PRELIMINARY REPORT

Stimulation of Cyclic Adenosine Monophosphate Formation by the Novel Vasorelaxant Peptide Adrenomedullin in Cultured Rat Mesangial Cells

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The present study was designed to examine the effect of synthetic adrenomedullin (AM), a novel vasorelaxant peptide originally isolated from human pheochromocytoma, on intracellular cyclic adenosine monophosphate (cAMP) formation in cultured rat mesangial cells. The effect of AM on cAMP formation in rat mesangial cells was compared with its effect in cultured rat vascular smooth muscle cells. cAMP levels were determined by radioimmunoassay after stimulation for 30 minutes with different concentrations (10^{-10} to 10^{-7} mol/L) of rat and human AM. Rat and human AM concentration-dependently (10^{-9} to 10^{-7} mol/L) stimulated cAMP formation in cultured mesangial cells. This stimulatory effect of rat AM was significantly greater than human AM. The stimulatory effect of rat AM in mesangial cells was significantly weaker than its potency in vascular smooth muscle cells. These preliminary data suggest that mesangial cells, as well as vascular smooth muscle cells, possess AM receptors functionally coupled to adenylate cyclase.

A NOVEL POTENT vasorelaxant peptide, adrenomedullin (AM), has recently been isolated from the acid extract of human pheochromocytoma.[1] This 52–amino acid peptide has one intramolecular disulfide bond and shows homology with calcitonin gene-related peptide.[1] Intravenous injection of AM causes a potent and long-lasting hypotensive effect in anesthetized rats and binds to specific receptors on platelet membranes to increase intracellular cyclic adenosine monophosphate (cAMP).

Recently, rat vascular smooth muscle cells were shown to possess specific AM receptors functionally coupled to adenylate cyclase.[2] In addition, it has been demonstrated that AM-like immunoreactivity is present not only in human adrenal medulla, but also in kidney.[3] Accordingly, the present study was designed to examine the effect of AM on intracellular cAMP formation in cultured rat glomerular mesangial cells. The effect of AM on cAMP formation in rat mesangial cells was compared with its effect in rat vascular smooth muscle cells.

MATERIALS AND METHODS

Materials

Angiotensin II and 3-isobutyl-1-methylxanthine were purchased from Sigma (St Louis, MO). Rat and human AM were purchased from Peptide Institute (Osaka, Japan). RPMI 1640 medium, Dulbecco's modified Eagle's medium, trypsin, Versene, and fetal calf serum were purchased from GIBCO Laboratories (Grand Island, NY). Flasks were purchased from Becton-Dickinson (Oxnard, CA). The cAMP assay kit was purchased from Yamasa Shoyu (Chiba, Japan).

Cell Culture

Rat glomerular mesangial cells were isolated and identified as described previously.[4] Glomeruli from Sprague–Dawley rats weighing 50 to 100 g were isolated by sieving with stainless steel and nylon meshes under sterile conditions. The isolated glomeruli were then cultured in RPMI 1640 medium containing 20% fetal calf serum and antibiotics. The identity of the mesangial cells was confirmed using the following criteria[5]: (1) morphology; (2) typical microfilaments seen by transmission electron microscopy; (3) survival in a medium containing D-valine substituted for L-valine, indicating the existence of D-amino acid oxidase; (4) resistance to puromycin aminonucleoside (10 μg/mL) but susceptibility to mitomycin C (10 μg/mL); (5) presence of receptors specific to Angiotensin II and contraction in response to Angiotensin II; and (6) absence of immunofluorescence with factor VIII antibody.

Rat vascular smooth muscle cells were grown from the explants of Sprague–Dawley rat aorta and cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, as previously described.[6] Cells were identified as vascular smooth muscle cells according to their morphological and growth characteristics.[7]

The cultures were maintained at 37°C with atmospheric air and 5% CO_2, and subculture was performed after treatment with Versene followed by trypsin. Mesangial cells and vascular smooth muscle cells obtained after three to seven passages were used for this experiment.

cAMP Measurement

After preincubation, cell monolayers were washed twice with serum-free medium and then stimulated for 30 minutes with rat or human AM dissolved in medium that contained 0.5 mmol/L 3-isobutyl-1-methylxanthine. The reaction was stopped by rapid aspiration and the addition of 2 mL ice-cold 65% ethanol as previously described.[6,7] After evaporation by a centrifugal evaporator (Model RD-31, Yamato Scientific, Tokyo, Japan), the dry residue

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was dissolved in an assay buffer according to the reported method in our laboratory.[6,7] cAMP levels were determined by radioimmunoassay with the cAMP assay kit.

Calculations and Statistical Analysis

The statistical significance of differences in the results was evaluated by ANOVA, and P values were calculated by Scheffe's method.[8] Values are expressed as the mean ± SD.

RESULTS AND DISCUSSION

The effects of rat and human AM on cAMP formation in cultured rat glomerular mesangial cells are shown in Fig 1. Rat and human AM concentration-dependently (10^{-9} to 10^{-7} mol/L) stimulated cAMP formation in these cells. The potency of rat AM to induce cAMP formation was significantly greater than that of human AM in these cells.

The effect of rat AM on cAMP formation in cultured rat glomerular mesangial cells was compared with its effect in cultured rat vascular smooth muscle cells derived from the same rat strain. Rat AM concentration-dependently (10^{-9} to 10^{-7} mol/L) stimulated cAMP formation in both mesangial cells (control, 6.1 ± 0.7 pmol/30 min per 5×10^5 cells; 10^{-9} mol/L AM, 13.0 ± 1.1; 10^{-8} mol/L AM, 44.7 ± 4.0; 10^{-7} mol/L AM, 72.8 ± 7.8) and vascular smooth muscle cells (control, 5.9 ± 1.0 pmol/30 min per 5×10^5 cells; 10^{-9} mol/L AM, 14.8 ± 1.3; 10^{-8} mol/L AM, 79.5 ± 5.0; 10^{-7} mol/L AM, 121.3 ± 15.0). The potency of rat AM to induce cAMP formation in cultured rat mesangial cells was significantly ($P < .05$) weaker than its potency in cultured rat vascular smooth muscle cells at concentrations of 10^{-7} and 10^{-8} mol/L, respectively.

Although these data are preliminary, we showed for the first time that rat AM potently stimulates cAMP formation in cultured rat mesangial cells, as well as in cultured rat vascular smooth muscle cells, and that the stimulatory effect of rat AM is significantly greater than that of human AM in rat mesangial cells. These results are in agreement with a recent report by Eguchi et al[2] that vascular smooth

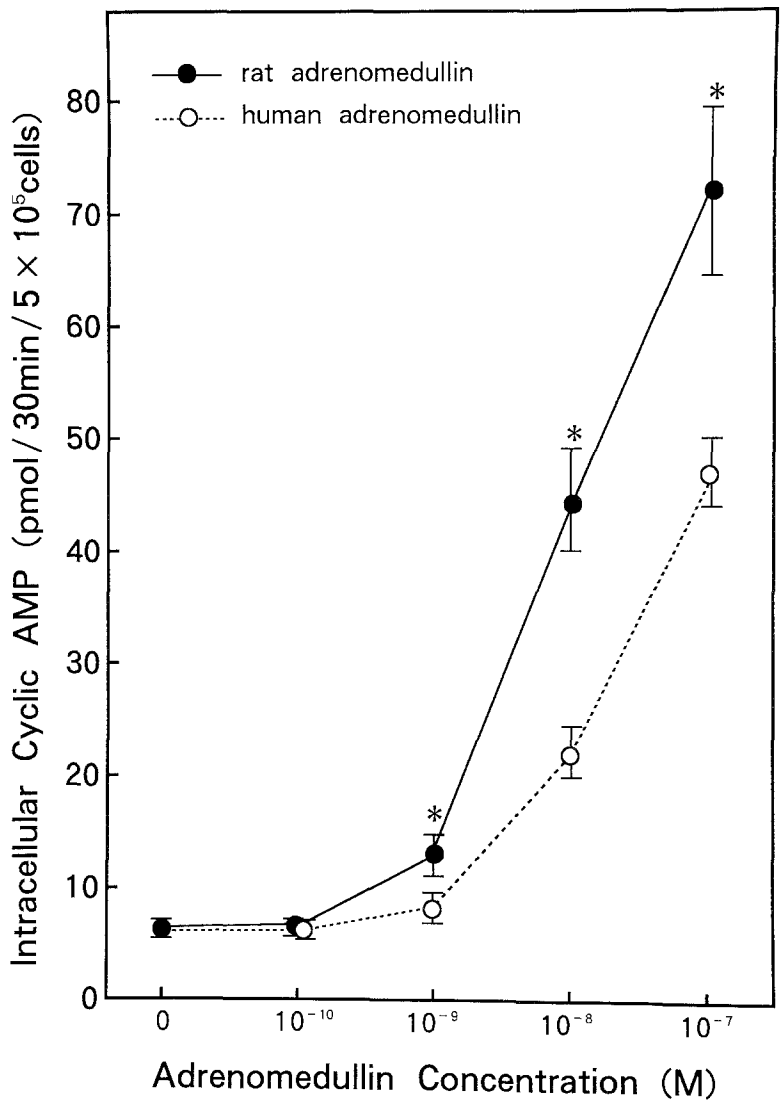

Fig 1. Effects of rat and human AM on cAMP formation in cultured rat glomerular mesangial cells. Cells were stimulated for 30 minutes with rat and human AM dissolved in medium that contained 0.5 mmol/L 3-isobutyl-1-methylxanthine. *$P < .05$ *v* cAMP levels induced by human AM.

muscle cells possess AM receptors functionally coupled to adenylate cyclase. cAMP is thought to play an important role as an intracellular mediator in the regulation of glomerular function. It is therefore possible that AM is related to the regulation of glomerular hemodynamics or function through its potent stimulatory effect on cAMP formation in glomerular mesangial cells. However, further studies will be required to clarify the physiological role of this peptide in the regulation of glomerular function.

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