a few years later, several groups observed that including ATP in the reaction mixture dramatically increased ANP-dependent cyclase activity (163–165). Because AMP-PNP, a nonhydrolyzable ATP analog that presumably cannot substitute for ATP in protein kinase reactions, also increased ANP-dependent activity, it was suggested that ATP directly binds and activates NPR-A (164). Subsequent reports found that ATP was absolutely required for NPR-A activation (166–168). Similar data were reported for NPR-B (169). This led to a two-stage activation model for natriuretic peptide receptors where natriuretic peptide binding to the extracellular domain facilitates ATP binding to the kinase homology domain, which ultimately brings the catalytic domains together to form an active site.

More recent studies suggest that the ATP-dependent regulation of the kinase homology domains of NPR-A and NPR-B also involves changes in their phosphorylation state, a process that is required for natriuretic peptide receptor activation (87, 121). For instance, mutations that disrupt the putative ATP regulator module in NPR-B reduce the phos-

phate content of the receptor (121). Additionally, ATPγS was found to sensitize NPR-A to subsequent activation by ANP and AMPPNP, indicating that in broken cell assays ATP is serving as a substrate for the protein kinase that phosphorylates NPR-A (170). Recently, we reported that NPR-A or NPR-B in membranes prepared in the presence of phosphatase inhibitors is activated up to 200-fold in the absence of ATP (171). Importantly, the addition of ATP did not increase initial enzymatic rates, but did increase activities measured at longer time periods. These data indicate that ATP stabilizes, but does not activate, natriuretic peptide receptors. Whether ATP binds directly to the receptors or to other regulatory proteins is not known.

V. Desensitization of NPR-A and NPR-B

Hormone-dependent guanylyl cyclase activities of both NPR-A and NPR-B are reduced due to chronic exposure to ligand, a process known as homologous desensitization. Incubation of HEK293 cells stably expressing NPR-A or NPR-B in the presence of ANP or CNP, respectively, leads to a time-dependent reduction in ligand-dependent guanylyl cyclase activity (desensitization) that correlates with receptor dephosphorylation (95, 160, 161, 172). *In vitro* treatment of membranes with a purified protein phosphatase also results in NPR-A dephosphorylation and inhibition (160). Tryptic phosphopeptide analyses of NPR-A or NPR-B isolated from cells treated in the presence or absence of hormones are qualitatively similar (172, 173). Thus, the dephosphorylation cannot be attributed to the loss of a specific phosphopeptide(s) despite a clear decrease in receptor-associated phosphate. One explanation for this apparent contradiction is that ligand exposure results in complete dephosphorylation of a receptor population subset, whereas the rest of the receptor population is not dephosphorylated. Another possibility is that a specific site(s) is dephosphorylated in response to ligand binding, but the phosphopeptide that contains this site is lost during the purification process. Hence, this phosphopeptide does not appear on the tryptic phosphopeptide maps of NPR-A or NPR-B from either control or desensitized cells.

To test the absolute requirement of dephosphorylation in hormone-dependent NPR-A desensitization, a mutant version of NPR-A was constructed where the known NPR-A phosphorylated residues were replaced with glutamate to mimic the negative charge of phosphate. This mutant was approximately one fifth as hormonally responsive as the wild type but was resistant to the effects of microcystin and ANP-dependent desensitization (174). These data indicate that dephosphorylation is a mechanism of NPR-A and NPR-B desensitization.

Classic heterologous desensitization, *i.e.*, the ability of other cGMP-elevating enzymes to desensitize NPR-A, does not seem to occur (175). These data suggest that cGMP elevations are not sufficient for homologous desensitization, which are consistent with studies showing that whole cell exposure to cGMP analogs does not affect NPR-A activity (173).

VI. Inhibition of NPR-A and NPR-B (Receptor Cross-Talk)

In general, hormones or growth factors that stimulate vasoconstriction or promote cellular growth or proliferation antagonize the actions of natriuretic peptides. Examples of factors that inhibit NPR-A and/or NPR-B are: angiotensin II, AVP, lysophosphatidic acid, sphingosine-1-phosphate, platelet-derived growth factor, basic fibroblast growth factor, and endothelin (115–117, 176–180). These agents bind either a tyrosine kinase or serpentine receptor that activates phospholipase C, converting phosphotidylinositol 1,4-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). Diacylglycerol is a direct activator of the classical and novel protein kinase C (PKC) isoforms, whereas IP_3 binds receptors on the sarcoplasmic or endoplasmic reticulum to increase intracellular calcium concentrations.

Initially, PKC was implicated in the mechanism of heterologous desensitization because treatment of cells expressing NPR-A or NPR-B with phorbol 12-myristate 13-acetate (PMA), a pharmacological PKC activator, markedly decreased whole cell hormone-dependent cGMP elevations and membrane-associated guanylyl cyclase activity but did not alter receptor numbers (173, 181). Subsequently, the PMA-dependent decrease in NPR-A activity was correlated with receptor dephosphorylation, and a relatively specific PKC inhibitor was shown to block both the PMA-dependent desensitization and dephosphorylation (173). The specific PKC isozyme involved in natriuretic peptide receptor inhibition has not been reported. In contrast to tryptic phosphopeptide maps associated with natriuretic peptide-dependent (homologous) desensitization, PKC activation results in the dephosphorylation of a single or small subset of the total phosphorylation sites (173, 181). Tryptic phosphopeptide mapping analysis of NPR-B isolated from HEK293 cells treated with or without PMA indicated that Ser-523 is dephosphorylated and that the phosphorylation of Ser-518 is increased in response to PKC activation (181). The mutation of Ser-523 to glutamate prevented the inhibition, indicating that dephosphorylation was required for PKC-dependent desensitization of NPR-B (181).

Evidence for a PKC-independent NPR-B desensitization pathway also exists. Incubation of A10 vascular smooth muscle cells that endogenously express NPR-B with AVP causes a decrease in intracellular cGMP synthesis and a reduction of guanylyl cyclase activity (115). These effects are independent of PKC because neither the PKC inhibitor, GF-109203X, nor the chronic down-regulation of PKC was able to block the desensitization. These observations suggest that, in addition to the diacylglycerol-PKC arm of the phospholipase C pathway, the inositol triphosphate-calcium arm also plays a role in the desensitization of NPR-B. In fact, AVP exposure elevates intracellular calcium concentrations in these cells (115). Furthermore, ionomycin, a calcium-ionophore, mimics the effects of AVP; and a cell-permeable calcium-chelator blocks the AVP-dependent desensitization. Together, these data suggest that calcium elevations, not PKC activation, are required for the AVP-dependent inhibition of NPR-B. Interestingly, calcium-dependent NPR-B desensitization has also been observed for the serum components lysophosphatidic

acid (182) and sphingosine-1-phosphate (176) as well as in response to hyperosmotic stimuli (182). In the latter scenario, calcium elevations were shown to stimulate NPR-B dephosphorylation (182).

The mechanisms for PKC- and calcium-dependent desensitization of NPR-B are unique. The former results from reduced phosphorylation of a known site and primarily affects the affinity of NPR-B for CNP and GTP (183). The latter is associated with reductions in maximal velocities by a mechanism that does not involve inhibition of NPR-B phosphorylation and requires a process in addition to the dephosphorylation of the known sites (183). Growth factor-dependent inhibition of NPR-B is also correlated with receptor dephosphorylation, but the involvement of individual phosphorylation sites in this process has not been reported (117).

VII. Internalization of NPR-A and NPR-B

Ligand-mediated internalization and degradation are also mechanisms for terminating surface receptor-mediated signaling. For natriuretic peptide signaling, there is some controversy as to whether internalization and degradation of NPR-A and NPR-B occur, whereas it is widely accepted that NPR-C internalizes and recycles back to the plasma membrane (see *Section III.C*). Early studies conducted on PC-12 pheochromocytoma cells suggested that both NPR-A and NPR-C internalize ANP and that both receptors are recycled back to the cell surface (184). Pandey and colleagues (185–188), using Leydig, Cos, and 293 cell lines, reported that ANP binding to NPR-A stimulates its internalization, which results in the majority of the receptors being degraded with a smaller portion being recycled to the plasma membrane. In contrast, Maack and co-workers (159, 189) reported that NPR-A in primary kidney or stably expressing Chinese hamster ovary cells is a constitutively membrane resident protein that neither undergoes endocytosis nor mediates lysosomal hydrolysis of ANP. Similarly, Jewett *et al.* (158) found that 293 cells expressing NPR-A bound less ANP over time but concluded that the reduced binding was due to a diminished affinity of NPR-A for ANP and not to decreased amounts of NPR-A at the cell surface. Finally, Fan *et al.* (138) failed to observe internalization of NPR-A, recycling of NPR-A, or significantly degraded ANP products in the media bathing NPR-A-expressing 293 cells. Only one study has addressed the receptor trafficking properties of NPR-B; it found no evidence for receptor internalization or recycling (138).

VIII. Degradation of Natriuretic Peptides

All three natriuretic peptides are degraded through two accepted processes: 1) NPR-C-mediated internalization followed by lysosomal degradation as discussed above; and 2) enzymatic degradation by neutral endopeptidase 24.11 (neprilysin), a zinc-dependent enzyme expressed on the plasma membrane that has broad substrate specificity and tissue distribution. In sheep, the enzymatic and receptor-mediated processes contribute equally to the degradation of ANP and BNP (190). Human BNP is more resistant to hydrolysis by neprilysin than ANP (191). Phosphoramidon, a potent inhibitor of this neutral endopeptidase, blocked the degradation of ANP in 293 cells expressing NPR-A but not NPR-C, indicating that NPR-C and neutral endopeptidase employ different degradation mechanisms (138). Addition of phosphoramidon to murine kidney slices increased the EC_{50} for ANP-dependent, but not BNP-dependent, activation of NPR-A, suggesting that ANP is a better neutral endopeptidase substrate than BNP (192). Targeted deletion of neutral endopeptidase 24.11 (193) does not lead to skeletal overgrowth like the targeted deletion of NPR-C (77), which suggests that CNP concentrations in the growth plate are primarily controlled by NPR-C in mice.

IX. Receptor-Specific Agonists and Antagonists

ANP, BNP, and CNP bind NPR-C and NPR-A or NPR-B. In an effort to identify a specific ligand for the guanylyl cyclase receptors, a phage library was screened for ANP variants that preferentially bind human NPR-A over human NPR-C (194). A variant was identified that has 1,000- to 10,000-fold greater affinity for NPR-A than for NPR-C. This analog was used to demonstrate that NPR-A, not NPR-C, is required for ANP-dependent inhibition of aldosterone synthesis in a human glomerulosa cell line (195). A similar approach was used to identify an ANP variant that had a 200-fold binding preference for rat NPR-A over rat NPR-C. Infusion of this analog into rats resulted in greater renal effects than the same concentration of natural ANP, presumably due to its reduced ability to be degraded through the NPR-C internalization pathway (196).

The best-studied antagonist of natriuretic peptides is a microbial polysaccharide known as HS-142-1 (197, 198). It inhibits ligand binding and activation of both NPR-A and NPR-B through a novel allotopic (allosteric), not simple competition, mechanism (199). It has no effect on natriuretic peptide binding to NPR-C (198). Current availability of HS-142-1 is unknown. Two other peptide-based antogonists to NPR-A, A71915 (200) and A74186 (201), also have been reported. The effect of these peptides on NPR-B activity is not known. To our knowledge, there is no specific antagonist that completely blocks the guanylyl cyclase activity of either NPR-A or NPR-B.

X. Physiological Effects of Natriuretic Peptides

Natriuretic peptides are often described simply as peptides involved in the regulation of blood pressure and volume. However, the effects of these peptides are widespread, and their levels change in response to a variety of pathological conditions (Table 1). The changes shown in Table 1 may be due to changes in intravascular volume or cardiac adrenergic tone as a result of the disease state or may be due to compensatory mechanisms causing increased production of the peptides. The levels of each peptide—ANP, BNP, or CNP—are also regulated by receptor activity, which can be altered by genetic mutation. Therefore, the downstream effects of each natriuretic peptide and the regulation of their

circulating levels is likely to be much more complex than originally anticipated.

ANP and BNP have overlapping functions when administered iv to mammals, including humans. However, studies in murine knockout models clearly demonstrate separate functions for ANP and BNP. Nevertheless, their roles in human physiology remain to be definitively defined. This section will describe in more detail where and how the natriuretic peptides are acting in the body as well as describe some of the implications of these actions (Fig. 4).

A. Cyclic GMP binding effectors

Natriuretic peptides elicit their physiological responses through the synthesis of cGMP, a classic intracellular second messenger that was originally identified in rat urine in 1963 (202). There are three known cGMP binding proteins: cGMP-dependent protein kinases (PKG), cGMP binding phosphodiesterases (PDEs), and cyclic nucleotide-gated ion channels (Fig. 5). The best-studied cGMP signaling effects occur through PKGs, serine and threonine kinases that are activated by cGMP binding (203, 204). There are two PKG genes. The PKGI gene is alternatively spliced to produce α and β isoforms that differ in their amino termini. Both PKGI isozymes are mostly cytosolic and are highly expressed in platelets, smooth muscle, cardiomyocytes, and brain. Deletion of functional PKGI by homologous recombination in mice results in loss of cGMP-dependent vascular smooth muscle relaxation and juvenile (205), but not adult (204), hypertension. PKGII is myristoylated at glycine-2, and therefore, mostly membrane bound. It is found in high concentrations in the intestine, kidney, brain, chondrocytes, and bone (206). Deletion of functional PKGII in mice (207) or rats (208) results in normotensive animals with dwarfism and resistance to infection by heat-stable enterotoxin from *Escherichia coli*.

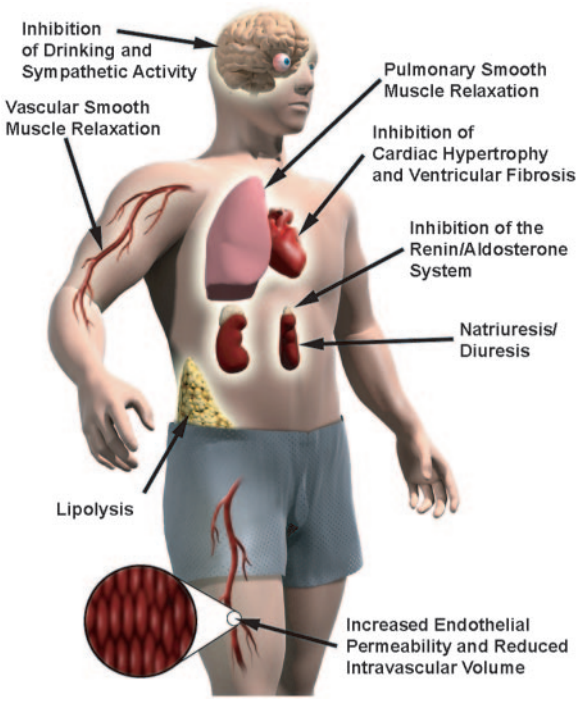

FIG. 4. Physiological consequences associated with NPR-A activation. See *Section X* for detailed description of physiological processes that are regulated by NPR-A.

Cyclic nucleotide PDEs are crucial regulators of cyclic nucleotide signaling because they degrade cyclic nucleotides into inactive 5′-nucleotide monophosphates. Hence, PDEs regulate concentrations of intracellular second messengers. There are 11 different families of PDEs, containing at least 25 different mammalian proteins (209, 210). The families are organized according to their substrate specificity (whether they degrade cAMP, cGMP, or both) and how they are activated or inhibited. For example, PDE1, -2, -3, -10, and -11 degrade both cGMP and cAMP; PDE4, -7, and -8 specifically hydrolyze cAMP; whereas PDE5, -6, and -9 only degrade cGMP (211). Some PDEs are regulated allosterically by cGMP. For instance, cGMP binding to PDE5 (the target of Viagra, Levitra, and Cialis) increases its activity and accelerates cGMP degradation in a feed-forward mechanism (Fig. 5). Alternatively, allosteric activation can facilitate cross-talk between the cGMP and cAMP pathways. For example, cGMP binding activates PDE2, which results in decreased intracellular cAMP concentrations, whereas cGMP binding inhibits PDE3 activity, resulting in increased cAMP levels.

cGMP mediates cellular responses through the regulation of cyclic nucleotide-gated (CNG) ion channels, a family of nonselective cation channels containing a carboxyl-terminal cyclic nucleotide-binding domain that binds cAMP or cGMP (212). They are most noted for their ability to control the dark cycle in photoreceptor cells, but they are also found in chemosensory cells, brain, airway epithelial cells, and the kidney. There are six known human genes encoding CNG channels, which can be broken into two groups designated A and B. The former group is comprised of those subunits that can form functional channels on their own, whereas the latter group cannot. To our knowledge, data linking CNG channels to specific natriuretic peptide functions have not been reported.

B. Effects of the ANP/NPR-A system on blood pressure

Mice completely lacking ANP (44) or NPR-A (109, 110) have blood pressures 20 to 40 mm Hg higher than normal, whereas animals transgenically expressing higher than normal amounts of ANP (213) or BNP (48) have blood pressures 20–30 mm Hg lower than normal. These data clearly indicate that the ANP/NPR-A system regulates basal blood pressures in mice. Some of the most striking data on this issue come from members of Smithies' group (214) who demonstrated that ANP-dependent guanylyl cyclase activities and blood pressures are directly proportional to NPR-A gene dosage over a range of 0 to 4 alleles. Although ANP was initially suggested to regulate blood pressure in a salt-sensitive manner, more recent data suggest that this is not the case (45, 109). Its combined effects on intravascular volume, vasorelaxation, natriuresis, and diuresis mediate the hypotensive nature of ANP as discussed below.

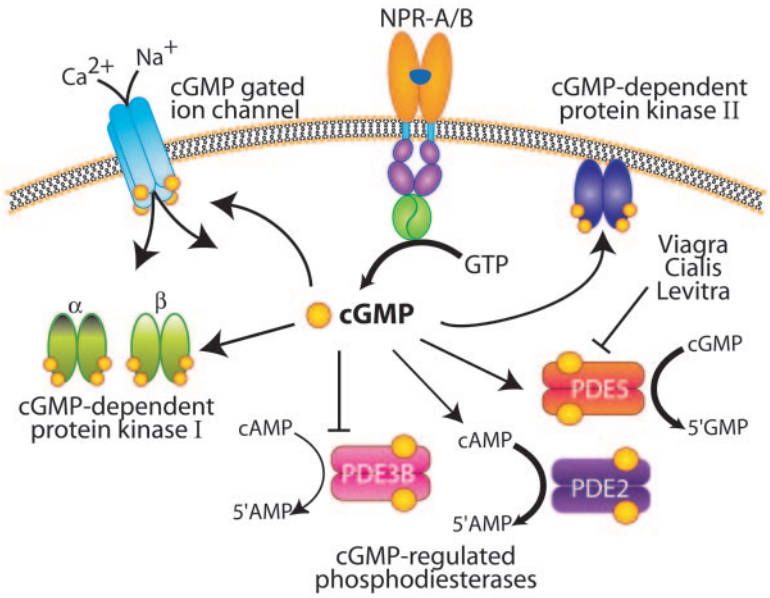

FIG. 5. Cyclic GMP effectors. cGMP mediates its effects by binding three known classes of proteins: cGMP-gated ion channels, cGMP-dependent protein kinases (type Iα, Iβ or type II) and phosphodiesterases (PDEs). Binding of cGMP to the different families of PDEs can induce degradation of cGMP (PDE5, the target of Viagra, Cialis, and Levitra), activate degradation of cAMP (PDE2), or inhibit degradation of cAMP (PDE3B), thereby regulating both cGMP and cAMP levels in cells.

C. Effects of ANP/NPR-A on endothelium permeability and intravascular volume

In the original article of De Bold *et al.* (2) describing the discovery of ANP, vascular atrial extract injections were shown to increase hematocrit levels (2). Subsequent studies indicated that the ANP-dependent vascular volume contraction does not require the natriuretic or diuretic effects of ANP because it precedes urination and occurs in nephrectomized animals (215–217). Additional experiments indicated that ANP increased capillary hydraulic conductivity (218) and permeability of the endothelium to macromolecules like albumin (219) (Fig. 4). However, data from cell culture-derived models are conflicted on this latter issue, with some reports suggesting that cGMP increases (220) and other reports suggesting that it decreases permeability (221). Consistent with ANP increasing cell permeability, mice specifically lacking NPR-A in their vascular endothelium are slightly hypertensive (10–15 mm Hg) and are volume expanded by 11–13% (222). This suggests that NPR-A in the endothelium accounts for about one third of the total hypotensive effects of ANP because animals completely lacking NPR-A are severely hypertensive (30–40 mm Hg) and volume expanded by 30%. Wild-type mice cleared radioiodinated serum albumin from their circulation in an ANP-dependent manner, whereas animals deficient in endothelial NPR-A did not (222). Strikingly, ANP increased the hematocrit levels in wild-type but not mutant animals, indicating that the ability of ANP to increase hematocrit levels absolutely requires endothelial NPR-A. Together, these data suggest that ANP regulates chronic transvascular fluid balance by increasing microvascular permeability. The mechanism for this phenomenon is currently unknown.

D. Effects of ANP and BNP on cardiac hypertrophy and fibrosis

ANP and BNP have direct effects on the heart. Mice lacking ANP (44) or NPR-A (110, 223) have enlarged hearts, whereas animals overexpressing ANP (213, 224) have smaller hearts. Initially, it was unclear whether the cardiac hypertrophy observed in the knockout animals resulted from prolonged exposure to systemic hypertension or from the loss of a local inhibitory effect on heart growth; it is likely that both processes lead to cardiac hypertrophy. The first evidence supporting a local effect involved NPR-A knockout mice that were treated with antihypertensive drugs from birth (111). These animals were normotensive but still had cardiac hypertrophy. In a separate study, the selective transgenic replacement of NPR-A in the heart of NPR-A knockout animals reduced cardiomyocyte size without affecting hypertension (225). Conversely, in a third elegant study, the selective deletion of NPR-A from the heart using Cre/lox technology resulted in mice with decreased blood pressure but mild cardiac hypertrophy (226). The reason for the reduced blood pressures in these animals likely results from elevated cardiac and plasma levels of ANP and BNP, which provides evidence for a local NPR-A-dependent feedback regulatory system for cardiac natriuretic peptide synthesis and/or secretion.

Early studies indicated that BNP inhibits the proliferation of cardiac fibroblasts in culture (227). This observation was validated *in vivo* when mice lacking BNP were shown to display pressure-sensitive ventricular fibrosis (52). The mechanism involved in the BNP-dependent regulation of fibroblasts is controversial. One group using a BNP transgene model system suggested that BNP attenuates angiotensin II-dependent fibrosis by inhibiting MAPK activity (228) whereas another group found that BNP inhibits transforming growth factor β-dependent fibrotic processes by activating MAPKs (229). Recent evidence suggests that the cardiac fibrosis involves matrix metalloproteinases (MMPs) because both ANP and BNP regulate MMP levels (229–231). Mice lacking NPR-A (*Npr1*$^{-/-}$) have increased expression and activity of MMP-2 and MMP-9. Furthermore, increased activity correlates with increased expression of nuclear factor-κB (NF-κB) (232). Several reports indicate that the ANP/

BNP/NPR-A system inhibits pressure-induced cardiac remodeling as well (111, 226, 233). Hence, drugs that activate this pathway or block the inactivation of this pathway may be of significant clinical benefit to patients with failing hearts.

E. Effects of ANP on natriuresis and diuresis

In the kidney, ANP increases glomerular filtration rate, inhibits sodium and water reabsorption, and reduces renin secretion (Fig. 6). ANP-dependent diuresis and natriuresis are mediated exclusively by NPR-A in mice because these effects are completely lost in NPR-A knockout animals (234). ANP increases the glomerular filtration rate by elevating the pressure in the glomerular capillaries through coordinated afferent arteriolar dilation and efferent arteriolar constriction (235). In addition to these hydraulic effects, ANP inhibits sodium and water reabsorption throughout the nephron. In the proximal tubules, ANP inhibits angiotensin II-stimulated sodium and water transport (236). In collecting ducts, it reduces sodium adsorption by inhibiting an amiloride-sensitive cation channel (237). The effect of ANP on both transport processes is cGMP-dependent.

F. Effects of ANP and CNP on vascular relaxation and remodeling

The ability of the cardiac natriuretic peptides to relax precontracted aortic rings requires NPR-A because preparations from animals lacking this receptor are unresponsive to ANP and BNP (238). CNP relaxes aortic rings by a process that does not require NPR-A, presumably by activating NPR-B (238, 239). Unlike wild-type animals, mice selectively lacking NPR-A in vascular smooth muscle cells as a result of Cre/lox technology do not undergo an acute reduction in blood pressure in response to a bolus injection of ANP (240). However, resting blood pressures in these Cre/loxed mice do not differ from their wild-type littermates, indicating that NPR-A-stimulated vasorelaxation is important for acute, but not chronic, blood pressure regulation.

The mechanism of ANP-dependent vasorelaxation has been well studied (Fig. 7). Consistent with the requirement of PKGI in this pathway, PKGI knockout mice do not vasodilate in response to cGMP-elevating agents like ANP or nitric oxide generators (205). Interestingly, the adult animals are normotensive (204), which suggests that the hypertensive phenotype of the ANP or NPR-A knockout animals must result from a PKGI-independent effect. PKGI stimulates vascular smooth muscle cell relaxation by decreasing intracellular calcium levels and by decreasing the calcium sensitivity of the contractile system. To lower calcium concentrations, PKGI acts on several calcium channels. PKGIα directly phosphorylates and activates (opens) calcium-activated potassium channels (241, 242), which increases potassium efflux and causes membrane hyperpolarization. The hyperpolarization then inhibits calcium influx through nearby voltage-gated calcium channels. PKGI is also thought to directly inhibit the voltage-gated calcium channels through phosphorylation of the channel or an associated regulatory protein. At the endoplasmic reticulum, PKGI directly phosphorylates the inositol (1, 4, 5) trisphosphate receptor (243) and the inositol (1, 4, 5) trisphosphate receptor-associated PKGI substrate to inhibit calcium release from this storage vesicle (244). PKGI also activates the calcium/ATPase membrane-associated pump via an unknown mechanism to pump calcium out of the cell, thus reducing intracellular calcium lev-

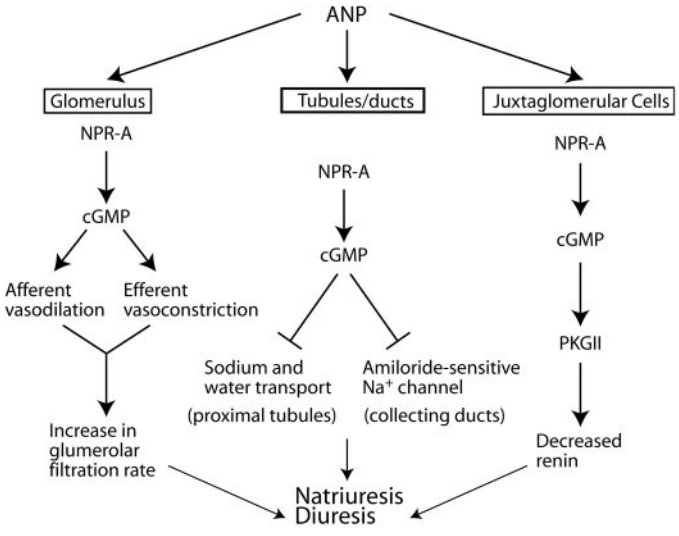

FIG. 6. ANP regulation of the kidney. Renal function is modulated by ANP in at least three ways. First, ANP increases the glomerular filtration rate by differentially regulating the tone of glomerular afferent and efferent blood vessels. Second, it decreases sodium reabsorption in the proximal tubules and collecting duct through cGMP-dependent modulation of sodium channels and transporters. Third, it decreases renin secretion from the juxtaglomerular cells via a PKGII-dependent process. Together, these processes reduce natriuresis, diuresis, and renin secretion.

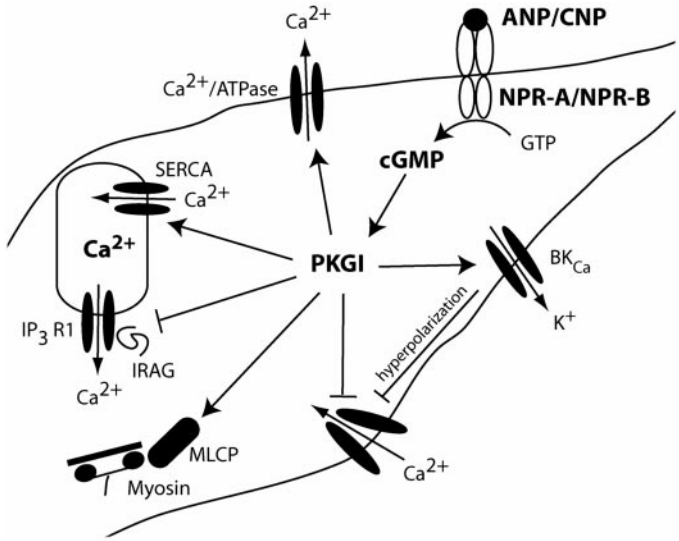

FIG. 7. Natriuretic peptide-dependent smooth muscle relaxation. ANP and CNP stimulation of their cognate receptors, NPR-A and NPR-B, respectively, increases intracellular cGMP concentrations. cGMP activates protein kinase GI (PKGI), which phosphorylates target proteins. PKGI inhibits the IP_3 receptor and stimulates the plasma membrane calcium/ATPase, the sarcoplasmic reticulum calcium/ATPase (SERCA), and the potassium/calcium channel (BK_{Ca}) to decrease intracellular calcium concentrations. PKGI phosphorylation and activation of myosin light chain phosphatase (MLCP) increases the calcium levels necessary for contraction, which lowers calcium sensitivity.

els. PKGI phosphorylates phospholamban, which activates the calcium/ATPase (SERCA), resulting in calcium sequestration into the sarcoplasmic reticulum (245). However, mice lacking phospholamban vasodilate normally in response to cGMP-elevating agents (246). Finally, PKGIα decreases the calcium sensitivity of the contractile system by phosphorylating and activating myosin light chain phosphatase (247), which decreases myosin light chain phosphorylation. Together, these effects stimulate vascular smooth muscle relaxation (Fig. 5) (reviewed in Refs. 248 and 249).

CNP also is a vasodilator and is released in response to vascular injury (56). NPR-B is present in aortic vascular smooth muscle and mediates CNP relaxation of precontracted rat aorta (239). Furthermore, CNP inhibits vascular smooth muscle proliferation (250) and oxidized low-density lipoprotein-induced migration of cultured human coronary artery smooth muscle cells (251) in a cGMP-dependent manner. In recent years, the therapeutic potential of CNP-dependent regulation of vascular tone has been explored. CNP is expressed in rat carotid neointimal but not medial vascular smooth muscle cells (252), suggesting that it may act in a paracrine fashion to regulate neointimal formation. Consistent with this idea, CNP suppresses intimal growth caused by several types of arterial injury (253–255). Finally, recent reports suggest that CNP is the long sought after "endothelium-derived hyperpolarizing factor" (256) and that it inhibits myocardial ischemia/reperfusion injury (257) as well as platelet-leukocyte interactions (258). Interestingly, these processes were suggested to require signaling through NPR-C, not NPR-B.

G. Effects of natriuretic peptides in the lung

All three natriuretic peptide receptors are highly expressed in the lung. ANP stimulates the dilation of pulmonary airways and blood vessels. Infusion or inhalation of ANP stimulates bronchodilation in normal and asthmatic patients (reviewed in Ref. 259). ANP and BNP are elevated in patients with pulmonary hypertension and are indicative of increased right ventricular strain (260–262). Mice overexpressing ANP are resistant to hypoxia-induced hypertension (263), whereas ANP-deficient mice exhibited increased pulmonary hypertension in response to chronic hypoxia (264). CNP also reduces pulmonary hypertension (265) and fibrosis (266).

H. ANP-dependent antagonism of the renin-aldosterone system

ANP regulates blood pressure, in part, through the inhibition of the renin-angiotensin II-aldosterone system (Fig. 6). Renin is a protease secreted from renal juxtaglomerular cells. It cleaves angiotensinogen to angiotensin I, which is subsequently converted to angiotensin II by angiotensin-converting enzyme in the pulmonary vascular endothelium. Angiotensin II then stimulates vasoconstriction and the release of aldosterone, the major hormone responsible for regulating sodium reabsorption in the renal cortical collecting ducts. High doses of ANP do not reduce renin levels in humans, presumably because of compensatory responses associated with the dramatic decreases in arterial blood pressure. However, physiological doses of ANP suppress both renin and aldosterone levels (267). In dogs, intrarenal ANP infusion markedly inhibits the renin secretion rate (268). Inhibition of cAMP-stimulated renin secretion requires PKGII (269). Mice lacking PKGII, but not PKGI, have higher renin expression than their wild-type littermates and are resistant to 8-bromo-cGMP-dependent inhibition of basal and forskolin-induced renin secretion (270). At birth, NPR-A null mice have elevated kidney renin and angiotensin II levels, which is consistent with the known antagonizing effects of ANP and NPR-A on the renin-angiotensin-aldosterone system (271). However, in the adult male NPR-A$^{-/-}$ mice, the renal and systemic levels of renin are decreased, whereas adrenal renin activity and aldosterone levels remain elevated, suggesting that the reduced renal and systemic renin levels result as a compensatory mechanism to increased blood pressure.

In addition to inhibiting renin secretion, ANP directly inhibits aldosterone production in the adrenal gland (Fig. 8). In the adrenal glomerulosa, ANP reduces ACTH-stimulated, angiotensin II-stimulated, and basal aldosterone levels (272–276). The involvement of cGMP in ANP-dependent inhibition of aldosterone production has been controversial because in some studies the ANP effect was mimicked by a cell-permeable cGMP analog (277), but in other studies, cGMP analogs were ineffective (278). Additional experiments indicated that the ability of ANP to reduce aldosterone levels could be mimicked with a NPR-C-specific ligand and blocked by the Gi/Go inhibitor, pertussis toxin. However, recent reports employing a natriuretic peptide variant that

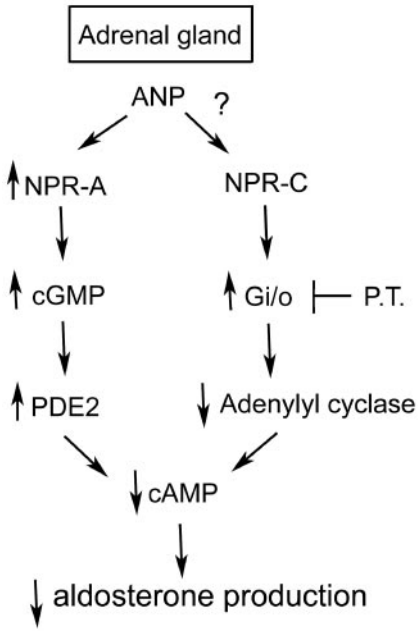

FIG. 8. ANP regulation of the adrenal gland. ANP-dependent decreases in aldosterone secretion from the adrenal gland require reductions in cAMP concentrations. There are two proposed mechanisms for this effect. One involves ANP-dependent activation of NPR-A, which produces cGMP and stimulates cAMP-hydrolyzing PDE2, whereas another involves NPR-C-dependent inhibition of adenylyl cyclase via a pertussis toxin-sensitive G protein-dependent pathway. P.T., Pertussis toxin.

has a 1000-fold higher binding constant for NPR-A compared with NPR-C (195) or catalytically active or inactive guanylyl cyclase C receptors (279) suggests that NPR-A is responsible for the ANP-dependent reductions in aldosterone levels. Consistent with this assessment, mice lacking NPR-A have plasma aldosterone levels about 2-fold higher than wild-type littermates (271). The mechanism for the ANP-dependent reductions in aldosterone may involve PDE2, a cGMP-activated PDE that is highly expressed in the glomerulosa layer of the adrenal gland (Fig. 8) (280). In this scenario, ANP binds to NPR-A causing intracellular cGMP elevations and PDE2 activation. Activated PDE2 then degrades cAMP, which is the major intracellular determinant for aldosterone synthesis. An alternative possibility involves the steroidogenic acute regulatory protein because ANP inhibits the synthesis (281) and phosphorylation (282) of the steroidogenic acute regulatory protein in adrenal glomerulosa cells as well.

I. Effects of ANP on fat metabolism

Many studies have shown an association between obesity and hypertension; however, the effect of natriuretic peptides on fat tissue is only now beginning to emerge. In the late 1980s, ANP-dependent cGMP elevations were measured in rat mammary gland fat cells (283) and rat brown adipose tissue (284). However, in both studies, scientists were unable to demonstrate ANP-stimulated lipolysis. In a later study, ANP-stimulated lipolysis was demonstrated both in isolated human fat cells and *in vivo* by peptide infusion (285). Subsequently, it was determined that ANP-stimulated lipolysis is specific to primates (286) presumably because primates contain a higher NPR-A to NPR-C ratio.

The mechanism and the pathophysiological relevance of these observations are beginning to be elucidated. ANP stimulation of lipolysis is mimicked by 8-bromo-cGMP and is independent of PDE3B, the main enzyme involved in the degradation of cAMP in the adipocyte (285). This suggests that ANP-stimulated lipolysis involves cGMP, but not cAMP elevations as is required for epinephrine-induced lipolysis. A recent study suggested that PKGI is the cGMP effector in the ANP-dependent lipolytic response because pharmacological inhibition of PKGI decreases ANP-dependent lipolysis in primary human preadipocytes (287). Similar to cAMP-dependent lipolysis, the ANP/NPR-A/cGMP-dependent pathway stimulated the phosphorylation of hormone-sensitive lipase, the major regulated enzyme in fat responsible for the hydrolysis of triglycerides into free fatty acids. Increased phosphorylation of lipid droplet-binding protein, perilipin, in response to ANP was also observed. Whether the same sites are phosphorylated on these enzymes in response to cGMP as are phosphorylated in response to cAMP is not known.

Recent papers have examined the metabolic role of ANP-dependent lipolysis to establish a possible link between obesity and hypertension. Unlike catecholamine-dependent dysregulation of lipolysis, which is associated with obesity, the lipid-mobilizing effects of ANP are not related to obesity in young men (288). However, obese women have increased ANP- and isoproterenol-dependent lipolysis when fed a low-caloric diet (289).

J. Neurological effects of natriuretic peptides

All natriuretic peptides and natriuretic peptide receptors have been found in the brain, although CNP and NPR-B appear to be particularly abundant. Consistent with the systemic volume-depleting effects, injection of ANP into the third ventricle of the hypothalamus inhibits water intake induced by overnight dehydration or angiotensin II exposure (Fig. 4) (290). Intracerebroventricular infusion of ANP suppresses salt appetite (291) as well as AVP release from the hypothalamus (292). ANP-dependent suppression of sympathetic activity in the brain stem also has been observed (293, 294). Specifically, ANP was shown to sensitize vagal afferents and dampen the arterial baro receptor response (293, 295, 296). Finally, CNP and cell permeable cGMP analogs have been reported to stimulate GH release in rat anterior pituitary cells (297) and pituitary-derived GH_3 cells (298).

K. Immunological effects of natriuretic peptides

Natriuretic peptides and their receptors are found in many immune cells; however, the significance of these peptides in the immune system is only now emerging. Current evidence suggests a role for ANP in the allergen response of asthma and in immune-related postischemic damage.

The most-studied role of natriuretic peptides in the immune response has been observed in macrophages and dendritic cells. ANP elicits its antiinflammatory effect by reducing production of proinflammatory cytokines (TNF-α and IL-12) while enhancing production of IL-10 (299, 300). ANP increases neutrophil migration *in vitro* (301), and NPR-A knockout mice exhibit decreased neutrophil infiltration to cardiac tissue after injury compared with wild-type mice by decreasing activation of the transcription factor NF-κB (302). Excessive neutrophil infiltration after ischemia can lead to further tissue damage, thus lending a cardioprotective function to blocking ANP signaling after ischemia. NPR-A knockout mice also exhibit decreased eosinophil accumulation in the lungs after allergic challenge with ovalbumin (303), suggesting that ANP signaling may play a role in asthma.

L. The CNP/NPR-B/cGMP/PKGII system and long bone growth

The most obvious physiological effect of CNP is to stimulate long bone growth (Fig. 9). It regulates many types of bone cells, but its major target appears to be the chondrocyte as described below. In a mouse osteoclast model, 1,25-dihydroxyvitamin D3 stimulated CNP expression, cGMP elevations, and osteoclast bone resorptive activity (304). In osteoblasts, CNP elevated differentiation markers like alkaline phosphatase and increased the mineralization of nodules (305). In chondrocytes, CNP elevated cGMP concentrations (53), and in fetal mouse tibia cultures, CNP induced endochondrial ossification (306).

The genetic data supporting the CNP/NPR-B/cGMP bone growth system in mice are striking. Inactivating mutations in the genes coding for CNP (70) or NPR-B (124, 125) cause dwarfism, whereas superphysiological levels of natriuretic peptides resulting from transgenic overexpression (74, 75,

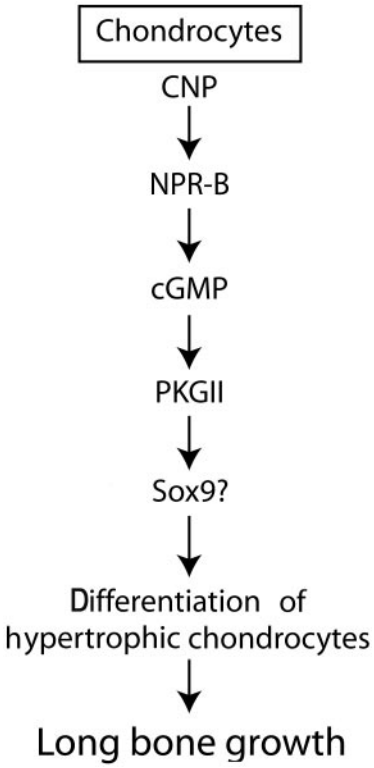

FIG. 9. CNP-dependent long bone growth. CNP-dependent long bone growth requires CNP binding and activation of NPR-B, cGMP binding, and activation of PKGII and PKGII-dependent increases in the proliferation of hypertrophic chondrocytes. The substrate(s) for PKGII in this process has not been identified. One possible substrate is the chondrocyte differentiation factor, Sox9.

307) or reduced clearance (76, 77) cause skeletal overgrowth. No growth abnormalities are observed in any of these mutant animals at birth, suggesting that the CNP/NPR-B/cGMP system only stimulates postpartum bone growth. Growth plates from animals lacking functional CNP or NPR-B (70, 124) are thinner due to reductions in the proliferative and hypertrophic zones, whereas growth plates in the transgenic mice are expanded (74, 307).

Targeted deletion of PKGII by homologous recombination in mice (207) or spontaneous loss of function mutations of PKGII in rats (208) also causes dwarfism. However, unlike the CNP and NPR-B knockout animals, the growth plates of these animals are expanded. One explanation for the growth plate differences is that other cGMP effectors besides PKGII also are required for normal CNP-dependent long bone growth, but this remains to be determined. The substrate(s) of PKGII that mediate its bone growth-promoting properties is not known. However, a recent report suggests that the "master" inhibitor of chondrocyte differentiation, SOX9, is a reasonable candidate because PKGII expression inactivates SOX9 by causing its translocation from the nucleus to the cytoplasm (208). Neither the sites of phosphorylation nor the identity of the kinase (PKGII or other) that phosphorylates SOX9 has been determined.

Multiple putative loss of function mutations in the gene encoding NPR-B were recently identified in human patients with the autosomal recessive disease, acromesomelic dysplasia, type Maroteaux (127). The frequency of this disease is rare (1/2000,000); but because carriers are shorter than matched controls, the effect of these mutations on the stature of the general population is significant. The most common form of human dwarfism, achondroplasia, results from autosomal dominant mutations in the gene coding for fibroblast growth factor-3 (FGF3) receptor, which causes constitutive activation of the signal transducer and activator of transcription 1 and MAPK pathways in chondrocytes (308). Mice expressing constitutively active FGF3 receptors are dwarfed, and their growth plates resemble those of mice lacking CNP or NPR-B (309). In contrast, mice lacking a functional version of the FGF3 receptor exhibit skeletal overgrowth, similarly to mice overexpressing CNP (309). Transgenic overexpression of CNP in growth plates partially reverses the dwarfism phenotype of mice expressing a constitutive active FGF3 receptor (75). The ability of CNP to stimulate bone growth in mice expressing the constitutively active FGF3 receptor mutant may result from its ability to inhibit MAPK signaling because mice expressing the CNP transgene had reduced MAPK kinase, but not signal transducer and activator of transcription 1, phosphorylation (75). The mechanism involved in the CNP-dependent MAPK inhibition is unknown.

XI. Therapeutic Applications of Natriuretic Peptides

As hormone/paracrine factors that regulate intravascular volume, blood pressure, natriuresis, diuresis, and long bone growth, the potential use of the natriuretic peptides for therapeutic benefit is promising. A search of the literature yields a list of over 100 reviews discussing the therapeutic use and potential of these peptides. Initial studies tested the use of ANP and BNP as potential therapeutic agents for the treatment of congestive heart failure, hypertension, and renal failure. The infusion of synthetic ANP, clinically known as anaritide and by the trade name Carperitide, into patients with hypertension (310) or chronic heart failure (41, 311) resulted in elevated sodium and water excretion and decreased blood pressure. Long term (48 h) anaritide infusions of patients with acute heart failure resulted in beneficial hemodynamic responses without tolerance, suggesting that ANP injections may be a clinically useful treatment for heart failure (312).

Anaritide was also used to treat patients with acute renal failure. However, data from these trials has proven contradictory. An early report found that ANP treatment did not improve the dialysis-free survival rate in critically ill patients with acute tubular necrosis (313). In fact, it was associated with decreased survival. However, a subsequent report found that administration of ANP to patients with acute ischemic renal failure resulting from complicated cardiac surgery significantly increased renal function and decreased the need for dialysis (314).

Human recombinant BNP, clinically known as nesiritide and by the trade name Natrecor, mimics the actions of endogenous BNP and has been shown to cause potent vasorelaxation accompanied with increases in natriuresis and diuresis, as well as decreases in plasma aldosterone and endothelin levels in patients with acute heart failure (re-

viewed in Ref. 315). Thus, BNP has emerged as a new tool to manage heart failure (261, 316, 317). The U.S. Food and Drug Administration approved the use of BNP (nesiritide) for the treatment of acutely decompensated heart failure in 2001. Because the half-life of nesiritide (BNP) is significantly longer than that of anaritide (ANP), it is thought to be the better of the two drugs (Table 1). Unfortunately, the widespread use of nesiritide has recently come under scrutiny due to the increased risk of renal dysfunction and mortality in patients undergoing BNP treatment (318, 319). Additional clinical trials are necessary to evaluate this situation and to more narrowly define the benefits, risks, and parameters required for optimal BNP treatment in humans.

The other clinical benefit of natriuretic peptides comes from their diagnostic use. ANP and BNP levels are increased in patients with heart failure and in many patients with hypertension and chronic renal failure (320–322). Because BNP plasma levels correlate more closely than ANP levels with left ventricular function, a common indicator of heart disease, BNP is considered a better diagnostic marker of heart failure. Immunoassays that measure the level of BNP or pro-BNP are commonly used clinically (322). The measurement of BNP levels in both emergency and primary care settings has been used to rule out or confirm a heart failure diagnosis in patients. In emergency care, patients presenting shortness of breath were evaluated for BNP level, and its correlation with heart failure was used to rule out heart failure *vs.* other pulmonary causes of dyspnea (323). Elevated BNP levels correlate with poor prognoses from other diseases as well. For example, BNP levels have been successfully used to predict poststroke mortality (324), postcardiac surgery atrial fibrillation (325), as well as the risk of death in patients with heart failure (326). Finally, based on its ability to effectively measure the benefit of various treatments in patients with right ventricular overload and pulmonary hypertension, BNP has been suggested to be a better guide for optimal treatment of heart failure than classical clinical measurements (261, 322).

Although many of the clinically therapeutic roles for natriuretic peptides have been centered on the current and potential uses of ANP and BNP, the therapeutic uses of CNP have yet to be explored. One potential use for CNP therapy is in the treatment of dwarfism, especially acromesomelic dysplasia type Maroteaux, which is the result of loss of function mutations in NPR-B. Activation of the CNP/NPR-B pathway downstream of the mutation could stimulate the expansion of the growth plates as was seen in the transgenic mice models. Another potential use of the CNP pathway may be to speed the healing of bone fractures, because the bones of rats lacking functional PKGII heal much slower than those from wild-type animals (208). Finally, CNP may hold promise as a cardiovascular drug because recent evidence indicates that it can prevent cardiac remodeling after myocardial infarction in mice (327).

XII. Concluding Comments and Future Directions

Over the past 25 yr, natriuretic peptides and their cognate receptors were discovered and purified, and the genes encoding these proteins were cloned and "knocked out." Additional studies investigated the structure and regulation of the individual participants in these signal transduction pathways; ultimately yielding a tremendous body of literature on natriuretic peptide-dependent regulation of physiological and pathophysiological processes as well as at least two drugs, anaritide and nesiritide.

During the next decade, we anticipate that further discoveries will be made regarding the molecular nature of these pathways as well as their clinical applications. Regarding the former, structural information on the guanylyl cyclase and kinase homology domains of NPR-A and NPR-B would be informative, as would be the identity of molecules such as kinases and phosphatases that regulate these receptors. Identifying downstream participants in ANP-dependent lipolysis and CNP-dependent bone growth pathways also will be of extraordinary importance, as would be the identification of physiological events that are specifically regulated by NPR-C. On the clinical side, we anticipate the discovery of small molecule activators or inhibitors (antagonist) of these pathways that may be used to treat diseases like systemic hypertension, obesity, pulmonary hypertension, heart failure, and skeletal growth disorders as well as diseases yet to be associated with these pleiotropic signaling systems.

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