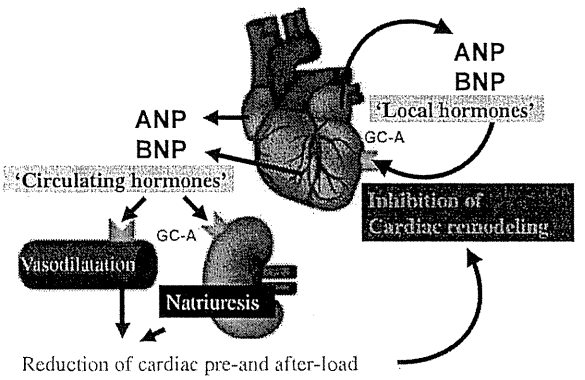

Fig. 1. ANP and BNP, the cardiac natriuretic peptides, protect the heart in not only an endocrine but also a paracrine fashion. Because ANP and BNP have potent diuretic, natriuretic and vasodilatory actions, augmentation of the ANP and BNP/GC-A signaling leads to a decrease in cardiac pre- and after-load, and their mobilization during cardiac failure is considered one of the compensatory mechanisms activated in response to heart damage. In addition to the hemodynamic effects of their actions as circulating hormones, recent evidence suggests that ANP and BNP also exert local cardioprotective effects by acting as autocrine/paracrine hormones.

Since the diuretic, natriuretic and vasorelaxant activities of ANP and BNP lead to reduction of the cardiac pre- and after-load, these results suggest that the cardiac natriuretic peptides/GC-A signaling exerts its cardioprotective actions in both an endocrine and an autocrine/paracrine fashion. These mechanisms are schematically depicted in Fig. 1.

The molecular mechanism of GC-A-mediated inhibition of cardiac hypertrophy

To identify the molecular mechanism underlying cardiac hypertrophy seen in GC-A-deficient mice, DNA microarrays were used to identify genes upregulated in the hypertrophied heart [45]. Among several genes known to be upregulated in cardiac hypertrophy (e.g. α-skeletal actin, ANP and BNP), it has been found that the expression of the gene encoding myocyte-enriched calcineurin-interacting protein (MCIP1) is also increased. The *MCIP1* gene is reportedly regulated by calcineurin, a critical regulator of cardiac hypertrophy. Thus, it was hypothesized that the calcineurin activity is enhanced in the heart of GC-A-deficient mice. To test this hypothesis, cultured neonatal cardiomyocytes were used to determine whether pharmacological inhibition of GC-A would increase calcineurin activity, which it did not [45]. On the other hand, stimulation of GC-A with ANP inhibited calcineurin activity, suggesting that it is by inhibiting the calcineurin pathway that cardiac GC-A signaling (activated by locally secreted natriuretic peptides) exerts its anti-hypertrophic effects. In fact, chronic treatment with FK506, which in combination with FK506-binding protein inhibits the phosphatase activity of calcineurin, significantly reduces the heart weight to body weight ratio, cardiomyocyte size and collagen volume fraction in GC-A-deficient mice compared with the wild-type mice [45]. A further study using microarray analysis and real-time PCR analysis revealed that, in addition to the calcineurin–nuclear factor of activated T-cells (NFAT) pathway, the calmodulin–CaMK–Hdac–Mef2 and PKC–MAPK–GATA4 pathways may also be involved in the cardiac hypertrophy seen in the GC-A-null mice [46].

Role of regulator of G-protein signaling in CG-A cardioprotective actions

Recently, it has been elegantly demonstrated that cGMP-dependent protein kinase (PKG) Iα attenuates signaling by the thrombin receptor protease-activated receptor (PAR) 1 through direct activation of regulator of G-protein signaling (RGS) 2 [47]. PKG-Iα binds directly to and phosphorylates RGS-2, which significantly increases the GTPase activity of $G\alpha_q$, thereby terminating PAR-1 signaling. Given that cGMP is an intracellular second messenger for natriuretic peptides, RGS might mediate the cardioprotective effect of the GC-A signaling. To test this hypothesis, the role of RGS-4, which is the predominant RGS in cardiomyocytes under physiological conditions, was examined. In cultured cardiomyocytes, ANP stimulated the binding of PKG-Iα to RGS-4 as well as the phosphorylation of RGS-4 and its subsequent association with $G\alpha_q$ [48]. In addition, cardiomyocyte-specific overexpression of RGS-4 in GC-A-null mice significantly rescued the cardiac phenotype of these mice. On the contrary, overexpression of a dominant-negative form of RGS-4 blocked the inhibitory effects of ANP on cardiac hypertrophy [48]. Therefore, GC-A may activate cardiac RGS-4, which then inhibits the activity of $G\alpha_q$ and its downstream hypertrophic effectors. The endogenous cardioprotective mechanism meditated by ANP/BNP, GC-A and RGS-4 is depicted schematically in Fig. 2.

Very recently, PKG activation reflecting chronic inhibition of cGMP-selective phosphodiesterase 5 has been shown to suppress maladaptive cardiac hypertrophy by inhibiting $G\alpha_q$-coupled stimulation, and the effect was not observed in mice lacking RGS-2 [49]. This suggests that RGS2 mediates the cardioprotective actions of PKG in pathological conditions such as

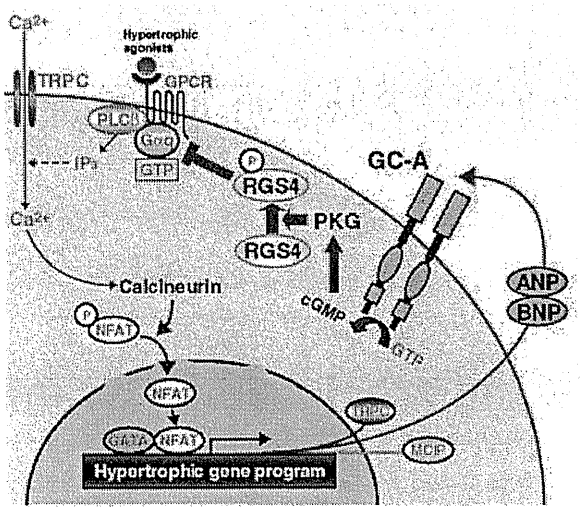

Fig. 2. Inhibitory mechanism of cardiac hypertrophy by the local natriuretic peptide system. Cardiac hypertrophy agonists such as angiotensin II, catecholamines and endothelins stimulate G-protein coupled receptor. Subsequent production of inositol triphosphate (IP3) promotes elevation of intracellular Ca^{2+} levels, which results in activation of the calcineurin/nuclear factor of activated T cells (NFAT) pathway. Cooperatively with the family of GATA transcription factors, NFAT activates the hypertrophic gene program, which includes the ANP- and BNP-coding genes. In an autocrine or paracrine fashion, ANP and BNP stimulate their receptor GC-A and exert their anti-hypertrophic actions via the activation of the RGS, which consequently results in an increase in the GTPase activity of the α subunit of the guanine nucleotide binding protein ($G\alpha_q$) and in a decrease in the activity of the downstream signaling mediators (adapted from [48]).

pressure overload or excessive $G\alpha_q$ activation due to hypertrophic stimuli. In fact, RGS-2 is also implicated in the anti-hypertrophic action of cardiac GC-A [50].

The role of GC-A in myocardial infarction

It is well known that plasma levels of ANP and BNP are dramatically elevated early after myocardial infarction [51]. To examine the significance of this upregulation, experimental myocardial infarction by ligation of the left coronary artery was induced in mice lacking GC-A [52]. GC-A-deficient mice exhibited significantly higher mortality rate than wild-type mice, reflecting a higher incidence of acute heart failure. Four weeks after infarction, left ventricular remodeling, including myocardial hypertrophy and fibrosis, and impairment of the left ventricular systolic function were significantly more severe in mice lacking GC-A than in wild-type mice [52]. GC-A activation by endogenous cardiac natriuretic peptides may protect against acute heart failure and attenuate chronic cardiac remodeling after acute myocardial infarction.

Role of GC-A in peripheral arterial disease

A role of the natriuretic peptide system in peripheral arterial diseases has also been suggested. Activation of the natriuretic peptides–cGMP–PKG pathway was found to accelerate vascular regeneration and blood flow recovery in a murine model of peripheral arterial disease, in which leg ischemia was induced by femoral arterial ligation [53]. Recently, it has been reported that intraperitoneal injection of carperitide, a recombinant human ANP, accelerated blood flow recovery with increasing capillary density in the ischemic legs [54], indicating the role of exogenously administered ANP and BNP in angiogenesis. When the hindlimb ischemia model was performed in GC-A-deficient mice, autoamputation or ulcers were more severe in GC-A-deficient mice than in their wild-type counterparts [55]. Laser Doppler perfusion imaging revealed that the recovery of blood flow in the ischemic limb was significantly inhibited in GC-A-null mice compared with wild-type mice. In addition, vascular regeneration in response to critical hindlimb ischemia was severely impaired [55]. Similar attenuation of ischemic angiogenesis was observed in mice with conditional, endothelial-cell-restricted GC-A deletion. On the other hand, smooth-muscle-cell-restricted GC-A ablation did not affect ischemic neovascularization [56], suggesting that it is the endothelial GC-A that stimulates endothelial regeneration after induction of ischemia. Taken together, the evidence suggests that the natriuretic peptide pathway significantly contributes to peripheral vascular remodeling during ischemia.

Role of the CNP/GC-B pathway in bone formation

In a 1998 study, mice with transgenic overexpression of the *BNP* gene, especially those exhibiting high expression levels, unexpectedly displayed deformed bony skeletons characterized by kyphosis, elongated limbs and paws, and crooked tails, which resulted from a high turnover of endochondral ossification accompanied by overgrowth of the growth plate [57]. Even after crossing with GC-A-null mice, transgenic mice overexpressing BNP continued to exhibit marked longitudinal growth of the vertebrae and long bones [58]. Therefore, the effect of excess amount of BNP on endochondral ossification is independent of GC-A, and so signaling through another receptor was suggested.

In 2001, CNP-deficient mice were reported to show severe dwarfism as a result of impaired endochondral ossification [59], thus indicating that CNP acts locally as a positive regulator of endochondral ossification. In 2004, the phenotype of mice lacking GC-B was reported [60]. The GC-B-null animals exhibited dramatically impaired endochondral ossification and attenuation of longitudinal vertebral or limb bone growth. Therefore, it appears that GC-B is the receptor mediating the CNP action in inducing longitudinal bone growth. Furthermore, homozygous C-receptor-null mice also have skeletal deformities associated with a considerable increase in bone turnover [28], an opposite phenotype to that observed in the mice deficient for CNP. Since CNP is the only natriuretic peptide expressed in bone, it is suggested that one function of the C receptor is to clear locally synthesized CNP from bone and modulate its effects.

Since pharmacological amounts of BNP can stimulate GC-B, these results suggest that activation of the CNP/GC-B pathway in transgenic mice with elevated plasma concentrations of BNP or in mice lacking the C receptor for natriuretic peptides results in skeletal overgrowth. By contrast, inactivation of the CNP/GC-B pathway in mice lacking CNP, GC-B or cGMP-dependent protein kinase II (a downstream mediator of the CNP/GC-B pathway) results in dwarfism caused by defects in endochondral ossification.

Summary

As stated above, studies using genetically engineered animals revealed physiological and pathophysiological roles of the natriuretic peptides/receptor signaling pathways in the regulation of blood pressure/volume, maintenance of the cardiovascular system, and development of the longitudinal bone, acting as not only a circulating hormonal system but also a local regulatory system. Recent evidence also suggests roles for the natriuretic peptide system in renal [61] and neuronal [62] morphology and function. In addition, genetic defects of each component of the system in humans may cause diseases that are also observed in the genetically engineered animals. Furthermore, an interesting hypothesis that needs verification is that these observed phenomena could be the recapitulation of early developmental mechanisms. More studies at tissue, cellular and molecular levels are needed to clarify the mechanisms underlying the intriguing phenotypes observed in transgenic animal models. In addition, more studies at clinical and population levels are needed to elucidate the potential importance of the natriuretic peptide system in humans.

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Disclosures

The authors have nothing to disclose.

References

1 de Bold AJ, Borenstein HB, Veress AT & Sonnenberg H (1981) A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci* **28**, 89–94.
2 Flynn TG, de Bold ML & de Bold AJ (1983) The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. *Biochem Biophys Res Commun* **117**, 859–865.
3 Thibault G, Garcia R, Seidah NG, Lazure C, Cantin M, Chrétien M & Genest J (1983) Purification of three rat atrial natriuretic factors and their amino acid composition. *FEBS Lett* **164**, 286–290.
4 Kangawa K & Matsuo H (1984) Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (alpha-hANP). *Biochem Biophys Res Commun* **118**, 131–139.
5 Misono KS, Fukumi H, Grammer RT & Inagami T (1984) Rat atrial natriuretic factor: complete amino acid sequence and disulfide linkage essential for biological activity. *Biochem Biophys Res Commun* **119**, 524–529.
6 Currie MG, Geller DM, Cole BR, Siegel NR, Fok KF, Adams SP, Eubanks SR, Galluppi GR & Needleman P (1984) Purification and sequence analysis of bioactive atrial peptides (atriopeptins). *Science* **223**, 67–69.
7 Kangawa K, Tawaragi Y, Oikawa S, Mizuno A, Sakuragawa Y, Nakazato H, Fukuda A, Minamino N & Matsuo H (1984) Identification of rat atrial natriuretic polypeptide and characterization of the cDNA encoding its precursor. *Nature* **312**, 152–155.
8 Sudoh T, Kangawa K, Minamino N & Matsuo H (1988) A new natriuretic peptide in porcine brain. *Nature* **332**, 78–81.
9 Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, Shirakami G, Jougasaki M, Obata K, Yasue H *et al.* (1991) Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest* **87**, 1402–1412.

10 Nakagawa O, Ogawa Y, Itoh H, Suga S, Komatsu Y, Kishimoto I, Nishino K, Yoshimasa T & Nakao K (1995) Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocyte hypertrophy. Evidence for brain natriuretic peptide as an 'emergency' cardiac hormone against ventricular overload. *J Clin Invest* **96**, 1280–1287.
11 Mukoyama M, Nakao K, Saito Y, Ogawa Y, Hosoda K, Suga S, Shirakami G, Jougasaki M & Imura H (1990) Increased human brain natriuretic peptide in congestive heart failure. *N Engl J Med* **323**, 757–758.
12 Sudoh T, Minamino N, Kangawa K & Matsuo H (1990) C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. *Biochem Biophys Res Commun* **168**, 863–870.
13 Suga S, Itoh H, Komatsu Y, Ishida H, Igaki T, Yamashita J, Doi K, Chun TH, Yoshimasa T, Tanaka I *et al.* (1998) Regulation of endothelial production of C-type natriuretic peptide by interaction between endothelial cells and macrophages. *Endocrinology* **139**, 1920–1926.
14 Naruko T, Ueda M, van der Wal AC, van der Loos CM, Itoh H, Nakao K & Becker AE (1996) C-type natriuretic peptide in human coronary atherosclerotic lesions. *Circulation* **94**, 3103–3108.
15 Fuller F, Porter JG, Arfsten AE, Miller J, Schilling JW, Scarborough RM, Lewicki JA & Schenk DB (1988) Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clones. *J Biol Chem* **263**, 9395–9401.
16 Anand-Srivastava MB, Sehl PD & Lowe DG (1996) Cytoplasmic domain of natriuretic peptide receptor-C inhibits adenylyl cyclase. Involvement of a pertussis toxin-sensitive G protein. *J Biol Chem* **271**, 19324–19329.
17 Chinkers M, Garbers DL, Chang MS, Lowe DG, Chin HM, Goeddel DV & Schulz S (1989) A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature* **338**, 78–83.
18 Chang MS, Lowe DG, Lewis M, Hellmiss R, Chen E & Goeddel DV (1989) Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases. *Nature* **341**, 68–72.
19 Schulz S, Singh S, Bellet RA, Singh G, Tubb DJ, Chin H & Garbers DL (1989) The primary structure of a plasma membrane guanylate cyclase demonstrates diversity within this new receptor family. *Cell* **58**, 1155–1162.
20 Koller KJ, Lowe DG, Bennett GL, Minamino N, Kangawa K, Matsuo H & Goeddel DV (1991) Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* **252**, 120–123.
21 Suga S, Nakao K, Kishimoto I, Hosoda K, Mukoyama M, Arai H, Shirakami G, Ogawa Y, Komatsu Y, Nakagawa O *et al.* (1992) Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology* **130**, 229–239.
22 Steinhelper ME, Cochrane KL & Field LJ (1990) Hypotension in transgenic mice expressing atrial natriuretic factor fusion genes. *Hypertension* **16**, 301–307.
23 Ogawa Y, Itoh H, Tamura N, Suga S, Yoshimasa T, Uehira M, Matsuda S, Shiono S, Nishimoto H & Nakao K (1994) Molecular cloning of the complementary DNA and gene that encode mouse brain natriuretic peptide and generation of transgenic mice that overexpress the brain natriuretic peptide gene. *J Clin Invest* **93**, 1911–1921.
24 John SW, Krege JH, Oliver PM, Hagaman JR, Hodgin JB, Pang SC, Flynn TG & Smithies O (1995) Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science* **267**, 679–681.
25 Lopez MJ, Wong SK, Kishimoto I, Dubois S, Mach V, Friesen J, Garbers DL & Beuve A (1995) Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide. *Nature* **378**, 65–68.
26 Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL, Pandey KN, Milgram SL, Smithies O & Maeda N (1997) Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci USA* **94**, 14730–14735.
27 Oliver PM, John SW, Purdy KE, Kim R, Maeda N, Goy MF & Smithies O (1998) Natriuretic peptide receptor 1 expression influences blood pressures of mice in a dose-dependent manner. *Proc Natl Acad Sci USA* **95**, 2547–2551.
28 Matsukawa N, Grzesik WJ, Takahashi N, Pandey KN, Pang S, Yamauchi M & Smithies O (1999) The natriuretic peptide clearance receptor locally modulates the physiological effects of the natriuretic peptide system. *Proc Natl Acad Sci USA* **96**, 7403–7408.
29 Yoshimoto T, Naruse M, Naruse K, Shionoya K, Tanaka M, Tanabe A, Hagiwara H, Hirose S, Muraki T & Demura H (1996) Angiotensin II-dependent down-regulation of vascular natriuretic peptide type C receptor gene expression in hypertensive rats. *Endocrinology* **137**, 1102–1107.
30 Kishimoto I, Yoshimasa T, Suga S, Ogawa Y, Komatsu Y, Nakagawa O, Itoh H & Nakao K (1994) Natriuretic peptide clearance receptor is transcriptionally down-regulated by beta 2-adrenergic stimulation in vascular smooth muscle cells. *J Biol Chem* **269**, 28300–28308.
31 Tamura N, Ogawa Y, Chusho H, Nakamura K, Nakao K, Suda M, Kasahara M, Hashimoto R, Katsuura G, Mukoyama M *et al.* (2000) Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci USA* **97**, 4239–4244.
32 Sabrane K, Kruse MN, Fabritz L, Zetsche B, Mitko D, Skryabin BV, Zwiener M, Baba HA, Yanagisawa M &

Kuhn M (2005) Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide. *J Clin Invest* **115**, 1666–1674.
33 Holtwick R, Gotthardt M, Skryabin B, Steinmetz M, Potthast R, Zetsche B, Hammer RE, Herz J & Kuhn M (2002) Smooth muscle-selective deletion of guanylyl cyclase-A prevents the acute but not chronic effects of ANP on blood pressure. *Proc Natl Acad Sci USA* **99**, 7142–7147.
34 Kishimoto I, Dubois SK & Garbers DL (1996) The heart communicates with the kidney exclusively through the guanylyl cyclase-A receptor: acute handling of sodium and water in response to volume expansion. *Proc Natl Acad Sci USA* **93**, 6215–6219.
35 Shi SJ, Vellaichamy E, Chin SY, Smithies O, Navar LG & Pandey KN (2003) Natriuretic peptide receptor A mediates renal sodium excretory responses to blood volume expansion. *Am J Physiol Renal Physiol* **285**, F694–F702.
36 Potter LR, Abbey-Hosch S & Dickey DM (2006) Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev* **27**, 47–72.
37 Curry FR (2005) Atrial natriuretic peptide: an essential physiological regulator of transvascular fluid, protein transport, and plasma volume. *J Clin Invest* **115**, 1458–1461.
38 Nakao K, Itoh H, Saito Y, Mukoyama M & Ogawa Y (1996) The natriuretic peptide family. *Curr Opin Nephrol Hypertens* **5**, 4–11.
39 Kishimoto I, Rossi K & Garbers DL (2001) A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-A) inhibits cardiac ventricular myocyte hypertrophy. *Proc Natl Acad Sci USA* **98**, 2703–2706.
40 Kuhn M, Holtwick R, Baba HA, Perriard JC, Schmitz W & Ehler E (2002) Progressive cardiac hypertrophy and dysfunction in atrial natriuretic peptide receptor (GC-A) deficient mice. *Heart* **87**, 368–374.
41 Knowles JW, Esposito G, Mao L, Hagaman JR, Fox JE, Smithies O, Rockman HA & Maeda N (2001) Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor A-deficient mice. *J Clin Invest* **107**, 975–984.
42 Kishimoto I, Tokudome T, Horio T, Garbers DL, Nakao K & Kangawa K (2009) Natriuretic peptide signaling via guanylyl cyclase (GC)-A: an endogenous protective mechanism of the heart. *Curr Cardiol Rev* **5**, 45–51.
43 Holtwick R, van Eickels M, Skryabin BV, Baba HA, Bubikat A, Begrow F, Schneider MD, Garbers DL & Kuhn M (2003) Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-A. *J Clin Invest* **111**, 1399–1407.
44 Molkentin JD (2003) A friend within the heart: natriuretic peptide receptor signaling. *J Clin Invest* **111**, 1275–1277.
45 Tokudome T, Horio T, Kishimoto I, Soeki T, Mori K, Kawano Y, Kohno M, Garbers DL, Nakao K & Kangawa K (2005) Calcineurin-nuclear factor of activated T cells pathway-dependent cardiac remodeling in mice deficient in guanylyl cyclase A, a receptor for atrial and brain natriuretic peptides. *Circulation* **111**, 3095–3104.
46 Ellmers LJ, Scott NJ, Piuhola J, Maeda N, Smithies O, Frampton CM, Richards AM & Cameron VA (2007) Npr1-regulated gene pathways contributing to cardiac hypertrophy and fibrosis. *J Mol Endocrinol* **38**, 245–257.
47 Tang KM, Wang GR, Lu P, Karas RH, Aronovitz M, Heximer SP, Kaltenbronn KM, Blumer KJ, Siderovski DP, Zhu Y *et al.* (2003) Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure. *Nat Med* **9**, 1506–1512.
48 Tokudome T, Kishimoto I, Horio T, Arai Y, Schwenke DO, Hino J, Okano I, Kawano Y, Kohno M, Miyazato M *et al.* (2008) Regulator of G-protein signaling subtype 4 mediates antihypertrophic effect of locally secreted natriuretic peptides in the heart. *Circulation* **117**, 2329–2339.
49 Tang KM, Wang GR, Lu P, Karas RH, Aronovitz M, Heximer SP, Kaltenbronn KM, Blumer KJ, Siderovski DP, Zhu Y *et al.* (2009) Regulator of G protein signaling 2 mediates cardiac compensation to pressure overload and antihypertrophic effects of PDE5 inhibition in mice. *J Clin Invest* **119**, 408–420.
50 Klaiber M, Kruse M, Völker K, Schröter J, Feil R, Freichel M, Gerling A, Feil S, Dietrich A, Londoño JE *et al.* (2010) Novel insights into the mechanisms mediating the local antihypertrophic effects of cardiac atrial natriuretic peptide: role of cGMP-dependent protein kinase and RGS2. *Basic Res Cardiol* **105**, 583–595.
51 Morita E, Yasue H, Yoshimura M, Ogawa H, Jougasaki M, Matsumura T, Mukoyama M & Nakao K (1993) Increased plasma levels of brain natriuretic peptide in patients with acute myocardial infarction. *Circulation* **88**, 82–91.
52 Nakanishi M, Saito Y, Kishimoto I, Harada M, Kuwahara K, Takahashi N, Kawakami R, Nakagawa Y, Tanimoto K, Yasuno S *et al.* (2005) Role of natriuretic peptide receptor guanylyl cyclase-A in myocardial infarction evaluated using genetically engineered mice. *Hypertension* **46**, 441–447.
53 Yamahara K, Itoh H, Chun TH, Ogawa Y, Yamashita J, Sawada N, Fukunaga Y, Sone M, Yurugi-Kobayashi T, Miyashita K *et al.* (2003) Significance and therapeutic potential of the natriuretic peptides/cGMP/cGMP-dependent protein kinase pathway in vascular regeneration. *Proc Natl Acad Sci USA* **100**, 3404–3409.
54 Park K, Itoh H, Yamahara K, Sone M, Miyashita K, Oyamada N, Sawada N, Taura D, Inuzuka M, Sono-

yama T *et al.* (2008) Therapeutic potential of atrial natriuretic peptide administration on peripheral arterial diseases. *Endocrinology* **149**, 483–491.
55 Tokudome T, Kishimoto I, Yamahara K, Osaki T, Minamino N, Horio T, Sawai K, Kawano Y, Miyazato M, Sata M *et al.* (2009) Impaired recovery of blood flow after hind-limb ischemia in mice lacking guanylyl cyclase-A, a receptor for atrial and brain natriuretic peptides. *Arterioscler Thromb Vasc Biol* **29**, 1516–1521.
56 Kuhn M, Völker K, Schwarz K, Carbajo-Lozoya J, Flögel U, Jacoby C, Stypmann J, van Eickels M, Gambaryan S, Hartmann M *et al.* (2009) The natriuretic peptide/guanylyl cyclase – a system functions as a stress-responsive regulator of angiogenesis in mice. *J Clin Invest* **119**, 2019–2030.
57 Suda M, Ogawa Y, Tanaka K, Tamura N, Yasoda A, Takigawa T, Uehira M, Nishimoto H, Itoh H, Saito Y *et al.* (1998) Skeletal overgrowth in transgenic mice that overexpress brain natriuretic peptide. *Proc Natl Acad Sci USA* **95**, 2337–2342.
58 Chusho H, Ogawa Y, Tamura N, Suda M, Yasoda A, Miyazawa T, Kishimoto I, Komatsu Y, Itoh H, Tanaka K *et al.* (2000) Genetic models reveal that brain natriuretic peptide can signal through different tissue-specific receptor-mediated pathways. *Endocrinology* **141**, 3807–3813.
59 Chusho H, Tamura N, Ogawa Y, Yasoda A, Suda M, Miyazawa T, Nakamura K, Nakao K, Kurihara T, Komatsu Y *et al.* (2001) Dwarfism and early death in mice lacking C-type natriuretic peptide. *Proc Natl Acad Sci USA* **98**, 4016–4021.
60 Tamura N, Doolittle LK, Hammer RE, Shelton JM, Richardson JA & Garbers DL (2004) Critical roles of the guanylyl cyclase B receptor in endochondral ossification and development of female reproductive organs. *Proc Natl Acad Sci USA* **101**, 17300–17305.
61 Das S, Au E, Krazit ST & Pandey KN (2010) Targeted disruption of guanylyl cyclase-A/natriuretic peptide receptor-A gene provokes renal fibrosis and remodeling in null mutant mice: role of proinflammatory cytokines. *Endocrinology* **151**, 5841–5850.
62 Kishimoto I, Tokudome T, Horio T, Soeki T, Chusho H, Nakao K & Kangawa K (2008) C-type natriuretic peptide is a Schwann cell-derived factor for development and function of sensory neurones. *J Neuroendocrinol* **20**, 1213–1223.
63 Yasoda A, Komatsu Y, Chusho H, Miyazawa T, Ozasa A, Miura M, Kurihara T, Rogi T, Tanaka S, Suda M *et al.* (2004) Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. *Nat Med* **10**, 80–86.
64 Kake T, Kitamura H, Adachi Y, Yoshioka T, Watanabe T, Matsushita H, Fujii T, Kondo E, Tachibe T, Kawase Y *et al.* (2009) Chronically elevated plasma C-type natriuretic peptide level stimulates skeletal growth in transgenic mice. *Am J Physiol Endocrinol Metab* **297**, E1339–E1348.
65 Wang Y, de Waard MC, Sterner-Kock A, Stepan H, Schultheiss HP, Duncker DJ & Walther T (2007) Cardiomyocyte-restricted over-expression of C-type natriuretic peptide prevents cardiac hypertrophy induced by myocardial infarction in mice. *Eur J Heart Fail* **9**, 548–557.
66 Schmidt H, Stonkute A, Jüttner R, Koesling D, Friebe A & Rathjen FG (2009) C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons. *Proc Natl Acad Sci USA* **106**, 16847–16852.
67 Langenickel TH, Buttgereit J, Pagel-Langenickel I, Lindner M, Monti J, Beuerlein K, Al-Saadi N, Plehm R, Popova E, Tank J *et al.* (2006) Cardiac hypertrophy in transgenic rats expressing a dominant-negative mutant of the natriuretic peptide receptor B. *Proc Natl Acad Sci USA* **103**, 4735–4740.