Role of adenosine and adenine nucleotides in the biological system
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CHAPTER 42

Adenosine control of the renal collecting tubule: receptors and signaling

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When adenosine binds to plasma-membrane receptors on a variety of cell types in the kidney, it stimulates functional responses that span the entire spectrum of renal cellular physiology, including alterations in hemodynamics, hormone and neurotransmitter release, and tubular reabsorption (Table 1). This array of diverse responses appears to represent a means by which the kidney and its constituent cell types can regulate the metabolic demand such that it is maintained at an appropriate level for the prevailing metabolic supply. With the increased recognition of this wide array of renal cellular actions, and the continuing development of relatively specific adenosine receptor agonist and antagonist ligands, investigators have undertaken the task of assigning the different renal actions of adenosine to the known adenosine receptor types, by comparison of relative agonist and antagonist potencies. It is apparent from the inspection of a list of the renal actions of adenosine that not only does adenosine control a variety of functions, but appears to have a 'dual-control' over many aspects of renal function mediated by separate receptors.

With the exception of their ability to respond to adenosine and adenosine analogs, nothing as yet has been described that distinguishes adenosine receptors from the wide variety of receptors that modify adenylate cyclase activity and are therefore likely members of a large class of hormone receptors that, like the visual pigment rhodopsin, are coupled to their intracellular effector systems by guanine nucleotide binding proteins. In some systems, however, it has been impossible to correlate physiological responses to adenosine with changes in levels of cAMP, and therefore it has been proposed that adenosine may be coupled to other signal transduction systems as well. In the kidney, several of the actions of adenosine associated with

activation of the A_1 receptor (i.e. vasoconstriction, renin release inhibition, and inhibition of neurotransmitter release) are effects that have been proposed to be mediated by changes in cytosolic calcium [1].

Adenosine receptor activation in rabbit collecting tubule cells

As a nephron segment that regulates water and sodium reabsorption in response to the circulating levels of vasopressin and aldosterone, the mammalian collecting tubule plays an important role in the regulation of extracellular fluid volume and composition. In conjunction with circulating hormonal effectors, locally produced factors are known to modulate function in this terminal nephron segment. To investigate the possibility that adenosine receptor activation results in altered collecting tubule function, we isolated highly purified populations of collecting tubule cells from rabbit renal cortex using a monoclonal antibody (IgG_3-*rct* 30) as an immunoaffinity reagent [2]. A clonal cell line (RCCT-28A) was established from primary cultures of purified collecting tubule cells, by infection with an SV-40/adenovirus-12 hybrid [3]. Measurement of cyclic AMP production in response to adenosine receptor agonist ligands revealed that adenylate cyclase in these cells is coupled to both A_1 and A_2 adenosine receptors [2]. In addition, measurements

TABLE 1

Renal actions of adenosine

Effect	Receptor
Hemodynamic (↓ GFR)	
Vasoconstriction (preglomerular)	A_1
Vasodilation (postglomerular)	A_2
Hormonal/neurotransmitter	
Renin release	
Inhibition	A_1
Stimulation	A_2
Erythropoietin	
Inhibition	A_1
Stimulation	A_2
Adrenergic transmission	
Inhibition (presynaptic)	A_1
Tubular	
Collecting Tubule	
↑ hydraulic conductivity	A_2
↓ cyclic AMP	A_1

of cytosolic free calcium [4] and inositol phosphate production [3] by the 28A cells revealed that adenosine activation of a pertussis toxin-sensitive pathway resulted in the activation of phospholipase C (Figs. 1 and 2). This response was inhibited by the adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Fig. 3 and 4). In addition, the elevation of cytosolic free calcium by adenosine agonists shows stereoselectivity for the enantiomers of PIA. Despite

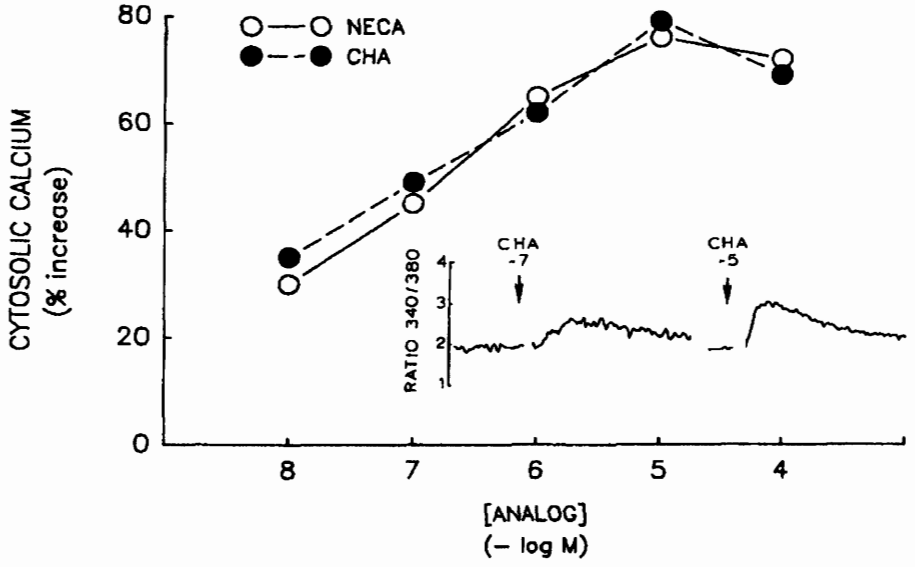

Fig. 1. Changes in cytosolic free calcium of RCCT-28A cells in response to adenosine analogs 5′-*N*-ethylcarboxamidoadenosine (NECA) and N^6-cyclohexyladenosine (CHA), presented as percent increase in calcium from control. Inset: representative fluorescent tracing of response of 28A cells to CHA (0.1 and 10 μM). Presented as ratio of fluorescence at excitation wavelength of 340 nm to fluorescence at excitation wavelength of 380 nm.

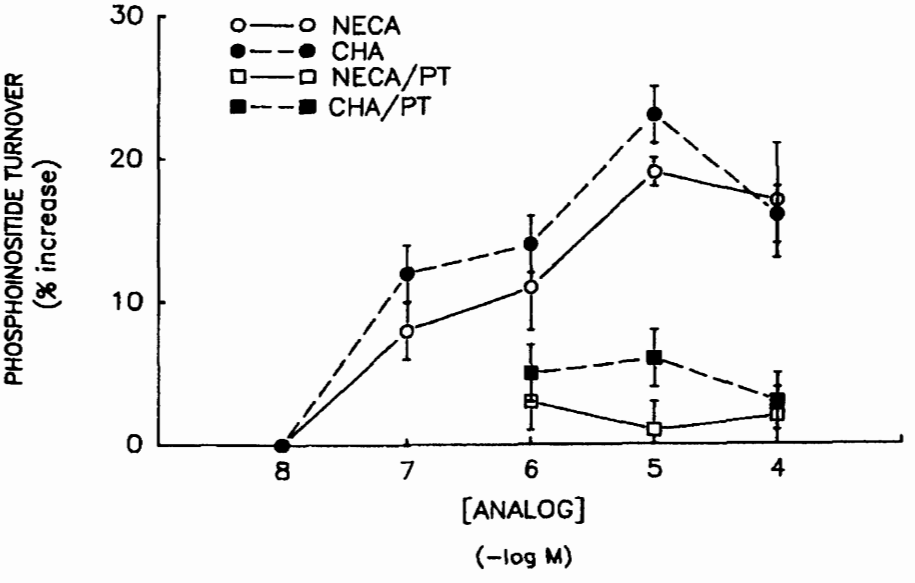

Fig. 2. Effect of pertussis toxin on responses to adenosine analogs, CHA and NECA. RCCT-28A cells were pretreated with and without pertussis toxin (PT) for 12 h with 1 μg/ml toxin before measurements; presented as percent increase from control. See Fig. 1 for definitions of abbreviations.

numerous similarities between the adenosine A_1 receptor-induced decrease in cyclic AMP production, a fundamental difference is the similarity in the potency of 5'-substituted adenosine analogs as compared to N^6-substituted analogs in the receptor-mediated increase in phospholipase C activity (Figs. 1 and 2; Table 2).

The presence of two different mechanisms associated with the adenosine A_1 receptor raises several important questions. The first and most obvious is whether two classes of A_1 receptors exist. One possibility is that both the inhibition of adenylate cyclase and the acceleration of inositol polyphosphate production are pro-

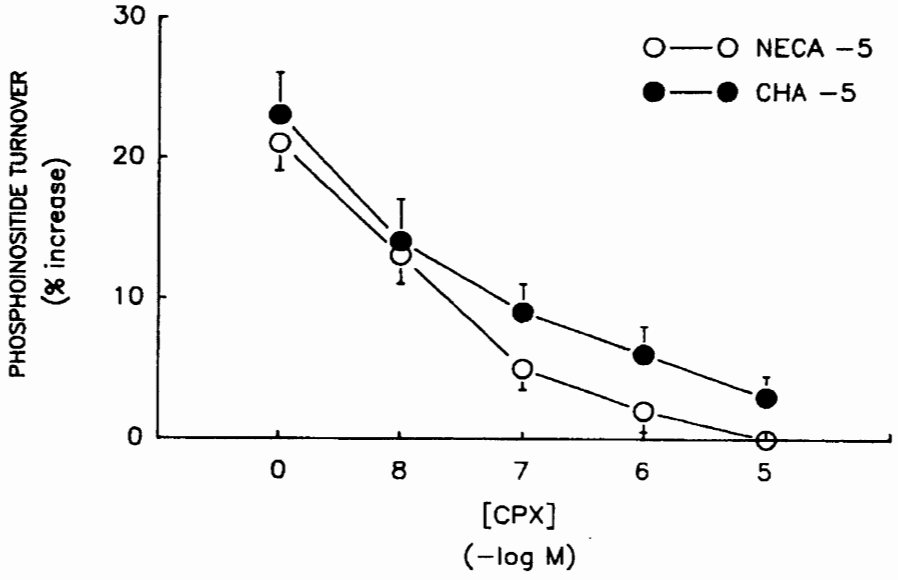

Fig. 3. Effect of A_1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) on increase in phosphoinositide turnover produced by NECA and CHA. See Fig. 1 for definitions of abbreviations.

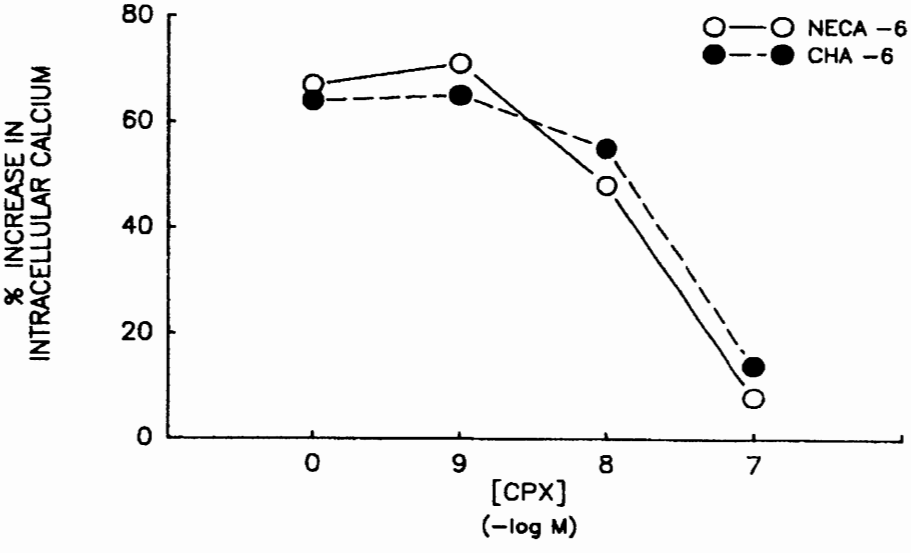

Fig. 4. Effect of A_1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) on increase in cytosolic free calcium by NECA and CHA. See Fig. 1 for definitions of abbreviations.

TABLE 2

Comparison of A_1 and 'A_{PLC}' receptor activation in rabbit cortical collecting tubule cells

	EC_{50} (nM)	R-PIA/NECA	R-PIA/S-PIA	DPCPX	Pertussis T sensitive
A_1	10	R-PIA > NECA	R-PIA >> S-PIA	+	+
'A_{PLC}'	500	R-PIA = NECA	R-PIA >> S-PIA	+	+

voked by a single receptor population via divergent coupling mechanisms. Alternatively, each response may be evoked by independent adenosine receptor populations.

Radioligand binding analysis of adenosine A_1 receptors in 28A cells

To determine whether a single population of A_1 receptors is coupled to divergent signaling pathways, we have measured radioligand binding of [^{3}H]DPCPX to plasma membranes from rabbit renal medulla and the RCCT-28A cells (28A cells). Saturation binding of [^{3}H]DPCPX in 28A membranes (Fig. 5), analyzed by non-linear curve fitting, gave a one-site model with an apparent K_D value of 1.4 nM and a maximum number of binding sites (B_{MAX} value) of 64 fmol/mg protein. Scatchard analysis of the saturation curve gave a linear plot, indicating the presence of a homogeneous population of binding sites. The non-specific binding was 20 – 30%

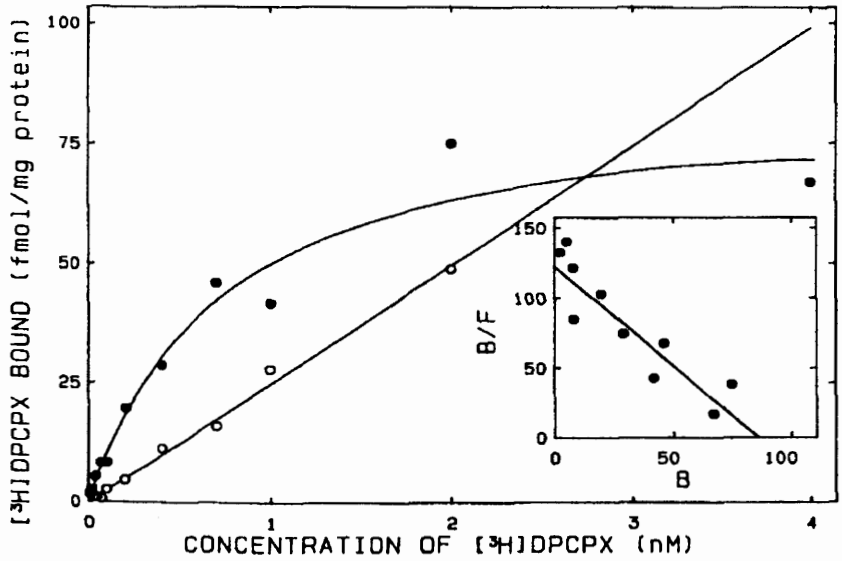

Fig. 5. Saturation binding of [^{3}H]DPCPX to RCCT-28A cell membranes. Data are given as specific (closed circles) and non-specific binding (open circles). The inset shows the Scatchard plot from the data.

of the total at the K_D, and saturation of specific binding was reached with 2 nM [^{3}H]DPCPX.

Competition of several agonists for the [^{3}H]DPCPX binding was measured to confirm that [^{3}H]DPCPX binds to the A_1 receptor. Competition of adenosine agonists for [^{3}H]DPCPX binding resulted in biphasic displacement curves (Table 3), indicating the presence of two affinity states for the agonists, with approximately one-half of the binding sites being in the high-affinity state and the other half in the low-affinity state. The K_i values for the various adenosine receptor agonists exhibit the typical pharmacological profile for A_1 receptors and the marked stereoselectivity for the PIA enantiomers.

Agonist binding was further characterized by measuring the competition of R-PIA for [^{3}H]DPCPX binding in the presence and absence of GTP (100 μM). In the absence of GTP the competition of [^{3}H]DPCPX by R-PIA resulted in a biphasic displacement curve with an apparent K_D value of 0.5 nM and B_{MAX} value of 16.1 pmol/mg protein for the high-affinity state and a low-affinity K_D value of 10.5 nM and B_{MAX} value of 20.2 fmol/mg protein. When the competition experiment was carried out in the presence of 100 μM GTP, a monophasic curve was obtained, indicating a single affinity state with a K_D value of 17.7 nM and a B_{MAX} value of 54.1 fmol/mg protein. Control binding (100%) increased from 36.3 to 54.1 fmol/mg protein with the addition of 100 μM GTP.

These binding data confirm the previously reported functional data, that cells of the cortical collecting tubule have adenosine A_1 receptors coupled through GTP-binding proteins. Furthermore, these binding data fail to provide support for the hypothesis that the inhibition of adenylate cyclase and the stimulation of phospholipase C are coupled to two sub-populations of the A_1 receptor, although it is recognized that this conclusion may be a function of the inability of currently available ligands to differentiate between the A_1 receptor subtypes. Furthermore, a final caveat to the above conclusion is that binding analysis can only reveal the nature of the binding domain of the receptor. It is possible that the ligand binding domain of the two receptor populations is the same, but the receptors are structurally different, for example in the G-protein coupling domains, allowing for separate or non-promiscuous signal activation.

TABLE 3

Pharmacological profile of [^{3}H]DPCPX binding to RCCT-28A membranes

	K_i^H (nM)	K_i^L (nM)
R-PIA	0.5	7.0
NECA	1.8	47
S-PIA	3.1	275

In conclusion, in the absence of evidence of sub-populations of the A_1 receptor, it appears that activation of a single A_1 receptor population, at least as defined by radiolabeled ligand binding analysis, results in the inhibition of adenylate cyclase and the activation of phospholipase C. Although GTP-binding proteins link receptor occupancy to changes in both inhibition of cyclase and the acceleration of inositol phosphate production, the identity of the GTP-binding proteins involved in vivo and the mechanisms are not certain. Finally, it remains to be determined which of the possible signaling events induced by occupancy of receptors linked to the inhibition of adenylate cyclase and/or phospholipase C are causal in mediating a given physiological event, which are permissive, and which are without any functional consequence in a given setting.

References

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Discussion

J. Linden: Have you tried yet to determine if adenosine can activate phospholipase C in broken or permeabilized cells?

W.S. Spielman: It's a good idea but we haven't as yet tried it.

I.L.O. Baxton: Not all would agree that a G_i protein couples receptors to regulation of phosphoinositide turnover in noncirculating (non-blood-borne cells) tissues. I wonder if the effect of a pertussis toxin sensitive stimulation of PI-turnover is the result of the viral transfection you have used to immortalize your cell line, or, conversely, that the effect is due to crosstalk produced by high agonist concentration that allows G_i-protein sub-units to influence another G-protein-mediated pathway? That is, that $G_i\alpha$ or $\beta\gamma$ could influence the activity of a Gp-GTP binding protein?

W.S. Spielman: As for the possibility that the pertussis toxin sensitivity is a function of the viral transformation of the cells, we are sure this is not the explanation as we see the same response in freshly isolated cells and cells in primary culture. In regard to the possibility that liberated $\beta\gamma$ subunits from some other G_i-protein is responsible for the effect on phospholipase C activity is an interesting idea, but it seems to us that if true that would inhibit, rather than stimulate PLC activity.

B.B. Fredholm: Some time ago Dr. Häggblad and I reported that in the rat vas deferens adenosine had little effect by itself on IP formation whereas it did enhance the IP formation induced by an α_1-agonist. Is it possible that also in your system the effect of adenosine on PI-turnover is an indirect rather than a direct consequence of adenosine receptor activation? An effect due to interaction with a parallel signal transduction pathway may also explain the unusual dose-response relationship.

S.J. Mustafa: Have you measured the changes in cyclic nucleotides with both NECA and R-PIA. This might help to understand the type of A_1 receptor and its coupling to the second messenger systems.

W.S. Spielman: Yes, we have determined full concentration-response curves for CHA, R-PIA and NECA on cyclic AMP. What we see is a pattern of response that is entirely in keeping with the concept of a classical high-affinity A_1 and low-affinity A_2 receptor system coupled to the inhibition and stimulation of adenylate cyclase respectively.

L. Belardinelli: Is the magnitude of the PTX inhibition of adenosine-induced stimulation of PLC and inhibition of cAMP production the same?

W.S. Spielman: I can only say that when we treat our cells with 1 μg/ml pertussis toxin for 12 h, both responses are completely inhibited. Perhaps if we used a series of lower concentration of the toxin we could uncover a difference between the sensitivity of the two systems.

D.M.F. Cooper: (1) How does the A_1 stimulation of $[Ca^{2+}]_i$ compare in magnitude with that elicited by e.g. bradykinin. (2) Does cAMP influence the A_1 effect on $[Ca^{2+}]_i$ – this question is raised to consider the possibility that you are actually getting an A_2 stimulation of $[Ca^{2+}]_i$, based on your low potency of PIA in mediating the effect: NECA being relatively impotent at A_1 receptors might then be equipotent with PIA at eliciting the effect, given that PIA concentration would have to be increased to levels that overcame the inhibition of cAMP.

W.S. Spielman: (1) The maximal increase in cytosol Ca^{2+} in response to adenosine agonist ligands is approximately 100%. This is substantially less than we observe with bradykinin. (2) This is an interesting possibility that we haven't investigated fully, but I don't think it is the explanation because recall that pertussis toxin pretreatment inhibits the mobilization of cytosolic calcium but actually enhanced the ability of the NECA and other agonist to simulate cyclic AMP production, presumably by eliminating the inhibitory input.

J.W. Daly: Is the potency of 8-cyclopentyl-1,3-dipropylxanthine versus A_1-receptor-mediated inhibition of cyclic AMP formation similar or different from its potency versus 'A_1'-receptor-mediated stimulation of phosphoinositide breakdown in RCCT28A cells?

W.S. Spielman: I can't tell you off-hand the exact IC_{50} values for DPCPX on the two responses but I can say we don't observe any major differences. If differences do exist, they are relatively small.