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A_2 adenosine receptors in human glomerular mesangial cells

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Abstract. A_2 adenosine receptors were characterized in human glomerular mesangial cells using [^{3}H] 5'-N-ethylcarboxamidoadenosine (NECA) as a tracer. There was a single group of receptor sites with a K_D of 184 nM, and a number of sites of 317 fmol/mg of cell protein. Adenosine agonists increased 5'-nucleotidase activity via A_2 receptor stimulation. The specific A_2 agonist-NECA, at 0.1 and 1 μm, was a potent inhibitor of DNA synthesis.
Key words. Adenosine; A_2 adenosine receptors; mesangial cells; ecto-5'-nucleotidase.

Adenosine plays an important role in the regulation of kidney function, including renal blood flow and glomerular filtration rate, hormone and neurotransmitter release, and tubular reabsorption[1]. The adenosine receptors have been functionally localized in rabbit glomeruli[2], rabbit cortical collecting tubule (RCCT) cells[3], and a RCCT-28A cell line[4].

Previous studies by Olivera et al. have provided indirect evidence of A_1 and A_2 adenosine receptors in rat mesangial cells[5, 6]. Exposure of mesangial cells to adenosine analogs such as NECA, 2-choroadenosine (2-CADO), R-N^6-phenyl-isopropyladenosine (R-PIA), and N^6-cyclohexyl-adenosine (CHA), stimulated 5'-nucleotidase activity at low concentrations of A_2 analog[7]. It has also been established that adenosine upregulates 5'-nucleotidase, the enzyme responsible for its formation, via A_2 receptor stimulation. This effect was associated with inhibition of cell growth as measured by [^{3}H] thymidine incorporation[7, 8].

Adenosine receptors have not been studied in human glomerular mesangial cells. In view of the marked species-specific properties of the glomerular mesangium[9], we characterized A_2 adenosine receptors in human glomerular mesangial cells. We found a lesser density of A_2 adenosine receptors in human mesangial cells in comparison with rat mesangial cells, and a discrete upregulation of 5'-nucleotidase activity after exposure to A_2 analogs.

Materials and methods

Culture of mesangial cells. Human glomerular mesangial cells were isolated and characterized as previously described[10]. Glomeruli were prepared by differential sieving and centrifugation from the cortex of human cadaver kidney. After collagenase treatment, glomeruli were seeded in Petri dishes and cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum, and buffered with 20 mM HEPES at pH 7.4. Cell cultures were grown at 37 °C in a humidified 5% CO_2-95% air atmosphere. Mesangial cells which grew from glomeruli fragments were purified by successive subculture and studied after 3–5 passages.

[^{3}H]-NECA binding studies. Binding studies were performed essentially as previously described[3]. Cells in 12-well plates were incubated with 30–300 nM [^{3}H]-NECA (The Radiochemical Center, Amersham, UK) and the appropriate unlabelled reagents in final volume of 500 μl of phosphate-buffered saline (PBS) pH 7.4, supplemented with 1 mM $MgCl_2$, at 4 °C for 60 min. Non-specific binding was measured by incubating cells in the presence of 1 mM unlabelled NECA. Specific binding was calculated by subtracting nonspecific binding from total binding. Results were expressed as fmol [^{3}H]-NECA bound per mg of protein per 60 min.

[^{3}H] thymidine incorporation. Mesangial cells cultured with adenosine agonists for 48 h were treated with [^{3}H] thymidine (Dositek, Orsay, France) 0.5 μCi/well, 17 h before harvesting. Details are given elsewhere[8].

Ecto-5'-nucleotidase assay. Enzyme activity was measured on intact cells in culture, in medium containing 30 mM Tris-HCl buffer (pH 7.4), 130 mM NaCl, 5 mM $MgCl_2$, and 5.5 mM glucose. Incubation was started upon addition of 3 mM 5'-AMP to the medium and continued at 37 °C for 10–20 min. The amount of liberated inorganic phosphate was determined according to the method of Gomori[11]. Enzyme activity is expressed as nmol of inorganic phosphate liberated per min and per mg of cell protein.

Cell protein was determined according to the method of Lowry et al.[12] using bovine serum albumin as a standard. Results are expressed as mean ± SD. Comparisons between groups were made using analysis of variance or Student's *t*-test.

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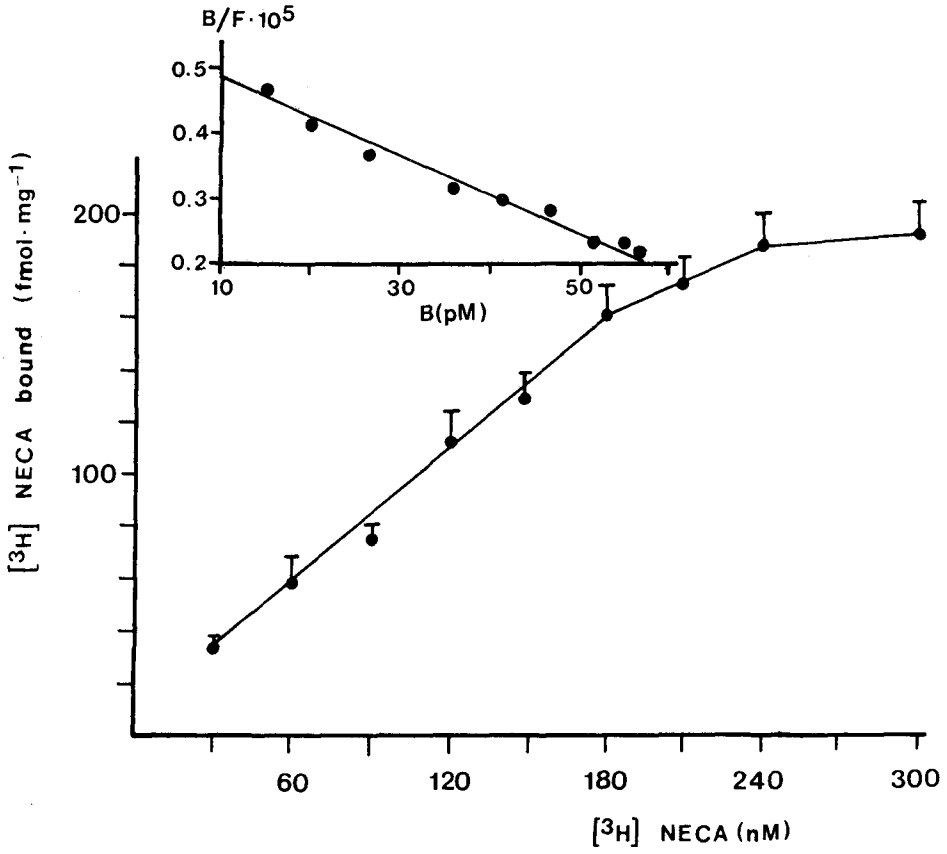

Figure. Concentration dependency of [^{3}H]-NECA binding to human mesangial cells. Specific [^{3}H]-NECA binding at 4 °C for 60 min is given as means ± SD of 4 determinations. Scatchard analysis of the data (inset) indicates a single class of binding sites with a K_D of 184 nM and a B_{max} of 317 fmol/mg of cell protein.

Table 1. Effect of adenosine agonists on ecto-5'-nucleotidase activity of human mesangial cells.

Agonist	Agonist concentration, μm			
	0	0.1	1	10
NECA	100 ± 7.9	114 ± 6.4	121 ± 8.9[a]	128 ± 7.5[a]
2-CADO	100 ± 5.9	111 ± 9.7	116 ± 5.5[a]	128 ± 8.3[a]
R-PIA	100 ± 8.4	105 ± 8.4	107 ± 7.5	110 ± 6.9
CHA	100 ± 10.9	104 ± 7.7	107 ± 7.9	111 ± 10.6

Mesangial cells were cultured with the agonists indicated for 48 h. Values are means ± SD of 4 experiments, expressed as percent of basal value. Basal 5-nucleotidase activity was 84.1 ± 7.2 nmol/min/mg. One-way analysis of variance (ANOVA) showed that ecto-5'-nucleotidase activity varied significantly ($p < 0.05$) with NECA and 2-CADO concentration. The effects of different analog concentrations were compared with control (0 concentration) using Student's *t*-test.
[a]vs 0 concentration $p < 0.05$.

Results

First, [^{3}H]-NECA binding was studied at 4 °C as a function of time. A plateau was reached within 60 min of incubation. Nonspecific binding was less than 30% of total binding at equilibrium. The concentration-dependency of [^{3}H]-NECA binding to human mesangial cells was studied over the range from 30 to 300 nM of the ligand (fig.). [^{3}H]-NECA binding followed a curvilinear ascending curve. The Scatchard plot of the data was linear, suggesting the presence of a single class of NECA binding sites. The maximum binding (B_{max}) and the K_D value derived from the Scatchard analysis were 317 fmol/mg of cell protein and 184 nM, respectively.

A modest stimulation of 5'-nucleotidase activity was observed after 48 h treatment of mesangial cells by adenosine analogs (table 1). 5'-Nucleotidase activity was significantly stimulated with NECA and 2-CADO, but not with the A_1 analogs R-PIA and CHA. NECA at concentrations of 0.1 and 1 μm markedly inhibited [^{3}H] thymidine incorporation into mesangial cells. A small but non-significant inhibition with 1 μm 2-CADO,

Table 2. Effect of adenosine agonists on [^{3}H] thymidine incorporation in human mesangial cells.

Agonist	Agonist concentration, μm			
	0	0.1	1	10
NECA	100 ± 11.4	79.0 ± 7.0[a]	76.3 ± 10.5[a]	67.9 ± 9.8[b]
2-CADO	100 ± 9.2	103.8 ± 5.0	89.6 ± 11.2	46.7 ± 7.4[c]
R-PIA	100 ± 3.8	99 ± 5.4	96 ± 2.8	65.7 ± 2.9[c]
CHA	100 ± 8.4	102 ± 10.9	91 ± 11	56 ± 4.5[c]

Mesangial cells were incubated with the agonists shown for 48 h. Values are means ± SD of 4 experiments, expressed as percent of basal value. Basal [^{3}H] thymidine incorporation was 31,034 ± 2631 cpm/well. One-way analysis of variance (ANOVA) showed that [^{3}H] thymidine incorporation varied significantly ($p < 0.05$) with NECA concentration. The effects of different analog concentrations were compared with control (0 concentration) using Student's *t*-test.
[a]vs 0 concentration $p < 0.05$.
[b]vs 0 concentration $p < 0.01$.
[c]vs 0 concentration $p < 0.001$.

CHA and R-PIA was observed. At a concentration of 10 μm all four adenosine analogs inhibited DNA synthesis of human mesangial cells (table 2).

Discussion

Evidence is presented in this work that human glomerular mesangial cells in culture have adenosine A_2 receptors. The adenosine analog NECA, a specific A_2-receptor agonist, was used to characterize these receptors in human mesangial cells. There was a single group of receptor sites with a K_D value of 0.184 μM, and the number of sites was 317 fmol/mg of cell protein. The K_D value for [^{3}H]-NECA in human mesangial cells is close to those reported with other preparations: 0.24; 0.3; 0.46 and 0.53 μM in rat type II pneumocytes[13], human placenta[14], rabbit alveolar macrophages[15] and rat mesangial cells[7], respectively. The number of binding sites in human mesangial cells is somewhat lower than in rat mesangial cells[7] or other cell preparations[13–15].

As in rat mesangial cells, adenosine A_2 analogs stimulated ecto-5′-nucleotidase activity, an A_2 receptor-mediated effect involving cAMP accumulation[7]. In human mesangial cells, however, this effect is very moderate, as the number of A_2 receptors per cell is lower than in rat mesangial cells.

[^{3}H] thymidine incorporation into human mesangial cells was inhibited by NECA over the same range of concentrations that stimulated 5′-nucleotidase activity. Therefore, the present study raises the question whether induction of 5′-nucleotidase activity and inhibition of cell growth, two cAMP-dependent events, are linked.

It should be noted that NECA is not a truly specific A_2 adenosine agonist. In fact, binding studies of [^{3}H]-NECA with adenosine receptor antagonists revealed a low inhibitory potency of PD 116,948, an A_1 antagonist and a high inhibitory potency of PD 115,190, an A_2 receptor antagonist[7].

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