Circulating C-Type Natriuretic Peptide (CNP) Rescues Chondrodysplastic CNP Knockout Mice from Their Impaired Skeletal Growth and Early Death

Toshihito Fujii, Yasato Komatsu, Akihiro Yasoda, Eri Kondo, Tetsuro Yoshioka, Takuo Nambu, Naotestu Kanamoto, Masako Miura, Naohisa Tamura, Hiroshi Arai, Masashi Mukoyama, and Kazuwa Nakao

Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan

C-type natriuretic peptide (CNP) is a potent stimulator of endochondral bone growth through a subtype of membranous guanylyl cyclase receptor, GC-B. Although its two cognate natriuretic peptides, ANP and BNP, are cardiac hormones produced from heart, CNP is thought to act as an autocrine/paracrine regulator. To elucidate whether systemic administration of CNP would be a novel medical treatment for chondrodysplasias, for which no drug therapy has yet been developed, we investigated the effect of circulating CNP by using the CNP transgenic mice with an increased circulating CNP under the control of human serum amyloid P component promoter (SAP-Nppc-Tg mice). SAP-Nppc-Tg mice developed prominent overgrowth of bones formed through endochondral ossification. In organ culture experiments, the growth of tibial explants of SAP-Nppc-Tg mice was not changed from that of their wild-type littermates, exhibiting that the stimulatory effect on endochondral bone growth observed in SAP-Nppc-Tg mice is humoral. Then we crossed chondrodysplastic CNP-depleted mice with SAP-Nppc-Tg mice. Impaired endochondral bone growth in CNP knockout mice were considerably and significantly recovered by increased circulating CNP, followed by the improvement in not only their longitudinal growth but also their body weight. In addition, the mortality of CNP knockout mice was greatly decreased by circulating CNP. Systemic administration of CNP might have therapeutic potential against not only impaired skeletal growth but also other aspects of impaired growth including impaired body weight gain in patients suffering from chondrodysplasias and might resultantly protect them from their early death. (Endocrinology 151: 0000–0000, 2010)

Recent studies have elucidated that C-type natriuretic peptide (CNP) is a crucial regulator of endochondral bone growth (1, 2). The biological actions of CNP are thought to be mediated by the production of intracellular second-messenger cGMP through a subtype of membranous guanylyl cyclase receptor, guanylyl cyclase (GC)-B (3). We have exhibited that both CNP and GC-B are expressed in the proliferative and prehypertrophic chondrocyte layers of the growth plate (1) and that CNP or GC-B knockout mice develop severely short stature phenotype owing to their impaired endochondral bone growth (1, 4). On the contrary, mice with targeted overexpression of CNP in the growth plate by using type II collagen promoter exhibit prominent skeletal overgrowth (5, 6).

After these discoveries, we planned to translate this strong stimulatory effect of the CNP/GC-B system on bone growth into clinical treatment for patients suffering from diseases with impaired skeletal growth. Chondrodysplasias are a group of genetic disorders characterized by impaired skeletal growth. The many different forms of chondrodysplasias add to produce a significant number of affected individuals with significant morbidity and mortality (7). Nevertheless, no efficient drug therapy has been developed to date for the treatment of chondrodysplasias.
In our previous report, we achieved targeted overexpression of CNP in the growth plate of a mice model of achondroplasia (8), the most common form of chondrodysplasias with a constitutive active mutation in the fibroblast growth factor receptor 3 gene (9), and successfully treated its impaired skeletal growth and short stature phenotype (5).

In contrast to atrial natriuretic peptide and brain natriuretic peptide, the two cognate natriuretic peptides of CNP that act as cardiac hormones produced predominantly from atrium and ventricle of heart, respectively (10, 11), CNP is thought to be an autocrine/paracrine regulator, rather than an endocrine regulator (12, 13). Because we have to evaluate the effect of circulating CNP on endochondral bone growth in case we use CNP as a drug for chondrodysplasias via systemic administration, we generated CNP transgenic mice with increased circulating CNP as a model of systemic administration of CNP (14): these transgenic mice carried the human serum amyloid P (SAP) component promoter/mouse CNP fusion gene (SAP-Nppc-Tg), and the expression of the transgene was targeted to the liver (15). SAP-Nppc-Tg mice exhibited prominent overgrowth of bones formed through endochondral ossification (14), and furthermore, we successfully rescued achondroplastic model mice from their impaired bone growth by crossing them with SAP-Nppc-Tg mice (16).

In the present study, we further investigated the effect of circulating CNP by using SAP-Nppc-Tg mice. At first, to certify the humoral effect of the overexpressed CNP in SAP-Nppc-Tg mice on endochondral bone growth, we performed organ culture experiments by using tibial explants from SAP-Nppc-Tg mice and compared them with those from cartilage-targeted CNP transgenic mice under the control of type II collagen promoter (Col2-Nppc-Tg mice) (5). Then we studied the effects of circulating CNP on the chondrodysplastic CNP knockout (Nppc<sup>-/-</sup>) mice by crossing them with SAP-Nppc-Tg mice.

To generate Nppc<sup>-/-</sup> mice carrying SAP-Nppc transgene, male Nppc<sup>+/</sup> mice were mated with female SAP-Nppc-Tg mice, and female F1 offspring heterozygous for both the transgene and the Nppc allele ablation were mated with male F1 offspring heterozygous only for the Nppc allele ablation to generate Nppc<sup>-/-</sup> mice with the transgene expression (Nppc<sup>-/-</sup>/SAP-Nppc-Tg) male. For generation of homozygous SAP-Nppc-Tg mice, male and female heterozygous SAP-Nppc-Tg mice were mated, and the genotype of the resultant transgenic mice was determined by quantifying SAP-Nppc transgene using StepOnePlus real-time PCR systems (Applied Biosystems Inc., Foster City, CA).

The care of the animals and all experiments were conducted in accordance with the institutional guidelines of Kyoto University Graduate School of Medicine.

Organ culture
Tibias from fetal SAP-Nppc-Tg mice and their wild-type littersmates (on d 16 of pregnancy), newborn Col2-Nppc-Tg mice and their wild-type littersmates, and newborn Nppc<sup>-/-</sup>/SAP-Nppc-Tg mice and their Nppc<sup>-/-</sup> littersmates were dissected out and cultured for 4 d in Biggers, Gwatkin, Judah tissue culture medium for bone and cartilage (Invitrogen, Carlsbad, CA) with BSA (6 mg/ml; Wako Pure Chemical Industries, Ltd., Osaka, Japan), ascorbic acid (150 µg/ml; Wako), and penicillin/streptomycin (10,000 U/ml; Wako) in 12-well plates. Tibias from newborn Nppc<sup>-/-</sup> mice were incubated with vehicle or CNP at the dose of 10<sup>-9</sup>, 10<sup>-8</sup>, or 10<sup>-7</sup> M for 4 d. At the end of the culture period, the longitudinal length of tibial explants was measured using a linear ocular scale mounted on a dissecting microscope at ×10 magnification.

Skeletal analysis
Mice were subjected to soft x-ray analysis (30 kVp, 5 mA for 1 min; Softron type SRO-M5; Softron, Tokyo, Japan), and the lengths of bones were measured on the soft x-ray film.

Histological analysis
For light microscopy, sections were cut from paraffin-embedded specimens. For Alcian Blue-hematoxylin and eosin (HE) staining, sections were deparaffinized with xylene and rehydrated through an ethanol series and distilled water. The sections were treated with 3% acetic acid for 3 min and Alcian Blue (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) for 20 min. Then they were treated with hematoxylin (Muto) for 2 min, eosin alcohol (Muto) for 1 min, dehydrated, and then mounted with malinol (Muto). As for in situ hybridization analyses for type II and type X collagens, 414- and 658-bp DNA fragments corresponding to the nucleotide positions 138-551 and 2893-3550 of mouse Col2a1 and Col10a1 cDNA (GenBank accession no. NM_031163 and 009925), respectively, were subcloned into pGEMT-Easy vector (Promega, Madison, WI) and were used for the generation of sense or antisense RNA probes. Digoxigenin (DIG)-labeled RNA probes were prepared with DIG RNA labeling mix (Roche, Stockholm, Sweden). Paraffin-embedded sections were hybridized with DIG-labeled RNA probes at 60°C for 16 h. The bound label was detected using 4-nitro blue tetrazolium chloride-5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt, an alkaline phosphatase color substrate. The sections were counterstained with Kernectrot (Muto).

For immunohistochemical detection of proliferating cell nuclear antigen (PCNA), tissue sections were incubated with mouse
monoclonal anti-PCNA antibody (Dako, Glostrup, Denmark), and immunostaining was performed using Histofine mouse stain kit (Nichirei Corp., Tokyo, Japan) according to the manufacturer’s instructions. Under the microscope (×400), three visual fields in the proliferative chondrocyte zone of the growth plate were randomly selected, and all cells and PCNA-positive cells in each field were counted. Then labeling index was calculated as the mean of these three values. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling staining was performed using in situ apoptosis detection kit (Takara Bio Inc., Otsu, Japan) according to the manufacturer’s instruction.

Results

Organ culture experiments using tibial explants from SAP-Nppc-Tg mice

We generated two lines of CNP transgenic mice under the control of an SAP promoter, and both of them exhibited prominent skeletal overgrowth phenotype (14). We used one of them with milder skeletal phenotype as the SAP-Nppc-Tg mice for further experiments. To confirm whether the effect of SAP-Nppc-transgene on skeletal growth is humoral, we performed organ culture experiments by using tibias from SAP-Nppc-Tg mice and compared them with those from CNP transgenic mice with targeted overexpression of CNP in the cartilage by using mouse type II collagen promoter (Col2-Nppc-Tg mice) (5).

At the end of the 4-d culture period, the length of tibial explants from SAP-Nppc-Tg mice was not changed from that of their wild-type littermates, whereas the length of tibial explants from Col2-Nppc-Tg mice was about 13% larger than that from their wild-type littermates (Fig. 1, A and B). Histological analyses revealed that the widths of both nonhypertrophic and hypertrophic chondrocyte layers of the growth plates in SAP-Nppc-Tg explants, shown to express type II and type X collagens by in situ hybridization analyses, were not changed from those in wild-type explants, whereas they were larger in Col2-Nppc-Tg explants (Fig. 1C).
In addition, in situ hybridization analyses exhibited that the patterns and intensities of the staining for type II and type X collagens as the differentiation markers for nonhypertrophic and hypertrophic chondrocytes, respectively, were not different between in SAP-Nppc-Tg and wild-type explants. Furthermore, the proliferation of the growth plate chondrocytes in SAP-Nppc-Tg explants, estimated by immunohistochemical staining for PCNA, was almost the same as that in wild-type explants (labeling index: 60.4 ± 3.4% vs. 60.0 ± 2.4%). These results exhibit that CNP generated by SAP-Nppc-transgene affects endochondral bone growth in an endocrine manner.

The impaired endochondral bone growth of Nppc−/− mice was recovered by circulating CNP

Next we investigated the effect of circulating CNP on the chondrodysplastic phenotype of CNP knockout mice by crossing them with SAP-Nppc-Tg mice. Because Nppc−/− mice are thought to be infertile, we crossed Nppc−/− mice with SAP-Nppc-Tg mice and obtained Nppc+−/−/SAP-Nppc-Tg mice. Then these Nppc+−/−/SAP-Nppc-Tg mice were crossed with Nppc+− mice to generate Nppc−/−/SAP-Nppc-Tg mice.

At the first week after birth, Nppc−/−/SAP-Nppc-Tg mice were smaller than their wild-type littermates, and the nasoanal length of Nppc−/−/SAP-Nppc-Tg mice was almost the same as that of Nppc−/− mice (Fig. 2A). But they gradually became larger than Nppc−/− mice and became close to their wild-type littermates (Fig. 2, A and B). The nasoanal length of Nppc−/−/SAP-Nppc-Tg mice was significantly larger than that of Nppc−/− mice at the age of 3 wk and at the age of 4 wk in female (male: 56.6 ± 1.1 mm and 51.9 ± 1.3 mm, respectively, n = 15 and 11 each, P < 0.01, and female: 63.3 ± 1.2 mm and 53.8 ± 0.7 mm, respectively, n = 10 and 10 each, P < 0.01). In accordance with the above observation, most bones formed through endochondral ossification in Nppc−/−/SAP-Nppc-Tg mice grew longer than those in Nppc−/− mice. At the age of 3 wk, lumbar spine, radius, femur, and tibia of Nppc−/−/SAP-Nppc-Tg mice were significantly longer than those of Nppc−/− mice, although they were still significantly shorter than those of their wild-type littermates (Fig. 3).

Histological analysis revealed that the width of the growth plate of tibia from Nppc−/−/SAP-Nppc-Tg mice was significantly larger than that from Nppc−/− mice and was comparable with that from wild-type mice (Fig. 4, A and B). Width of every zone of the growth plate, especially that of hypertrophic chondrocyte zone expressing type X collagen as shown by in situ hybridization analysis, was significantly larger in Nppc−/−/SAP-Nppc-Tg tibia than that in Nppc−/− tibia and was comparable with that in wild-type tibia (Fig. 4, A, C, and D).

The intensities or patterns of the staining for both type II and type X collagens by in situ hybridization were not different between that in Nppc−/−/SAP-Nppc-Tg and that in Nppc−/− tibias, indicating that the differentiation for nonhypertrophic and hypertrophic chondrocytes in Nppc−/− growth plate was not affected by circulating CNP (Fig. 4C). Furthermore, immunohistochemical detection of PCNA revealed that the rate of PCNA-positive chondrocytes in Nppc−/−/SAP-Nppc-Tg growth plate was not changed from that in Nppc−/− growth plate (labeling index: 23.0 ± 7.3% vs. 25.4 ± 1.4%), exhibiting that the proliferation of the chondrocytes in Nppc−/− growth plate was not altered by circulating CNP. In addition, we
could scarcely find out the difference in the state of apoptosis of the growth plate chondrocytes between that in Nppc−/−/SAP-Nppc-Tg and that in Nppc−/−/−/SAP-Nppc-Tg mice. To further confirm whether the SAP-Nppc-transgene product humorally affects the endochondral bone growth in Nppc−/− mice, organ culture experiments using tibial explants from neonatal Nppc−/−/−/SAP-Nppc-Tg and Nppc−/−/− mice were performed. At the end of the 4-d culture period, longitudinal length of tibial explants from Nppc−/−/−/SAP-Nppc-Tg mice was not changed from that from Nppc−/−/− mice (Fig. 5A). Histological analyses revealed that the widths of both nonhypertrophic and hypertrophic chondrocyte layers of the growth plate, expressing type II and type X collagens, respectively, were not different between in Nppc−/−/−/SAP-Nppc-Tg and Nppc−/−/−/− explants (Fig. 5B). Neither the differentiation (estimated by in situ hybridization analyses for type II and type X collagens, Fig. 5B) nor the proliferation (evaluated by PCNA analysis, labeling index: 41.0 ± 3.3 vs. 44.9 ± 3.0%) of the growth plate chondrocytes was different between that in Nppc−/−/−/SAP-Nppc-Tg and that in Nppc−/−/− mice.

To investigate whether the stimulatory effect of circulating CNP on the endochondral bone growth of Nppc−/− mice is dose dependent, we studied the effect of CNP on the growth of tibial explants from neonatal Nppc−/− mice in organ culture experiment. As shown in Fig. 6A, the growth of tibial explants from Nppc−/− mice was stimulated by addition of CNP in a dose-dependent manner. Furthermore, we generated SAP-Nppc-Tg mice with homozygous SAP-Nppc transgene to confirm a dose-dependent effect of circulating CNP on endochondral bone growth in vivo.

At the age of 3 wk, soft x-ray analyses revealed that the longitudinal body length and the growth of every bone formed through endochondral bone growth were promoted in accordance with the copy number of SAP-Nppc transgene, indicating the dose-dependent effect of circulating CNP on endochondral bone growth in vivo (Fig. 6B). Collectively, these results suggest that circulating CNP would cure the impaired skeletal growth of Nppc−/− mice in a dose-dependent manner in vivo.

Effects of increased circulating CNP on the body weight gain and the survival rate of Nppc−/− mice

We also investigated the effects of circulating CNP on other aspects of the impaired growth of chondrodysplastic Nppc−/− mice. The body weight of Nppc−/−/−/SAP-Nppc-Tg mice was smaller than that of their wild-type littermates and was comparable with that of their Nppc−/−/− littermates at the age of 1 wk (Fig. 7A). However, Nppc−/−/−/SAP-Nppc-Tg mice gradually became heavier
than their Nppc<sup>−/−</sup> littermates (Fig. 7A), and the body weight of Nppc<sup>−/−</sup>/SAP-Nppc-Tg mice was significantly larger than their wild-type littermates at the age of 4 wk in males and 3 wk in females (males: 9.3 ± 0.5 g and 7.3 ± 0.9 g, respectively, n = 12 and 7 each, P < 0.05, and females: 5.4 ± 0.1 g and 4.7 ± 0.2 g, respectively, n = 11 and 12 each, P < 0.05). On the other hand, there was no difference in body weight between the SAP-Nppc-Tg and wild-type mice, albeit SAP-Nppc-Tg mice became larger than the wild-type mice in nasoanal length (Figs. 2A and 6).

We have previously reported that the survival rate of Nppc<sup>−/−</sup> mice greatly drops before adulthood, albeit the genotype ratio of Nppc<sup>−/−</sup> mice on d 16.5 of pregnancy is in accord with Mendelian proportion (1). In this study, analysis of intercrosses between Nppc<sup>+/+</sup>/SAP-Nppc-Tg mice and Nppc<sup>+/−</sup> mice revealed that the genotype ratios of wild type to Nppc<sup>−/−</sup> to Nppc<sup>−/−</sup> and SAP-Nppc-Tg to Nppc<sup>−/−</sup>/SAP-Nppc-Tg to Nppc<sup>−/−</sup>/SAP-Nppc-Tg at weaning (3 wk of age) are 1:2.78:1 and 1:2.71:1.24 (total n = 104 and 110), respectively, indicating expected Mendelian proportions. As have we previously reported, the survival rate of Nppc<sup>−/−</sup> mice dropped to about 40% before adulthood (Fig. 7B). However, the survival rate of Nppc<sup>−/−</sup>/SAP-Nppc-Tg mice was greatly improved compared with that of Nppc<sup>−/−</sup> mice (Fig. 7B).

**Discussion**

In the present study, we investigated the endocrine effects of CNP on chondrodysplastic CNP knockout mice by using SAP-Nppc-Tg mice.

In the organ culture experiments, the growth of SAP-Nppc-Tg mice was not changed from that of wild-type mice, whereas the growth of Col2-Nppc-Tg mice was strongly promoted compared with that of wild-type mice.

This result confirms that the growth stimulating effect of bones formed through endochondral ossification in SAP-Nppc-Tg mice is not autocrine/paracrine but endocrine effect of CNP, which is produced by the SAP-Nppc transgene. Because we expected that we would observe the effect of circulating CNP on endochondral bone growth clearly in a state without basal CNP effect, we then investigated whether or not elevation of circulating CNP could recover the impaired endochondral bone growth caused by depletion of CNP in mice in vivo. Decreased width of the growth plate observed in Nppc<sup>−/−</sup> mice was recovered in Nppc<sup>−/−</sup>/SAP-Nppc-Tg mice, and accordingly, impaired endochondral bone growth observed in Nppc<sup>−/−</sup> mice was considerably and significantly recovered in Nppc<sup>−/−</sup>/SAP-Nppc-Tg mice.

The endocrine effect of CNP produced by the SAP-Nppc transgene in Nppc<sup>−/−</sup>/SAP-Nppc-Tg mice was further confirmed by the organ culture experiments in that the growth of Nppc<sup>−/−</sup>/SAP-Nppc-Tg mice was not changed from that of Nppc<sup>−/−</sup> mice. These results clearly indicate that CNP can humorally affect endochondral bone growth. Furthermore, the result of the organ culture experiment using Nppc<sup>−/−</sup> bones (Fig. 6A) and the gene-dose effect of SAP-Nppc transgene on bone growth in vivo (Fig. 6B, C) suggest that the endocrine effect of CNP on endochondral bone growth is dose dependent.

Chondrodysplasia is composed of many different forms of genetic disorders characterized by impaired endochondral bone growth (7, 17). Because the CNP/GC-B system plays a crucial role in endochondral bone growth, loss of function mutations in the genes coding for molecules related to the CNP/GC-B system could cause chondrodysplasia. In fact, recent studies have revealed that mutations in the gene encoding human GC-B cause one form of chondrodysplasia, acromesomelic dysplasia type Maroteaux (18, 19).

In mice, loss of function mutations in the GC-B gene cause impaired skeletal growth in spontaneous mutant cnln/cnln and short-limbed dwarfism (slsh/slsh) mice (20, 21). As for spontaneous mutations in other genes related to the CNP/GC-B system, a mutation in the gene coding for cGMP-dependent protein kinase type II, an important downstream mediator of the CNP/GC-B system, causes impaired endochondral bone growth in Komeda miniature rat Ishikawa (22, 23). Furthermore, recent studies have elucidated that a spontaneous loss of function mutation in the murine CNP gene causes impaired skeletal growth observed in the long bone abnormality (lhab/lbab) mice (24–26).
Just as in the case with rodents, any forms of human chondrodysplasia might be caused by mutations in the cGMP-dependent protein kinase type II or CNP gene, albeit they are not yet discovered. In case a form of human chondrodysplasia caused by a mutation in the CNP gene is discovered in future, CNP knockout mice would be a novel mice model of human chondrodysplasia. On the other hand, spontaneous GC-B mutant (cn/cn and slw/slw) mice and GC-B knockout mice are regarded as mice models of acromesomelic dysplasia type Maroteaux, and impaired skeletal growth of these mice would not be recovered by crossing them with SAP-Nppc-Tg mice. This notion is supported by the result of the organ culture experiment, in which tibial explants from fetal GC-B knockout mice are not increased in length by addition of CNP (4).

We previously reported that the impaired skeletal growth of achondroplastic model mice was almost completely recovered by crossing them with SAP-Nppc-Tg mice (16). The impairment of skeletal growth of the achondroplastic model mice that we used in our previous study was considerably mild compared with that of Nppc−/− mice: the nasoanal length of the achondroplastic model mice was about 10% shorter than that of wild-type mice at the age of 10 wk (14), whereas the nasoanal length of Nppc−/− mice was about 30% shorter than that of wild-type mice. The reason that the impaired skeletal growth of Nppc−/− mice was not completely rescued in Nppc−/−/SAP-Nppc-Tg mice in our present study might be because the low graded elevation of the plasma CNP concentrations in SAP-Nppc-Tg mice (about 1.8 times higher than those in wild-type mice) was not sufficient for the complete rescue of severe skeletal phenotype of Nppc−/− mice, whereas it was enough to cure the mild skeletal impairment of the achondroplastic model mice. Although about 2 times of elevation of plasma CNP concentrations can stimulate bone growth in SAP-Nppc-Tg mice (14) or human with a chromosomal translocation (27), higher plasma concentration of CNP might be needed for the complete treatment of impaired bone growth in chondrodysplasia.

As for the mechanism of the skeletal rescue of CNP knockout mice by circulating CNP, the differentiation and the proliferation of the growth plate chondrocytes of Nppc−/−/SAP-Nppc-Tg mice were not changed from those of Nppc−/− mice. This result coincides with our previous observation that CNP does not so strongly affect differentiation and proliferation of the growth plate chondrocytes in vivo (5, 14). On the other hand, proteoglycan synthesis is greatly increased in the growth plate of SAP-Nppc-Tg mice (14), so we speculate that the shortened Nppc−/− growth plate is restored by circulating CNP in Nppc−/−/SAP-Nppc-Tg mice through the recovery of matrix synthesis, resulting in the recovery of endochondral bone growth.

The impaired growth of Nppc−/− mice was recovered not only longitudinal length but also body weight, and furthermore, the mortality of Nppc−/− mice was greatly decreased, by circulating CNP. Together with our previous results that targeted overexpression of CNP in the cartilage of Nppc−/− mice improved not only their impaired longitudinal growth but also their impaired body weight gain and that prolonged their survival (1), we consider that the recovery from the impaired endochondral bone growth in Nppc−/− mice by circulating CNP resulted in the recovery of overall growth and also in longevity. The mechanisms through which recovery in skeletal growth results in the recovery of overall growth and the prolonged survival are not yet elucidated. One of the possibilities is that the malformation in the maxillofacial region of Nppc−/− mice, which is caused by impaired endochondral ossification, may disturb their teeth coming together correctly: this condition may prevent them from eating enough and lead them to malnutrition. Further investigation of the craniofacial phenotype of Nppc−/− mice is now ongoing in our laboratory (Nakao, K., Y. Okubo, N. Koyama, K. Osawa, M. Miura, A. Yasoda, K. Nakao, and K. Bessho, manuscript in preparation).

In conclusion, we have revealed that circulating CNP rescues the impaired growth and early death of chondrodysplastic CNP knockout mice through the recovery of endochondral bone growth. We have started to apply the strong stimulatory effect of the CNP/GC-B system on endochondral bone growth to the treatment of chondrodysplasias (16) for those no effective drug therapy is available to date. The results of our present paper suggest that systemic administration of CNP or its analog, which would stimulate GC-B, might have therapeutic potential against not only impaired skeletal growth but also other aspects of impaired growth including impaired body weight gain in patients suffering from chondrodysplasias and might resultantly protect them from their early death.

Acknowledgments

We thank Chugai Pharmaceutical Co. for SAP-Nppc-Tg mice and Asubio Pharma Co. for Col2-Nppc-Tg mice.

Address all correspondence and requests for reprints to: Akihiro Yasoda, M.D., Ph.D., 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan. E-mail: yasoda@kuhp.kyoto-u.ac.jp.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare of Japan and the Ministry of Education, Culture, Sports, Sciences, and Technology of Japan (Grant 19591075).

References


26. Yoder AR, Kruse AC, Earhart CA, Ohlendorf DH, Potter LR 2008 Reduced ability of C-type natriuretic peptide (CNP) to activate natriuretic peptide receptor B (NPR-B) causes dwarfism in lbab mice. Peptides 29:1573–1581