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Expression of adrenomedullin and its receptor by chondrocyte phenotype cells

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Abstract

For clarifying a process of de-differentiation in culturing chondrocytes, the present study was undertaken to investigate the secretion of adrenomedullin (AM) by chondrocyte phenotype cells and whether or not AM effects this proliferation in a cAMP-dependent fashion. Chondrocyte phenotype cells expressed AM and the AM receptor, and secreted high concentration of AM into the culture medium. When added to cultures, AM increased the intracellular cAMP level and decreased the number of these cells in a similar concentration-dependent fashion. Addition of forskolin and dibutyryl-cAMP caused a significant decrease in the number of these cells. Furthermore, the effect of AM was inhibited by a cAMP-dependent protein kinase A inhibitor (H89). The present findings indicate that AM has an autocrine/paracrine type of anti-proliferative effect on these cells mediated via a cAMP-dependent pathway and raise the possibility that AM plays a role in the local modulation of a process of de-differentiation by culturing chondrocyte phenotype cells.

Keywords: Adrenomedullin; Chondrocyte phenotype; cAMP; Receptor

Adrenomedullin (AM) is a 52-amino acid peptide, which was originally identified in a human phaeochromocytoma by investigating the cause of increased cAMP activity in rat platelets [1]. AM exerts a potent hypotensive effect in several species via its vasodilatory action. AM has also been shown to inhibit the growth of several types of human cells, such as vascular smooth muscle cells [2]. In addition to platelets, AM increases intracellular cAMP levels in several other types of cell [3]. AM circulates in the blood and is synthesized in numerous tissues, including those of the vascular system and bone [4–6]. A previous immunohistochemical study has also shown that normal human articular chondrocytes are able to produce AM [7]. Following the pioneering studies of Benya and his coworkers [8–10] on the phenotypic changes in cultured rabbit chondrocytes, the modulation of morphological and biosynthetic phenotypes of cartilage cells has been the subject of intense investigation. Numerous studies have examined the plasticity of the chondrocyte phenotype, employing cells of human, avian, lapine, and rodent origin. It has become apparent from these collective studies that culturing chondrocytes invariably leads to de-differentiation, in which cells acquire fibroblastic morphology and lose the chondrocyte-specific gene-expression pattern. When chondrocytes are cultured, there is prompt down-regulation of expression of cartilage-specific genes, including those encoding type-2, -9, and -11 collagens and aggrecan, as well as concomitant initiation or up-regulation of expression of fibroblast-associated genes, including those for type-1, -3, and -5 collagens and versican [8–18]. However, the molecular mechanisms governing this process are not well understood.

It is known that cAMP exerts different effects on mitogenesis, depending on cell type. Cyclic AMP acts as a second messenger in various tissues, including fibroblasts and chondrocytes, and in cultured fibroblasts and

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chondrocytes, cAMP analogs decrease thymidine incorporation into DNA [12,19,20]. The observation that AM increases intracellular cAMP levels in several cultured cell lines suggests that it may influence growth of the chondrocyte phenotype. However, production of AM by chondrocyte phenotype cells and its effects on these cells have yet to be investigated.

The present study was therefore performed to clarify the influence of AM on chondrocyte phenotype cells. The reverse transcriptase polymerase chain reaction (RT-PCR) was used to amplify receptor activity modifying protein (RAMP)-1, -2, and -3, as well as the calcitonin receptor-like receptor (CRLR), after which products were detected by electrophoresis. It has been reported that CRLR and RAMP1 function as the CGRP receptor, while CRLR and RAMP2 act as the AM receptor [21–23]. We demonstrated increased secretion of immunoreactive AM (ir-AM) by cultured chondrocyte phenotype cells when compared with other human cells. We then examined whether synthetic AM was able to inhibit chondrocyte phenotype cell proliferation and evaluated the role of cAMP as an intracellular second messenger. Finally, the actions of endogenous AM secreted by chondrocyte phenotype cells were examined using an anti-AM monoclonal antibody.

Materials and methods

Chemicals. Chondrocyte basal medium (CBM: serum-free) and chondrocyte growth medium (CGM: containing R3-IGF-1, bFGF, insulin, transferrin, fetal bovine serum (FBS), and gentamicin/amphotericin-B) (CC-3216) were purchased from Clonetics (San Diego, CA, USA). Normal human articular chondrocytes (NHAC-kn) (CC-2550) were also obtained from Clonetics. Tetracolor One, which consists of 5 mM of 2-[2-methoxy-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt (WST-8), 0.2 mM of 1-methoxy-5-methyl phenazinium methylsulfate (1-methoxy PMS), and 150 mM NaCl, was obtained from Seikagaku (Tokyo, Japan). Human AM was purchased from Peptide Institute (Osaka, Japan). Forskolin, 3-isobutyl-1-methylxanthine (IBMX), dibutyryl cyclic AMP, H89 (a PKA inhibitor), and H85 (a negative control for H89) were purchased from Sigma Chemical (St. Louis, MO, USA).

Cell culture. Normal human articular chondrocyte (NHAC-kn) cells were cryopreserved, seeded onto a 100-mm dish, and maintained in CGM at 37 °C in a humidified atmosphere of 95% air/5% CO_2. After reaching 90% confluence, cells were washed twice in phosphate-buffered saline without calcium and magnesium [PBS (−)] and then were passaged using 0.5% (w/v) trypsin–0.002% EDTA in phosphate-buffered saline. This cell line de-differentiates in two or three passages and we determined the characterized gene expression for collagen types in the chondrocyte phenotype.

Expression of AM, CRLR, and RAMP1, -2, and -3. Total RNAs were extracted from chondrocyte phenotype cells using Total RNA Isolation Reagent (Invitrogen) and then reverse-transcribed using SuperScript reverse transcriptase (Invitrogen), yielding the respective cDNAs. Primers were designed to be specific for CRLR, RAMP1, -2, or -3 (Table 1) and to minimize cross-hybridization with any other known sequences. Each PCR mixture contained 10× PCR buffer, 5 μL, dNTP 4 μL, rTaq 0.5 μL, and dH_2O. Final volumes were 50 μL including 2 μL cDNA solution. PCRs initiated by the specific primer sets for CRLR, RAMP1, -2, and -3 were carried out with cDNA. PCRs were carried out on a Program Temp. Control PC 700 (ASTEL, Fukuoka, Japan) in the following manner: an initial denaturation step at 95 °C for 3 min was followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 45 s at 72 °C. Reactions were terminated by a 5 min elongation step at 72 °C. PCR products were loaded onto a 3% agarose gel containing 0.5 μg/mL ethidium bromide and the size of amplified products was verified by co-electrophoresis of an appropriate nucleotide marker (Takara, Tokyo, Japan). Reverse transcriptase negative controls were performed for each mRNA extract by substituting the reverse transcriptase enzyme in the reaction mixture with nuclease free water.

Extraction of AM in conditioned medium and cells. Chondrocyte phenotype cells were seeded onto 6-well plates at a density of 96,000 cells/well and were maintained in CGM. After reaching confluence, cells were washed twice with 2 mL/well of PBS (−) and were incubated with another 3 mL CBM (FBS-free, containing gentamicin/amphotericin-B) at 37 °C for 1, 3, 6, 12, 24, or 48 h in a humidified atmosphere of 95% air/5% CO_2 prior to the experiment.

The serum-free medium of the cultured chondrocyte phenotype cells was collected, immediately acidified with acetic acid to a final concentration of 1.0 M, and then boiled for 10 min to inactivate the proteases. The boiled medium was applied to a Sep-Pak C18 cartridge (Millipore–Waters, Milford, MA). After the cartridge was washed with 10% CH_3CN in 0.1% trifluoroacetic acid, the absorbed materials were eluted with 50% CH_3CN in 0.1% trifluoroacetic acid.

In order to measure the intracellular AM level, chondrocyte phenotype cells were scraped into 1.0 M acetic acid and sonicated for 10 min. The sample was then boiled for 10 min, cell extract was cen-

Table 1
Oligonucleotide sequences used for quantitative PCR

Gene	Primer/probe	Sequence (5′–3′)
Human AM	Foward primer (397–416)	GAA-GAC-AGC-AGT-CCG-GAT-GC
	Reverse primer (301–322)	CGT-TGT-CCT-TGT-CCT-TAT-CTG-TGA
Human CRLR	Foward primer (1458–1484)	CTG-TAC-ATG-AAA-GCT-GTG-AGA-GCT-ACT
	Reverse primer (1571–1597)	TGG-AAG-TGC-ATA-AGG-ATG-TGC-ATG-ATG
Human RAMP1	Foward primer (189–209)	GAG-ACG-CTG-TGG-TGT-GAC-TGG
	Reverse primer (329–350)	GAT-GGG-GCA-GCT-CCT-GAA-GTA-G
Human RAMP2	Foward primer (414–438)	GCA-GAG-AGG-ATC-ATC-TTT-GAG-ACT-C
	Reverse primer (542–569)	CCT-CCA-TAC-TAC-AAG-AGT-GAT-GAG-GAA-G
Human RAMP3	Foward primer (208–232)	CCG-AGT-TCA-TCG-TGT-ACT-ATG-AGA-G
	Reverse primer (301–322)	CTG-TGG-ATG-CCG-GTG-ATG-AAG-C

trifuged for 10 min at 3000 rpm, and supernatant was applied to a Sep-Pak C18 cartridge. Extraction of the cartridge was carried out as described above. Cell extracts as well as conditioned media were lyophilized, reconstituted, and subjected to a specific radioimmunoassay (RIA) for human AM.

Radioimmunoassay for AM. The incubation buffer for RIA was 0.05 M sodium phosphate buffer (pH 7.4), containing 0.5% BSA, 0.5% Triton X-100, 0.08 M NaCl, 0.025 M EDTA 2Na, 0.05% NaN_3, and 500 KIU/mL trasylol. Disposable plastic tubes (10×75 mm) were used for assays. All assay procedures were performed at 4 °C. Either standard AM or unknown sample (100 μL) was incubated with anti-AM antiserum diluents (200 μL) for 12 h and then tracer solution (^{125}I-AM, 18,000–20,000 cpm in 100 μL) was added. After incubation for 16 h, anti-rabbit IgG goat serum diluent (100 μL) was added. This solution was left to stand for 24 h, after which the tubes were centrifuged at 3000 rpm for 30 min at 4 °C, and the radioactivity of the precipitate was measured in an Aloka ARC-600 gamma counter.

Characterization of secreted AM. In order to examine the molecular forms of immunoreactive-AM (ir-AM), the extracts of cells cultured on a 100-mm dish in 15 mL of conditioned medium (1% FBS) were analyzed by reverse phase high performance-liquid chromatography (HPLC) with a column of TSK ODS 120 A (Tosoh, Tokyo, Japan). A linear gradient of 10–60% acetonitrile was run in 0.1% trifluoroacetic acid for 60 min and the ir-AM level in each fraction was measured by RIA. Recovery of ir-AM from this HPLC system was greater than 70%.

Measurement of intracellular cyclic AMP. Confluent chondrocyte phenotype cells in 24-well plates were washed twice with 500 μL/well of assay buffer (Hanks' solution containing 0.1% bovine serum albumin (BSA), 20 mM Hepes, and final pH 7.4) prior to the experiment. Cells were preincubated with 0.5 mL assay buffer containing 1 mM of 1-methyl 3-isobutylxanthine (IBMX) at 37 °C for 15 min. The reaction was initiated by adding various concentrations of AM and incubation was conducted for a further 15 min. Aspiration and addition of 1 N acetic acid containing 20 mM HCl (0.5 mL/well) terminated the reaction. After incubated cells were scraped off, each sample was sonicated for 10 min and then boiled for 10 min. Cell extract was centrifuged for 10 min at 3000 rpm and the supernatant was succinylated and measured by specific RIA.

Radioimmunoassay for cAMP. Cyclic AMP antibody was prepared in our laboratory according to previously described methods [24], and ^{125}I-succinyl-cAMP tyrosine methyl ester was prepared using the peroxidase method and purified by reverse phase HPLC as described elsewhere [25]. The incubation buffer for radioimmunoassay was 50 mM sodium acetate buffer (pH 6.2) containing 0.1% BSA, 0.01% Triton X-100, 1 mM EDTA 2Na, and NaN_3. RIA incubation mixture consisted of 50 μM of either standard or sample solution, 100 μL of antiserum at a dilution of 1:5000, and 50 μL ^{125}I-labeled ligand (18,000 cpm) in the standard buffer. The above mixture was placed in a plastic tube (7.5×78 mm), mixed well, and then equilibrated at 4 °C for 24 h, after which 50 μL of 1% bovine γ-globulin and 500 μL of 25% polyethylene glycol in standard buffer were added. After vigorous shaking, the mixture was incubated at 4 °C for 30 min. The supernatant was aspirated and radioactivity in the pellets was determined in a gamma counter. All assays were performed in duplicate.

Measurement of cell number. We used Tetracolor One (tetrazolium salt) for cell viability assay [26]. Chondrocyte phenotype cells were seeded onto 96-well plates with conditioned medium (1% FBS) at a density of 3200 cells/well. Conditioned medium is CBM containing 1% FBS and gentamicin/amphotericin-B. On day two, various concentrations of peptides were added to each well. On day four, cell number was determined. After adding 10 μL Tetracolor One solution to each well, cells were incubated for 4 h at 37 °C. A microplate reader (Malitiskan Bichromatic, Labsystems) was then used to measure the absorbance using a test wavelength of 450 nm and a reference wavelength of 620 nm.

Effect of an anti-AM monoclonal antibody. Chondrocyte phenotype cells were seeded onto 96-well plates containing conditioned medium (1% FBS) at a density of 3200 cells/well. On day two, anti-AM monoclonal antibody donated by Dr. Tsuji [27,28], or as a control, mouse IgG (Sigma) was added and cells were incubated for three days. On day four, cell number was measured as described above.

Statistical analysis. Multiple comparisons were assessed by one-way ANOVA followed by Scheffe's test. All results were expressed as means ± SD and $p < 0.05$ was considered significant. All experiments were repeated at least three times using cells isolated separately from various sources of chondrocyte phenotype cells.

Results

Expression of AM, CRLR, and RAMPs

We examined the RT-PCR products of AM, RAMP1, -2, and -3, and CRLR by electrophoresis. Agarose gel electrophoresis of the RT-PCR products gave bands of the expected size (positive control shown Fig. 1) and that corresponded to the present mRNA encoding CRLR as well as RAMP1 and RAMP2 in chondrocyte phenotype cells. However, an RT-PCR product for RAMP3 was not detectable in chondrocyte phenotype cells. No bands were seen in the negative controls, which did not include the reverse transcriptase enzyme.

Ir-AM in chondrocyte phenotype cells

AM specific RIA measured the ir-AM level in the conditioned media and in chondrocyte phenotype cells. When chondrocyte phenotype cells were cultured in serum-free medium, cells actively secreted AM in a time-dependent manner, and AM reached a concentration of 8.8 ± 0.4 fmol/1×10^5 cells at 24 h (Fig. 2). However,

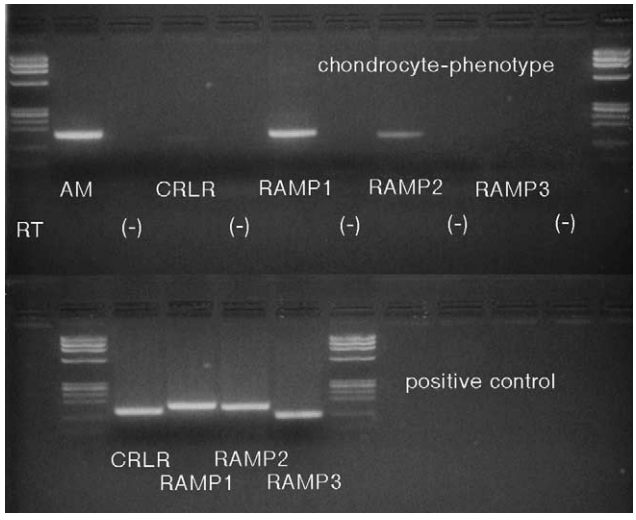

Fig. 1. Expression of AM, CRLR, RAMP1, RAMP2, and RAMP3 in chondrocyte phenotype cells, as detected by RT-PCR. RT: reverse transcriptase. Bands corresponding to mRNAs encoding CRLR as well as RAMP1 and RAMP2 are evident in lanes representing chondrocyte phenotype cells. No band is seen for RAMP3. No bands are seen in negative controls where mRNA was not reverse transcribed prior to amplification. Positive controls show bands of the expected size.

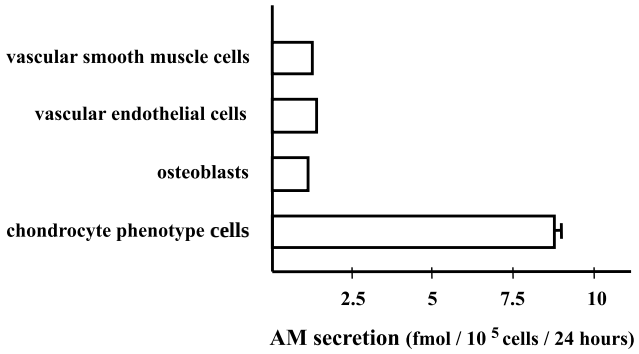

Fig. 2. Secretion of AM from cultured human cell lines. We demonstrated the secretion of AM by human endothelial cells ([19], including our data), human vascular smooth muscle cells [20], osteoblast (NHOst) (our data), and chondrocyte phenotype cells. These cells secreted AM into the medium (fmol/10^5 cells/24 h).

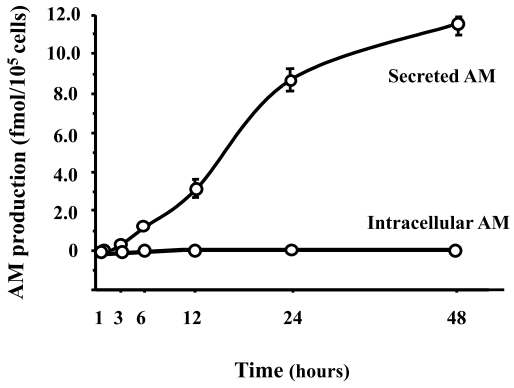

Fig. 3. Time course curve for secretion of AM and intracellular AM. Cells were incubated for the indicated time-periods in serum free medium and AM was determined by RIA, as described in Materials and methods. Each value represents means ± SEM of six wells examined.

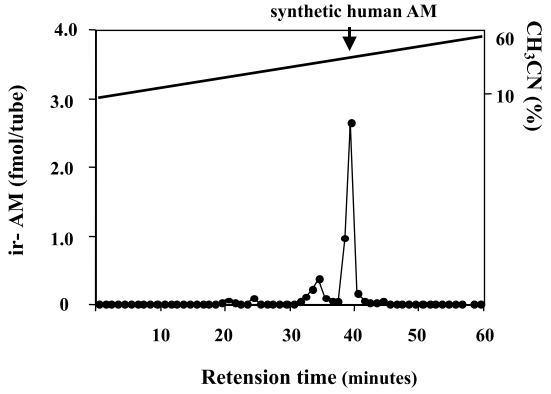

Fig. 4. Analysis by reverse-phase HPLC of immunoreactive adrenomedullin (ir-AM) secreted into the media. A linear gradient of 10–60% acetonitrile in 0.1% trifluoroacetic acid was run for 60 min at a flow rate of 1.0 mL/min. The arrow indicates the elution fraction of synthetic human AM.

the intracellular AM concentration remained below detectable limits (Fig. 3). The molecular form of ir-AM secreted into the medium was characterized by reverse phase HPLC. As shown in Fig. 4, the majority of the ir-AM was eluted in the same fraction as authentic human AM (1–52)-NH_2. In addition to the major peak, a minor peak was observed in an earlier fraction.

Effect of AM on cyclic AMP production

Human AM increased intracellular cAMP levels in a dose-dependent fashion. The increase in cAMP was maximal when human AM was added at 10^{-6} mol/L, as shown in Fig. 5. The ED50 value of AM was estimated to be 2×10^{-7} M. Fig. 6 shows the time course of cAMP

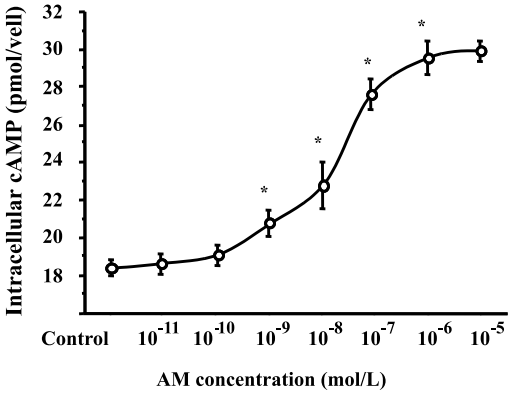

Fig. 5. Augmentation of cAMP production by human AM in cultured chondrocyte phenotype cells. These cells were incubated with the peptide in the presence of 1 mM IBMX, as described in Materials and methods. Data represent mean values of triplicate determinations from three separate experiments in chondrocyte phenotype cells. $^*p < 0.05$.

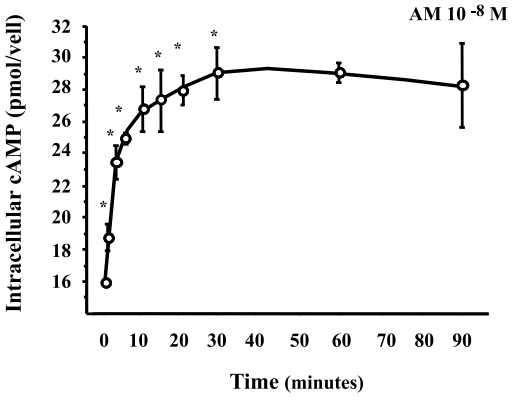

Fig. 6. Time course of cAMP production in chondrocyte phenotype cells by human AM. Cells were exposed to a concentration of 10^{-8} M of AM during increasing time periods, up to 90 min. Cells were incubated for the indicated time-periods in serum-free media with 1 mM IBMX and cAMP was determined by RIA, as described in Materials and methods. Data represent mean values of triplicate determinations from three separate experiments in chondrocyte phenotype cells. $^*p < 0.05$.

production in response to human AM (10^{-8} M). AM caused a rapid increase in the intracellular cAMP concentration, which peaked after 30 min.

Effect of synthetic AM on proliferation of chondrocyte phenotype cells via a cyclic AMP-dependent pathway

The biological action of AM was further investigated by examining its effects on proliferation of chondrocyte phenotype cells. AM inhibited the proliferation of these cells in a dose-dependent manner (Fig. 7).

In addition to AM (10^{-8} M), forskolin (a phosphodiesterase inhibitor: 10^{-7} mol/L) and dibutyryl cyclic AMP (1 mmol/L) caused significant inhibition of chondrocyte phenotype cell proliferation (Fig. 8). Furthermore, H89, an inhibitor of cAMP-dependent protein kinase A (PKA), blocked the inhibitory effects of AM on proliferation of chondrocyte phenotype cells, while H85 (the negative control for H89) did not block the effects of AM (Fig. 9).

Action of endogenous AM

In order to further investigate the hypothesis that AM acts on chondrocyte phenotype cells in an autocrine/paracrine fashion, we performed in vitro neutralization experiments using a purified anti-AM monoclonal antibody [28]. Addition of 10 μg/ml (final concentration in the medium) of purified anti-AM monoclonal antibody significantly increased the cell population when compared with mouse IgG-treated cultures ($P < 0.05$) (Fig. 10).

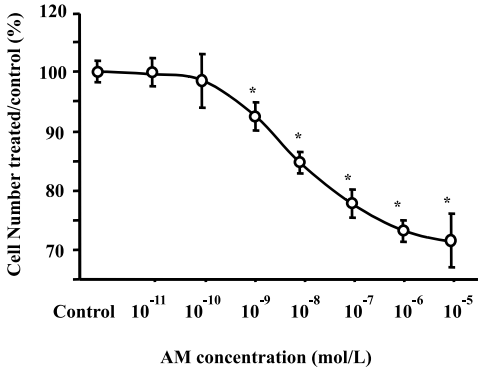

Fig. 7. Effects of various AM doses in cultured chondrocyte phenotype cells. Cell proliferation was measured as described in Materials and methods. Values are means ± SEM of six wells examined. Each was compared with cells incubated in 1% FBS media (control). Each set of experiments was repeated three times and identical results were obtained. *$p < 0.05$.

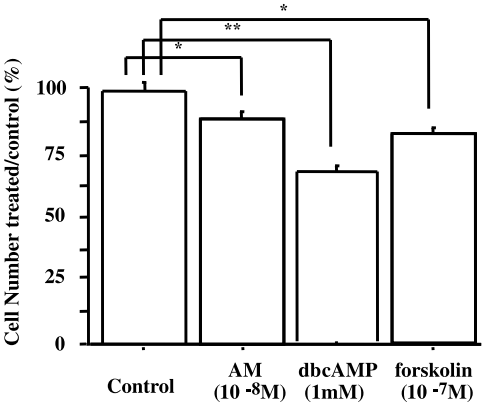

Fig. 8. Comparison of chondrocyte phenotype cell proliferation by AM (10^{-8} M), dibutyryl cAMP (dbcAMP) (1 mM), and forskolin (10^{-4} M). Cell proliferation was measured as described in Materials and methods. Values are means ± SEM of six wells examined. Each was compared with cells incubated in 1% FBS medium (control). Each set of experiments was repeated three times and identical results were obtained. *$p < 0.05$. **$p < 0.01$.

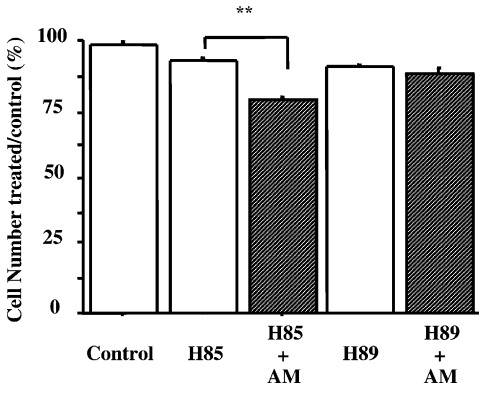

Fig. 9. Effect of AM for cell proliferation inhibited by H89 in chondrocyte phenotype cells. These cells were incubated with a PKA inhibitor (H89) in the absence (open bars) or presence (solid bars) of 10^{-8} M AM for four days. Values are means ± SEM of six wells examined. Each was compared with the cells incubated in 1% FBS (control). Each set of experiments was repeated three times and identical results were obtained. **$p < 0.01$.

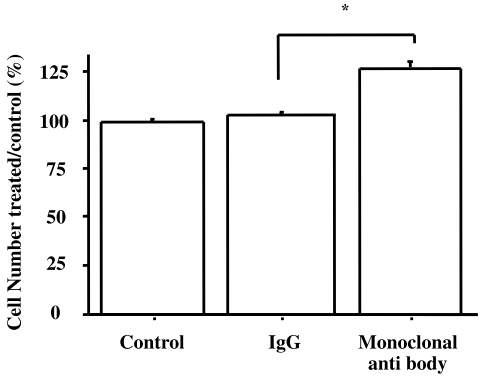

Fig. 10. Effects of anti-AM monoclonal antibody on control of cell proliferation. Serum-starved chondrocyte phenotype cells were incubated with either 10 μg/ml of purified anti-AM monoclonal antibody or 10 μg/ml of mouse IgG (SIGMA). Values are means ± SEM of six wells examined. Each was compared with the cells incubated in 1% FBS media (control). Each set of experiments was repeated three times and identical results were obtained. *$p < 0.05$.

Discussion

A previous immunohistochemical study demonstrated that normal human articular chondrocytes could produce AM [7], but there have been no reports of AM expression by chondrocyte phenotype cells. The present study revealed that cultured chondrocyte phenotype cells expressed mRNA for AM and also actively secreted AM (Fig. 1). The active secretion of AM by chondrocyte phenotype cells seems to be similar to that by endothelial cells [29,30]. Indeed, the level of AM secretion by chondrocyte phenotype cells was 6-fold greater than that by normal human osteoblasts (Fig. 2). Ir-AM in the culture medium appeared to be authentic AM because reverse-phase HPLC revealed that most of the AM secreted into the medium was eluted in the same fraction as human AM (1–52), which was the full-length human AM peptide. The minor peak eluted earlier (Fig. 4) was believed to be oxidized AM containing methyl sulfoxide. The intracellular AM concentration remained extremely low in chondrocyte phenotype cells (Fig. 3), suggesting that these cells constitutively secrete AM. Therefore, chondrocyte phenotype cells secrete AM rapidly after synthesis, with little intracellular storage.

The present study also revealed that AM inhibits proliferation of cultured chondrocyte phenotype cells. As is the case for vascular smooth muscle cells, AM stimulated intracellular cAMP levels, and thus, inhibited cell growth [31]. In chondrocyte phenotype cells, cAMP concentration was increased by AM from 10^{-9} mol/L and the maximal effect was observed at 10^{-6} mol/L. It should be emphasized that these values are similar to the inhibitory effects of AM shown in Fig. 7, which were detected at concentrations between 10^{-9} and 10^{-6} mol/L. We further demonstrated that the addition of forskolin (10^{-7} mol/L) and dibutyryl-cAMP (1 mmol/L) caused a significant decrease in cell population ($P < 0.05$) (Fig. 8). Therefore, AM activity is likely to be primarily mediated via the cAMP pathway and involves inhibition of the mitogen-activated protein kinase cascade, as reported for mesangial cells and vascular smooth muscle cells [32,33]. This hypothesis is supported by the observation that H89, a PKA inhibitor, blocked the effects of AM on chondrocyte phenotype cells (Fig. 9). However, Miller et al. reported that AM stimulates proliferation of other cell types, such as tumor cells, in a cAMP-dependent manner [34,35]. These apparently contradictory actions of cAMP reportedly depend upon the relative intracellular levels of two distinct cAMP-dependent protein kinase A isoforms; RI, which promotes growth and RII, which inhibits growth [36].

We further demonstrated that neutralization of AM using a monoclonal antibody significantly increased proliferation of chondrocyte phenotype cells. This result indicates that AM may be an important autocrine/paracrine regulator of chondrocyte phenotype cell growth. However, in vivo, modulation of the morphological and biosynthetic phenotypes of cartilage cells may be mediated by several factors, including AM. Our results suggest that AM is at least an important autocrine/paracrine regulator of chondrocyte phenotype cell growth.

Numerous studies have examined the plasticity of the chondrocyte phenotype, employing cells of human, avian, lapine, and rodent origin. It has become apparent from these collective studies that culturing chondrocytes as an adherent monolayer invariably leads to de-differentiation whereby cells acquire a fibroblastic morphology and lose the chondrocyte-specific gene-expression pattern [37,38]. AM is likely to be important as an autocrine or paracrine factor in the process of de-differentiation. It is not known what variety of factors promotes or inhibits growth of chondrocyte phenotype cells. Therefore, interaction between AM and such factors should be further investigated in order to better understand the de-differentiation of chondrocytes.

In the initial part of this study, we demonstrated that the AM receptor is present in chondrocyte phenotype cells as a combination of CRLR, RAMP1, and RAMP2. It has been reported that CRLR and RAMP1 function as the CGRP receptor, while CRLR and RAMP2 form the AM receptor [39,40]. Because RAMP3 was not detectable in chondrocyte phenotype cells, the functioning AM receptor is likely to consist of CRLR and RAMP2. However, further experiments are essential to precisely identify the AM receptor in chondrocyte phenotype cells, because AM activates cAMP production via the CGRP receptor, which consists of CRLR and RAMP1 [22,23].

In conclusion, we demonstrated that AM is actively secreted by cultured human chondrocyte phenotype cells and that secreted AM inhibits proliferation of these cells via a cAMP-dependent pathway. It is possible that AM is capable of modulating growth of human chondrocyte phenotype cells in an autocrine or paracrine manner.

Acknowledgments

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