ELSEVIER

European Journal of Pharmacology 315 (1996) 319–325

ejp

Adrenomedullin-sensitive receptors are preferentially expressed in cultured rat mesangial cells

Akihiko Osajima [a,*], Yasuhito Uezono [b], Masahito Tamura [a], Kazuo Kitamura [c], Yoshinobu Mutoh [a], Yoichi Ueta [d], Kenji Kangawa [e], Masaru Kawamura [f], Tanenao Eto [c], Hiroshi Yamashita [d], Futoshi Izumi [b], Masayuki Takasugi [a], Akio Kuroiwa [a]

[a] *Second Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807, Japan*

[b] *Department of Pharmacology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807, Japan*

[c] *First Department of Internal Medicine, Miyazaki Medical College, Kihara Kiyatake, Miyazaki 889-16, Japan*

[d] *Department of Physiology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807, Japan*

[e] *National Cardiovascular Center Research Institute, Fujishirodai, Suita, Osaka 565, Japan*

[f] *Department of Biology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807, Japan*

Received 4 March 1996; revised 4 July 1996; accepted 9 July 1996

Abstract

By using cultured rat mesangial cells, we compared the effects on cyclic nucleotide levels of adrenomedullin with those of the structurally related peptides, calcitonin gene-related peptide (CGRP) and amylin. Adrenomedullin potently increased cAMP levels 7-fold in a time- and concentration-dependent manner. Its EC_{50} was 3×10^{-9} M. CGRP was less potent (2-fold) with an EC_{50} of 10^{-7} M, and amylin had no effect on cAMP levels. All three peptides failed to increase cGMP levels. Treatment of cells with near maximal concentrations of adrenomedullin (10^{-7} M) and CGRP (10^{-6} M) had no additive effect on cAMP levels. Human adrenomedullin-(22–52)-NH_2, a putative adrenomedullin receptor antagonist, inhibited the production of cAMP elicited by adrenomedullin (IC_{50}: 7×10^{-8} M) and CGRP (IC_{50}: 5×10^{-8} M). Human CGRP-(8–37), a CGRP receptor antagonist, conversely, reduced the cAMP elevation caused by these peptides with a lower potency (IC_{50}: 10^{-6} M for both peptides). This demonstrated that human adrenomedullin-(22–52)-NH_2 was a more effective antagonist for adrenomedullin- and CGRP-specific receptors than human CGRP-(8–37). Results suggest that receptors sensitive to adrenomedullin are preferentially expressed in cultured rat mesangial cells. Immunohistochemical study showed almost no immunoreactive adrenomedullin and CGRP, if any, in the cells. Adrenomedullin may regulate mesangial function as either a paracrine or circulating hormone via a cAMP- but not a cGMP-dependent mechanism.

Keywords: Adrenomedullin; Amylin; CGRP (calcitonin gene-related peptide); cAMP; Mesangial cell, cultured, rat; Immunohistochemistry

1. Introduction

Glomerular mesangial cells regulate glomerular function through their contraction or relaxation (Schor et al., 1981). Cyclic nucleotides are believed to mediate this relaxation, thereby increasing the glomerular filtration rate (Singhal et al., 1986, 1989). For example, calcitonin gene-related peptide (CGRP) elicits relaxation of mesangial cells through the generation of cAMP (Kurtz et al., 1989). Similarly, the natriuretic peptides, atrial natriuretic peptide and brain natriuretic peptide, relax the cells by increasing the cGMP levels (Singhal et al., 1989; Kohno et al., 1993). Thus, vasoactive peptides play a key role in the regulation of mesangial cell function (Schor et al., 1981).

Adrenomedullin, a novel peptide that was isolated from human pheochromocytoma, displays a slight homology with CGRP and amylin (Kitamura et al., 1993). All of these compounds exert their function by increasing the levels of cAMP (Muff et al., 1995). Adrenomedullin increases the glomerular filtration rate (Ebara et al., 1994; Jougasaki et al., 1995), probably due to an increase in cAMP levels (Osajima et al., 1995) in a manner similar to CGRP (Gnaedinger et al., 1989). Kohno et al. (1995)

* Corresponding author. Tel.: (81-93) 603-1611; Fax: (81-93) 691-6913.

PII S0014-2999(96)00557-2

recently reported that adrenomedullin stimulated cAMP formation in cultured rat mesangial cells. It appears that in mesangial cells, adrenomedullin and CGRP receptors are expressed and that these peptide ligands regulate cellular function. However, no comparative studies have carried out on the modulation of cyclic nucleotide levels. Thus, we used the specific receptor antagonists, human adrenomedullin-(22–52)-NH_2, a newly discovered adrenomedullin receptor antagonist, and human CGRP-(8–37), a CGRP receptor antagonist (Chiba et al., 1989; Eguchi et al., 1994), to evaluate this relationship. Accordingly, in cultured rat mesangial cells, we compared the cyclic nucleotide modulatory effects of adrenomedullin with those of CGRP and amylin, with and without their specific receptor antagonists.

Immunohistochemical studies have shown the presence of CGRP in rat renal cortex nerve fibers including juxtaglomerular apparatus and peritubules (Kurtz et al., 1988). Jougasaki et al. (1995) showed that adrenomedullin is present in glomerulus and tubules in canine kidney. In contrast, a recent report by Washimine et al. (1995) showed that adrenomedullin-immunoreactive cells were not detectable in rat, human and porcine kidney. Hence it is still not certain whether adrenomedullin and CGRP are localized in kidney, in particular, in mesangial cells. Therefore, we performed an immunohistochemical study for adrenomedullin and CGRP in cultured rat mesangial cells. In addition, LLC-PK1 cell line that expresses a renal tubule phenotype (Cantau et al., 1990) was examined as being representative of renal tubule cells.

2. Materials and methods

2.1. Cell cultures

Rat mesangial cells were obtained from the intact glomeruli of 4-week-old Wistar rats by using a sieving procedure as previously described (Wolthuis et al., 1992). The correct cell type was confirmed by performing the contraction reaction with angiotensin II (Ausiello et al., 1980). Subsequently, the cells were suspended in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 5 mg/ml bovine insulin, and 25 mM Hepes buffer, and cultured (2×10^5 cells/dish, Falcon 35 mm) at 37°C under a 5% CO_2 atmosphere. All experiments were performed by using cells between the fifth and ninth passages. LLC-PK1 cells (ATCC No. CL101) and bovine adrenal medulla cells were cultured as previously described (Cantau et al., 1990; Wada et al., 1983).

2.2. cAMP and cGMP measurements

cAMP and cGMP accumulation in the cells was determined by using the modified method of Kurtz et al. (1989). Briefly, cells were washed with phosphate buffered saline (PBS) buffer (pH 7.4) and incubated at 37°C for up to 30 min with or without varying amounts of adrenomedullin, CGRP, amylin, human CGRP-(8–37), or human adrenomedullin-(22–52)-NH_2 in 1 ml of PBS buffer (pH 7.4) containing 0.5 mM or 5 mM 3-isobutyl-1-methyl-xanthine (IBMX). After this reaction, the cells were rapidly scraped from the culture dish in 1 ml 0.1 M HCl, boiled at 95°C for 5 min, and centrifuged at 12000 rpm for 10 min. The supernatants were assayed for cAMP and cGMP by using specific radioimmunoassay kits (Yamasa, Chiba, Japan). Proteins were measured according to the method of Lowry et al. (1951).

2.3. Immunohistochemistry

Cultured rat mesangial (2×10^5 cells/dish), LLC-PK1 (2×10^5 cells/dish) and bovine adrenal medulla cells (2×10^6 cells/dish), 4 or 5 days after plating, were used for immunohistochemistry. The cells were washed 3 times with PBS, fixed with 4% paraformaldehyde, and washed 3 times with autoclaved H_2O. The cells were then immunostained with antibodies against adrenomedullin (rabbit polyclonal adrenomedullin antibody, 1:2000 dilution) or CGRP (1:1000 dilution; Affinity Research Products, Nottingham, UK), and a streptavidin/biotin detection system was used according to the manufacturer's instructions (Dako LSAB(R), K0681). Negative controls were performed by running a preabsorption test (Ueta et al., 1995) with cultured bovine adrenal medulla cells. The avidin-biotin complexed peroxidase was visualized after a 5–10-min incubation with 0.02% diaminobenzidine in Tris buffer containing 0.05% H_2O_2. The cells were then air dried and studied by light microscopy.

2.4. Drugs and chemicals

Drugs and chemicals were obtained from the following sources: Dulbecco's modified Eagle's medium and bovine insulin (Gibco BRL, New York, NY, USA); fetal calf serum (Bioserum, Victoria, Australia); penicillin, streptomycin, and paraformaldehyde (Nacalai Tesque, Kyoto, Japan); 3-isobutyl-1-methyl-xanthine (Sigma, St. Louis, MO, USA); adrenomedullin, CGRP, amylin, human CGRP-(8–37), human adrenomedullin-(22–52)-NH_2 and angiotensin II (Peptide Institute, Osaka, Japan); diaminobenzidine (Doujindo, Kumamoto, Japan) and hydrogen peroxide (Santoku, Tokyo, Japan).

2.5. Statistical analysis

Data are expressed as means ± S.D. Data were evaluated by analysis of variance. If significant F values were found, Scheffé's test for multiple comparisons was carried out to identify the differences among the groups. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Concentration-dependent increases in cAMP formation

Fig. 1 shows the dose-dependent formation of cAMP caused by adrenomedullin, CGRP and amylin. Adrenomedullin at concentrations above 10^{-12} M increased cAMP levels in a concentration-dependent manner with an EC_{50} of 3×10^{-9} M. At 10^{-6} M, adrenomedullin elevated cAMP levels 7-fold over the basal value. CGRP increased cAMP approximately 2-fold at 10^{-6} M with an EC_{50} of 10^{-7} M. Amylin failed to increase cAMP at any concentration.

3.2. cAMP formation

The time-dependent changes in cAMP formation caused by adrenomedullin and CGRP are shown in Fig. 2. In the presence of 0.5 mM IBMX, basal cAMP levels increased slightly over 20 min; thereafter, these levels decreased gradually (data not shown). Within 10 min of incubation, adrenomedullin and CGRP increased cAMP levels, but at 20 min these levels had gradually decreased to the basal values. This may have been due to the presence of potent phosphodiesterase activity in our mesangial cell preparations. In the presence of 5 mM IBMX, a more pronounced formation of cAMP was observed, and remained stable for up to 30 min (Fig. 2).

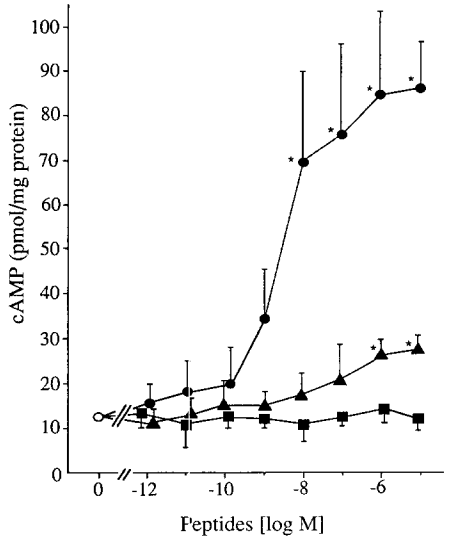

Fig. 1. Concentration-dependent increases in cAMP formation by adrenomedullin, CGRP or amylin. Cultured rat mesangial cells were incubated with or without various concentrations of adrenomedullin (●), CGRP (▲) or amylin (■) for 10 min at 37°C in the presence of 0.5 mM IBMX. The cAMP generated was measured by radioimmunoassay. Data are expressed as means ± S.D. of 3 separate experiments, each carried out in duplicate. * $P < 0.05$ vs. basal value.

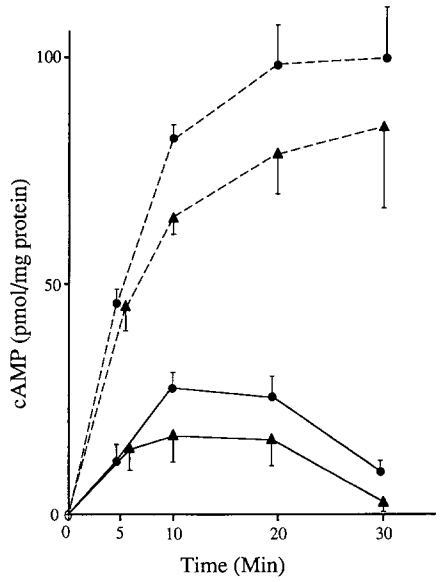

Fig. 2. Time course of cAMP formation caused by EC_{50} concentrations of adrenomedullin and CGRP. Cultured rat mesangial cells were incubated with 3×10^{-9} M adrenomedullin (●) or 10^{-7} M CGRP (▲) for the indicated times at 37°C in the presence of 0.5 mM (solid line) or 5 mM (broken line) IBMX. cAMP levels obtained without peptide were subtracted from each data point. Data are expressed as means ± S.D., where $n = 4$.

3.3. Combined effects of adrenomedullin and CGRP on cAMP levels

To evaluate whether adrenomedullin and CGRP share common receptors, thereby increasing cAMP levels, we simultaneously treated the cells with near maximal concen-

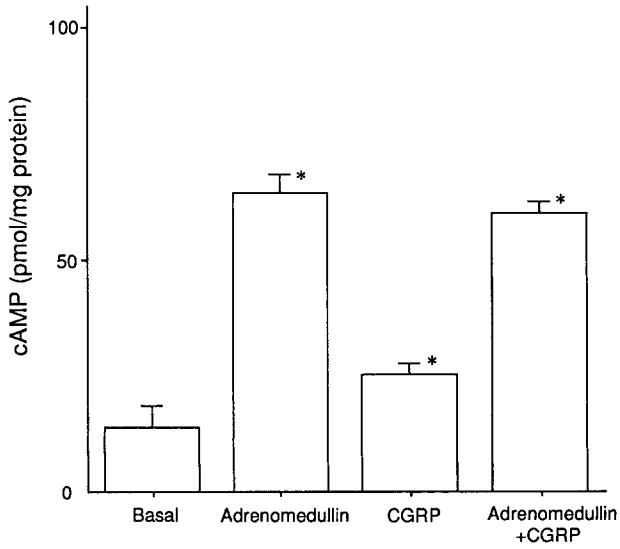

Fig. 3. Lack of additive effects of adrenomedullin (10^{-7} M) and CGRP (10^{-6} M) on cAMP levels. Data are expressed as means ± S.D., where $n = 3$. * $P < 0.05$ vs. basal value.

trations of adrenomedullin (10^{-7} M) and CGRP (10^{-6} M). Adrenomedullin and CGRP caused no additive increase in the cAMP levels (Fig. 3).

3.4. Antagonistic effects of human adrenomedullin-(22–52)-NH_2 or human CGRP-(8–37) on cAMP formation stimulated by adrenomedullin and CGRP

Fig. 4A summarizes the effects of human adrenomedullin-(22–52)-NH_2, an adrenomedullin receptor antagonist, on adrenomedullin- and CGRP-induced cAMP formation. In the presence of EC_{50} concentrations of adrenomedullin (3×10^{-9} M) or CGRP (10^{-7} M), human adrenomedullin-(22–52)-NH_2 inhibited both the adrenomedullin- and CGRP-induced cAMP production with IC_{50} values of 7×10^{-8} M and 5×10^{-8} M, respectively. Fig. 4B summarizes the effects of human CGRP-(8–37) on peptide-induced cAMP levels as well. This antagonist less potently inhibited the adrenomedullin- and CGRP-induced cAMP increase than did human adrenomedullin-(22–52)-NH_2. The IC_{50} value for human CGRP-(8–37) was 10^{-6} M for both peptides. The above two antagonists had no effect on basal levels of cAMP (data not shown). In the absence of human adrenomedullin-(22–52)-NH_2 or human CGRP-(8–37), 3×10^{-9} M adrenomedullin and 10^{-7} M CGRP increased cAMP levels from 13.8 ± 1.6 to 41.7 ± 9.3 and 20.8 ± 5.3 pmol/mg protein, respectively ($n = 3$, means ± S.D.).

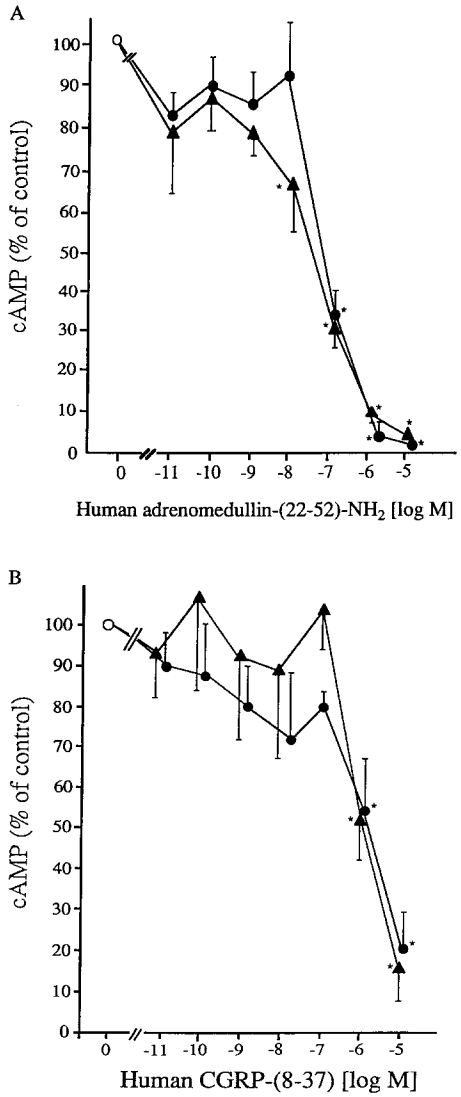

Fig. 4. The antagonistic effects of (A) human adrenomedullin-(22–52)-NH_2 or (B) human CGRP-(8–37) on cAMP levels stimulated by EC_{50} concentrations of adrenomedullin and CGRP. Cultured rat mesangial cells were incubated with 3×10^{-9} M adrenomedullin (●) or 10^{-7} M CGRP (▲) for 10 min at 37°C in the presence of 0.5 mM IBMX. Basal values (13.8 ± 1.6 pmol/mg protein) without peptide were subtracted from the data and 100% represents the value obtained with peptide in the absence of each antagonist. Data are expressed as means ± S.D. of 3 separate experiments, each carried out in duplicate. * $P < 0.05$ vs. control value.

Table 1
Effect of adrenomedullin, CGRP, and amylin on cGMP levels in cultured rat mesangial cells

	cGMP (fmol/mg protein)
Basal	21 ± 10
Adrenomedullin (10^{-7})	16 ± 7
CGRP (10^{-6} M)	10 ± 3
Amylin (10^{-6} M)	12 ± 4
Atrial natriuretic peptide (10^{-7} M)	3003 ± 54 [a]
Brain natriuretic peptide (10^{-7} M)	3341 ± 307 [a]

Cultured rat mesangial cells were incubated with or without (basal) adrenomedullin (10^{-7} M), CGRP (10^{-6} M), amylin (10^{-6} M), atrial natriuretic peptide (10^{-7} M), or brain natriuretic peptide (10^{-7}) for 10 min at 37°C in the presence of 0.5 mM IBMX.

3.5. Effects of adrenomedullin, CGRP, and amylin on cGMP levels

We measured the cGMP levels induced by adrenomedullin, CGRP, and amylin. The cGMP levels elicited by atrial natriuretic peptide and brain natriuretic peptide, peptides known to increase cGMP concentrations (Singhal et al., 1989; Kohno et al., 1993), were also measured. Adrenomedullin and CGRP, at concentrations that caused significant cAMP production, failed to increase cGMP levels. However, both 10^{-7} M atrial natriuretic peptide and 10^{-7} M brain natriuretic peptide increased cGMP approximately 150–165-fold above basal levels. Amylin at 10^{-6} M also did not increase cGMP (Table 1 and Fig. 1).

3.6. Immunohistochemical analysis of adrenomedullin and CGRP

In cultured bovine adrenal medulla cells, significant immunoreactivity for both peptides was observed (Fig.

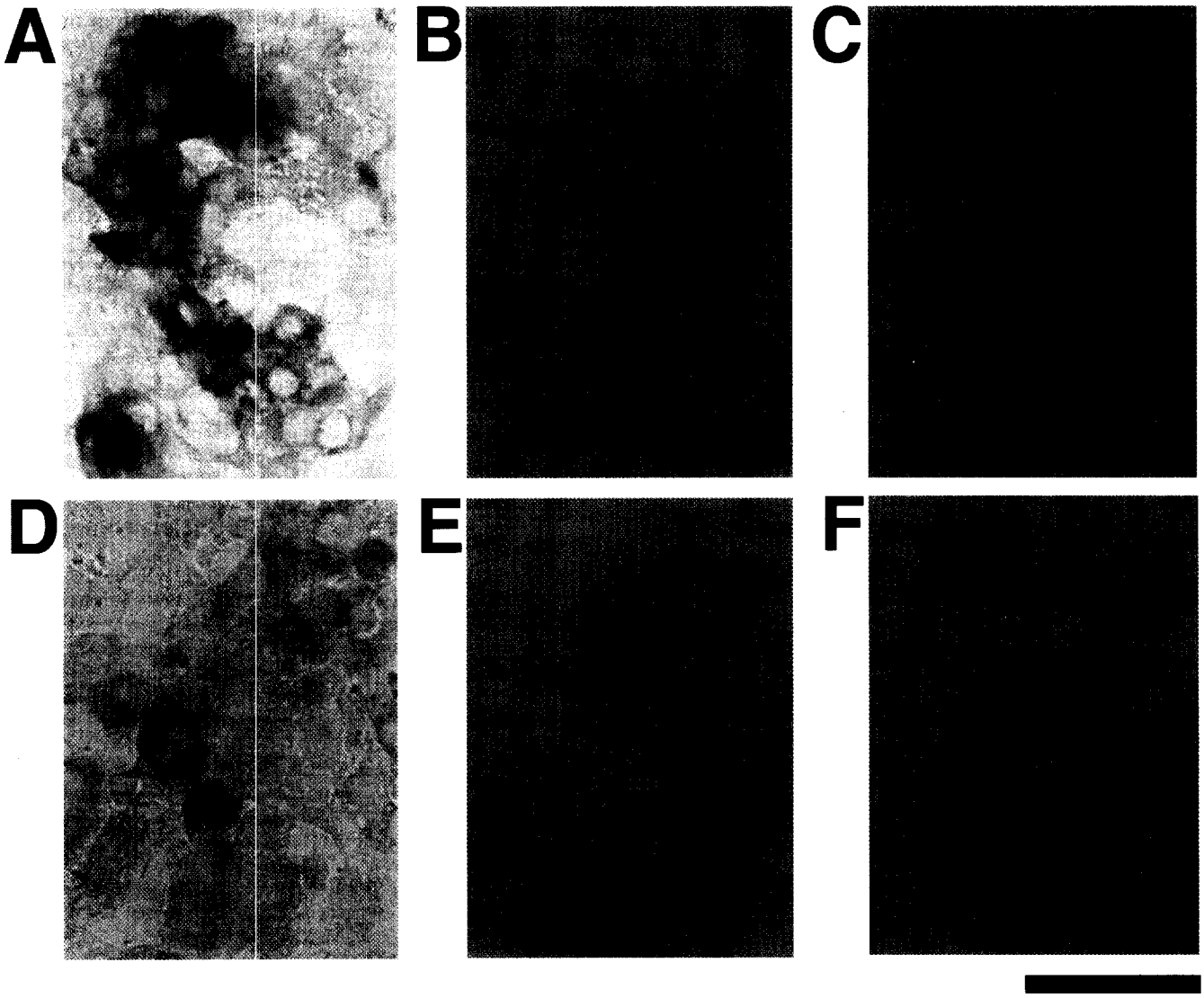

Fig. 5. Photomicrographs showing adrenomedullin-immunoreactivity (upper panel) and CGRP-immunoreactivity (lower panel) in cultured bovine adrenal medulla cells (A,D), LLC-PK1 cells (B,E) and cultured rat mesangial cells (C,F). Bar = 50 μm.

5A,D). LLC-PK1 cells were weakly stained for adrenomedullin (Fig. 5B) but were not stained for CGRP (Fig. 5E). Conversely, there was almost negligible immunoreactivity for adrenomedullin and CGRP in our cultured rat mesangial cell preparations (Fig. 5C,F).

4. Discussion

Mesangial cells are involved in regulating the glomerular filtration rate through their contractility (Singhal et al., 1986). Vasoactive peptides cause contraction or relaxation of these cells, leading to a change in the capillary surface area and thereby affecting the glomerular filtration rate (Schor et al., 1981). It is known that agents that raise intracellular cAMP levels prevent the contraction of mesangial cells thereby increasing the glomerular filtration (Kreisberg et al., 1985). It was previously reported that CGRP-stimulated cAMP formation reduced the angiotensin II-mediated contraction of cultured rat mesangial cells (Kurtz et al., 1989). Adrenomedullin has been reported to stimulate cAMP formation in cultured rat mesangial cells (Kohno et al., 1995), which suggests a role for adrenomedullin in mesangial cells. Our results showing that adrenomedullin more potently increased cAMP levels relative to CGRP suggest that adrenomedullin may be the predominant effector of mesangial cell function. Furthermore, cAMP production by the two peptides was not additive (Fig. 1 and Fig. 3), suggesting that they elevate cAMP levels by the same pathway. We also found that human adrenomedullin-(22–52)-NH_2 was a more potent antagonist for adrenomedullin- and CGRP-induced responses than human CGRP-(8–37) (Fig. 4A,B), indicating that adrenomedullin-sensitive receptors are preferentially expressed in cultured rat mesangial cells. These results raise the possibility that CGRP may bind to adrenomedullin

receptors with a lower affinity than adrenomedullin, or perhaps partially share adrenomedullin receptors in these cells. Binding studies using radiolabelled adrenomedullin and CGRP will clarify the classification of these receptors.

Immunohistochemical studies with kidney sections have shown that CGRP is localized in rat renal cortex nerve fibers (Kurtz et al., 1988). Our data showed that neither mesangial cells nor LLC-PK1 cells were stained for CGRP (Fig. 5), suggesting that CGRP is locally released from nerve fibers to exert its effects on the glomeruli and renal tubules (Kurtz et al., 1989). A recent immunohistochemical study indicated that the existence of adrenomedullin in kidney, in particular in the mesangial cells, is still unclear (Jougasaki et al., 1995; Washimine et al., 1995). Our immunohistochemical results showed that there was almost negligible adrenomedullin reactivity, if any, in cultured mesangial cells whereas very weak immunoreactivity was observed in LLC-PK1 cells (Fig. 5). Since adrenomedullin is synthesized and secreted by the endothelial cells of rat thoracic aorta, bovine carotid artery, and bovine brain capillaries (Sugo et al., 1994), the adrenomedullin-immunoreactive cells in the glomerulus reported by Jougasaki et al. (1995) might have been capillary epithelial or endothelial cells adjacent to mesangial cells in glomeruli. Hence, it is likely that adrenomedullin produced by epithelial or endothelial cells works on neighboring mesangial cells in a paracrine manner. In addition, significant concentrations of compounds having immunoreactivity similar to adrenomedullin and CGRP are present in human and rat plasma (Ishimitsu et al., 1994; Zaidi et al., 1985). Taken together, it may be possible that both peptides cause cAMP production as either a paracrine effector or a circulating hormone acting on mesangial cells. Further studies will be required to clarify this possible endocrine-like mechanism.

Adrenomedullin is a potent natriuretic peptide (Ebara et al., 1994; Jougasaki et al., 1995), as is CGRP (Gnaedinger et al., 1989; Kurtz et al., 1989). It is believed that CGRP acts as a natriuretic peptide by elevating the cAMP levels in the renal tubules (Edwards and Trizna, 1990) and in the mesangium (Kurtz et al., 1989). We have previously shown that adrenomedullin increases cAMP 100-times more potently than does CGRP and amylin in rat renal tubule basolateral membranes. From these data we have proposed adrenomedullin may modulate renal tubule function by elevating cAMP levels (Osajima et al., 1995) as well as CGRP (Edwards and Trizna, 1990). The present study showed that adrenomedullin-sensitive receptors are preferentially expressed in renal mesangial cells, suggesting that adrenomedullin modulates mesangial function by affecting cAMP levels as a paracrine and/or a circulating hormone.

Acknowledgements

The authors thank Ms A. Sugimoto for her technical assistance. This work was supported in part by a grant-in-aid for scientific research (No. 07770914) from the Ministry of Education, Science and Culture of Japan (A.O.).

References

Ausiello, D.A., J.I. Kreisberg, C. Roy and M.J. Karnovsky, 1980, Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin, J. Clin. Invest. 65, 754.

Cantau B., J.N. Barjon, D. Chicot, P.P. Baskevitch and S. Jard, 1990, Oxytocin receptors from LLC-PK1 cells: expression in *Xenopus* oocytes, Am. J. Physiol. 258, F963.

Chiba T., A. Yamaguchi, T. Yamatani, A. Nakamura, T. Morishita, T. Inui, M. Fukase, T. Noda and T. Fujita, 1989, Calcitonin gene-related peptide receptor antagonist human CGRP-(8–37), Am. J. Physiol. 256, E331.

Ebara, T., K. Miura, M. Okumura, T. Matsuura, S. Kim, T. Yukimura and H. Iwao, 1994, Effect of adrenomedullin on renal hemodynamics and functions in dogs, Eur. J. Pharmacol. 263, 69.

Edwards, R.M. and W. Trizna, 1990, Calcitonin gene-related peptide: effects on renal arteriolar tone and tubular cAMP levels, Am. J. Physiol. 258, F121.

Eguchi, S., Y. Hirata, H. Iwasaki, K. Sato, T.X. Watanabe, T. Inui, K. Nakajima, S. Sakakibara and F. Marumo, 1994, Structure-activity relationship of adrenomedullin, a novel vasodilatory peptide, in cultured rat vascular smooth muscle cells, Endocrinology 135, 2454.

Gnaedinger, M.P., D.E. Uehlinger, P. Weidmann, S.G. Sha, R. Muff, W. Born, W. Rascher and J.A. Fischer, 1989, Distinct hemodynamic and renal effects of calcitonin gene-related peptide and calcitonin in men, Am. J. Physiol. 257, E848.

Ishimitsu, T., T. Nishikimi, Y. Saito, K. Kitamura, T. Eto, K. Kangawa, H. Matsuo, T. Omae and H. Matsuoka, 1994, Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure, J. Clin. Invest. 94, 2158.

Jougasaki, M., C.-M. Wei, L.L. Aarhus, D.M. Heublein, S.M. Sandberg and J.C. Burnett, Jr., 1995, Renal localization and actions of adrenomedullin: a natriuretic peptide, Am. J. Physiol. 268, F657.

Kitamura, K., K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakamura, H. Matsuo and T. Eto, 1993, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma, Biochem. Biophys. Res. Commun. 192, 553.

Kohno, M., T. Horio, M. Ikeda, K. Yokokawa, T. Fukui, K. Yasunari, K. Murakawa, N. Kurihara and T. Takeda, 1993, Natriuretic peptides inhibit mesangial cell production of endothelin induced by arginine vasopressin, Am. J. Physiol. 264, F678.

Kohno, M., K. Yokokawa, K. Yasunari, H. Kano, T. Horio and T. Takeda, 1995, Stimulation of cyclic adenosine monophosphate formation by the novel vasorelaxant peptide adrenomedullin in cultured rat mesangial cells, Metabolism 44, 10.

Kreisberg, J.I., M. Venkatachalam and D. Troyer, 1985, Contractile properties of cultured glomerular mesangial cells, Am. J. Physiol. 249, F457.

Kurtz, A., R. Muff, W. Born, J.M. Lundberg, B.-I. Millberg, M.P. Gnadinger, D.E. Uehlinger, P. Weidmann, T. Hokfelt and J.A. Fischer, 1988, Calcitonin gene-related peptide is a stimulator of renin secretion, J. Clin. Invest. 82, 538.

Kurtz, A., H.-J. Schurek, W. Jelkmann, R. Muff, H.-P. Lipp, U. Heckmann, K.-U. Eckardt, H. Scholz, J.A. Fischer and C. Bauer, 1989, Renal mesangium is a target for calcitonin gene-related peptide, Kidney Int. 36, 222.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

Muff, R., W. Born and J.A. Fischer, 1995, Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions, Eur. J. Endocrinol. 133, 17.

Osajima, A., Y. Mutoh, Y. Uezono, M. Kawamura, F. Izumi, M. Takasugi and A. Kuroiwa, 1995, Adrenomedullin increases cyclic AMP more potently than CGRP and amylin in rat renal tubular basolateral membranes, Life Sci. 57, 457.

Schor, N., I. Ichikawa and B.M. Brenner, 1981, Mechanisms of action of various hormones and vasoactive substances on glomerular ultrafiltration in the rat, Kidney Int. 20, 442.

Singhal, P.C., L.A. Scharschmidt, N. Gibbons and R.M. Hays, 1986, Contraction and relaxation of cultured mesangial cells on a silicone rubber surface, Kidney Int. 30, 862.

Singhal, P.C., S. Decandido, J.A. Satriano, D. Schlondorff and R.M. Hays, 1989, Atrial natriuretic peptide and nitroprusside cause relaxation of cultured rat mesangial cells, Am. J. Physiol. 257, C86.

Sugo, S., N. Minamino, K. Kangawa, K. Miyamoto, K. Kitamura, J. Sakata, T. Eto and H. Matsuo, 1994, Endothelial cells actively synthesize and secrete adrenomedullin, Biochem. Biophys. Res. Commun. 201, 1160.

Ueta, Y., K. Kitamura, T. Isse, I. Shibuya, N. Kabashima, S. Yamamoto, K. Kangawa, H. Matsuo, T. Eto and H. Yamashita, 1995, Adrenomedullin-immunoreactive neurons in the paraventricular and supraoptic nuclei of the rat, Neurosci. Lett. 202, 37.

Wada, A., N. Yanagihara, F. Izumi, S. Sakurai and H. Kobayashi, 1983, Trifluoperazine inhibits $^{45}Ca^{2+}$ uptake and catecholamine secretion and synthesis in adrenal medullary cells, J. Neurochem. 40, 481.

Washimine, H., Y. Asada, K. Kitamura, Y. Ichiki, S. Hara, Y. Yamamoto, K. Kangawa, A. Sumiyoshi and T. Eto, 1995, Immunohistochemical identification of adrenomedullin in human, rat, and porcine tissue, Histochemistry 103, 251.

Wolthuis, A., A. Boes, H.P. Rodemann and J. Grond, 1992, Vasoactive agents affect growth and protein synthesis of cultured rat mesangial cells, Kidney Int. 41, 124.

Zaidi, M., P.J.R. Bevis, S.I. Girgis, C. Lynch, J.C. Stevenson and I. MacIntyre, 1985, Circulating CGRP comes from the perivascular nerves, Eur. J. Pharmacol. 117, 283.