Alteration of cGMP metabolism during chondrogenic differentiation of chondroprogenitor-like EC cells, ATDC5

Kotomi Fujishige, Jun Kotera, Noriyuki Yanaka, Hiroyuki Akatsuka, Kenji Omori *

Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-50 Kawagishi-2-chome, Toda, Saitama 335-8505, Japan

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Abstract

Guanosine 3′,5′-cyclic monophosphate (cGMP) has been recently reported to be involved in bone formation. ATDC5 cells were used to investigate cGMP metabolism during chondrogenic differentiation. Natriuretic peptide receptor (NPR)-A and NPR-B coupled with guanylate cyclase (GC) mediate biological functions of NPs, whereas NPR-C uncoupled with GC is thought to be the clearance receptor for NPs. The amounts of NPR-A, NPR-B, and CNP transcripts were increased but the amount of NPR-C transcripts was decreased in association with the chondrogenic differentiation of ATDC5 cells. CNP, a specific ligand for NPR-B lets ATDC5 cells accumulate great amounts of cGMP, revealing NPR-B as a dominant biological receptor through differentiation. cGMP hydrolytic activities of PDE1 and PDE5 existed in ATDC5 cells, and the activity of PDE1, which is stimulated by Ca²⁺ and calmodulin (CaM) was major of them. Total cGMP hydrolytic activities as well as the amounts of PDE1 and PDE5 transcripts were enhanced during chondrogenic differentiation. Therefore, cGMP production and hydrolysis, cGMP metabolism was considered to be activated in association with chondrogenic differentiation of ATDC5 cells. These observations may lead to a better understanding of cGMP in the chondrocytes where bone formation occurs. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Guanosine 3′,5′-cyclic monophosphate (cGMP) is involved in many physiological functions, such as smooth muscle relaxation, retinal phototransduction, and intestinal secretion. Intracellular cGMP levels are regulated by guanylate cyclases (GCs) and phosphodiesterases (PDEs). GCs produce cGMP in response to natriuretic peptides (NPs) and nitric oxide, whereas PDEs hydrolyze cGMP and cAMP.

NPs act as potent regulators in cardiovascular homeostasis through their function such as vasorelaxation, diuresis, and natriuresis [1–3]. The peptides form a family of at least three polypeptide hormones: atrial-NP (ANP), brain-NP (BNP), and C-type-NP (CNP) [4,5]. The biological effects of these peptides are mediated through membrane-bound receptors (NP receptor (NPR)-A and NPR-B). These receptors are glycoproteins of ~120 kDa with GC [6,7]. ANP is a selective ligand for NPR-A, and CNP possesses the highest affinity for NPR-B but the low-
est affinity for NPR-A [8]. Although BNP binds to NPR-A rather than to NPR-B, the selective receptor for BNP has not been identified. On the other hand, NPR-C, a homodimer of ~70 kDa, has a shorter intracellular tail lacking the cyclase domain and is therefore uncoupled from cGMP [9,10]. NPR-C has a very high selectivity for all members of the NP family [8]. Because NPR-C is the most abundant NPR in the tissues and cells that receive a large fraction of the cardiac output, including vascular endothelial [11] and smooth muscle cells [12], the expected function of NPR-C was thought to remove NPs from the blood circulation by binding and internalization [13].

Intracellular cGMP is metabolized by many kinds of PDEs. PDEs possessing cGMP hydrolytic activity have been classified based on their amino acid sequence homology, biochemical properties, and inhibitor profiles [14,15]. PDE1 is Ca²⁺/calmodulin-dependent, hydrolyzing both cAMP and cGMP. PDE2 is stimulated by cGMP and hydrolyzes cAMP and cGMP. PDE5 is a cGMP-specific PDE. PDE6 is a photoreceptor cGMP PDE. PDE9, which was recently identified [16,17], is a cGMP PDE and insensitive to a nonspecific PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX). Recently, we identified a novel PDE: PDE10, which is a cAMP and a cAMP-inhibited cGMP PDE [18].

Longitudinal bone formation occurs through the endochondral ossification, that is chondrocytes differentiate sequentially from the proliferating stage to the hypertrophic stage, next to the calcified stage, and then are replaced by osteoblasts at the growth plate. Several lines of evidence have suggested that NPs may be involved in bone formation. For instance, cGMP produced in response to ANP and CNP inhibits the proliferation and promotes the differentiation of osteoblast-like cells from newborn rat calvaria [19]. CNP is also a stimulator of the differentiation of clonal osteoblastic MC3T3-E1 cells [20]. Moreover, involvement of cGMP in bone formation was reported using knockout and transgenic mice. Mice deficient in the cGMP-dependent protein kinase II show dwarfism caused by a defect in endochondral ossification at the growth plate [21]. The heights of both proliferating and hypertrophic zones of the growth plate cartilage are increased in transgenic mice that overexpress BNP [22]. Thus, NPs and their second messenger cGMP is expected to be one of key factors in bone formation.

Chondrocytes in three differentiating stages, such as the proliferative, hypertrophic, and calcified stages, exist at the growth plate. The calcified chondrocytes are replaced by osteoblasts, resulting in the endochondral bone formation. The clonal mouse embryonic cell line ATDC5 cells keep track of the multistep differentiation process from chondroprogenitor mesenchymal cells to calcified chondrocytes [23,24]. On the other hand, only terminal differentiation, which is from proliferating cells to hypertrophic and calcified cells, can be observed in the primary chondrocyte culture. Therefore, ATDC5 cells are considered suitable for the analysis of initial differentiation, that is, from mesenchymal cells to proliferating chondrocytes. We demonstrated that the expression of NPR-C is decreased in association with initial chondrogenic differentiation of the rat cartilage and of ATDC5 cells in a previous study [25]. In the present study, we have focused on the alteration of cGMP production and hydrolysis during initial chondrogenic differentiation using ATDC5 cells. These studies have suggested that cGMP metabolism may become more active in the differentiated ATDC5 cells and it perhaps also in the chondrocytes.
differentiation medium (maintenance medium supplemented with 10 μg/ml bovine insulin; Wako Pure Chemical, Osaka, Japan) to induce differentiation of the cells. ATDC5 cells were maintained in a controlled atmosphere of 5% CO₂/95% air at 37°C.

2.3. Reverse transcriptase–polymerase chain reaction (RT–PCR) analysis

The RT reaction was performed using mRNAs from ATDC5 cells to identify expression of differentiated marker (type II collagen) transcripts. RT was carried out using random hexamers and 1 μg of mRNA as a template at 42°C for 60 min according to the manufacturer’s instruction of GeneAmp RNA PCR Core kit (PE Applied Biosystems). Sense and antisense primers for PCR were prepared as described previously [26]. PCR was carried out through 20 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min, under conditions that each PCR did not reach saturation. Mouse β-actin transcripts were also detected by RT–PCR as a control for the RNA quantity among different samples. β-Actin cDNA fragment was amplified by PCR using the specific primer set (5′-TTAACCTGTAAACCAACTGGAAGCAGATATGG-3′ plus 5′-AGAAGCCCTTGATCTT-GATCTTCTAGGGC-3′).

PDE1A, PDE1B, and PDE1C transcripts were investigated by RT–PCR under different conditions. RT was under the same condition as described above and PCR was carried out through 20 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min, under conditions that each PCR did not reach saturation. Mouse β-actin transcripts were also detected by RT–PCR as a control for the RNA quantity among different samples. β-Actin cDNA fragment was amplified by PCR using the specific primer set (5′-TTAACCTGTAAACCAACTGGAAGCAGATATGG-3′ plus 5′-AGAAGCCCTTGATCTT-GATCTTCTAGGGC-3′).

2.4. Northern blot analysis

ATDC5 cells were cultured in a maintenance medium for 4 days and in differentiation medium for 20 days. Total RNAs were isolated from the cells by ISOGEN (Nippon Gene, Japan) and mRNA was purified using an mRNA separator kit (Clontech). mRNAs were subjected to electrophoresis in 1% agarose/0.66 M formaldehyde gels. The blots were transferred onto Hybond-N+nylon membrane and fixed by ultraviolet irradiation using a UV cross-linker (Stratagene). DNA probes for the hybridization analysis were obtained by RT–PCR using mRNAs from mouse lung or ATDC5 cells as a template and sense and antisense primers designed based on the nucleotide sequences of NPR-A [30] and NPR-B [7,31]. The cDNA fragments encoding a part of NPR-A and NPR-B were produced using the specific primer sets (5′-CCGCCAGCCCTTTCAGGCTG-3′ plus 5′-GGAGAAAGTTGCTTGGGTGC-3′) and (5′-CCCTGAGGGACCAAACCTC-3′ plus 5′-AGGCACTGATAAGCAGGCG-3′), respectively. PCR was carried out through 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min. The amplified fragments were subcloned into pGEM-T easy and their nucleotide sequences were confirmed by the dideoxy-chain-termination method, using an Applied Biosystems model 373A automated sequencer and a dye primer cycle sequencing kit (PE Applied Biosystems). 32P-Labeled DNA probes were prepared using a random primer labeling kit (Takara Shuzo, Japan). The probe for NPR-C was prepared as described previously [32]. The probe for PDE5 was obtained by RT–PCR using the mouse lung cDNA library (Clontech) as described previously [33]. Hybridization was performed in 50% formamide, 5× SSC, 0.5% SDS, 5× Denhardt’s, 100 μg/ml salmon sperm DNA, and the probe at 42°C for 16 h. All blots were washed finally in 0.2× SSC and 0.1% SDS at 60°C for 30 min. The membranes were exposed to X-ray films at −70°C. The resultant X-ray films were scanned by ScanJet 4c (Hewlett Packard) and DeskScan II program, and quantitated using the NIH Image program.

2.5. Measurements of the accumulation of intracellular cGMP

ATDC5 cells cultured for 4 days in maintenance medium in 12-well plates and for 20 days in differ-
entiation medium in 24-well plates were washed twice with PBS. Then, the cells were incubated in maintenance medium containing 0.1% BSA instead of 5% FCS, 0.5 mM IBMX, and one of three NPs for 30 min. After incubation, the cells were washed twice with PBS and intracellular cGMP were eluted in 50% ethanol. The elution was dried up and then cGMP contents were measured using the cGMP, Biotrak enzyme immunoassay system (Amersham Pharmacia Biotech).

2.6. RT–PCR and Southern blot analysis

To detect CNP-22 transcripts in the undifferentiated and the differentiated ATDC5 cells, RT–PCR was performed using 1 μg of mRNA prepared from the cells. Sense and antisense primers were designed based on the nucleotide sequences [34]. The cDNA fragments encoding a part of CNP were produced using the specific primer sets 5'-CACCATG-CACCTCTCCAGC-3' plus 5'-ATGGAGCCGATCCGGTCCAG-3'. PCR was carried out through 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min. The RT–PCR products were subjected to 2% agarose gel electrophoresis, then the DNA fractions were transferred onto Hybond-N+nylon membrane. The blots were fixed by ultraviolet irradiation. To detect PCR products by Southern blot analysis, 32P-labeled oligonucleotide probes homologous to a part of CNP-22 sequence, which do not contain the primer sequences for RT–PCR mentioned above, were prepared. Their nucleotide sequences are 5'-ACCACCGAAGTCCCGAGAAC-3' for CNP. Hybridization was performed in 6×SSC, 0.5% SDS, 5×Denhardt’s, 100 μg/ml salmon sperm DNA, and the 32P-labeled probe at 65°C for 16 h. All blots were washed finally in 1×SSC and 0.1% SDS at 50°C for 20 min. The membranes were exposed to X-ray film at −70°C.

2.7. DEAE–Sepharose chromatography

ATDC5 cells were cultured for 4 days in maintenance medium and for 20 days in differentiation medium. 6×10^7 cells each were used for the following experiments. The cells were washed with PBS twice and incubated in collagenase solution (1 mg/ml) for 15 min at 37°C. The cells were scraped and washed with PBS again. The collected cells were disrupted in ice-cold homogenization buffer (20 mM Tris–HCl (pH 7.5), 2 mM Mg acetate, 0.3 mM CaCl₂, 1 mM dithiothreitol, 40 μM leupeptin, 1.3 mM benzamidine, 0.2 mM phenylethylsulfonylfluoride, and 1 mM NaN₃) by a sonicator (TOMY Seiko, Japan) for 15 s (three times with 1-min intervals) and homogenates were centrifuged at 100,000×g for 60 min. The resultant supernatants were applied to a DEAE–Sepharose column (Amersham Pharmacia Biotech) equilibrated in elution buffer (20 mM Tris–HCl (pH 7.5), 1 mM dithiothreitol, 1 mM CaCl₂, 2 μM leupeptin, and 5 mM benzamidine). The column was washed with 10 ml of elution buffer, and the proteins were then eluted from the column by running a linear NaCl gradient (0–1000 mM, 50 ml) in elution buffer. Fractions (1 ml each) were collected on ice and assayed for PDE activity.

2.8. PDE assay

The PDE assay was performed by the radiolabeled nucleotide method [35]. The assay buffer contained 50 mM Tris–HCl (pH 8.0), 5 mM MgCl₂, 4 mM 2-mercaptoethanol, either 1 μM unlabeled cGMP or cAMP, and either 22 nM [3H]cGMP or [3H]cAMP. In order to observe the activation of cGMP hydrolytic activity, either 1 mM EGTA or 20 U CaM and 2 mM CaCl₂ was added to the assay buffer. All assays were performed for 30 min at 37°C. After boiling for 1.5 min, the mixtures were added by 100 μl of 1 mg/ml solution of Crotalus atrox snake venom and incubated for 30 min at 37°C. The reaction was stopped by the addition of 500 μl of methanol and then resultant solutions were applied to a Dowex (1×8–400) column. Aqueous scintillation cocktails were added to each elute and counted.

3. Results

3.1. Induction of chondrogenic differentiation of ATDC5 cells

ATDC5 cells retain the properties of chondrogenic progenitor cells [23]. The proliferation of ATDC5 cells as well as other cell lines halts at the confluent stage.
However, the proliferation restarts in the presence of insulin and then chondrogenic differentiation occurs through a cellular condensation process, resulting in the formation of cartilage nodule like cell aggregates [23]. In order to know the alteration of cGMP metabolism during chondrogenesis, chondrogenic differentiation of ATDC5 cells was induced. ATDC5 cells were cultured for 4 days in maintenance medium and for 20 days in differentiation medium. Type II collagen is known as the cartilage-characteristic extracellular matrix, and the amounts of this transcripts become higher in the differentiated cells [26]. Type II collagen and β-actin transcripts in ATDC5 cells were analyzed by the RT–PCR method as a differentiation marker and a control for mRNA quantities, respectively (Fig. 1). Although type II collagen transcripts were barely detectable on day 4, they became easily detectable on day 20. Because the amounts of β-actin transcripts were unchanged, chondrogenic differentiation was certainly induced in ATDC5 cells cultured for 20 days in differentiation medium.

3.2. Expression of the NPR transcripts during chondrogenesis of ATDC5 cells

Northern blot analysis was performed using mRNAs isolated from ATDC5 cells on day 4 and on day 20 to investigate expression patterns of NPR transcripts during chondrogenic differentiation (Fig. 2). The amount of NPR-A transcripts was increased from undetectable to barely detectable level. NPR-B transcripts were already detectable on day 4 and were produced to a greater extent on day 20. The increase was approximately 9-fold. On the contrary, the amount of NPR-C transcripts was decreased by half on day 20. From these observations, intracellular cGMP production through the NP sys-

![Graph A](image1)

![Graph B](image2)

Fig. 1. Induction of type II collagen transcripts in the differentiated ATDC5 cells. mRNAs were isolated from ATDC5 cells on day 4 (lane 1) and day 20 (lane 2). One µg of mRNAs was used for RT–PCR analysis. PCR was carried out through 20 cycles with primer sets for type II collagen and β-actin. PCR products were subjected to 4% polyacrylamide gel and the gel was stained with ethidium bromide.

![Graph C](image3)

Fig. 2. Expression of the NPR transcripts during chondrogenic differentiation of ATDC5 cells. Northern blot analysis was performed using mRNAs isolated from ATDC5 cells on day 4 (lane 1) and day 20 (lane 2). mRNAs (2 µg/lane) were subjected to 1% agarose/0.66 M formamide gel and transferred onto the membrane. The blots were hybridized with 32P-labeled DNA fragments encoding a part of NPR-A, NPR-B, NPR-C, and β-actin.

![Graph D](image4)

Fig. 3. Intracellular cGMP accumulation in the undifferentiated and the differentiated ATDC5 cells. ATDC5 cells cultured for 4 days (A) and for 20 days (B) were exposed to various concentrations of ANP (squares), BNP (triangles), and CNP (circles) for 30 min. Intracellular cGMP contents were measured. Data points and bars represent mean ± S.E. (n = 3).
tem was considered to be enhanced in the differentiated ATDC5 cells.

3.3. Intracellular cGMP accumulation by NPs

The amounts of intracellular cGMP by NP stimulation were measured in ATDC5 cells to compare the contribution of NPR-A to cGMP production with that of NPR-B. In the undifferentiated cells (Fig. 3A), CNP-22 was the most potent stimulator and approximately 240-fold higher intracellular cGMP over the basal level was accumulated by stimulation of 10^{-7} M of CNP-22. By contrast, increase of cGMP levels by stimulation of 10^{-7} M of ANP and BNP-45 was only 20- and 12-fold, respectively. In the differentiated cells (Fig. 3B), CNP-22 was also more potent than other two peptides. 10^{-7} M of ANP, BNP-45 and CNP-22 showed increase of approximately 5-, 20-, and 390-fold intracellular cGMP over the basal level, respectively. These results demonstrated that NPR-B is a dominant biological receptor through the differentiation of ATDC5 cells.

3.4. Expression of CNP transcripts during chondrogenesis of ATDC5 cells

Expression of CNP transcripts was examined in ATDC5 cells. Because the transcripts could not be detected in Northern blot analysis, we employed a combination of RT–PCR and Southern blot analysis (Fig. 4). To distinguish between RT–PCR products from mRNA and PCR products from contaminated genomic DNA, specific primers designed to span a genomic intron were used for PCR. The predicted RT–PCR products derived from mRNAs were subjected to Southern blot analysis using 32P-labeled oligonucleotides homologous to a part of nucleotide sequences of CNP.

Fig. 5. Elution profile of DEAE-Sepharose chromatography of cytosolic extracts of ATDC5 cells. The cytosolic extracts of the undifferentiated ATDC5 cells on day 4 (A) and the differentiated cells on day 20 (B) were loaded onto a DEAE-Sepharose column and eluted with buffers containing 0–1 M NaCl. Each fraction was used for the assay of PDE activity. One μM cGMP or cAMP was used as a substrate. Circles represent the cGMP hydrolytic activity in the presence of Ca2+/CaM (open) and EGTA (closed). Squares represent the cAMP hydrolytic activity in the absence (open) and presence (closed) of cGMP.

Fig. 4. Detection of CNP transcripts by RT–PCR and Southern blot analysis. mRNAs prepared from ATDC5 cells on day 4 (lane 1) and day 20 (lane 2) were used for RT–PCR analysis. PCR was carried out through 30 cycles with a primer set for CNP. The RT–PCR products derived from mRNAs were subjected to Southern blot analysis using 32P-labeled oligonucleotides homologous to a part of nucleotide sequences of CNP.

from mRNA and PCR products from contaminated genomic DNA, specific primers designed to span a genomic intron were used for PCR. The predicted RT–PCR products are 366 bp for CNP in length. RT–PCR products from mRNA and the PCR products from contaminated genomic DNA can be recognized as different bands by 2% agarose gel electrophoresis. Although RT–PCR products from CNP transcripts were undetectable in the undifferentiated
cells, they were increased to be detectable in the differentiated cells. Therefore, CNP transcripts were revealed to be mainly expressed in the differentiated cells, and cGMP may be produced by autocrine regulation in the differentiated cells.

3.5. Properties of cGMP PDEs in ATDC5 cells

DEAE–Sepharose column chromatography was carried out to investigate which kinds of cGMP PDEs are present in ATDC5 cells. The cytosolic extract of the undifferentiated cells displayed PDE5 and PDE1 activities resolved by DEAE–Sepharose column chromatography (Fig. 5A). Fractions 21–24 hydrolyzed cGMP with a high degree of selectivity and the cGMP hydrolytic activities were not stimulated by the addition of Ca\(^{2+}/\)CaM. E4021, which is a PDE5-specific inhibitor, inhibited the activities with IC\(_{50}\) of 11 nM. By contrast, the cGMP hydrolytic activities of fractions 29–38 were stimulated by Ca\(^{2+}/\)CaM. Therefore, we considered that fractions 21–24 and fractions 29–38 corresponded to PDE5 and PDE1, respectively. The cAMP hydrolytic activity was not stimulated by cGMP. Thus, PDE2 activity was not detected in the cells. The cytosolic extract of the differentiated cells displayed also PDE5 and PDE1 activities but not PDE2 activity (Fig. 5B). Fractions 21–24 and fractions 25–44 corresponded to PDE5 and PDE1 as in the case of the undifferentiated cells mentioned above. Total cGMP hydrolytic activities were apparently increased in the differentiated cells. These findings indicated that cGMP hydrolytic activities of PDEs were enhanced during chondrogenic differentiation of ATDC5 cells.

3.6. Expression of PDE1 and PDE5 transcripts during chondrogenesis of ATDC5 cells

PDE5 transcripts were detected by Northern blot analysis (Fig. 6A). Their amount was increased during chondrogenic differentiation. PDE1 transcripts were detected by RT–PCR analysis (Fig. 6B). Three isoforms of the PDE1 family, PDE1A, PDE1B, and PDE1C, are reported. Specific PCR primers for each PDE1 isozyme were designed and RT–PCR analysis was performed. The length of amplified DNA fragments and their nucleotide sequence agreed with those predicted. The levels of transcripts encoding these PDEs were revealed to be elevated during chondrogenic differentiation. Therefore, the amounts of PDE1 and PDE5 transcripts were augmented, as was their cGMP hydrolytic activities in the differentiated cells.

4. Discussion

In the previous study, we demonstrated that the
amounts of the NPR-C protein are decreased in as-
sociation with the initial differentiation of ATDC5 cells. This suggests the involvement of NP regulation leading to control of cGMP production on chondro-
genic differentiation. We have investigated the alter-
ation of cGMP metabolism including cGMP produc-
tion by NP system and cGMP hydrolysis by PDEs during chondrogenic differentiation of ATDC5 cells. Interesting changes are reduction of the level of NPR-C transcripts as well as that of the NPR-C protein, and increases of the levels of GC-coupled receptors and CNP transcripts during differentiation. NPR-C lacking GC activity regulates the biological effects by eliminating NPs. Therefore, in the differentiate
ted cells, a larger amount of cGMP may be produced by autocrine or paracrine regulation. NPs are known to inhibit the proliferation and to pro-
mote the differentiation of osteoblast-like cells via
cGMP production [19]. Thus, cGMP produced in the differentiated ATDC5 cells may inhibit the pro-
liferation and promote the next differentiation, termi
nal differentiation. On the other hand, NPR-C was re
ported to be expressed in the osteoblastic cells, and mice deficient in NPR-C showed skeletal deformities associated with a considerable increase in bone turn-
over [36]. These results suggested that NPR-C may have a signaling function of its own in osteoblastic cells and may play a role on bone formation.

CNP that is a specific ligand for NPR-B lets the
cells accumulate a great deal of intracellular cGMP
in contrast to other NPs both in the undifferentiated and in the differentiated ATDC5 cells. Thus, the
CNP/GC-B pathway was considered to be physiologi-
significant in ATDC5 cells, in primary chon-
drocytes, and in an organ culture of tibias [19,37].
The undifferentiated ATDC5 cells were equipped
with the CNP/GC-B pathway, suggesting that the
pathway may be present in chondroprogenitor cells in vivo.

cGMP hydrolytic activities in the cytosolic extract
of ATDC5 cells were separated by DEAE-Sepharose
column chromatography, resulting in two peaks both in the undifferentiated cells and in the differentiated
cells. A minor peak is derived from PDE5 and a
major peak from PDE1. The cytosolic extracts of
MC3T3-E1 cells that have osteoblastic properties and
cause osteoblastic differentiation also yield
cGMP hydrolytic activity of PDE1 (unpublished data). ATDC5 cells and MC3T3-E1 cells differentiate
during culture and are often used for the study of endochondral bone formation. It is intriguing that
cGMP hydrolytic activity that is regulated by Ca²⁺/calmodulin was displayed in those cells, suggesting the involvement of the controllable cGMP hydrolytic
activity in differentiation. Three isozymes, PDE1A, PDE1B, and PDE1C, are present in the PDE1 fam-
ily. Expression of PDE1C is specifically induced when smooth muscle cells dedifferentiate to the pro-
liferative cells [38]. In ATDC5 cells, however, the three PDE1 isozyme transcripts were produced in
higher amounts during differentiation, and specific change of PDE1C expression in association with dif-
ferentiation was not observed.

Total cGMP hydrolytic activities were augmented
during chondrogenic differentiation of ATDC5 cells.
Because cGMP is known to be toxic against the cells
[39] in addition to promoting differentiation, it is
likely that increased cGMP-hydrolytic activities func-
tion to degrade cGMP after a cGMP-mediated signal
has been transduced. On the other hand, cAMP hy-
drolytic activities which did not overlap with cGMP
hydrolytic activities were shown in fractions 35–45
(Fig. 5). The cAMP hydrolytic activities are probably
derived from PDE4 and/or PDE7 (cAMP-specific
PDEs), but not PDE1. Thus, these cAMP-specific
PDEs may hydrolyze cAMP in cooperation with
PDE1 in ATDC5 cells.

Although involvement of cAMP in chondrogenic
differentiation has been investigated in many reports,
few reports concerning cGMP are available. We have
demonstrated the alteration of cGMP metabolism
during chondrogenic differentiation of ATDC5 cells.
These results suggest involvement of cGMP-mediated
signal transduction in initial chondrogenic dif-
ferentiation. Further investigations will elucidate the
physiological functions of cGMP in endochondral
bone formation.

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