
Longitudinal Growth in Rheumatologic Conditions: Current and Emerging Treatments of Growth Delay in Children with Chronic Autoimmune Diseases

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Abstract

Chronic rheumatologic, inflammatory diseases of childhood, such as juvenile idiopathic arthritis (JIA), Crohn's disease (CD), and systemic lupus erythematosus (SLE), affect both trabecular bone formation and remodeling and longitudinal bone growth resulting in short stature and causing bone developmental deformities. Inflammation alone or together with poor nutritional intake and chronic glucocorticoid therapy are major factors in growth retardation seen in children with chronic inflammatory diseases. When the growing process is continuous, acute or chronic inflammation causes dysregulation of both central endocrine and local paracrine secretion of the growth factors and hormones, impairing bone growth in children. In this chapter, we review major growth factors such as growth hormone that affect longitudinal growth and how they are affected by inflammation in childhood rheumatologic diseases. We also review a recently described growth factor, CNP, and its potential therapeutic role in chronic inflammatory diseases.

Keywords: juvenile idiopathic arthritis, natriuretic peptides, dwarfism, growth factor, atrial natriuretic peptide, B-type natriuretic peptide, C-type natriuretic peptide, natriuretic peptide receptor 1, -2, -3

1. Introduction

Longitudinal growth is a continuous process under the influence of multiple complex factors starting from prenatal life until end of puberty. Major factors that control longitudinal growth can be summarized as genetic background, nutrition, and endocrine growth factors. Major

endocrine factors that control longitudinal growth are growth hormone (GH) and insulin-like growth factor (IGF), thyroid stimulating hormone (TSH), parathyroid hormone (PTH), insulin, and sex steroids. There are also multiple growth factors in the growth plates and other organs that are involved in processing the growth factors in the regulation of longitudinal bone growth. Some of these growth factors are found and secreted by the growing cartilage and bone tissue and under the influence of the endocrine hormones. These are insulin-like growth factor 1 (IGF-1), insulin-like growth factor-binding protein 3 (IGFBP-3), fibroblast growth factors (FGF), Indian hedgehog (Ihh), parathyroid hormone-like receptor protein (Pthrp), and C-type natriuretic peptide (CNP).

1.1. How do growth hormones and factors affect longitudinal skeletal growth?

Skeletal growth and development follows two different pathways: chondrogenesis and osteogenesis (both endochondral and membranous bone growth). Longitudinal growth is mainly controlled by endochondral ossification which is orchestrated by a complex network of endocrine and paracrine growth hormones and factors that control growth plate cartilage and bone tissue.

While membranous bone such as those in our skull forms as a result of direct mechanism of mesenchymal cell differentiation into osteoblasts, endochondral osteogenesis follows an initial mesenchymal stem cell differentiation into chondrocytes, and the chondrogenesis process is later replaced by bone tissue [1–4]. During endochondral growth, mesenchymal cells (See **Figure 1**) first condense in the growth plate and then with interactions between cells via local transcription factors such as Sox9 and other extracellular molecules such as collagen II differentiate gradually into chondrocytes. Chondrocytes proliferate and organize in columns making stacks which are perpendicular to gravity. They gradually stop proliferating and become pre-hypertrophic with increased matrix synthesis (**Figure 1**) [5, 6]. Eventually, these cells stop proliferating and start terminally differentiating into hypertrophic chondrocytes. Finally, the hypertrophic zone of the growth plate becomes mineralized. Then the vascular system merges into this hypertrophic chondrocyte region and with more signaling the mineralized tissue is possibly resorbed by osteoclasts that originate from hematopoietic stem cells. Eventually, mineralized tissue is replaced by bone tissue which is made by osteoblasts that differentiate from mesenchymal cells. Thus, endochondral bone growth combines together chondrogenesis, extracellular matrix formation, mineralization, and osteogenesis process. These processes are synchronized by a series of systemic growth hormones such as growth hormone (GH), thyroid-stimulating hormone (TSH), glucocorticoids and local growth factors such as parathyroid hormone-related peptide (Pthrp) and members of the transforming growth factor β (TGF- β), fibroblast growth factors (FGF), Indian hedgehog (Ihh), and Wnt's [7, 8] (**Figure 1**). Intracellular pathways that are activated by the orchestra of factors are yet to be determined. Sox9 and Runx2, transcription factors, have been shown to regulate chondrogenesis and hypertrophic differentiation [9, 10].

During linear growth, endocrine hormones such as GH, IGF-1, glucocorticoids, and thyroid stimulating hormone first interact at the level of hypothalamus and pituitary [11, 12] and

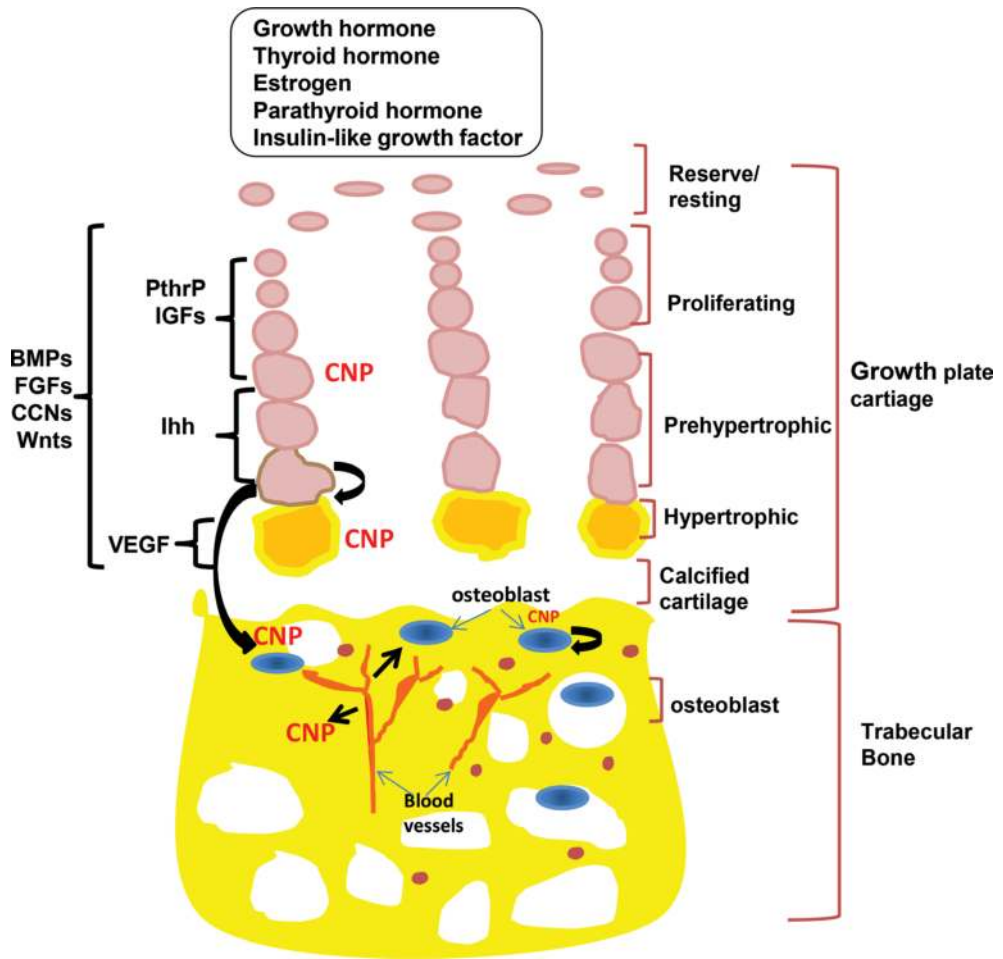


Figure 1. Longitudinal growth is regulated by multiple systemic (in bubble) and local growth factors expressed differentially in the proliferating, hypertrophic chondrocyte zones of the growth plate (black text on the left of the figure). CNP seems to be expressed and plays a role in the growth plate cartilage, in the osteoblasts and in the endothelial cells of vessel walls.

then, they act directly on peripheral target tissues, such as liver [13, 14], heart [15], kidney and growth plates [14, 16].

GH action regulates growth plates using both direct and indirect mechanisms. While GH directly stimulates chondrocyte proliferation on the growth plate [17], it indirectly stimulates the production of IGF-1 that promotes chondrocyte hypertrophy, which in turn exerts its effects directly on the growth plate.

Various danger signals or stimuli, such as TNF- α , LPS, or low-oxygen tension, increase the expression of IGF-1, vascular endothelial growth factor (VEGF), and FGF-2 with mechanisms

dependent on NF- κ B activation and result in bone resorption, osteopenia [11]. Excess of TNF- α during systemic arthritis has been found to be responsible for periarticular osteopenia most probably due to the same mechanism. GH action has both direct and indirect effects on the growth plate. GH acts indirectly, stimulating the production of IGF-1 that promotes chondrocyte hypertrophy, which in turn exerts its effects on the growth plate. The direct effect of GH on the growth plate stimulates chondrocyte proliferation [18]. Most recently, nitric oxide (NO) and C-type natriuretic peptide (CNP) have been identified as new regulators of endochondral bone growth, as they both stimulate chondrogenesis and both act through a common mediator, cyclic guanosine monophosphate (cGMP) [19].

While it is important to study anabolic effects of growth factors and hormones that promote chondrogenic differentiation in the growth plate, it is also very important to recognize the effects of factors that play roles in remodeling such as factors that control osteoclastic differentiation or activity. Osteoclasts are major cells that degrade bones for remodeling. The balance between bone degradation and bone building is critical for physiological bone homeostasis. Factors such as NF- κ B and cytokines that are controlled by this factor may cause an imbalance during systemic inflammatory diseases such as JIA [20, 21]. NF- κ B activation is a relevant component for osteoclast development, differentiation, and survival, cooperating with other pro-inflammatory cytokines [22]. Loss of NF- κ B signaling prevents osteoclastogenesis [23]. NF- κ B knockout mice showed severe osteopetrosis [24].

2. Growth delay in chronic inflammatory diseases

In chronic inflammatory childhood diseases such as inflammatory bowel diseases, mainly Crohn's disease, juvenile idiopathic arthritis, systemic lupus erythematosus, or other diseases in which there are excess number of circulating cytokines, it is suggested that growth hormone signaling pathways are disrupted [25–27].

Out of all factors that affect growth during chronic inflammatory diseases, growth hormone GH/IGF axis has been studied the most. Multiple steps of the growth hormone and its effector IGF-1 axis may be interrupted; these are poor signal transduction of growth hormone in the liver and in the growth plate and diminished IGFBP concentrations, which directly affect the growth plate and suppress the sensitivity of IGF-1.

After the growth hormone is secreted by the pituitary gland, it stimulates the hepatic tissue to generate IGF-1 which increases the growth plate chondrocyte proliferation.

Pro-inflammatory cytokines play a critical role in the disruption of the IGF-1/GH axis. Many studies have shown that pro-inflammatory cytokines such as IL-6, TNF- α , and IL-1 β interact with the IGF-1/GH system. Transgenic mice for IL-6 develop low serum level IGF-1 and develop severe growth delay [25]. There is also evidence that suggests a role for suppressors of the cytokine signaling (SOCS) family proteins in IL-6 dysregulation [26]. The K \times B/N transgenic mouse model for arthritis has also been described to have growth delay during our studies [27]. K \times B/N mouse has increased serum levels of IL-1 β and TNF- α and they develop systemic inflammatory

arthritis [28]. Thus, high levels of any pro-inflammatory cytokine are sufficient to arrest the growth process in developing organisms with open growth plates.

The IGF-1 signaling pathway is altered in chondrocytes during chronic inflammatory conditions by pro-inflammatory cytokine activities (TNF- α , IL-6, and IL-1). These pro-inflammatory mediators work via disruption of intracellular MAPK/extracellular signal-regulated kinases (ERKs) and phosphoinositide 3-kinase (PI3K) [29, 30].

Besides the inhibition of the MAPK pathway, there are also debates about the potential of disrupted miRNA effect on overexpression of proteins involved in the regulation of GH/IGF-1 axis. miRNA deregulation previously has been reported during childhood chronic inflammatory diseases such as IBD and JIA [21, 31].

In juvenile idiopathic arthritis (JIA), bone growth abnormalities are seen as either or both short stature and bone deformities. The prevalence of juvenile rheumatoid arthritis is as high as 20 per 100,000 people per year. Growth delay in generalized linear growth occurs predominantly in the systemic onset juvenile arthritis population and to a lesser degree in those with poly-articular onset JIA associated with RF positivity [32]. During active disease in JIA, elevated serum levels of cytokines may modify target cell's sensitivity by down-regulating the GH receptor (GHR) gene expression, leading to short stature as an adult [33]. Therefore, the shortcoming of GH function during JIA is explained more as resistance to growth hormones than deficiency in growth hormone secretion.

Growth hormone (GH) treatment by providing excess GH in the circulation can overcome growth hormone resistance and improve growth velocity and prevent development of short stature in children affected from JIA.

Recent studies suggest that early initiation of GH treatment helps in maintaining normal growth in children with JIA [34, 35]. Thus, recombinant growth hormone treatment has been the mainstream since no other medications that induce skeletal growth are available to be used in pediatrics [36, 37]. Nevertheless, even with GH treatment, catch-up growth is variable and is more dependent on the severity of the inflammatory state, duration, and additional corticosteroid treatment [34, 37–41].

Another childhood disease studied for its growth delay complication is Crohn's disease, an inflammatory bowel disease (IBD). Almost, one-third of the children affected by Crohn's disease (CD) develop longitudinal growth delay. Unlike JIA, Crohn's disease patients do not develop bony deformities since the major inflammatory target is not the joint cartilage but the intestinal system. Additional to the pro-inflammatory cytokine excess that directly affects the growth plate during active disease in Crohn's disease, other factors such as malnutrition, mal-absorption of the nutrients, and central nervous system were also blamed for longitudinal growth delay. Especially those patients affected more with jejunum inflammation have poor nutrition and severe deficiency in energy metabolism as well as a chronic inflammation state which contributes to the growth delay [42]. In Crohn's disease it has been suggested that chronic inflammation interferes with both central and peripheral growth hormone/factor secretion causing hormonal deficiency and/or resistance. While inflammatory

cytokines directly affect appetite centers, they also disrupt growth hormone signal transduction and proteolyze IGFBP-3 and inhibit the IGF-1 expression in the growth plate [43].

3. Other growth factors that affect longitudinal growth: C-type natriuretic peptide

C-type natriuretic peptide NP is anabolic in the growth plate, articular cartilage, and in bone tissue: We and others have shown that CNP is anabolic in the growth plate and that CNP/natriuretic peptide receptor-B (NPR-B)/cyclic guanosine monophosphate (cGMP) signaling regulates linear bone growth/endochondral bone formation through the cGMP-dependent protein kinase II (cGK-2). CNP induces chondrocyte proliferation, differentiation, and extracellular matrix (ECM) production.

We have recently shown that transgenic mice that overexpress CNP under the control of the type-II collagen promoter had increased endochondral bone growth with thick and matrix-rich articular joint cartilage. Most importantly, we have also shown that in an animal model of inflammatory arthritis, CNP overexpression in chondrocytes protects the articular cartilage integrity and prevents subchondral bone defects [27]. Our transgenic mice that overexpressed CNP on cartilage developed dense trabeculation under the subchondral bone supporting in vitro experiments showing increased matrix secretion by osteoblastic cells [44]. In addition, there is data about CNP enhancing ECM secretion in cultured articular chondrocytes seeded on a type-II collagen-coated scaffold [45]. CNP has a unique dual anabolic effect on chondrocytes and osteoblasts for matrix synthesis. Together, these findings suggest that CNP is an ideal growth factor to be used in TE for osteochondral defects since it may promote both cartilage and bone regeneration.

CNP improves vasculogenesis and graft survival: Vascular endothelial cells also express and secrete CNP, and CNP has a major role in embryonic vasculogenesis and graft vasculogenesis [46, 47].

Angiogenesis is essential for bone formation during embryonic life and after fracture, indicating a further role in bone fracture healing for CNP.

3.1. CNP signaling pathway

Natriuretic peptides are one of the main classes of cGMP inducers which are known as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). Natriuretic peptides are secreted proteins that control cell behavior through activation of two major transmembrane receptors, natriuretic peptide receptor 1 and 2 (NPR1 and NPR2) [48–50]. Receptors NPR1 and NPR2 have guanylyl cyclase activity and synthesize cGMP in response to ligand binding. ANP and BNP signal mainly through NPR1/GC-A, while CNP predominantly activates NPR2/GC-B. All three ligands also bind to a third receptor, NPR3, which is known as the clearance receptor that does not have an intracellular signaling part from the molecule and limits ligand availability once attached and limits natriuretic peptide signaling.

Once CNP gene and its signaling were disrupted in mice, those mice suffered from postnatal dwarfism [51]. While CNP knock-out mice developed normal membranous ossification,

endochondral ossification was severely impaired and all long bones and vertebrae were significantly shorter. About 70% of null mice die in the first 100 days after birth. When crossed with transgenic mice that overexpressed CNP in cartilage, knock-out mice phenotype was completely rescued.

Further organ culture experiments (femur) confirmed CNP's effect as the potent stimulator of endochondral bone growth. Other mice models were present in which *Npr2* was knocked out and similar dwarf phenotype was observed, thus further confirming the importance of CNP signaling pathways. A loss-of-function mutation in the cGMP-dependent protein kinase II (*cGKII* gene) has also recently been identified as the cause of dwarfism in mice which is a downstream effector of CNP. When *Npr3* is knocked out, an opposite phenotype of skeletal over growth is observed [52, 53].

All natriuretic peptides (ANP, BNP, and CNP) have the ability to bind NPRA, NPRB, and NPRC, while CNP has the most affinity to bind NPRB which seems to control endochondral bone growth the most. Mice deficient for ANP, BNP, or NPR1 genes were reported not to develop dwarfism or abnormal skeletal phenotypes [54, 55], suggesting that these genes play only minor supportive roles during endochondral bone formation. Overexpression of BNP in transgenic mice developed skeletal overgrowth, but this was explained as overstimulation of NPR2 by excess levels of BNP [56, 57].

4. CNP's role in cartilage homeostasis

Transgenic mice that overexpress CNP (*CNPcol2a1TG*) in cartilage develop skeletal overgrowth and increased bone density: in order to further study the effects of CNP in skeletal growth and bone architecture *in vivo*, we generated transgenic mice by cloning human CNP cDNA (450 bp) into a construct that contained mouse collagen type II (*Col2a1*) promoter (GenBank #m65161) to specifically overexpress CNP in chondrocytes. Growth plates of *CNPcol2a1TG* mice showed increased numbers of proliferative chondrocytes measured by BrdU uptake ($p < 0.05$) and increased numbers of enlarged hypertrophic chondrocytes



Figure 2. 20 weeks old male *CNPcol2a1TG* mice with kyphosis and excess growth of longitudinal and vertebral bones.

with increased proteoglycan deposition as evidenced by strong Safranin-O staining [27]. CNPcol2a1TG mice developed increased endochondral bone growth (30%) and developed kyphosis due to vertebral overgrowth by 18 weeks (**Figure 2**). In the skeletal histomorphology of CNPcol2a1TG mice, the most intriguing finding was the increased trabecular bone formation in proximity to the growth plate cartilage, subchondral (juxta-articular) bones, and in vertebrae.

5. CNP's role in bone homeostasis

C-type natriuretic peptide (CNP) has dual anabolic effects on cartilage and bone tissues. We have recently shown that CNP is a major contributor to post-natal skeletal growth in humans by its effects on the growth plate cartilage [58]. CNP also has an anabolic effect on bone morphogenesis [59]. Previous reports of in vitro experiments suggest that the CNP signaling system is an autocrine/paracrine regulator of osteoblast growth and differentiation, and CNP plays a role in bone remodeling [60, 61].

Although it is one of the major regulators of endochondral bone growth with its impact on cartilage tissue and its homeostatic role in the growing bone tissue, CNP's role in adult bone is unclear. Recent research in ewes (adult female sheep) showed that when estrogen was given CNP, content was increased the most in the estrogen-responsive trabecular bones (vertebrae and iliac bones) more than the longitudinal bones (tibia). The same study suggested that dexamethasone injections to ewes did not change the content of CNP in bone tissue, while plasma CNP peptides and bone alkaline phosphatase levels were significantly decreased.

Dwarf mice, that is. Npr2 knockout or Nppc KO, have not been reported for the lack of their bone mineral content or bone mineral density. Our observation in the CNPcol2a1TG mice that overexpressed CNP, particularly in the cartilage tissue, was that both vertebral bones and the metaphysis/epiphysis of long bones, around the growth plates, develop significantly increased trabeculation and mineralization [59, 62]. This may be because Nppc overexpression was more significant in the growth plate cartilage and somewhat in the joint cartilage in our CNPcol2a1TG mice. Others using different promoters that caused increased production of CNP (SAP-CNP-Tg mice) in serum showed the effect of CNP on bone turnover microcomputed tomography (CT) analysis revealed increased trabeculation and dense bones lumbar vertebrae in contrast to long bones such as femur. However, the fracture model showed that there is increased bone turnover and fracture healing in the SAP-CNP-Tg mice even in long bones with less trabecular ratio. Bone histomorphometric analysis of the tibiae from SAP-CNP-Tg mice showed that stabilized femoral fracture healing is advanced in SAP-CNP-Tg mice supporting the hypothesis that CNP regulates bone homeostasis and contributes to remodeling [63].

Osteoblastic cell culture experiments showed CNP's anabolic effect in osteoblastic activity.

6. CNP's role in vascular homeostasis

One of the most important roles of CNP is in the venous system. CNP via its NPR2 and NPR3 receptor signaling in the vascular wall regulates vasodilatation particularly on the venous wall. Enhanced osteoblastic and osteoclastic activities. In addition, serum levels of osteocalcin and tartrate-resistant acid phosphatase-5b, were elevated in the Tg mice. The same study showed that open and Vascular endothelial cells express and secrete CNP. CNP is suppressed by the VEGF secretion and is known to act as a vasodilator [64]. Also, CNP has been suggested to have a major role in angiogenesis [47, 65]. Once the growth plate is closed after puberty, the chondrogenic CNP will no longer be available. Then CNP needed for trabecular bone remodeling will then have to be secreted partially by osteoblasts as a paracrine/autocrine factor and/or by vascular endothelium. Steady serum levels of NT-proCNP may also be regulating the cartilage and bone homeostasis and the main source of NT-proCNP might only be the vascular endothelium. Due to the vascular wall expression of CNP and its vasodilator effect, CNP's role in hypertension, vasculitis and myocardial infarction has been studied.

7. Lack of CNP signaling in growth plate causes short stature and dwarfism

Heterozygous carriers of a mutation in NPR-B, the receptor for CNP, have idiopathic short stature suggesting a quantitative effect of the CNP pathway on skeletal growth [66]. Serum levels of CNP's N-terminal pro-peptide (NT-proCNP), the inactive form of CNP, were found to be highest at birth, gradually decreased by puberty, and plateau after 18 years of age [67]. The levels of NT-proCNP correlate with levels of alkaline phosphatase (bone formation markers) in humans [67, 68]. More importantly, serum NT-proCNP levels are maintained at a level in adults.

Individuals with acromesomelic dysplasia-type Maroteaux (AMDM), a type of human dwarfism, develop periarticular osteopenia, loss of trabecular bone structure. AMDM is caused by loss of function mutations in the CNP's receptor, natriuretic peptide-B (Npr2 gene). AMDM patients have disproportional growth retardation and abnormal development of bone tissue and appear to have (juxta-articular) metaphyseal flaring and osteopenia but do not have any other health problems [58, 69]. The trabecular bone loss is more significant in the juxta-articular area resembling osteopenia of inflammatory arthritis.

8. CNP in skeletal overgrowth

Before CNP and its effectors can be used as a remedy for short stature and growth delay, its effects in humans need to be studied well. Evidence for complications of excess systemic CNP

came after the description of two novel mutations that resulted in gain of function in humans. The C-type natriuretic peptide (CNP), encoded by NPPC gene, is located on chromosome 2q37.1. Two independent studies have described three patients with a Marfan-like phenotype presenting a de novo balanced translocation involving the same chromosomal region 2q37.1 and overexpression of NPPC [70]. One study reported on two partially overlapping interstitial 2q37 deletions. These two patients showed opposite phenotypes characterized by short stature and skeletal overgrowth, respectively. The patient with short stature presented a 2q37 deletion causing the loss of one copy of the NPPC gene with normal CNP plasma concentration. The deletion identified in the patient with a Marfan-like phenotype interrupted the DIS3L2 gene without involving the NPPC gene. In addition, a strongly elevated CNP plasma concentration was found in this patient with Marfanoid features and a tall stature [71, 72].

9. CNP roles in energy metabolism

CNP was first isolated from porcine brain and was expected to be a neuropeptide [73], but the physiological significance of the CNP/GC-B system has been established in the vascular and skeletal systems [53, 58, 71, 72, 74–78]. It was reported that CNP and GC-B are expressed in the central [79–83] and peripheral nervous systems [84, 85]. It has been shown that the hypothalamus is an important center to control food intake and energy expenditure [86]. CNP mRNA was detected in the rat hypothalamus indicating this peptide's role in numerous



Figure 3. CNPcol2a1TG mice (on the right) are tall and slender as compared to the wild type littermates (on the left).

neuroendocrine regulation [87]. CNP is suggested to play important roles in central energy expenditure and food intake via its effects on the central nervous system (**Figure 3**).

10. CNP during inflammatory disease activity

Initial reports about CNP's effect on inflammatory disease activity came from osteoarthritis disease models. Since both nitric oxide and CNP are the two main activators of cGMP in cartilage they were checked for their role in development of osteoarthritis. The production of nitric oxide (NO) regulates host defense and inflammation. Nitric oxide has vasodilation, cytotoxicity, and it has a role in cytokine-dependent tissue injury. NO effects have been blamed in the tissue injury of a variety of rheumatologic conditions including systemic lupus erythematosus, rheumatoid arthritis, and osteoarthritis. Pro-inflammatory effects of nitric oxide include vasodilation, edema, cytotoxicity, and the mediation of cytokine-dependent processes that can lead to tissue destruction. In contrast to NO effect in the cartilage, NO secretion from vascular wall is protective against neutrophil adhesion and related vascular injury. Thus, nitric oxide has been found as a clear role player in osteoarthritis pathogenesis but not in microvasculature injury [88, 89]. Although CNP is known to induce articular chondrocyte hypertrophy, it was never blamed to be involved in osteoarthritis pathogenesis, while it was considered to contribute to the disease progression. On the other hand, we were able to show in an inflammatory arthritis murine model that cartilage overexpression of CNP increased the chondrocyte number, matrix synthesis, and maintained a thicker hypertrophic cartilage in the growth plate and in the joints and thus was protective against the cartilage degenerative effects of inflammatory arthritis. The mouse model we used was for a K/BxN rheumatoid arthritis mouse model that developed severe inflammatory arthritis which was evident from first synovitis, pannus formation, and then secondary cartilage deterioration [28, 90]. Our transgenic mouse under Col2a1 promoter overexpressed CNP in the cartilage tissue mainly in the growth plate and in the joint cartilage. CNP transgenic mice developed thick growth plates with enlarged chondrocytes and wider growth plates with proliferating chondrocytes that produced a rich matrix. Furthermore, when CNP overexpressing mice was crossed with K/BxN mouse and developed systemic arthritis, we observed that cartilage matrix integrity and cartilage structure was protected against the inflammation. CNP transgenic mice did not develop severe complications of arthritis (**Figure 4**) [27, 91].

Another evidence for CNP's systemic anti-inflammatory effect was reported in a rat model of hemorrhagic shock and resuscitation. CNP infusion to this model lowered the myeloperoxidase activity and decreased the expression of TNF- α , IL-6, and IL-1 β in the kidneys. CNP treatment suppressed oxidative stress, ameliorated the inflammatory response, and caused acute kidney injury [92]. Investigators suggested that they demonstrated CNP infusion's inhibitory effect on the generation of reactive oxygen species (ROS) and pro-inflammatory cytokines after hemorrhagic shock induction and subsequently suppressed the activation, recruitment, and adherence of neutrophils in the kidney. Neutrophil recruitment is one of the fundamental pathways in hemorrhagic shock. It is suggested that neutrophil recruitment is also delayed after CNP treatment. In an earlier study, Chen et al. showed that CNP treatment

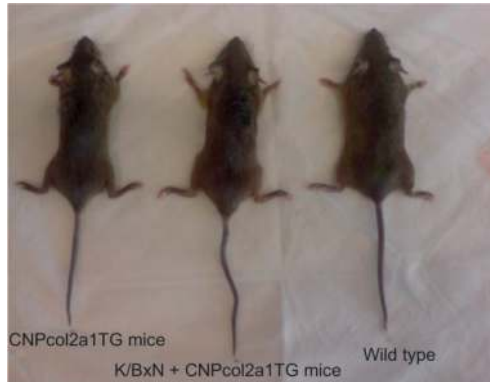


Figure 4. K/BxN+CNPcol2a1TG double transgenic mice do not develop growth retardation or severe complications of arthritis.

effectively attenuates lipopolysaccharide (LPS)-induced endothelial activation by eliminating intracellular ROS production, inhibiting the NF- κ B and MAPK p38 signaling pathways and activating the PI3 K/Akt/HO-1 pathway in human umbilical vein endothelial cells (HUVECs), [93] suggesting an anti-inflammatory effect for CNP.

Another study that showed CNP's effect in reducing the LPS-induced lung injury suggested that mechanism of action might involve downregulation of inflammatory cytokine expression in lung parenchyma and again downregulation of neutrophil migration in the lungs [94].

Finally, evidence for CNP and its derivate anti-inflammatory treatment potential were shown in a wounded cartilage explant model in steers. In this study, wounded explants were cultured with 0 or 10 ng/mL IL-1 β and/or microcapsules loaded with or without CNP for a period of 48 h. The presence of CNP microcapsules had a concentration-dependent effect with significant inhibition of NO release in response to IL-1 β at 2000 ($p < 0.01$), 10,000 ($p < 0.01$), and 50,000 microcapsules/well ($p < 0.001$) [45]. Others suggested that the effect of CNP on preventing the inflammatory effects of IL-1 β in chondrocytes depends on local protein concentration. While low concentrations (pM) were shown to promote a proliferative response, high concentrations (μ M) lead to anabolic effects such as matrix synthesis in chondrocytes [95, 96].

Acute inflammation and the inflammatory mediators seems to suppress the activity of CNP in growing organisms [97]. It is possible that in chronic inflammatory diseases, serum NT-proCNP levels are also low and contribute to the growth arrest during active disease in children.

11. Other conditions in which circulating CNP levels are affected

CNP regulates fat metabolism in adipogenic tissue, and adipogenic CNP transgenic mice is resistant to obesity when fed by high fat content. Natriuretic peptides regulate intracellular cGMP and phosphorylated vasodilator-stimulated phosphoprotein (VASP). Adipogenic CNP

transgenic mice showed a decrease in fat weight and adipocyte hypertrophy and increases in fatty acid β -oxidation, lipolysis-related gene expression, and energy expenditure during high fat diet (HFD)-induced obesity. Furthermore it seems like CNP overexpression diminished the inflammatory activity in adipogenic tissue. Adipogenic cell CNP transgenic mice were reported to have significantly decreased gene expression of TNF- α , interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and F4/80 in mature adipocytes [98]. Adipogenic CNP transgenic mice also developed better glucose tolerance and insulin sensitivity, which were found to be associated with enhanced insulin-stimulated Akt phosphorylation. It was suggested that CNP overexpression in adipocytes protects against adipocyte hypertrophy, excess lipid metabolism, inflammation, and decreased insulin sensitivity during HFD-induced obesity. An earlier report also suggested that overexpression of endothelial-specific CNP overexpression protects against visceral adipose tissue hypertrophy, systemic inflammation, and insulin resistance during the development of obesity due to the feeding of a high-fat diet (HFD) [99]. Overall current knowledge suggests that natriuretic peptides are new pathways controlling human adipose tissue lipolysis operating via a cGMP-dependent pathway.

One of the intriguing studies is one that examined serum levels of 53 patients with Behçet's disease and showed that all patients with active disease had lower levels of CNP indicating its suppression by inflammatory disease activity [100]. Thus, there is evidence that during chronic inflammatory diseases CNP serum levels might be suppressed which can impact the skeletal growth if the individuals affected have open growth plates and ongoing longitudinal growth process.

12. Current clinical use of CNP

Use of CNP and its analogues in achondroplasias: gain-of-function mutations in the FGFR3 gene result in achondroplasia. Achondroplasia is known as the most common form of dwarfism. In patients with achondroplasia there is impaired proliferation and differentiation of the chondrocytes in the growth plate cartilage that causes stunted longitudinal growth due to endochondral growth suppression and skull abnormalities due to membranous ossification disruption. In achondroplasia, FGFR3 mutations induce increased phosphorylation of the tyrosine kinase receptor FGFR3 and increase the mitogen-activated protein kinase (MAPK). It is known that C-type natriuretic peptide (CNP) suppresses FGFR3 downstream signaling by inhibiting the pathway of mitogen-activated protein kinase (MAPK) in vivo and in vitro. Mice overexpressing CNP rescues FGFR3 gain of mutation-related dwarfism. Exogenous administration of CNP has been challenging since it is rapidly cleared and degraded in vivo through receptor-mediated and proteolytic pathways such as proteolytic-neutral endopeptidase degradation. Therefore, multiple variants of CNP molecules have been tested for their efficacy. Recently, a variant of CNP called BMN111, neutral endopeptidase-resistant CNP analog, showed significant ability to stimulate signaling downstream of the CNP receptor, natriuretic peptide receptor B. Initial trial of continuous delivery of CNP through intravenous (IV) infusion in the form of BMN111 in 2014 showed normalization of dwarfism [101].

Since subcutaneous (SC) route of administration is preferred over continuous infusion in pediatric individuals, it is expected that BMN 111, a 39 amino acid CNP pharmacological analog, would be very effective in diseases where CNP signaling pathway is impaired. BMN 111 can be applied once daily via SC administration at physiological concentrations [101].

In the near future, CNP analogues might be used in other diseases where CNP signaling is impaired or blocked by chronic inflammation affecting cartilage and bone. Use of CNP analogues can be applicable to both adult and pediatric diseases.

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