Korean J Physiol Pharmacol
Vol 4: 283–289, August, 2000

Effect of Atrial Natriuretic Peptide on the Proliferation and Activity of Osteoblastic Cells

Jong-Ryeul Lee, Seon-Yle Ko[1], Jung-Keun Kim[1], and Se-Won Kim

Departments of Dental Pharmacology and [1]Oral Biochemistry, School of Dentistry, Dankook University, Cheonan 330–714, Korea

Natriuretic peptides comprise a family of three structurally related peptides; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). The present study was performed to investigate the effect of ANP on the proliferation and activity of ROS17/2.8 and HOS cells which are well-characterized osteoblastic cell lines. ANP dose-dependently decreased the number of ROS17/2.8 and HOS cells after 48-hour treatment. ANP generally increased the alkaline phosphatase activity of ROS17/2.8 and HOS cells after 48 hr treatment, regardless of the fact that basal activity of alkaline phosphatase was much lower in HOS cells compared to that of ROS17/1.8 cells. ANP increased the NBT reduction by ROS17/2.8 and HOS cells. ANP showed the variable but no significant effect on the nitric oxide production by ROS17/2.8 and HOS cells. ROS17/2.8 and HOS cells produced and secreted gelatinase into culture medium, and this enzyme was thought to be the gelatinase A type with the molecular weight determination. The gelatinase activity produced by ROS17/2.8 cells was increased by the treatment of ANP. However, the enzyme activity was not affected by ANP treatment in the HOS cell culture. In summary, ANP decreased the proliferation and increased the alkaline phosphatase activity and NBT reduction of osteoblasts. These results indicate that ANP is one of the important regulators of bone metabolism.

Key Words: Atrial natriuretic peptide, Osteoblast, Cell proliferation, Alkaline phosphatase, NBT reduction, Gelatinase

INTRODUCTION

Atrial natriuretic factor was discovered by the pioneering work of DeBold et al (1981). In their works, supernatants of atrial homogenates, when injected into rats, caused a potent natriuresis and diuresis. The factor involved was found to be a peptide hormone synthesized, stored, and released by mammalian atrial cardiocytes. Natriuretic peptides comprise a family of three structurally related peptides; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (Rosenzweig & Seidman, 1991; Samson, 1992). ANP and BNP act as cardiac hormones that are produced predominantly by atrium and ventricle, respectively (Mukoyama et al, 1991; Tamura et al, 1994). CNP occurs in a wide variety of tissues, where it acts as a neuropeptide as well as a local regulator (Komatsu et al, 1991; Vollmar et al, 1993). These natriuretic peptides can elicit vasorelaxing, natriuretic and diuretic responses (Brenner et al, 1990). Recently, these peptides were reported to be the potent regulators of cell growth (Appel, 1992; Hagiwara et al, 1994).

It is well recognized that natriuretic peptide receptors are expressed not only in the cardiovascular tissues, but in a variety of extracardiovascular tissues, suggesting that natriuretic peptides play important roles outside the cardiovascular system as well (Chinkers & Garbers, 1991). There are several reports that provided an incentive for investigating a possible role for natriuretic peptides in bone metabolism. In fact,

Corresponding to: Se-Won Kim, Department of Dental Pharmacology, School of Dentistry, Dankook University, San 7-1, Shinbu-dong, Cheonan 330-714, Korea. (Tel) 82-41-552-7648, (Fax) 82-41-552-7648, (E-mail) doban@anseo.dankook.ac.kr

avian chondroprogenitor cells and fetal rat bone cultures bear receptors for ANP (Pines & Hurwitz, 1988, 1989; Vargas et al, 1989). Specific, high affinity binding sites for ANP have also been identified in stromal cells derived from mouse bone marrow (Agui et al, 1992) and in osteoblast-rich cells prepared from neonatal rat calvaria as well as in clonal osteogenic sarcoma cells (UMR 106-01) which have an osteoblastic phenotype (Fletcher et al, 1986).

Previous studies using primary cultures of osteoblast-like cells and chondrocytes and osteoclast-containing bone marrow cultures revealed that natriuretic peptides can regulate the proliferation and differentiation of osteoblasts, chondrocytes, and osteoclasts (Holliday et al, 1995; Suda et al, 1996; Hagiwara et al, 1996). Several reports suggest a physiological significance of natriuretic peptides in the process of endochondral ossification (Suda et al, 1998; Yasoda et al, 1998). Hagiwara et al (1996) demonstrated that continuous culture of osteoblast-like cells from newborn rat calvariae in the presence of natriuretic peptides resulted in stimulation of bone formation as well as activation of alkaline phosphatase. Moreover, ANP has been shown to partially inhibit PGE_2-stimulated bone resorption without effect on basal or PTH-stimulated resorption in fetal rat bone organ cultures (Vargas et al, 1989). All these results suggest that natriuretic peptides could affect bone remodeling, but the physiological role of natriuretic peptides in this process has not been fully understood. This study was performed to explore the possible role of ANP in the regulation of osteoblast proliferation and activity.

METHODS

Cell culture

Two well-characterized osteoblastic cell line, ROS 17/2.8 and HOS, were used in this study. ROS17/2.8 cell line was derived from rat osteosarcoma and cultured with Dulbecco's modified Eagle medium (DMEM, BioWhittaker) containing 10% fetal bovine serum (FBS, Gibco). HOS cell line was derived from human osteosarcoma and cultured with F-12 medium (Gibco) containing 5% FBS.

Cell proliferation assay

Osteoblastic cells were plated in 24-well plate (2×10^4 cells/well) and cultured for 48 hours with DMEM containing 10% FBS or F-12 media containing 5% FBS. Media was replaced with fresh media or media containing various concentrations of ANP ($10^{-11}\sim10^{-7}$ M, Sigma) and cultured for additional 48 hours. After culture, adherent cells were detached by trypsinization and resuspended in FBS-containing media. Cell suspension was mixed with equal volume of 0.4% trypan blue solution in phosphate buffered saline and cells were counted and scored for dye exclusion in a hemocytometer under the light microscope.

Alkaline phosphatase assay

Alkaline phosphatase activity of osteoblastic cells was determined in ROS17/2.8 and HOS cultures after treatment with ANP. Osteoblastic cells were plated at a density of 2×10^4 cells/well in 24-well plate and cultured for 48 hours. When the cells reached about 70% confluence, media was changed with various concentrations of ANP. After 2 days of culture, media was discarded and enzymes were extracted with 0.1% Triton X-100/saline. Enzyme activity was measured by spectrophotometer using p-nitrophenyl phosphate (pNPP, 100 mM, Sigma) as a substrate. Protein concentration of each sample was measured using a commercial kit (BCA Protein Assay Kit, Pierce). The enzyme activity was calculated as nmol or μmole substrate cleaved/min/mg protein.

Nitro blue tetrazolium (NBT) reduction assay

To determine the production of superoxide by osteoblastic cells, NBT reduction assay was performed. Osteoblastic cells were grown to confluence in 96-well plates and media was replaced with fresh media containing various concentrations of ANP. After 2 days of culture, 20 μl of NBT solution (Grade III, 10 mg/ml, Sigma) was added to each well. After 2-hour incubation, media was discarded, and the formazan products formed during the culture were solubilized by adding 200 μl of 0.04 N HCl/isopropanol. Absorbance was read at 562 nm on a microplate absorbance reader (SLT 400 SFC, Austria). Data are expressed as percent absorbance of the cultured control [100 × (absorbance of treated wells/absorbance of control well)].

Nitrite assay

Osteoblastic cells were grown in 96-well plates and incubated with media containing various concentrations of ANP for 48 hours. Cell culture conditioned medium samples were assayed for nitrite, a stable end product of NO, using the Griess reaction (Green et al, 1982). Briefly, 100 μl of Griess reagent consisting of 1% sulphanilamide and 1% N-1-naphthylethylenediamine dihydrochloride in 2% phosphoric acid was mixed with 100 μl of conditioned medium. Absorbance was then measured at 530 nm with a microplate reader (SLT 400 SFC, Austria).

Type IV collagenase/gelatinase activity (zymogram)

Cells were grown to confluence in 24-well culture plate. Media was replaced with serum-free media containing various concentrations of ANP and cultured for additional 48 hours. After culture, conditioned media was concentrated about 5-fold with a 30,000 molecular weight cut-off device (Centricon, Amicon). Gelatin-degrading activity was assessed by SDS-PAGE with a zymogram gel containing 0.1% gelatin (NOVEX) using a mini-gel apparatus. After electrophoresis, the enzyme was renatured by soaking the gel in renaturing buffer (2.5% Triton X-100) for 30 min at room temperature and then in developing buffer (50 mM Tris-HCl, pH 8.3, 0.2 M NaCl, 6.7 mM $CaCl_2$, and 0.02% Brij 35) for 30 min. After overnight incubation at 37°C in fresh developing buffer, the gel was stained with 0.5% Coomassie blue R250 and destained in 10% methanol/10% acetic acid. Enzyme activity appeared as a clear band in a dark blue background. Molecular weight standards were run on the same gels.

Table 1. Effect of ANP on the proliferation of ROS17/2.8 and HOS cells

Treatment	Cell number ($\times 10^{-4}$)	
	ROS17/2.8	HOS
Control	35.4±1.37	41.9±1.62
ANP 10^{-11} M	31.1±1.29	37.9±1.56
ANP 10^{-10} M	29.4±1.98	39.3±5.28
ANP 10^{-9} M	29.2±1.77**	37.4±1.44
ANP 10^{-8} M	25.6±1.80**	33.9±1.17**
ANP 10^{-7} M	25.6±1.62**	36.6±1.63*

ROS17/2.8 and HOS cells were cultured in the presence or absence of ANP for 48 hours. Values are Mean±S.E. (n=8). *P<0.05, **P<0.01, significantly different from control.

RESULTS

Cell proliferation

Cell number was calculated after culture with ANP for 48 hours. ANP, at the concentration range of 10^{-11} ~10^{-7} M, decreased the number of ROS17/2.8 cells and statistically significant decrease was shown at 10^{-9} ~10^{-7} M ANP treatment. Total number of HOS cells was also decreased by ANP treatment. However, maximum decrease was observed at the 10^{-8} M ANP (Table 1).

Alkaline phosphatase activity

Alkaline phosphatase activity, one of the well-known osteoblast phenotype marker, was increased by ANP treatment. ANP increased the alkaline phosphatase activity of ROS17/2.8 cells and the maximum increase was shown in 10^{-8} M ANP treatment. Basal activity of alkaline phosphatase was very low in HOS cells compared to ROS17/2.8 cells. However, ANP also increased the alkaline phosphatase activity in HOS cell culture by 17% (Table 2).

Table 2. Effect of ANP on alkaline phosphatase activity of ROS17/2.8 and HOS cells

Treatment	ALP activity	
	ROS17/2.8	HOS
Control	1.69±0.05	29.8±1.19
ANP 10^{-10} M	1.65±0.08	30.3±0.83
ANP 10^{-9} M	1.74±0.15	32.3±0.88
ANP 10^{-8} M	2.03±0.27*	32.3±0.90
ANP 10^{-7} M	1.82±0.30	35.4±1.31**
ANP 10^{-6} M	2.02±0.36	34.8±0.95**

ROS17/2.8 and HOS cells were cultured in the presence or absence of ANP for 48 hours. The enzyme activity was calculated as μmole or nmole substrate cleaved/min/mg protein in ROS17/2.8 and HOS cells respectively. Values are Mean±S.E. (n=8). *P<0.05, **P<0.01, significantly different from control.

Table 3. Effect of ANP on the superoxide production in the ROS17/2.8 and HOS cell culture

Treatment	Absorbance (OD_{550})	
	ROS17/2.8	HOS
Control	0.147±0.001	0.150±0.001
ANP 10^{-11} M	0.158±0.001	0.149±0.001
ANP 10^{-10} M	0.158±0.002	0.151±0.002
ANP 10^{-9} M	0.190±0.002**	0.169±0.002**
ANP 10^{-8} M	0.192±0.002**	0.171±0.002**
ANP 10^{-7} M	0.177±0.001**	0.176±0.001**

ROS17/2.8 and HOS cells were cultured in the presence or absence of ANP for 48 hours. After dissolution of formazan granule, each well of the culture plate was read using a microplate reader at 550 nm. Values are OD_{550} and expressed as Mean±S.E. (n=10). **P<0.01, significantly different from control.

Superoxide production

NBT reduction due to superoxide production by ROS17/2.8 and HOS cells was measured after culture with ANP. Table 3 shows that ANP increased the NBT reduction by ROS17/2.8 cells and statistically significant increase was observed at 10^{-9}~10^{-7} M ANP treatment. The maximum stimulatory effect of ANP was found to be 31% at the 10^{-8} M ANP. Similarly, ANP increased the NBT reduction by HOS cells. However, the maximum stimulatory effect was lower than that of ROS17/2.8 cells.

Nitric oxide production

Nitrite level in conditioned media was measured by the spectrophotometric measurement. ANP showed the variable effect on the nitric oxide production by ROS17/2.8 and HOS cells (Table 4). However, basal amount of nitric oxide produced by these cells was very low, and the changes of NBT reduction by the treatment with ANP was negligible.

Type IV collagenase/gelatinase activity

Gelatinase, produced by osteoblastic cells and secreted into the culture medium, was detected with substrate gel electrophoresis (zymography). As shown in Fig. 1, one gelatinase band which had an apparent molecular weight of 65~68 KDa was detected. Although it is not conclusive that this enzyme activity represents a 72 KDa type IV collagenase (gelatinase A), the enzyme activity was increased by the addition of ANP in the ROS17/2.8 cell culture. However, the enzyme activity was not affected by ANP treatment in the HOS cell culture (Fig. 1).

Table 4. Effect of ANP on the nitric oxide production from the ROS17/2.8 and HOS cell culture

Treatment	Absorbance (OD_{530})	
	ROS17/2.8	HOS
Control	0.017±0.002	0.050±0.001
ANP 10^{-11} M	0.028±0.004	0.066±0.003
ANP 10^{-10} M	0.018±0.001	0.049±0.002
ANP 10^{-9} M	0.019±0.001	0.039±0.002
ANP 10^{-8} M	0.022±0.004	0.036±0.001
ANP 10^{-7} M	0.019±0.002	0.047±0.002

ROS17/2.8 and HOS cells were cultured in the presence or absence of ANP for 48 hours. Values are Mean±S.E. (n=10).

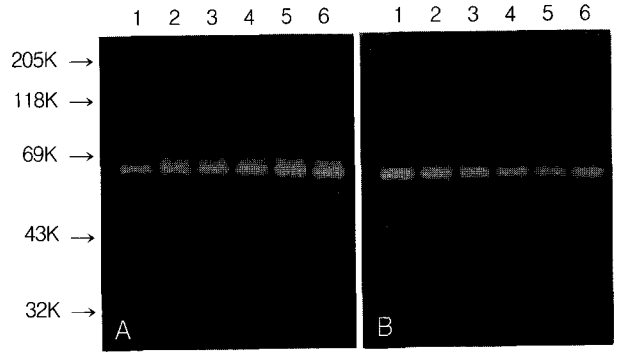

Fig. 1. Zymogram of concentrated conditioned media obtained from ROS17/2.8 (A) and HOS (B) cell culture. Concentrated conditioned media after culture with various concentrations of ANP was resolved in 10% zymogram gel containing 1 mg/ml gelatin. Numeral are molecular weight standard. Lane 1, control ; Lane 2-6, ANP 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7} M.

DISCUSSION

The control mechanism that allow for coordinated bone growth are poorly understood, but probably involve many systemic and local regulators. These in-

clude parathyroid hormone, 1,25-dihydroxy vitamin D_3, growth factors and cytokines as well as others yet unknown (Thompson et al, 1987; Antoz et al, 1989; Stashenko et al, 1989).

In this experiment, it was found that ANP inhibited the proliferation of ROS17/2.8 and HOS cells which retain much of the osteoblastic phenotype. ANP, at the concentration of $10^{-11} \sim 10^{-7}$ M, dose-dependently decreased the cell number of ROS17/2.8 cells after 48-hr treatment. ANP also inhibited the cell proliferation of HOS cells (Table 1). Pines and Hurwitz (1988) reported that ANP inhibited cell proliferation and PTH-stimulated [^{3}H]-thymidine incorporation into DNA of avian chondroprogenitor cells. In primary osteoblastic cells exposed to natriuretic peptides, a dose-dependent reduction in the rate of DNA synthesis was observed (Hagiwara et al, 1996). Our results are in accordance with these reports and ANP seems to have a antimitotic effect on the various kinds of cell types including osteoblasts (Appel, 1992). However, Suda et al (1996) reported that CNP dose-dependently decreased DNA synthesis of MC3T3-E1 cells, while ANP had little effects. This was due to the difference of osteoblastic cells used for the experiments.

ROS17/2.8 and HOS cells are widely used osteoblastic cell line, but they show a number of phenotypic differences. ROS17/2.8 cells exhibit high alkaline phosphatase activity and rapidly forms mineralized nodules in vitro (Majeska et al, 1985; Simmons & Grynpas, 1990), while HOS cells express relatively high levels of integrin subunits. In this study, basal level of alkaline phosphatase of ROS17/2.8 cells is higher than that of HOS cells. ANP increased the alkaline phosphatase activity of ROS17/2.8 cells and the maximal increase was shown in 10^{-8} M ANP treatment. ANP also increased the alkaline phosphatase activity in HOS cells cultures and statistically significant increase was shown in 10^{-8} and 10^{-7} M ANP treatment (Table 2). These results are in accordance with the report that ANP increased the alkaline phosphatase activity and the expression of mRNA of alkaline phosphatase and osteocalcin in primary osteoblastic cells (Hagiwara et al, 1996). Stein et al (1990) provided a model demonstrating the relationship between proliferation and differentiation during the development of rat osteoblasts. They proposed that cell proliferation is inhibited before events associated with differentiation of osteoblasts, such as increases in the rates of expression of genes for alkaline phosphatase and osteocalcin. Our results suggest the possibility that ANP may be a local regulator which inhibits cell proliferation while stimulates differentiation of osteoblasts.

To explore the effect of ANP on the superoxide production by osteoblastic cells, we performed the NBT reduction assay after treatment of ROS17/2.8 and HOS cells for 48-hrs. In control culture, we have observed that osteoblastic cells reduced yellow NBT dye to an insoluble formazan. NBT reduction was increased by treatment of ROS17/2.8 and HOS cells with ANP (Table 3). It has been known that ANP can modulate the phagocytosis and respiratory burst in murine macrophages. Vollmar et al (1997) reported that ANP increased the ingestion of opsonized fluorescent latex particles and enhanced the reactive oxygen production. Our results are in accordance with this report. However, there is little available information on the role of superoxide produced by osteoblasts in bone metabolism.

Recent studies have demonstrated that osteoblast produces NO in response to proinflammatory cytokines stimulation. This indicated that NO may play a role in the regulation of activity of osteoblasts and osteoclasts. There are many reports that ANP inhibits nitric oxide synthesis in different cells (McLay et al, 1995; Vollmar & Schulz, 1995; Kiemer & Vollmar, 1997). In this experiment, we studied the effect of ANP on the nitric oxide production by osteoblastic cells. As shown in Table 4, it was found that osteoblastic cells produced the nitric oxide. However, the basal level before cytokine stimulation was very low. ANP had variable but not significant effect on the nitric oxide production by osteoblastic cells.

Bone resorption involves the removal of both the mineral and organic matrix components of bone. However, the mechanism for degradation of collagen, the major structural protein of bone, remains controversial. Cystein proteinase, particularly cathepsins, and metalloproteinases such as interstitial collagenase (MMP-1) are capable of degrading native type I collagen (Wooley, 1984; Delaisse et al, 1991). Another member of the metalloproteinase family, type IV collagenase (gelatinase), is produced by bone cells (Lorenzo et al, 1992). Although the actual role for gelatinase in the bone remodeling process is unknown, it is believed to contribute to the final degradation of collagen. In our study, osteoblastic cells produced and secreted gelatinase, which had the molecular weight of 65~68 kDa, into the medium dur-

ing culture (Fig. 1). Because of proteolytic activation of latent gelatinase and shifting molecular weights of activated forms in concentrated conditioned media samples, it was not possible to conclude that this enzyme represented gelatinase A or gelatinase B by the zymogram method. Recent Western blot analysis with specific anti-gelatinase A and B antisera indicated that the main activity produced by osteoblast is gelatinase A (data not shown). Importantly, gelatinase activity is increased by the treatment of ANP in ROS17/2.8 cells. The gelatinase activity with the same molecular weight as the ROS17/2.8 culture was observed in HOS cell culture medium. However, this enzyme activity did not change after treatment of ANP (Fig. 1). These results indicate that ANP decreases the proliferation of osteoblast, while the activity of osteoblast is increased by ANP. Although the precise mechanism of ANP remains to be elucidated, ANP seems to be one of the important regulators of bone metabolism.

REFERENCES

Agui T, Yamada T, Legros G, Nakajima T, Clark M, Peschel C, Matsumoto K. Expression of receptors for atrial natriuretic peptide on the murine bone marrow-derived stromal cells. *Endocrinology* 130: 2487−2494, 1992

Antosz ME, Bellows CG, Aubin JE. Effects of TGF-β and epidermal growth factor on cell proliferation and the formation of bone nodules in isolated foetal rat calvaria cells. *J Cell Physiol* 140: 386−395, 1989

Appel RG. Growth-regulatory properties of atrial natriuretic factors. *Am J Physiol* 262: F911−F918, 1992

Brenner BM, Ballermann BJ, Gunning ME, Zeidel ML. Diverse biological actions of atrial natriuretic peptide. *Physiol Rev* 70: 665−699, 1990

Chinkers M, Garbers DL. Signal transduction by guanylyl cyclases. *Annu Rev Biochem* 60: 553−575, 1993

DeBold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci* 28: 89−94, 1981

Delaisse JM, Ledent P, Vaes G. Collagenolytic cysteine proteinases of bone tissue. *Biochem J* 279: 167−174, 1991

Fletcher AE, Allan EH, Casley DJ, Martin TJ. Atrial natriuretic factor receptors and stimulation of cyclic GMP formation in normal and malignant osteoblast. *FEBS Lett* 208: 263−268, 1986

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnock JS, Tannenbaum SR. Analysis of nitrite, and ^{15}N-nitrate in biological fluids. *Anal Biochem* 126: 131−138, 1982

Hagiwara H, Sakaguchi H, Itakura M, Yoshimoto T, Furuya M, Tanaka S, Hirose S. Autocrine regulation of rat chondrocyte proliferation by natriuretic peptide C and its receptor, natriuretic peptide receptor B. *J Biol Chem* 269: 10729−10733, 1994

Hagiwara H, Inoue A, Yamaguchi A, Yokose S, Furuya M, Tanaka S, Hirose S. cGMP produced in response to ANP and CNP regulates proliferation and differentiation of osteoblastic cells. *Am J Physiol* 270: C1311−C1318, 1996

Holliday LS, Dean AD, Greenwald JE, Glucks SL. C-type natriuretic peptide increases bone resorption in 1,25-dihydroxyvitamin D_3-stimulated mouse bone marrow cultures. *J Biol Chem* 270: 18983−18989, 1995

Kiemer AK, Vollmar AM. Effects of different natriuretic peptides on nitric oxide synthesis in macrophages. *Endocrinology* 138: 4282−4290, 1997

Komatsu Y, Nakao K, Suga S, Ogawa Y, Mukoyama M, Arai H, Shirakami G, Hosoda K, Nakagawa O, Hama N, Kishimoto I, Imura H. C-type natriuretic peptide (CNP) in rats and humans. *Endocrinology* 129: 1104−1106, 1991

Lorenzo JA, Pilbeam CC, Kalinowski JF, Hibb MS. Production of both 92- and 72-kDa gelatinase by bone cells. *Matrix* 12: 282−290, 1992

Majeska RJ, Nair BC, Rodan GA. Glucocorticoid regulation of alkaline phosphatase in the osteoblastic osteosarcoma cell line ROS17/2.8. *Endocrinology* 116: 170−179, 1985

McLay JS, Chatterjee PK, Jardine AG, Hawksworth GM. Atrial natriuretic factor modulates nitric oxide production: an ANF-C receptor-mediated effect. *J Hypertension* 13: 625−630, 1995

Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, Shirakami G, Jougasaki G, Obata K, Yasue H, Kambayashi Y, Inouye K, Imura H. 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, 1991

Pines M, Hurwitz S. The effect of parathyroid hormone and atrial natriuretic peptide on cyclic nucleotides production and proliferation of avian epiphyseal growth plate chondroprogenitor cells. *Endocrinology* 123: 360−365, 1988

Pines M, Hurwitz S. Atrial natriuretic peptide and sodium nitroprusside stimulate cGMP accumulation by avian skin fibroblasts and epiphyseal growth-plate chondroprogenitor cells. *J Endocrinol* 120: 319−324, 1989

Rosenzweig A, Seidman CE. Atrial natriuretic factor and related peptide hormones. *Annu Rev Biochem* 60: 229

−255, 1991

Samson WK. Natriuretic peptides: A family of hormones. *Trends Endocrinol Metab* 3: 86−90, 1992

Simmons DJ, Grynpas MD. Mechanism of bone formation in vivo. In: Hall BK ed, *Bone* vol. 1, Telford Press, Caldwell, N.L., p 193−302, 1990

Stashenko P, Obernesser MS, Oewhirst FE. Effect of immune cytokines on bone. *Immunol Invest* 18: 239−249, 1989

Stein GS, Lian JB, Owen TA. Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation. *FASEB* J 4: 3111−3123, 1990

Suda M, Tanaka K, Fukushima M, Natsui K, Yasoda A, Komatsu Y, Ogawa Y, Itoh H, Nakao K. C-type natriuretic peptide as an autocrine/paracrine regulator of osteoblast. Evidence for possible presence of bone natriuretic peptide system. *Biochem Biophys Res Commun* 223: 1−6, 1996

Suda M, Ogawa Y, Tanaka K, Tamura N, Yasoda A, Takigawa T, Uehira M, Nishimoto H, Itoh H, Saito Y, Shiota K, Nakao K. Skeletal overgrowth in transgenic mice that overexpress brain natriuretic peptide. *Proc Natl Acad Sci USA* 95: 2337−2342, 1998

Tamura N, Ogawa Y, Itoh H, Arai H, Suga S, Nakagawa O, Komatsu Y, Kishimoto I, Takaya K, Yoshimasa T, Shiono S, Nakao K. Molecular cloning of hamster brain and atrial natriuretic peptide cDNAs. Cardiomyopathic hamsters are useful models for brain and atrial natriuretic peptides. *J Clin Invest* 94: 1059−1068, 1994

Thompson BM, Mundy GR, Chambers TJ. Tumor necrosis factors α and β induce osteoblastic cells to stimulate osteoclastic bone resorption. *J Immunol* 138: 775−779, 1978

Vargas SJ, Holden SN, Fall PM, Raisz LG. Effects of atrial natriuretic factor on cyclic nucleotides, bone resorption, collagen and deoxyribonucleic acid synthesis, and prostaglandin E_2 production in fetal rat bone culture. *Endocrinology* 125: 2527−2531, 1989

Vollmar AM, Gerbes AL, Nemer M, Schulz R. Detection of C-type natriuretic peptide (CNP) transcript in the rat heart and immune organs. *Endocrinology* 132: 1872−1874, 1993

Vollmar AM, Schulz R. Atrial natriuretic peptide inhibits nitric oxide synthesis in mouse macrophages. *Life Sci* 56: 149−155, 1995

Vollmar AM, Forster R, Schulz R. Effects of atrial natriuretic peptide on phagocytosis and respiratory burst in murine macrophages. *Eur J Pharmacol* 319: 279−285, 1997

Wooley DE. Mammalian collagenase. In: Piez KA, Reddi AH ed, *Extracellular matrix biochemistry,* Elservier, New York, p 119−157, 1984

Yasoda A, Ogawa Y, Suda M, Tamura N, Mori K, Sakuma Y, Chusho H, Shiota K, Tanaka K, Nakao K. Natriuretic peptide regulation of endochondral ossification: Evidence for possible roles of the C-type natriuretic peptide/guanylyl cyclase-B pathway. *J Biol Chem* 273: 11695−11700, 1998