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Effects of adrenomedullin on cultured rat cardiac myocytes and fibroblasts

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Abstract

The direct effects of adrenomedullin, a novel vasorelaxant peptide, on protein synthesis and atrial natriuretic peptide release in myocytes and on DNA and collagen syntheses in fibroblasts were examined using cultured ventricular cardiocytes. The protein synthesis of cardiac myocytes was not affected by adrenomedullin under non-stimulated conditions. Endothelin-1-induced protein synthesis in myocytes was slightly but significantly elevated by adrenomedullin. Likewise, the secretion of atrial natriuretic peptide from myocytes stimulated by endothelin-1 was increased by adrenomedullin. In cardiac fibroblasts, adrenomedullin clearly inhibited DNA synthesis and collagen production in a dose-dependent manner under both basal and angiotensin II-stimulated conditions. DNA and collagen syntheses by cardiac fibroblasts were suppressed by both 8-bromo cAMP and forskolin. Furthermore, a cAMP-specific phosphodiesterase inhibitor decreased DNA and collagen syntheses in fibroblasts and enhanced the inhibitory effects of adrenomedullin on these syntheses. Our observations suggest that adrenomedullin has opposite effects on cultured cardiac myocytes and fibroblasts and that the effects of adrenomedullin at least on fibroblasts are probably mediated through a cAMP-dependent pathway. As adrenomedullin is produced and secreted from both types of cardiac cells, adrenomedullin may play a role as an autocrine/paracrine modulator in the process of cardiac remodeling, mainly by suppressing mitogenesis and collagen synthesis in fibroblasts.

Keywords: Adrenomedullin; Myocyte; Fibroblast; Hypertrophy; Proliferation; cAMP

1. Introduction

Adrenomedullin, a potent vasodilator and natriuretic peptide, may function as an endogenous autocrine and/or paracrine regulator of cardiac function, since adrenomedullin peptide, mRNA, and binding sites have been found in the heart (Kitamura et al., 1993; Sakata et al., 1994; Owji et al., 1995). A recent immunohistochemical study revealed that adrenomedullin immunoreactivity is markedly increased in failing human ventricles (Jougasaki et al., 1995). Our previous report (Nishikimi et al., 1997) showed that the gene expression and peptide levels of adrenomedullin are elevated in the hearts of rats with heart failure. These observations indicate that adrenomedullin is produced in normal hearts, and that its production is elevated in cardiac disorders. We demonstrated recently that both ventricular myocytes and fibroblasts synthesize and release adrenomedullin in cell cultures (Horio et al., 1998). We also showed that adrenomedullin increases the cellular cAMP levels in cultured cardiac myocytes and fibroblasts, probably via different receptors (Nishikimi et al., 1998). These results suggest that adrenomedullin produced by these cardiac cells may have some function as an autocrine/paracrine factor in the regulation of cardiac function. As for the cardiac effect of adrenomedullin, however, opposite data have been reported; i.e., a negative inotropic effect on isolated rabbit ventricular cardiocytes (Ikenouchi et al., 1997) and a positive inotropic effect on isolated rat heart (Szokodi et al., 1998). Besides, little is known about the direct action of adrenomedullin on cultured cardiac cells (Sato et al., 1997; Tsuruda et al., 1998). Therefore, we conducted this study to examine the direct effects of adrenomedullin on myocyte hypertrophy and atrial natriuretic peptide (ANP) release, and on fibroblast proliferation and collagen production, using the two types

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of ventricular cells purified and cultured from neonatal rats. We also investigated the participation of cAMP in the effects of adrenomedullin on cardiac myocytes and fibroblasts.

2. Materials and methods

2.1. Cell cultures

Primary cultures of neonatal ventricular myocytes and fibroblasts were prepared as described previously (Horio et al., 1998). Apical halves of cardiac ventricles from 1- to 2-day-old Wistar rats were separated and minced in a chilled balanced salt solution (116 mM NaCl, 20 mM HEPES, 12.5 mM NaH_2PO_4, 5.6 mM glucose, 5.4 mM KCl, and 0.8 mM $MgSO_4$, pH 7.35). Ventricular cardiocytes were dispersed in the balanced salt solution containing 0.1% collagenase type II (Worthington Biochemical, Freehold, NJ) with agitation for 6 min at 37°C. The digestion steps were repeated five to seven times until the tissues were completely digested. The cells were combined, centrifuged, and resuspended in chilled fetal calf serum (Life Technologies, Grand Island, NY). To separate myocytes from nonmyocytes, a discontinuous gradient of 40.5% and 58.5% Percoll (Sigma, St. Louis, MO) was prepared in the balanced salt solution, and ventricular cells were suspended in the layer of 58.5% Percoll. After centrifugation at 3000 rpm for 30 min, the upper layer consisted of a mixed population of nonmyocyte cell types, and the lower layer consisted almost exclusively of cardiac myocytes. Both myocytes and nonmyocytes were washed twice by centrifugation and resuspension to remove all traces of Percoll.

After myocytes were incubated twice on uncoated 10-cm culture dishes for 30 min to remove any remaining nonmyocytes, the nonattached viable cells (purified myocytes) were plated at a density of 1.2×10^5 cells/well on gelatin-coated 24-well culture plates and were cultured in Dulbecco's modified Eagle's medium (DMEM, Life Technologies) supplemented with 10% fetal calf serum and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin, ICN Biomedicals, Aurora, OH) at 37°C in humidified air with 5% CO_2. After 24–48 h of incubation in DMEM with fetal calf serum, the culture medium was changed to serum-free DMEM, and the experiments were performed 24 h later. This purification procedure has well been established (Zheng et al., 1994; Harada et al., 1997), and in fact more than 95% of the cells we obtained by this method were cardiomyocytes.

Nonmyocyte cells were resuspended in DMEM with 10% fetal calf serum and plated on uncoated 10-cm culture dishes for 30 min. After the plating period, nonadherent cells and debris were washed away and fresh medium was added. Cells were allowed to grow to confluence, trypsinized, and passaged 1:3. This procedure yielded cultures of cells that were almost exclusively fibroblasts by the first passage, as described by Villarreal et al. (1993). Fibroblasts at the second or third passage were plated at a density of 2×10^4 cells/well on 24-well plates and allowed to grow to confluence. After incubation in DMEM with fetal calf serum, the culture medium was changed to serum-free DMEM, and the experiments were performed 48 h later.

2.2. Analyses of protein, DNA, and collagen syntheses

The effects of various agents on protein, DNA, and collagen syntheses in cardiac myocytes and fibroblasts were evaluated by the incorporation of [^{14}C]phenylalanine, [^{3}H]thymidine, and [^{3}H]proline into cells, respectively. After the preconditioning period, the culture medium was replaced with fresh serum-free DMEM. Then, endothelin-1 (Peptide Institute, Osaka, Japan), angiotensin II (Peptide Institute), rat adrenomedullin (Peptide Institute), 8-bromo cAMP (Sigma), forskolin (Sigma), and/or 4-(butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724, Biomol Research Laboratories, Plymouth Meeting, PA) were added. For protein synthesis in cardiac myocytes or collagen synthesis in cardiac fibroblasts, either 0.2 μCi of [^{14}C]phenylalanine or 0.5 μCi of [^{3}H]proline was added, and then the plates were incubated for 24 h. For DNA synthesis in cardiac fibroblasts, 0.5 μCi of [^{3}H]thymidine was added 12 h after pharmacological treatments, and the cells were further incubated for 12 h. After completion of labeling, the cells were rinsed twice with cold phosphate-buffered saline and incubated with 10% trichloroacetic acid at 4°C for 30 min. The precipitates were washed twice with cold 95% ethanol and solubilized in 1 M NaOH. The radioactivity of aliquots of the trichloroacetic acid-insoluble material was determined using a liquid scintillation counter.

2.3. Measurement of cellular cAMP

After preincubation, myocytes or fibroblasts grown in 24-well plates were treated for 10 min with various concentrations of rat adrenomedullin in the presence of 5×10^{-4} M 3-isobutyl-1-methylxanthine (Nacalai Tesque, Kyoto, Japan), as described previously (Horio et al., 1995). The reaction was stopped by rapid aspiration of the medium and the addition of ice-cold 70% ethanol. After each ethanol sample was evaporated by a centrifugal evaporator, the dry residue was dissolved in an assay buffer. The cAMP levels were determined by a radioimmunoassay performed with a cAMP assay kit (Yamasa Shoyu, Chiba, Japan), as previously reported (Nishikimi et al., 1998).

2.4. Measurement of immunoreactive ANP

After cardiac myocytes were treated with rat adrenomedullin, 8-bromo cAMP, or forskolin for 24 h, the

Table 1
Effects of endothelin-1 and angiotensin II on protein, DNA, and collagen syntheses in cultured cardiac myocytes and fibroblasts
Values are given as the mean ± S.E. of 4–6 measurements. Control indicates vehicle alone.

	Myocytes	Fibroblasts	
	[^{14}C]phenylalanine uptake (%)	[^{3}H]thymidine uptake (%)	[^{3}H]proline uptake (%)
Control	100.0 ± 4.9	100.0 ± 4.3	100.0 ± 6.3
Endothelin-1 (10^{-7} M)	149.2 ± 3.6[a]	149.1 ± 11.6[a]	115.5 ± 4.9
Angiotensin II (10^{-6} M)	109.9 ± 4.6	150.9 ± 9.1[a]	149.2 ± 6.2[a]

[a] $P < 0.01$ vs. control.

culture medium was aspirated and stored at −80°C. The medium (100 μl) was acidified with acetic acid, boiled to inactivate intrinsic proteases, and lyophilized. The radioimmunoassay for rat ANP was performed as described previously (Horio et al., 1998). Assays were performed in duplicate.

2.5. Calculations and statistical analysis

The statistical significance of differences in the results was evaluated using an unpaired analysis of variance, and P values were calculated by Fisher's method. $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Stimulation by endothelin-1 and angiotensin II of protein, DNA, and collagen synthesis in cardiac myocytes and fibroblasts

The [^{14}C]phenylalanine incorporation level in cardiac myocytes was increased by endothelin-1 (10^{-7} M), but not by angiotensin II (10^{-6} M) (Table 1). In cardiac fibroblasts, angiotensin II (10^{-6} M) stimulated both [^{3}H]thymidine and [^{3}H]proline incorporation, whereas endothelin-1 (10^{-7} M) significantly increased only the incorporation of [^{3}H]thymidine. In later experiments, therefore, stimulation of protein synthesis in myocytes was performed with 10^{-7} M endothelin-1, and stimulations of DNA and collagen syntheses in fibroblasts were performed with 10^{-6} M angiotensin II.

3.2. Effect of adrenomedullin on cellular cAMP levels in cardiac myocytes and fibroblasts

The cellular levels of cAMP in cultured myocytes were increased dose-dependently after treatment with 10^{-9}–10^{-6} M adrenomedullin (Fig. 1A). In cardiac fibroblasts, adrenomedullin also increased the cAMP levels at concentrations of 10^{-8}–10^{-6} M (Fig. 1B). The maximum cAMP formation by adrenomedullin was about 5-fold greater in fibroblasts than in myocytes.

3.3. Effects of adrenomedullin and cAMP-related reagents on protein synthesis and ANP secretion in cardiac myocytes

The effect of adrenomedullin on protein synthesis in cultured cardiac myocytes under basal and endothelin-1-

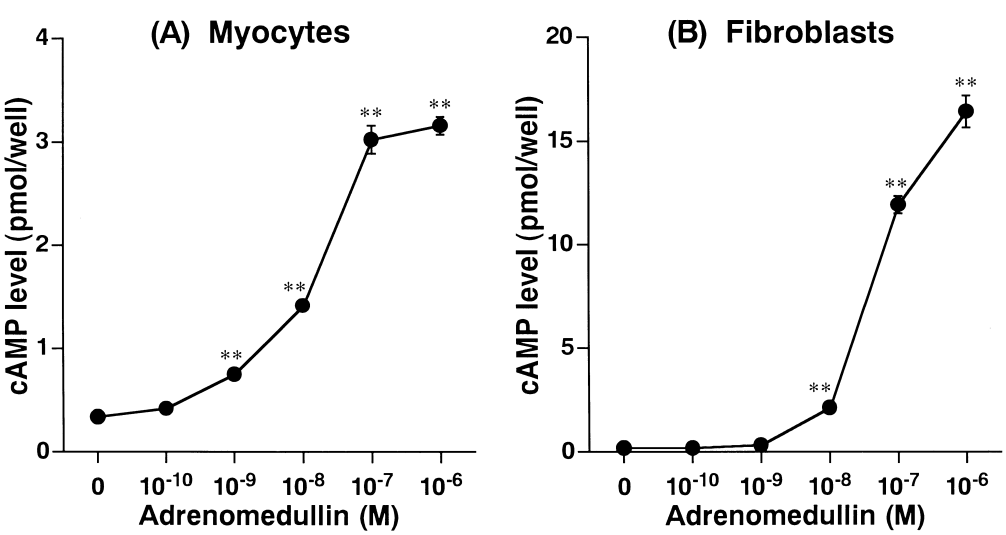

Fig. 1. Effect of adrenomedullin on the production of cellular cAMP in cultured cardiac myocytes (A) and fibroblasts (B). Values are the mean ± S.E. of 4 measurements. ** $P < 0.01$ vs. control.

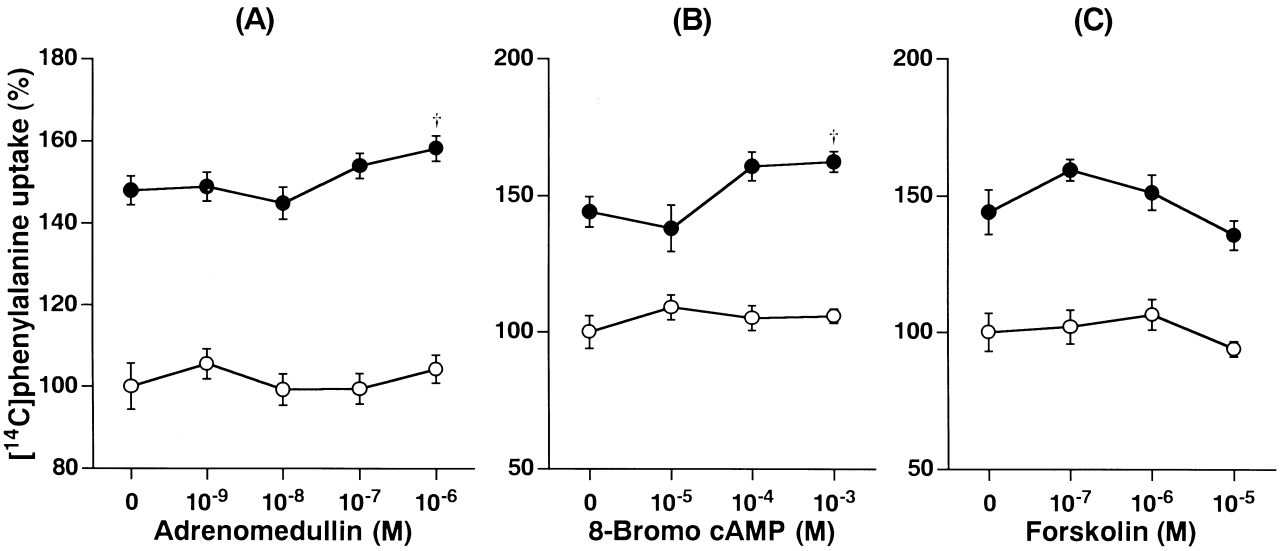

Fig. 2. Effect of adrenomedullin (A), 8-bromo cAMP (B), and forskolin (C) on protein synthesis in cultured cardiac myocytes under non-stimulated (○) and endothelin-1 (10^{-7} M)-stimulated (●) conditions. Values are the mean ± S.E. of 6–8 measurements. †$P < 0.05$ vs. endothelin-1 alone.

stimulated conditions is shown in Fig. 2A. The basal incorporation level of [^{14}C]phenylalanine was not affected by 10^{-9}–10^{-6} M adrenomedullin. Endothelin-1-induced [^{14}C]phenylalanine incorporation was slightly but significantly elevated by 10^{-6} M adrenomedullin. To elucidate whether this effect of adrenomedullin is causally linked to

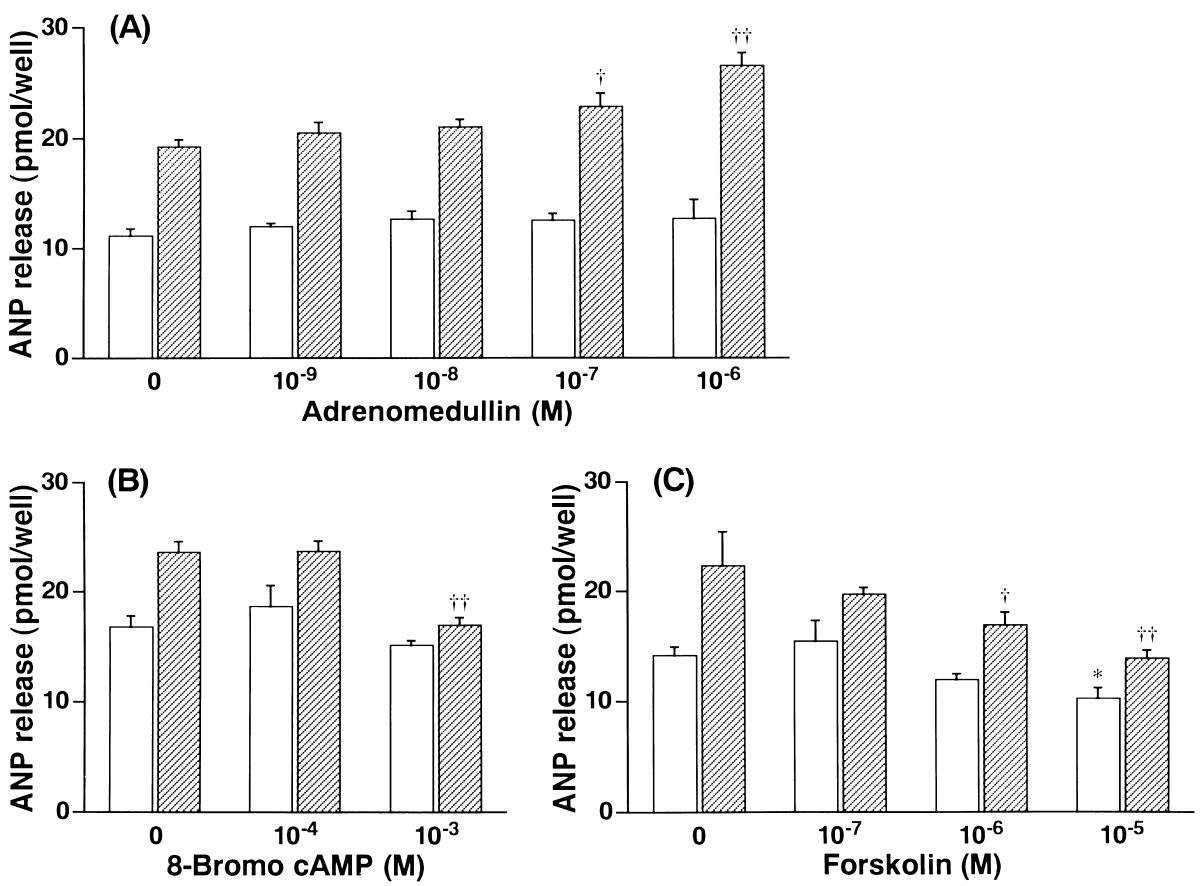

Fig. 3. Effect of adrenomedullin (A), 8-bromo cAMP (B), and forskolin (C) on ANP secretion in cultured cardiac myocytes under non-stimulated (open bars) and endothelin-1 (10^{-7} M)-stimulated (hatched bars) conditions. Values are the mean ± S.E. of 6 measurements. *$P < 0.05$ vs. control; †$P < 0.05$ and ††$P < 0.01$ vs. endothelin-1 alone.

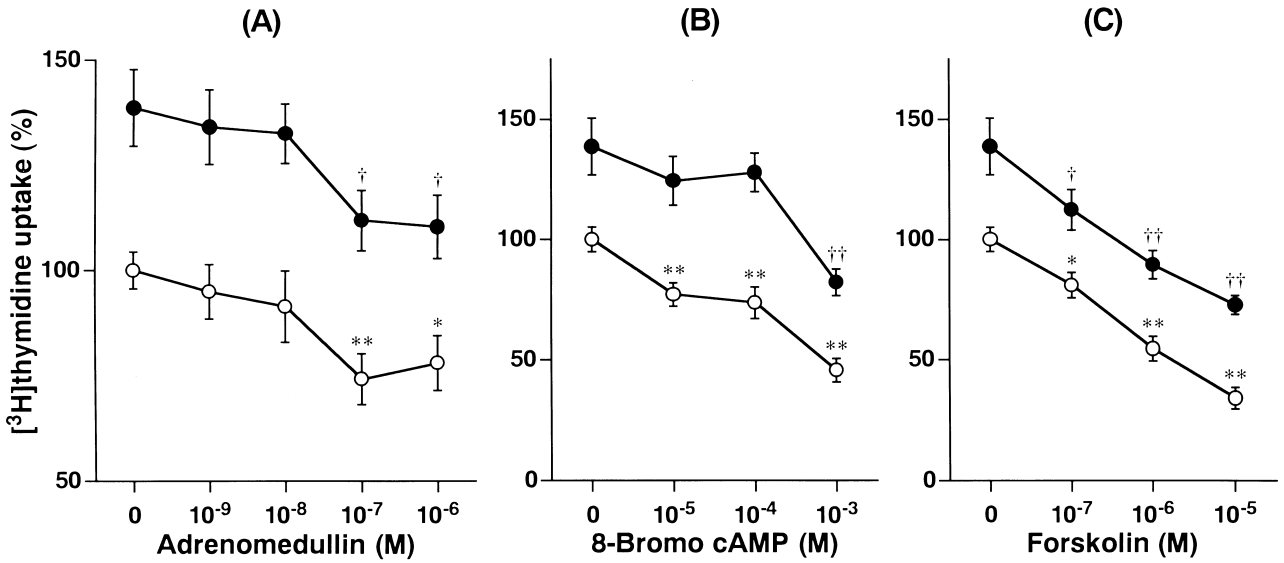

Fig. 4. Effect of adrenomedullin (A), 8-bromo cAMP (B), and forskolin (C) on DNA synthesis in cultured cardiac fibroblasts under non-stimulated (○) and angiotensin II (10^{-6} M)-stimulated (●) conditions. Values are the mean ± S.E. of 6–8 measurements. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $\dagger P < 0.05$ and $\dagger\dagger P < 0.01$ vs. angiotensin II alone.

the increase in cellular cAMP induced by adrenomedullin, we examine the effect of two cAMP-related compounds; that is, 8-bromo cAMP, a membrane-permeable analogue of cAMP, and forskolin, a stimulator of adenylate cyclase activity. As for the protein synthesis in cardiac myocytes, 10^{-3} M 8-bromo cAMP increased the [^{14}C]phenylalanine incorporation only under endothelin-1-stimulated conditions, similarly to adrenomedullin (Fig. 2B). Forskolin (10^{-7}–10^{-5} M) did not affect the control or endothelin-1-stimulated level of [^{14}C]phenylalanine incorporation (Fig. 2C).

Adrenomedullin had no significant effect on the basal secretion of ANP from ventricular myocytes (Fig. 3A). The secretion of ANP was increased by stimulation with 10^{-7} M endothelin-1, and the endothelin-1-stimulated release of ANP was further increased by 10^{-7}–10^{-6} M

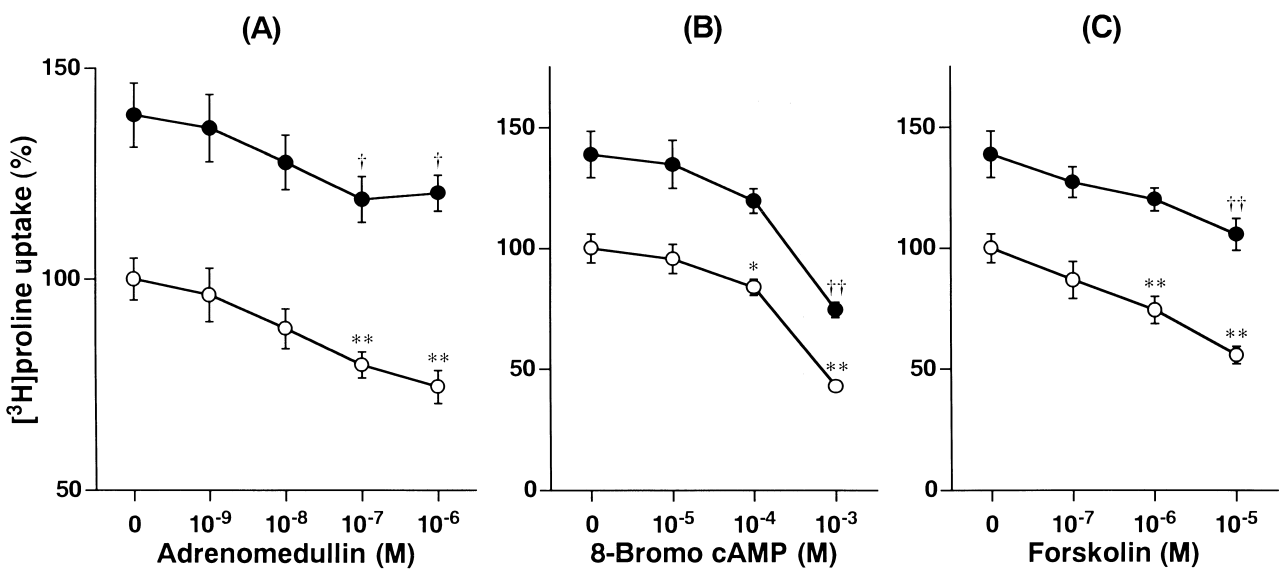

Fig. 5. Effect of adrenomedullin (A), 8-bromo cAMP (B), and forskolin (C) on collagen synthesis in cultured cardiac fibroblasts under non-stimulated (○) and angiotensin II (10^{-6} M)-stimulated (●) conditions. Values are the mean ± S.E. of 6–8 measurements. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $\dagger P < 0.05$ and $\dagger\dagger P < 0.01$ vs. angiotensin II alone.

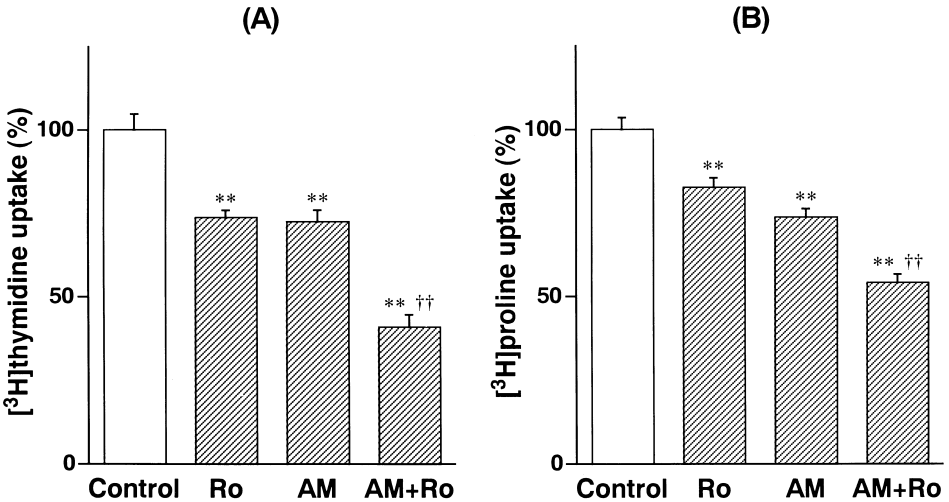

Fig. 6. Effects of Ro 20-1724 (Ro, 10^{-4} M) on DNA synthesis (A) and collagen synthesis (B) in cultured cardiac fibroblasts under basal and adrenomedullin (AM, 10^{-6} M)-treated conditions. Values are the mean ± S.E. of 4 measurements. Control indicates vehicle alone. $^{**}P < 0.01$ vs. control; ††$P < 0.01$ vs. adrenomedullin alone.

adrenomedullin. Whereas, 8-bromo cAMP and forskolin decreased the ANP secretion mainly under endothelin-1-stimulated conditions (Fig. 3B and C).

3.4. Effects of adrenomedullin and cAMP-related reagents on DNA and collagen syntheses in cardiac fibroblasts

The effect of adrenomedullin on DNA and collagen synthesis in cultured cardiac fibroblasts under basal and angiotensin II-stimulated conditions is shown in Figs. 4A and 5A. Adrenomedullin clearly inhibited the [^{3}H]thymidine uptake at concentrations of 10^{-7} and 10^{-6} M under both basal and angiotensin II-stimulated conditions (Fig. 4A). The [^{3}H]proline uptake into non-stimulated and stimulated cells was also inhibited by adrenomedullin in a concentration-dependent manner (Fig. 5A).

In cardiac fibroblasts, inhibition of basal and angiotensin II-induced[^{3}H]thymidine uptake by adrenomedullin could be mimicked by both 8-bromo cAMP (10^{-5}–10^{-3} M) and forskolin (10^{-7}–10^{-5} M) (Fig. 4B and C). These two compounds also reduced the [^{3}H]proline uptake concentration-dependently in non-stimulated and angiotensin II-stimulated cells (Fig. 5B and C).

3.5. Effects of phosphodiesterase inhibitor on protein, DNA, and collagen syntheses in cardiac myocytes and fibroblasts

We further examined the effect of a cAMP-specific phosphodiesterase inhibitor, Ro 20-1724, on protein synthesis in myocytes and DNA and collagen syntheses in fibroblasts. In cardiac myocytes, Ro 20-1724 did not affect the control level of [^{14}C]phenylalanine incorporation, and did not augment the effect of adrenomedullin on endothelin-1-induced incorporation (data not shown). In cardiac fibroblasts, Ro 20-1724 decreased the incorporation of [^{3}H]thymidine and [^{3}H]proline under basal conditions (Fig. 6). In addition, the inhibitory effects of adrenomedullin on [^{3}H]thymidine and [^{3}H]proline incorporation were enhanced by this agent.

4. Discussion

The present study has clearly demonstrated that adrenomedullin inhibits DNA and collagen syntheses in cultured cardiac fibroblasts under non-stimulated and angiotensin II-stimulated conditions. Adrenomedullin remarkably elevated intracellular cAMP levels in fibroblasts at concentrations of 10^{-7}–10^{-6} M, and the decrease in DNA and collagen syntheses by adrenomedullin appeared to parallel the marked increase in cAMP. A cAMP analogue and an activator of adenylate cyclase mimicked the adrenomedullin-induced inhibition of DNA and collagen syntheses in fibroblasts. In addition, a cAMP-specific phosphodiesterase inhibitor enhanced the suppression by adrenomedullin of these syntheses. These results suggest that the observed inhibitory effects of adrenomedullin on cardiac fibroblasts are probably mediated through a cAMP-dependent process.

Several reports have shown the effect of adrenomedullin on mitogenesis in cell types other than cardiac fibroblasts. In Swiss 3T3 cells and human keratinocytes, adrenomedullin exerts mitogenic effects through a cAMP-mediated mechanism (Withers et al., 1996; Kapas et al., 1997; Isumi et al., 1998). In vascular smooth muscle cells and endothelial cells, and glomerular mesangial cells, conversely, adrenomedullin suppresses cell proliferation via the cAMP-protein kinase A pathway (Chini et al., 1995,1997; Kano et al., 1996; Michibata et al., 1998). In

Rat-2 fibroblasts, adrenomedullin has been recently shown to increase cAMP levels and to decrease mitogen-activated protein kinase (MAPK) activity (Coppock et al., 1999). With regard to the effect of cAMP-elevating compounds other than adrenomedullin on mitogenesis in fibroblasts, contradictory findings have also been reported. 8-Bromo cAMP or forskolin inhibits growth factor-induced DNA synthesis in human foreskin and hamster lung fibroblasts (Heldin et al., 1989; Magnaldo et al., 1989). However, in Swiss 3T3 fibroblasts, these agents have been shown to elicit a positive mitogenic activity in the presence of insulin (Yamashita et al., 1986; Withers et al., 1995). Although the exact reason for this discrepancy remains unexplained, cAMP has been shown to have dual opposite actions, growth stimulation and growth suppression, depending on the relative cellular amounts of two distinct cAMP-dependent protein kinase A isoforms (Ishizuka et al., 1994). Furthermore, there is known to be a cell type-specificity in the effect of cAMP on the MAPK pathway (Calleja et al., 1997). As for the mechanism by which cAMP inhibits the MAPK activity, it is noted that cAMP blocks the activation by the small G protein Ras of serine/threonine kinase Raf-1, an activator of MAPK (Cook and McCormick, 1993). On the other hand, Vossler et al. (1997) have recently shown in PC12 cells that cAMP activates B-Raf, one of the Raf family, in a Ras-independent manner and subsequently activates MAPK.

Regarding collagen production in fibroblasts, there are no reports examining the effect of adrenomedullin on it other than our present study. The suppressive effect of adrenomedullin on extracellular matrix production in cardiac fibroblasts, probably via the cAMP-dependent pathway, observed in the present study seems to be consistent with the previous reports that cAMP inhibits collagen synthesis in corneal fibroblasts (Chao and Walkenbach, 1986) and decreases protein kinase C-stimulated fibronectin synthesis in human lung fibroblasts (Lee et al., 1997). Thus, increased adrenomedullin in the failing heart may act to attenuate the mitogenesis and collagen production in cardiac fibroblasts.

In the present study, adrenomedullin increased slightly the protein synthesis and also stimulated ANP secretion, a marker for hypertrophic response, in ventricular myocytes under endothelin-1-stimulated conditions. Calcitonin gene-related peptide, which has some homology with adrenomedullin, has been reported to exert hypertrophic effects on cardiac myocytes up-regulating ANP gene expression (Bell et al., 1995,1997). In contrast to these findings and our data, recent studies have shown that adrenomedullin inhibits ANP gene expression and secretion (Sato et al., 1997), and inhibits angiotensin II-induced protein synthesis in cardiac myocytes (Tsuruda et al., 1998). The reason for such discrepant findings is not clear, but it may be partly due to the difference in the methods of separation of myocytes from nonmyocytes and the purity of the myocyte cell culture used. In the preparation of cardiac myocytes, it appears to be difficult to avoid the contamination of fibroblasts without using the discontinuous Percoll gradient method. Harada et al. (1997) have clearly shown that angiotensin II-induced hypertrophic effect depends on the presence of cardiac fibroblasts in a cadiocyte culture and that even a relatively small number of fibroblasts can modulate hypertrophy of myocytes. Therefore, it is possible that adrenomedullin may indirectly elicit suppressive effects on protein synthesis and ANP secretion in cardiac myocytes through its direct negative actions on fibroblasts.

Yamazaki et al. (1997a,b) have demonstrated that protein kinase A and protein kinase C synergistically activate MAPK and protein synthesis in cultured cardiac myocytes, in contrast to the finding that the protein kinase C pathway suppresses the effects mediated by the protein kinase A pathway in fibroblasts (Döbbeling and Berchtold, 1996). The observed small increase in protein synthesis by adrenomedullin in myocytes only under stimulation with endothelin-1 may be the result of the enhancement of protein kinase A activity derived from the protein kinase C activation by endothelin-1. However, it has not been sufficiently proved whether this effect occurs through the cAMP-protein kinase A pathway, because forskolin or Ro 20-1724 did not have an additional effect on endothelin-1-stimulated protein synthesis of myocytes. Some reports (Church et al., 1994; Asai et al., 1996) have shown that in ventricular myocytes, forskolin exhibits concentration-dependent stimulatory and inhibitory properties or has cAMP-independent inhibitory actions. In addition, large or prolonged increases in cAMP caused by a high dose of forskolin or a phosphodiesterase inhibitor may be somewhat toxic to cultured myocytes. Alternatively, we cannot rule out the possibility that adrenomedullin has a cAMP-independent effect on cardiac myocytes, as previously shown in isolated perfused hearts (Szokodi et al., 1998). In fact, they also obtained the finding that the positive inotropic effect of adrenomedullin could not be enhanced in the presence of isobutyl methylxanthine, a phosphodiesterase inhibitor. As for the secretion of ANP from cardiac myocytes, the stimulatory effect of adrenomedullin under endothelin-1-induced conditions did not be reproduced by 8-bromo cAMP or forskolin. Far from that, these two reagents inhibited the ANP secretion, compatible with the previous observations (Shields and Glembotski, 1989; Gardner and Schultz, 1990; Sato et al., 1997). Therefore, adrenomedullin may enhance the ANP release stimulated by endothelin-1 at least partly via cAMP-independent mechanism, though the exact mechanism has not been elucidated in the present study.

Our previous reports (Nishikimi et al., 1995; Miyao et al., 1998; Yoshitomi et al., 1998) showed that plasma adrenomedullin concentrations increase in patients with congestive heart failure and with acute myocardial infarction. We also demonstrated augmented levels of the adrenomedullin transcript and peptide in the hearts of rats

with heart failure (Nishikimi et al., 1997). Although the pathophysiological roles of adrenomedullin in such cardiac disorders remain unclear, our present findings may suggest some roles. After myocardial infarction, areas of myocyte loss in the infarcted region are replaced by areas of fibroblasts and collagen deposition. Fibroblast proliferation is also involved in eccentric hypertrophy of the noninfarcted region. As we have shown in the rat models with myocardial infarction that the levels of adrenomedullin gene expression and peptide increase in both the infarcted and noninfarcted regions (unpublished observations), this augmented adrenomedullin may function as a local modulator against progressive ventricular remodeling after infarction by suppressing mitogenesis and collagen synthesis in fibroblasts. On the other hand, the hypertrophic effect of adrenomedullin on myocytes may be adapted to compensate for the loss of contractile cells, although the effect was additive and weak in the present study. These multiple effects of adrenomedullin on cardiac myocytes and fibroblasts may be probably cardioprotective in some pathological states. However, further investigations are necessary to clarify the physiological and pathophysiological significance of adrenomedullin in the heart.

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