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REVIEW

Natriuretic peptide receptor B signaling in the cardiovascular system: protection from cardiac hypertrophy

Ines Pagel-Langenickel · Jens Buttgereit · Michael Bader · Thomas H. Langenickel

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Abstract Natriuretic peptides (NP) represent a family of structurally homologous but genetically distinct peptide hormones involved in regulation of fluid and electrolyte balance, blood pressure, fat metabolism, cell proliferation, and long bone growth. Recent work suggests a role for natriuretic peptide receptor B (NPR-B) signaling in regulation of cardiac growth by either a direct effect on cardiomyocytes or by modulation of other signaling pathways including the autonomic nervous system. The research links NPR-B for the first time to a cardiac phenotype in vivo and underlines the importance of the NP in the cardiovascular system. This manuscript will focus on the role of NPR-B and its ligand C-type natriuretic peptide in cardiovascular physiology and disease and will evaluate these new findings in the context of the known function of this receptor, with a perspective on how future research might further elucidate NPR-B function.

Keywords Cardiac hypertrophy · Natriuretic peptide receptor B · C-type natriuretic peptide · Cardiovascular

I. Pagel-Langenickel
National Heart, Lung, and Blood Institute,
National Institutes of Health,
Bethesda, MD, USA

J. Buttgereit · M. Bader
Max Delbrueck Center for Molecular Medicine Berlin-Buch,
Berlin, Germany

T. H. Langenickel (✉)
Vascular Biology Section, Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health,
50 South Drive, Building 50, Room 4529,
Bethesda, MD 20892, USA
e-mail: langenit@mail.nih.gov

Ines Pagel Langenickel received her M.D. and doctorate from the Medical Faculty of the Humboldt University, Berlin, Germany. She is currently visiting fellow at the National Heart, Lung, and Blood Institute, Bethesda, USA. Her research interests include natriuretic peptide receptor signaling in heart failure and mitochondrial biology in diabetes and diabetic cardiomyopathy.

Thomas H. Langenickel received his M.D. and doctorate from the Medical Faculty of the Humboldt University, Berlin, Germany. He is currently visiting fellow at the National Human Genome Research Institute, Bethesda, USA. His research interests include natriuretic peptide receptor signaling in the vasculature and the role of cell cycle regulating proteins in vascular wound repair and pulmonary hypertension.

Abbreviations

CNP	C-type natriuretic peptide
NPR-B	natriuretic peptide receptor B

Natriuretic peptide system

de Bold et al. [1] demonstrated for the first time that atrial extracts exerted a long lasting natriuretic and diuretic response

after intravenous injection into rats. The peptide responsible for this observed effect was identified as atrial natriuretic peptide, ANP [2]. ANP was able to increase cyclic guanosine monophosphate (cGMP) concentration in tissue culture and in tissues and urine of rats, suggesting that cGMP is the second messenger of ANP [3]. Subsequently, other peptides with structural and functional homologies to ANP were purified and named brain natriuretic peptide, BNP, and C-type natriuretic peptide, CNP [4, 5]. Although CNP does not share the diuretic and natriuretic properties of ANP and BNP, it is highly conserved among species and considered the most ancient member of the natriuretic peptide family. Evolutionary studies suggest that ANP and BNP have diverged from CNP by gene duplication events most likely reflecting changes in osmoregulatory systems [6]. A key feature of all three peptides is a 17 amino acid (aa) ring structure that is formed by an intramolecular disulfide bridge [7]. Differences in the primary structure of natriuretic peptides (NP) are predominantly located at the C- and N-terminal end. Studies employing photoaffinity labeling and cross-linking studies of ANP led to identification of the natriuretic peptide receptors A (NPR-A) and C (NPR-C) [8, 9]. Chinkers et al. [10] reported the complementary DNA (cDNA) sequence of NPR-A derived from rat brain and showed ANP binding and generation of cGMP when NPR-A was overexpressed in mammalian cells. The second guanylyl cyclase (GC)-coupled receptor, natriuretic peptide receptor B (NPR-B), was discovered by cloning and sequencing from a human or rat cDNA library, respectively [11, 12]. Binding studies revealed different receptor binding properties of NP, with ANP and BNP having the highest affinity to NPR-A, whereas CNP binds preferentially to NPR-B [13]. All three peptides bind NPR-C with comparable affinity. A detailed insight into the complex interaction between the NP and NPR was recently provided by crystallographic analysis of the various receptor ligand combinations. Here, it was demonstrated that two pockets on the receptor surfaces function as anchoring sites for the hormone side chains and determine receptor selectivity of the NP [14].

It is important to note that the different receptor affinities for NP do not exclude binding of ANP and BNP to NPR-B or CNP to NPR-A. As supraphysiological concentrations were used in most in vitro experiments or were administered intravenously into intact animals, it is difficult to attribute the respective findings to the signaling and physiological function of one single NPR. To our knowledge, there is only one NPR-B selective peptide antagonist described in the recent literature [15]. It has, however, not been widely used yet to study NPR-B function. HS-142-1, the most used antagonist, blocks NPR-A and -B signaling through an allotropic mechanism [16, 17]. Genetic models have contributed to our understanding of the many physiological functions of NP and their receptors (Table 1). Experiments in mice with targeted disruption of ANP, BNP, or NPR-A suggested a role of this signaling pathway in regulation of blood pressure, cardiac hypertrophy, and fibrosis [18–20], while CNP and NPR-B knockout mice displayed severe dwarfism resulting in early death due to impaired endochondral ossification [21, 22]. Interestingly, transgenic rats expressing a dominant negative NPR-B mutant have a normal life span probably due to a minimally remaining receptor activity that is sufficient to blunt the skeletal phenotype. Cardiovascular phenotyping of these animals has suggested a role of NPR-B in growth modulation of cardiac myocytes and regulation of sympathetic nerve activity [23].

Table 1 Animal models resulting from genetic alterations of natriuretic peptides or their receptors

Genetic alteration	Cardiovascular phenotype	References
ANP deletion	Salt-sensitive hypertension, pulmonary hypertension	[18]
ANP overexpression	Arterial hypotension	[19]
BNP deletion	Cardiac fibrosis	[19]
BNP overexpression	Arterial hypotension, Skeletal overgrowth	[120, 121]
CNP deletion	None (altered endochondral ossification, dwarfism, early death)	[122]
NPR-A deletion	Salt-resistant hypertension, cardiac hypertrophy and fibrosis, enhanced cardiac remodeling after myocardial infarction	[20, 123, 124]
NPR-A overexpression	Arterial hypotension, protection against dietary salt	[125]
NPR-B deletion	None (dwarfism, accumulation of white adipose tissue, seizure attacks, infertility)	[22]
NPR-BΔKC overexpression	Concentric cardiac hypertrophy, normotension, increased sympathic nerve activity	[23]
NPR-C deletion	Hypotension, reduced ability to concentrate urine, mild diuresis, volume depletion	[126]

NPR Natriuretic peptide receptors; *NPR-BΔKC* dominant negative mutant of the catalytical receptor subunit.

C-type natriuretic peptide

In contrast to ANP and BNP, which are most abundant in the heart, CNP is predominantly expressed in the brain, chondrocytes, cytokine-stimulated endothelial cells, uterus, and ovaries [24]. CNP is released upon stimulation of endothelial cells with tumor necrosis factor alpha (TNF-α), transforming growth factor beta, interleukin-1, and shear stress [25–27]. Human CNP is synthesized as a 126 aa pre-pro peptide. After a signal peptide is removed by the signal peptidase, the resulting 103 aa pro-CNP is further cleaved by the endoprotease furin into the biologically active CNP-53 and a 50 aa pro peptide [28]. Both peptides are released from the cells and have been detected in several tissues. Another form of CNP, CNP-22, is similar to CNP-53 in terms of biological activity, although it shows different tissue distribution. CNP-53 is present in brain, heart, and endothelial cells, whereas CNP-22 is the predominant form in plasma and cerebral spinal fluid [29–31]. The mechanism by which CNP-22 is processed from the precursor protein has not yet been identified, but the cleavage of pro-CNP by furin is assumed to be the critical step in converting the precursor to the active hormone. Therefore, a differential regulation of furin expression in various tissues could effect the availability of CNP and NPR-B stimulation. Because the plasma concentration is very low as compared to ANP and BNP, CNP is thought to act mainly in a paracrine fashion [29]. CNP has been demonstrated to have potent vasorelaxing properties [32]. It negatively regulates smooth muscle cell proliferation [33, 34], but does not share the diuretic and natriuretic features of ANP and BNP. Several studies revealed that CNP exerts its vasodilating effects independent of endothelium via hyperpolarization of smooth muscle cell membranes [35, 36]. In fact, CNP, released from endothelial cells, accounts for the biological activity of the earlier described endothelium-derived hyperpolarizing factor [37]. The proposed mechanism involves binding of CNP to NPR-C, G_i protein activation, and G_i coupling to a smooth muscle potassium channel.

In pathophysiological conditions such as congestive heart failure, where circulating ANP and BNP are highly upregulated, data on the regulation of CNP are conflicting. Unlike early reports, recent studies have described increased myocardial production and elevated plasma concentration of CNP in patients with heart failure [38, 39]. Interestingly, increased CNP secretion into the coronary circulation of heart failure patients has been found [40], suggesting an involvement of CNP in pathophysiological cardiac remodeling. In addition, a mild increase in circulating CNP levels was detected in patients with chronic renal failure, and marked CNP elevation was found in patients with septic shock [41], possibly due to the presence of high levels of TNF-α and lipopolysaccharides that are known to modulate CNP secretion from endothelial cells.

Natriuretic peptide receptor B

NPR-B is expressed in the brain, chondrocytes, lung, vascular smooth muscle cells, fibroblasts, and uterus [12, 42] and represents the main natriuretic peptide receptor in the brain [43, 44]. NPR-A and -B share structural similarities. Both have an extracellular ligand-binding domain characterized by three loops formed by intramolecular disulfide bridges [45, 46]. A short transmembrane region anchors the receptor in the cell membrane. The intracellular domain consists of a regulatory kinase homology domain (KHD), a hinge region, and a GC. The KHD is able to bind ATP and is phosphorylated, thus, allowing ligand dependent activation of the GC [47]. Nonactivated NPR-B is present as an oligomer with very low GC activity [48]. Ligand binding to the receptor dimer leads to a rotational shift of the transmembrane region [49], which is translated into a conformational change of the intracellular receptor domain, reversing the inhibitory effect of the KHD on the C-terminal GC and subsequently leading to formation of 3′,5′-cGMP [10, 50]. The detailed crystal structure of NPR-B is not known, but a model of the NPR-B/CNP complex was recently proposed by He et al. [14] based on crystallographic analyses of interactions between the NP and NPR-A or NPR-C, respectively. Similar to the topology of NPR-A and -B, CNP binds to a NPR-B dimer as demonstrated for ANP and NPR-A [49, 51].

To further understand the biology of NPR signaling in vitro and in vivo and given that dimerization is a prerequisite for receptor activation, dominant negative receptors have been developed and studied in detail. Chinkers and Wilson [48] demonstrated that extracellular NPR domains were necessary and sufficient to form receptor heterodimers, including receptor mutants lacking either part of or the entire intracellular domain. An NPR-A mutant (NPR-AΔKC) lacking KHD and the GC itself was identified as a dominant negative mutant specifically interacting with NPR-A, not NPR-B. This concept has been applied to NPR-B by our group to generate transgenic rats with a functional downregulation of NPR-B signaling [23]. We demonstrated unaffected NPR-A signaling in cells derived from NPR-BΔKC transgenic rats to prove that the phenotype can be attributed to reduced NPR-B signaling.

Cardiovascular functions of CNP and NPR-B signaling

While the role of NPR-A on cardiac hypertrophy and blood pressure regulation has been extensively characterized, fewer data are available to provide insight into the function of NPR-B signaling in the cardiovascular system. Until recently, neither genetic models nor NPR-B specific pharmacological inhibitor existed that allowed the discrimination between NPR-A and NPR-B signaling. Because CNP possesses

relative receptor specificity [13], several CNP effects have been attributed to NPR-B stimulation. Furthermore, it has been revealed that also NPR-C plays a significant role in mediating CNP effects in several cell types [52]. Despite controversial data, recent research suggests a role for CNP/NPR-B signaling in regulation of cardiac growth, vascular remodeling, and autonomic nervous activity.

Effects on vascular tone

NPR-B is highly abundant in vascular endothelial cells and smooth muscle cells [33], implying its role in the regulation of vascular tone and blood pressure. Intravenous administration of CNP in dogs has resulted in a reduction in blood pressure, with a greater effect from CNP than ANP [53]. The effect of CNP was not associated with increased natriuresis as observed for ANP administration, indicating direct modulation of vascular tone, rather than a reduction in the circulating blood volume as the underlying mechanism. Several ex vivo models have strengthened this hypothesis.

CNP induced a dose-dependent relaxation in precontracted porcine coronary arteries and guinea pig aortas that was mediated by cGMP [32, 35]. The vasorelaxation was endothelium-independent and accompanied by hyperpolarization of smooth muscle cell membranes. Furthermore, it involved the activation of voltage-dependent and calcium-sensitive potassium (BKCa) channels [54]. A similar mechanism has been demonstrated for the vasorelaxation of isolated canine femoral veins [55]. The effects of CNP on arterial vasodilation are further modulated by the presence of nitric oxide (NO). As Wennberg et al. [56] showed, CNP release is involved in endothelium-dependent relaxation due to acetylcholine or bradykinin in isolated canine coronary arteries. Similarly, the inhibition of GC-coupled NPR with HS-142-1 blunted bradykinin-induced vasodilatation in porcine coronary arteries [35]. Another hint at a possible interaction between CNP and NO-induced vasorelaxation came from a study in endothelial NO synthase (eNOS) knockout mice. Here, CNP caused a dose-dependent vasorelaxation in aortas from wild-type mice that could be blocked by HS-142-1. The effect was enhanced in mice lacking eNOS or in the presence of the eNOS inhibitor L-NAME, suggesting a cross talk of the NO and NP system in regulating vascular tone [57]. In contrast, a study using porcine coronary rings revealed that vasorelaxation, upon CNP stimulation, was not affected by inhibition of NOS with L-NAME [35], raising the possibility that the degree of interaction between both systems is tissue and/or species specific.

Despite the relative specificity of CNP for the stimulation of NPR-B, the vasorelaxing effect of CNP in isolated renal arteries of NPR-A knockout mice was blunted compared to wild-type mice. Meanwhile, this response was unaffected in aortic rings [58]. This effect could neither be attributed to a change in NPR-B or -C expression levels nor to impaired downstream signaling. Differential expression of receptor subtypes in different sections of the vasculature provides a possible explanation for this phenomenon.

In animal models as well as in humans, CNP infusion lowered arterial blood pressure [53, 59, 60]. Igaki et al. [59] have shown that intravenously administered CNP in humans decreased systolic and diastolic blood pressure. In healthy volunteers, intra-arterial infusion of CNP led to increased forearm blood flow that was not dependent on NOS activity but attenuated by inhibition of calcium-sensitive potassium channels [36]. These data suggest that CNP, in accordance with in vitro effects, mediates arterial vasodilatation in vivo via potassium channel opening and hyperpolarization of vascular smooth muscle cells. In rat renal microvessels, CNP was able to dilate pre- and postglomerular vessels, while stimulation with the NPR-C specific ligand C-ANP was devoid of any vascular effect [61]. However, several studies suggest that the arterial vasodilating properties of CNP are at least partially mediated through NPR-C binding [62]. Thus, the precise role of NPR-B in the regulation of the arterial vascular tone is not entirely clear.

In contrast, CNP/NPR-B seems to be the responsible signaling pathway for venodilatory effects of CNP. Furthermore, when compared to ANP, CNP was more potent in dilating pulmonary veins but less capable of relaxing pulmonary arteries, suggesting distinct mechanisms for NPR-A and NPR-B in mediating vasodilation in arteries and veins [63]. NPR-B could, therefore, have a predominant role for the reduction in cardiac preload.

In most of these studies, however, CNP was applied at supraphysiological doses, and the observed effects cannot entirely be attributed to exclusive NPR-B stimulation. Hence, when NPR-B receptors in healthy volunteers were stimulated by a low-dose CNP infusion that led to a fourfold and tenfold increase in circulating CNP levels, respectively, neither cardiac output nor systemic blood pressure was affected [64]. In a similar study, CNP infusion did not have any additional effect on arterial response when coadministered with ANP [65]. Another limitation of supraphysiological doses is a possible competition for NPR-C leading to an increased efficiency of circulating ANP and BNP due to reduced clearance from circulation.

The generation of a mouse line lacking NPR-B by Tamura et al. [22] partially overcame this problem and promised a better insight into the role of NPR-B in the chronic regulation of blood pressure and vascular tone. These NPR-B null mice showed a significantly blunted cGMP response upon CNP stimulation on a cellular level. Main phenotypic findings included dwarfism due to impaired enchondral ossification and female infertility

resulting from abnormal uterine and ovarial development. Unexpectedly, blood pressure assessment under either low or high salt diet did not show any differences between NPR-B null and wild-type control mice. In addition, the suppression of aldosterone secretion by high salt diet was preserved in both genotypes. However, these data only reflect the blood pressure regulation in young animals because the majority of the NPR-B null mice died prematurely (before 100 days of age) as a consequence of skull deformation with compression of the medulla oblongata or seizures. Likewise, an earlier model of targeted disruption of CNP demonstrated a similar skeletal phenotype along with a reduced survival rate, and no evident cardiovascular phenotype was reported [21]. Therefore, a role of NPR-B for the long-term blood pressure regulation cannot be concluded from these studies.

A different approach was employed by our group using transgenic rats expressing a dominant negative mutant of the NPR-B mutant (NPR-BΔKC) that resulted in a functional knockdown of the receptor [23]. These rats were viable, had a normal life span, and only modest skeletal abnormalities. Under normal conditions, the arterial blood pressure, as assessed telemetrically, in conscious rats did not differ between NPR-BΔKC and wild-type animals. Given the increase in circulating levels of both ANP and BNP in the NPR-BΔKC rats, it is possible although unlikely that a hypertensive phenotype, resulting from a disrupted NPR-B signaling, is masked.

In conclusion, despite mediating a vasodilatory response upon CNP stimulation, NPR-B does not seem to play a significant role in long-term blood pressure regulation under physiological conditions. Its signaling may, however, prevent the manifestation of hypertension under pathophysiological conditions such as an activated renin–angiotensin–aldosterone system.

Effects on vascular remodeling

In vitro studies have shown that CNP mediates antiproliferative and antihypertrophic actions in several cell types including vascular smooth muscle cells and cardiac fibroblasts [33, 66]. Interestingly, while the vasorelaxing properties of CNP where inferior to ANP, its antiproliferative effects were superior. CNP was shown to be the most potent among the three NP regarding the inhibition of smooth muscle cell migration after stimulation with platelet-derived growth factor [67]. This effect was accompanied by an increase in cellular cGMP levels and mimicked by the cGMP analog 8-Br-cGMP, suggesting a NPR-B-dependent mechanism. These antiproliferative and antimigratory effects could play a role in vascular remodeling. In fact, CNP has reduced neointima formation and thrombosis in different vascular injury models [68, 69]. When CNP was administered locally, it prevented intima proliferation and endothelial dysfunction in rabbits without affecting endothelium-independent relaxation [70]. As observed for the vasodilatory effects, the involvement of NOS seems to play an important role in this process, as CNP caused a significant iNOS induction after carotid artery balloon injury in rabbits. Conversely, the antiproliferative effect could be blunted by inhibiting NOS with L-NAME [69]. Furthermore, CNP-mediated suppression of the antifibrinolytic enzyme plasminogen activator inhibitor-1 transcription, regulation of its release from VSMC in vitro, and its expression in neointima, media, and adventitia could have a beneficial effect in the pathogenesis of atherosclerosis [71, 72]. Similar results were obtained in a model of bleomycin-induced pulmonary fibrosis in mice [73]. Here, CNP attenuated the infiltration of monocytes and macrophages in the lung and reduced the collagen content and the cell proliferation in fibrotic lesions. In addition, a recent study by Scotland et al. [74] demonstrated that CNP was able to suppress basal and inflammation-stimulated leukocyte activation, inhibited platelet–leukocyte interaction, prevented thrombin-induced platelet aggregation in human blood, and reduced endothelial expression of P-selectin, an important regulator of leukocyte recruitment into tissue. Thus, due to the beneficial combination of antiproliferative, anti-inflammatory, and vasoactive properties and the lack of side effects on systemic hemodynamics, CNP might have therapeutic potential for the management of diseases involving pathological vascular remodeling, such as primary pulmonary hypertension.

To date, it has not been completely elucidated if the stimulation of NPR-C by CNP also contributes to the observed effects on vascular remodeling. While the expression of CNP and NPR-C were upregulated in parallel in the neointima after vascular injury in rabbits, NPR-B was not detectable in the neointima and did not significantly change in the media [75, 76]. A similar observation has been made in patients undergoing percutaneous coronary intervention. CNP was strongly upregulated in the neointima, but NPR-B expression was not regulated [77]. Of note, a recent study showed NPR-B messenger RNA (mRNA) in intermediate plaques and advanced atherosclerotic lesions in human coronary arteries, together with upregulated CNP expression that was localized in endothelial cells, smooth muscle cells, macrophages, and microvessels [78]. Hence, the possibility of NPR-B involvement in the process of vascular remodeling and development of atherosclerosis has to be considered despite inconsistent data regarding receptor regulation. Studies employing animals lacking a functional receptor are necessary to further clarify this issue. The antiproliferative effects of NP on smooth muscle cells raise the question of whether the CNP/NPR-B signaling could also prevent angiogenesis. On the contrary,

the vascular regeneration in a mouse model of hindlimb ischemia was improved as demonstrated by increased capillary density and perfusion rate when either BNP or the downstream target of the NP, guanylyl cyclase I (cGKI), was overexpressed [79]. This effect was mimicked by adenoviral gene transfer of CNP. Conversely, the recovery after surgery was blunted in cGKI knockout mice. The in vivo data were further complemented by the observation that ANP, BNP, and CNP stimulate the capillary network formation of human endothelial cells, with CNP being the most potent of the three NP. These data suggest that the CNP/NPR-B signaling pathway could play an important role in ischemia-induced angiogenesis. However, vascular endothelial growth factor (VEGF), a potent angiogenetic factor, has been demonstrated to suppress CNP mRNA expression and CNP secretion [80]. The interaction between VEGF and CNP-signaling has not been verified yet.

Effects on pathological cardiac hypertrophy and remodeling

The normal cardiac response to an increase in workload, as seen during exercise, involves the stimulation of insulin-like growth factor 1 and results in hypertrophy of cardiomyocytes and increased contractility as an adaptive mechanism [81]. By contrast, the presence of chronic overload as in arterial hypertension or after loss of viable myocardium due to infarction leads to pathological remodeling that is characterized by proliferation of fibroblasts with subsequent fibrosis, loss of cardiomyocytes by apoptosis or necrosis, and cardiac dysfunction. These effects are mediated by an activated renin–angiotensin–system, endothelin-1 (ET-1), and enhanced sympathetic tone.

The NP play an important role in counterbalancing the effects of hypertrophic stimuli such as angiotensin II, ET-1, vasopressin, and aldosterone on pathological cardiac growth. While the role of NPR-A has been analyzed in several in vitro as well as animal models, fewer data are available linking CNP/NPR-B signaling to the regulation of cardiac growth and hypertrophy (Fig. 1). A recent study by Tokudome et al. [82] demonstrated that CNP was able to reduce basal as well as ET-1 stimulated protein synthesis in cardiomyocytes. In parallel, a significant reduction in the expression levels of hypertrophy-associated genes, transcriptional activity of MEF2 and GATA4, diminished ANP secretion and Ca^{2+}/calmodulin-dependent kinase II activity were found. Conversely, the antihypertrophic properties of CNP were blunted by ET-1 involving a protein kinase C (PKC)-depending mechanism [66]. In isolated adult rat cardiomyocytes, the stimulation of NPR-B with CNP was as potent as ANP or BNP in inhibiting angiotensin II-induced hypertrophy [83]. This antihypertrophic action was mediated via cGMP, as the effect was abolished by either blocking the GC-coupled NPR with HS-142-1 or inhibiting the cGMP-dependent PK. Despite conflicting data regarding a possible role of NPR-C in mediating antiproliferative effects of CNP, specific NPR-C ligands did not mimic the CNP effect in these cells [33, 84].

It seems that, similar to its mode of action in the vasculature, CNP/NPR-B signaling plays a paracrine role in

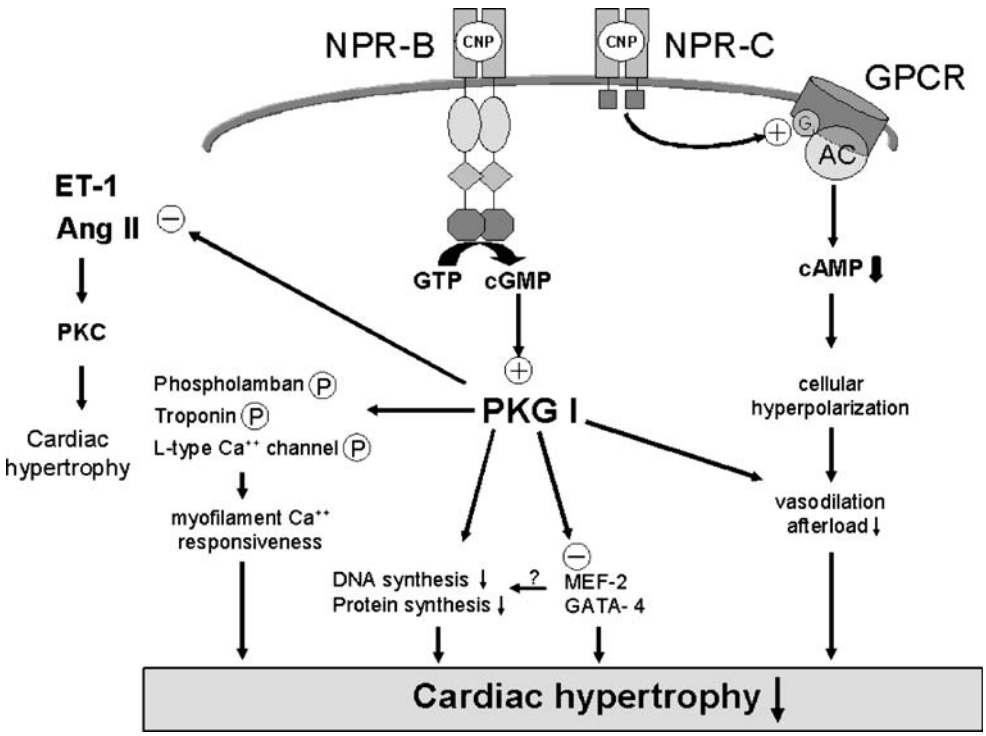

Fig. 1 Signaling pathways involved in antihypertrophic actions of CNP. CNP binds to NPR-B and activates the GC, leading to intracellular cGMP formation and subsequent activation of the cGMP-dependent protein kinase (*PKG*). PKG phosphorylates intracellular target proteins, inhibits activation of transcription factors facilitating cardiac growth such as MEF-2 and GATA-4, and impairs DNA as well as protein synthesis. In addition, PKG inhibits endothelin-1 (*ET-1*) and angiotensin II (*Ang II*) mediated cardiac hypertrophy. Another relevant pathway of CNP signaling involves binding to NPR-C that leads to activation of inhibitory G protein (G_i) and subsequent inhibition of adenylate cyclase (*AC*) at the G-protein coupled receptor (*GPCR*)

the heart, where endothelial cells and fibroblasts secrete CNP that targets NPR on cardiomyocytes, fibroblasts, and vascular smooth muscle cells [85]. In fact, synthesis and secretion of CNP from cardiac fibroblasts was recently demonstrated in cultured rat fibroblasts [66]. Here, the production of CNP was stimulated by TNF-β1, basic fibroblast growth factor, and ET-1, and CNP was able to suppress hypertrophy and DNA synthesis in the fibroblasts in a cGMP-dependent mode.

In addition to its antihypertrophic properties, NPR-B has been shown to mediate pro-apoptotic effects in neonatal cardiomyocytes [86]. The stimulation of apoptosis in these cells was also cGMP-dependent and antagonized by ET-1. In contrast to mice lacking NPR-A, which are characterized by cardiac hypertrophy, NPR-B knockout mice did not show any signs of heart hypertrophy or fibrosis. As mentioned above, these mice have a reduced life expectancy that limits a more comprehensive cardiovascular phenotyping; thus, it cannot be truly inferred from this model that NPR-B does not possess antihypertrophic effects in the heart in vivo.

Despite the fact that the nonmyocyte population in the heart constitutes the predominant localization of NPR-B [87], transgenic rats expressing the dominant negative receptor mutant (NPR-BΔKC) developed significant cardiac hypertrophy that could be attributed to an increased size of single cardiomyocytes without evident fibrosis (Fig. 2) [23]. Echocardiographic and histological analysis in these animals revealed concentric left ventricular hypertrophy which aggravated with age. However, no left ventricular dysfunction was observed in the transgenic rats up to 1 year of age. Furthermore, when cardiac hypertrophy was challenged by chronic volume overload, the hypertrophy was enhanced in the transgenic rats, and deterioration of left ventricular contractility was blunted in these animals. Because the blood pressure was unchanged in the NPR-BΔKC rats compared to wild-type rats, these results suggest a role for NPR-B in cardiac growth under both physiological and pathophysiological conditions. The lack of significant fibrosis was surprising, as CNP has been demonstrated to have an antiproliferative effect on fibroblasts, NPR-B is the predominant natriuretic receptor in cardiac fibroblasts, and cardiomyocytes mainly express NPR-A. A possible explanation for this phenomenon is the lack of complete disruption of NPR-B signaling in the transgenic rats. The remaining baseline receptor activity might be sufficient to prevent cardiac fibrosis in these animals and could also limit the extent of cardiac hypertrophy, meaning the antihypertrophic effect of NPR-B signaling might be underestimated in this model.

Given that antihypertrophic and antiproliferative effects of CNP via activation of NPR-B have been demonstrated in several in vitro experiments and in NPR-BΔKC transgenic rats, these features could be of potential clinical relevance in the context of cardiac remodeling. As a study by Soeki et al. [88] demonstrated, when infused for 2 weeks after an experimental myocardial infarction in rats, CNP prevented cardiac hypertrophy and dysfunction as determined by attenuated left ventricular enlargement and preserved left ventricular contractility and relaxation. These effects were observed using a CNP dose that did not affect the blood pressure. The effect on both cardiomyocytes and cardiac fibroblasts contributed to this phenotype, as the hearts were characterized by a reduced cardiomyocyte cross-sectional area, reduced levels of fibrosis markers collagen I, collagen III, and fibronectin along with lower collagen volume fraction compared to the vehicle-treated animals. Interestingly, the left ventricular CNP mRNA levels showed a dramatic upregulation 3 days after coronary ligation and returned to baseline levels within 3 weeks, suggesting a paracrine compensatory role for CNP/NPR-B signaling under these pathophysiological conditions.

In addition to cardiac remodeling after myocardial infarction, a possible involvement of NPR-B has been analyzed in the pathogenesis of diabetic cardiomyopathy [89]. The regulation of cardiac NPR-B was assessed in two different models of diabetes in mice resembling either type I (streptozotocin-treated mice) or type II diabetes (ob/ob mice). In both models, cardiac NPR-B mRNA was increased compared to control mice, while NPR-A and NPR-C mRNA levels did not change, suggesting that upregulation of NPR-B may counteract the development of cardiac fibrosis under these conditions. In addition, chronic CNP infusion ameliorated the development of pulmonary hypertension in monocrotaline-treated rats without affecting systemic blood pressure [90]. The reduction in right ventricular pressure and hypertrophy was accompanied by suppressed inflammatory response, blunted vascular remodeling, and improved survival compared to vehicle-treated animals. The possible compensatory role for CNP/NPR-B signaling in states of cardiac hypertrophy and heart failure is further underlined by a recent clinical report demonstrating that the myocardium is a site of CNP production in chronic heart failure [91]. Taken together, the close proximity of ligand and receptor-expressing cells points towards a paracrine role of the CNP/NPR-B signaling mechanism in controlling cellular growth and hypertrophy in the heart.

Effects on myocardial function

Positive effects of CNP on cardiac contractility, lusitropy, chronotropy, and dromotropy have been described in several studies using intact animals, isolated hearts, cardiac muscle preparations, and isolated cardiomyocytes [92], although conflicting data characterizing the effect of CNP on cardiac function have been published. When CNP was adminis-

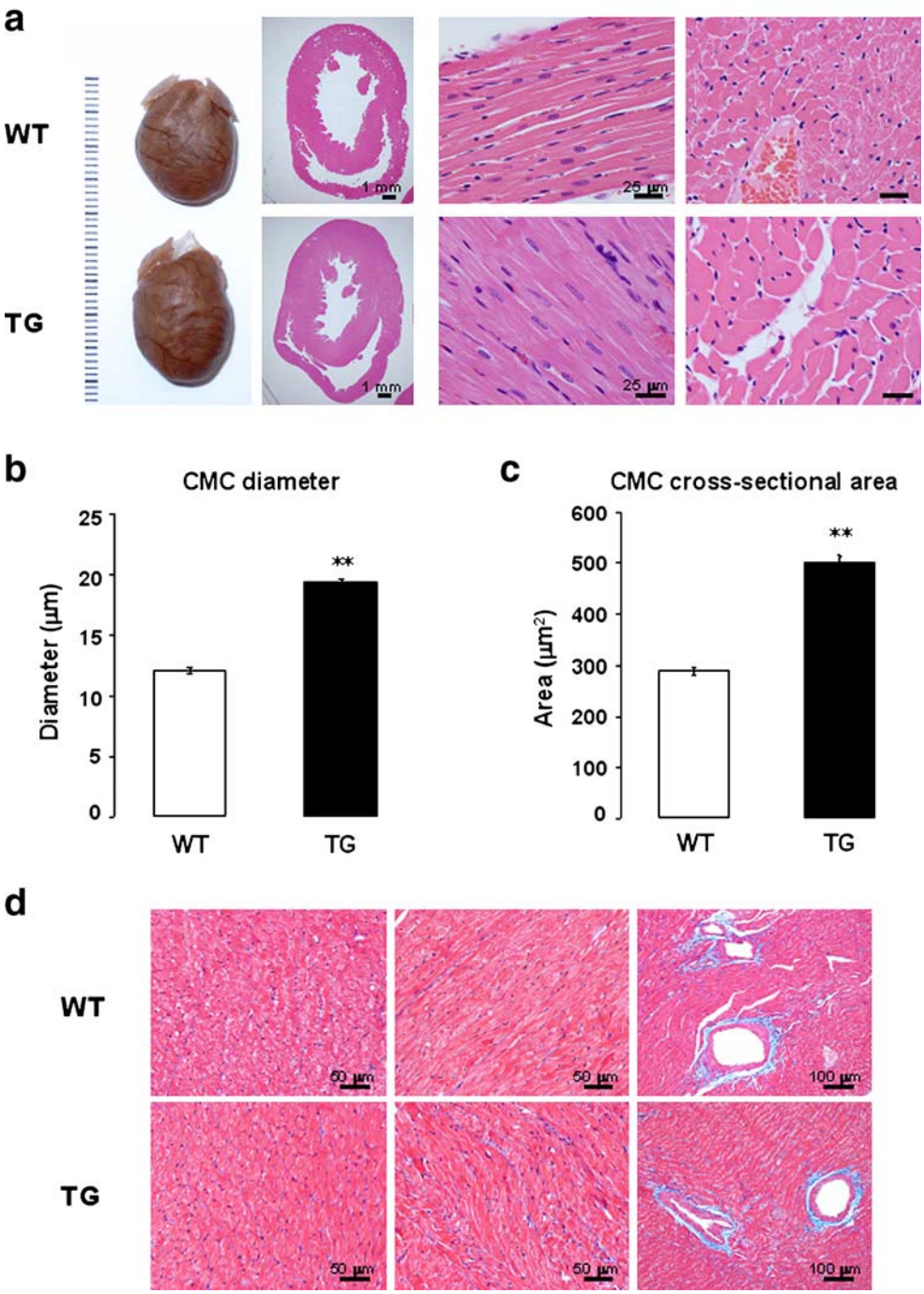

Fig. 2 Morphological analysis of cardiac hypertrophy and fibrosis in 6-month-old NPR-BΔKC transgenic animals. **a** Exemplary hearts and hematoxylin and eosin-stained cardiac cross and longitudinal sections of wild-type (*WT*) and transgenic (*TG*) rats. **b** Quantification of cardiomyocyte (*CMC*) diameter and **c** cross-sectional area revealed hypertrophy of cardiomyocytes in NPR-BΔKC transgenic animals. $^{**}p<0.01$ vs WT control; $n=5$ hearts, and 200 images per genotype for statistical analysis. **d** Masson's trichrome staining of cross and longitudinal left ventricular sections showed no increase in interstitial or perivascular fibrosis in NPR-BΔKC transgenic (*TG*) rats compared to wild-type (*WT*) controls ([23], Copyright (2006) National Academy of Sciences, U.S.A.)

tered intravenously in dogs, it slightly reduced end-diastolic volume and pressure along with an approximately 15-fold increase in circulating CNP levels [93]. In addition, cGMP levels increased significantly, suggesting a NPR-B mediated effect. These cardiac actions were abolished, however, in dogs with pacing-induced heart failure, in parallel with blunted cGMP response, suggesting an impaired receptor signaling under the conditions of heart failure.

CNP perfusion of isolated mouse hearts showed a biphasic regulation with an initial increase in contraction and relaxation rates, increased ventricular pressure, and reduced relaxation time, followed by a slow decline of these hemodynamic parameters to baseline levels [94, 95]. The positive inotropic and lusitropic effect in this model involved the generation of cGMP and the subsequent activation of cGMP-dependent protein kinase-I (PKG I), phosphorylation of phospholamban and intracellular Ca^{2+} release from the sarcoplasmatic reticulum. Concurrently, CNP caused a dose-dependent increase in atrial and ventricular contractility and slightly enhanced sinus rate in

isolated blood perfused dog heart preparations, involving the activation of guanylyl cyclase-coupled receptors [96]. Although it could be speculated that at higher concentrations CNP binds to and stimulates NPR-A, the cardiac responsiveness to CNP was even enhanced in NPR-A knockout mice [95], possibly due to enhanced expression of PKG I in these mice. In isolated mouse cardiomyocytes, CNP stimulation increased cell shortening and systolic Ca^{2+} levels in parallel with an accelerated Ca^{2+} decay [94]. In contrast, negative inotropic properties of CNP, mediated via NPR-B/PKG activation, have been described in rat papillary muscle [97] despite an increase in relaxation rate, in neonatal rat [98] and mouse cardiomyocytes [99], and rabbit ventricular cardiomyocytes [100]. Furthermore, the cGMP analog 8Br-cGMP reduced contractile force in mouse atrial and ventricular myocardial preparations [101]. In addition, in intact animals such as conscious sheep, a significant reduction in cardiac output after CNP infusion has been shown, while the arterial blood pressure remained unchanged in this experimental design [102]. In accordance with a possible negative inotropic CNP effect, NPR-BΔKC transgenic rats had enhanced ventricular contractility and showed a blunted left ventricular dysfunction after chronic volume overload [23]. However, the activation of NPR-C might also contribute to the observed negative inotropic effect of CNP. In two recent studies using either mouse sinoatrial or bullfrog atrial cells, CNP inhibited L-type Ca^{2+} current, thereby, slowing the pacemaker activity of these cells [103]. This effect was mimicked by either cANP, a NPR-C specific ligand, or a G_i protein activator but was not blocked by the NPR-A/-B antagonist HS-142-1, suggesting that NPR-C is the predominant receptor mediating CNP effects on cardiac chronotropy.

Effects on the autonomic nervous system

CNP is the major natriuretic peptide in the central nervous system and cerebrospinal fluid [104]. Large or intermediate NPR-B expression was observed in limbic cortex, neocortex olfactory bulb, hippocampus, amygdala, preoptic–hypothalamic neuroendocrine circuits, motor nuclei of cranial nerves, and in brainstem nuclei controlling autonomic function, suggesting a prominent physiological role of CNP and NPR-B in the central control of cardiovascular homeostasis [43, 105]. Several studies demonstrated the regulatory properties of NP by facilitating vagal or suppressing sympathetic neurotransmission. Upon injection into the lateral cerebroventricular area of the brain, CNP enhanced pancreatic fluid and protein secretion through a vagal pathway in rats [106]. Although injection of supraphysiological dosages of CNP may not exclude coactivation of NPR-A, this study suggests involvement of NPR-B in central regulation of autonomic nerve activity. In addition, ANP was able to mediate sympatho-inhibitory responses when injected locally into the anterior hypothalamic nucleus of conscious mice; however, CNP was not applied in this study [107]. When administered intravenously into conscious sheep, CNP, along with ANP and BNP, has been reported to enhance reflex bradycardia as induced by repetitive intravenous injection of a serotonin agonist [108]. Local positive chronotropic effects were found when CNP was injected directly into the sinus node artery of anesthetized dogs [109]. This was supported by another study on isolated, blood-perfused canine right atrial and left ventricular preparations, where CNP was found to increase myocardial contractile force along with sinus rate in a cGMP-dependent manner [96]. In isolated guinea pig right atrial vagal nerve preparations, both BNP and CNP facilitated heart rate response to vagal-induced bradycardia in a cGMP-dependent manner, further suggesting a local effect of CNP on cardiac vagal neurotransmission [110]. Because regulatory effects on autonomic nerve activity have been observed for ANP, BNP, and CNP, effects mediated by either NPR-A or -B were difficult to distinguish. Interestingly, NPR-BΔKC transgenic rats demonstrated an increased heart rate in parallel with enhanced sympathetic nerve activity [23], suggesting that the cardiac phenotype of NPR-BΔKC transgenic rats might also be a result of reduced CNP-mediated inhibition of the autonomic nervous system. In addition, NBR-BΔKC rats displayed elevated renin levels (own unpublished data), most likely as a result of enhanced sympathetic nerve activity in this model. This function seems to be specific for NPR-B, as it has not been found in genetic mouse models with altered ANP or NPR-A signaling [111, 112]. Further studies are necessary to determine whether CNP-mediated and NPR-B play an exclusive role in controlling cardiovascular functions by regulating the autonomic nervous system, including regulation of renin secretion, and to distinguish direct effects on cardiomyocytes from indirect effects involving modulation of vagal and sympathetic nerve activity.

Contribution of genetic alteration of CNP and NPR-B to cardiovascular disease

Loss-of-function mutations in the human NPR-B have been reported in patients with acromesomelic dysplasia, type Maroteaux [113]. Given the cardiovascular effects described in this review, however, information on genetic alterations of CNP and NPR-B and their association with cardiovascular diseases in humans are of great interest.

Ono et al. [114] performed an association study linking mutations in the CNP gene to essential hypertension in a Japanese population using data from 2006 subjects. Out of four genetic variants found in the CNP gene, a G2628A

polymorphism in the 3′-untranslated region was significantly associated with essential hypertension. The odds ratio was even higher when only a subpopulation of subjects age 65 and younger was studied. This study suggests that this CNP gene variant may contribute to development of hypertension. Rehemudula et al. [115] unraveled the structure of NPR-B and were able to find a CA/GT microsatellite repeat localized to intron 2 approximately 150 bp downstream of the exon-intron junction. Although only 103 patients with hypertension and 101 normotensive control subjects were enrolled in this study, a GT(11) microsatellite repeat distribution was significantly associated with essential hypertension in this Japanese population. Other mutations found in the NPR-B gene, such as a C2077T polymorphism in exon 11 or a 9-bp insertion/deletion in intron 18, were neither associated with essential hypertension nor myocardial or cerebral infarction [116–118]. These data show that mutations in CNP or NPR-B genes might be related to essential hypertension, however, are limited to some extent by sample size and ethnic background of the study population. To draw a more general conclusion, it would be of great interest to confirm these findings in larger study populations and diverse ethnic groups. The findings from these genetic studies also seem to contradict findings from the genetic mouse and rat models altering NPR-B signaling, where no effect on blood pressure could be demonstrated. This raises the question of whether compensatory mechanisms are activated in genetic animal models counteracting the loss of NPR-B signaling, such as increased NPR-A signaling by increased ANP and BNP plasma concentrations, or whether the finding from the genetic studies cannot be generalized due to methodical restriction. Further studies are necessary to fully elucidate and understand the role of genetic alterations of CNP or NPR-B in cardiovascular diseases.

Conclusion

CNP and NPR-B signaling play an important regulatory role in the cardiovascular system (Fig. 3). Significant progress has been made to understand underlying mechanisms, accelerated by recent publications of genetic mouse and rat models. Although CNP and NPR-B null mice suggest a major role of NPR-B signaling in endochondral ossification and development of female reproductive organs, the first in vivo evidence of cardiovascular functions of NPR-B was derived from transgenic rats overexpressing a dominant negative mutant of the receptor. These rats demonstrated cardiac hypertrophy, along with increased heart rate and sympathetic nerve activity. In contrast to ex vivo data, none of the genetically engineered animal models had elevated blood pressure, suggesting that CNP and NPR-B are not involved in long-term blood pressure regulation and that the observed cardiac hypertrophy is not a result of elevated blood pressure. Further research is needed to distinguish direct effects on cardiomyocytes from effects mediated by modulation of heart rate and autonomic nervous activity. Pharmacological approaches employing available genetic mouse and rat models as well as organ specific

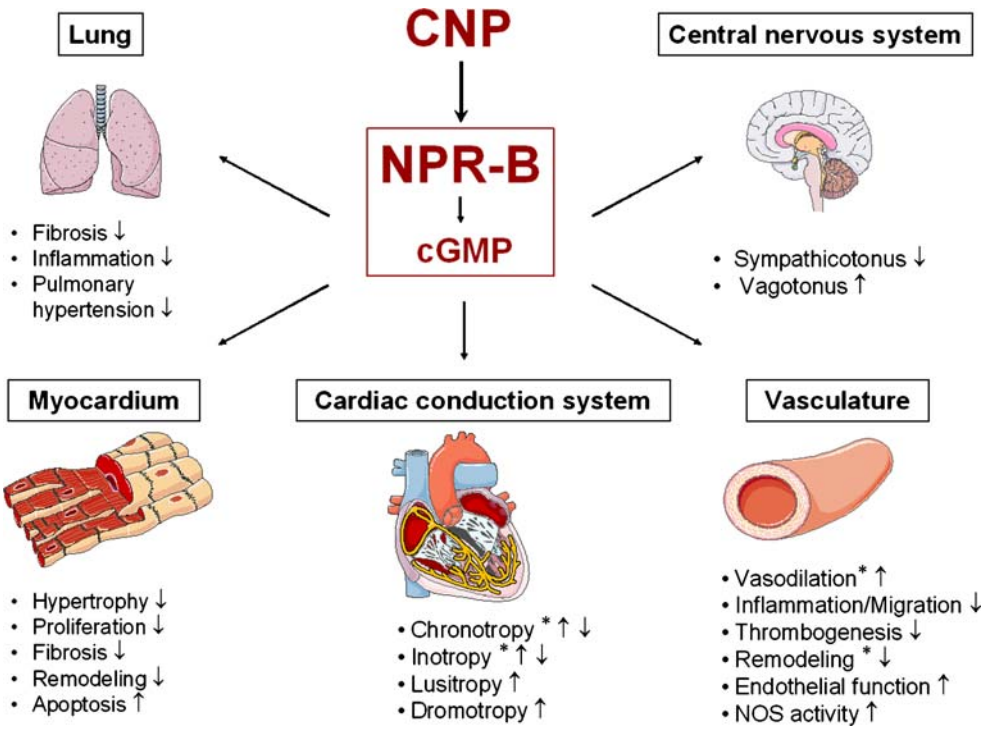

Fig. 3 Summary of cardiovascular functions of C-type natriuretic peptide (*CNP*) and natriuretic peptide receptor B (*NPR-B*). Recent findings link NPR-B signaling to control of cardiomyocyte growth, involving either direct cardiac effects or regulatory effects on the autonomic nervous system. *Asterisk* CNP possesses affinity to NPR-B and -C. Some of the described effects cannot be clearly attributed to signaling of either receptor, or there are conflicting data reported in the literature

deletion of NPR-B signaling in the heart and brain will be necessary. Moreover, other cardiovascular functions of CNP may be mediated in part by coactivation of NPR-C and will need to be further elucidated employing animal models lacking NPR-B signaling, including regulation of vascular smooth muscle cell proliferation during vascular wound repair and atherosclerosis, cardiac performance and remodeling, and the beneficial anti-inflammatory and antiproliferative effects of CNP in primary pulmonary hypertension. These questions are of fundamental interest not only to further understand the pathophysiological significance of CNP and NPR-B, but also to identify NPR-B as potential pharmacological target for treatment of cardiovascular diseases.

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