A_1 and A_2 Adenosine Receptors in Rabbit Cortical Collecting Tubule Cells

Modulation of Hormone-stimulated cAMP

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Abstract

Adenosine analogs were used to investigate the cellular mechanisms by which adenosine may alter renal tubular function. Cultured rabbit cortical collecting tubule (RCCT) cells, isolated by immunodissection, were treated with 5'-N-ethylcarboxamideadenosine (NECA), N^6-cyclohexyladenosine (CHA), and R-N^6-phenylisopropyladenosine (PIA). All three analogs produced both dose-dependent inhibition and stimulation of RCCT cell cyclic AMP (cAMP) production. Stimulation of cAMP accumulation occurred at analog concentrations of 0.1 μM to 100 μM with the rank order of potency NECA > PIA > CHA. Inhibition occurred at concentrations of 1 nM to 1 μM with the rank order of potency CHA > PIA > NECA. These effects on cAMP production were inhibited by 1,3-diethyl-8-phenylxanthine and isobutylmethylxanthine. CHA (50 nM) blunted AVP- and isoproterenol-stimulated cAMP accumulation. This modulation of hormone-induced cAMP production was abolished by pretreatment of RCCT cells with pertussis toxin. Prostaglandin E_2 production was unaffected by 0.1 mM CHA. These findings indicate the presence of both inhibitory (A_1) and stimulatory (A_2) receptors for adenosine in RCCT cells. Moreover, occupancy of the A_1 receptor causes inhibition of both basal and hormone-stimulated cAMP formation through an action on the inhibitory guanine nucleotide-binding regulatory component, N_i, of the adenylate cyclase system.

Introduction

Adenosine is an important intermediate in the pathway of purine nucleotide degradation and has been shown to modify a variety of renal functions (1–8). Adenosine can alter renal excretory function (1, 5, 7–9), but it is not clear to what extent these effects are the result of a direct tubular action or are an indirect result of altered renal hemodynamics. Recent studies by Forrest and coworkers on cultured amphibian epithelial (A6) cells (10), the rectal gland of the shark (11), and the rabbit colon (12) have demonstrated that adenosine can alter active transepithelial transport. Dillingham and Anderson (13) have shown that adenosine analogs inhibit vasopressin-stimulated water movement in rabbit collecting tubules. These studies provide important precedents for a direct action of adenosine on mammalian renal tubular epithelium, though relatively little is known regarding the cellular mechanisms underlying the tubular actions of adenosine. The present study was undertaken to determine the effect of adenosine analogs on the production of cyclic AMP (cAMP) by cultured rabbit cortical collecting tubule (RCCT)[1] cells isolated by immunodissection from the kidney, and to test the hypothesis that adenosine modulates hormone-stimulated cAMP production. Data are presented indicating the presence of both inhibitory (A_1) and stimulatory (A_2) receptors for adenosine in RCCT cells and that stimulation of the A_1 receptor inhibits hormone-stimulated cAMP formation. The inhibition is mediated by the inhibitory guanine nucleotide-binding regulatory component of the adenylate cyclase system and is not dependent on stimulation of prostaglandin production.

Methods

Materials. Trypsin (1:250), Dulbecco's modified Eagle's medium (DME), collagenase, fetal bovine serum (FBS), and bovine serum albumin (BSA) were purchased from Gibco Laboratories, Grand Island, NY. The adenosine analogs, 5'-N-ethylcarboxamideadenosine (NECA), N^6-cyclohexyladenosine (CHA), R-N^6-phenylisopropyladenosine (PIA), and 1,3-diethyl-8-phenylxanthine (DPX) were a gift from Warner Lambert Co., Milford, CN. The phosphodiesterase inhibitor, R0 20-1724, was a gift from Hoffmann-LaRoche Inc., Nutley, NJ. Arginine vasopressin (AVP) was from Peninsula Laboratories Inc., Belmont, CA. Pertussis toxin was from List Biological Laboratories, Inc., Campbell, CA. ^{125}I adenosine 3'5'cyclicmonophosphoric acid and ^3H prostaglandin E_2 were from ICN Biomedicals Inc., Irvine, CA. Other chemicals of reagent grade or better were obtained from standard sources.

Isolation and culture of RCCT cells. RCCT cells were isolated by immunodissection with a cell-specific mouse monoclonal antibody as described previously (14). Briefly, plastic culture dishes were coated with an IgG_3 (*rct*-30) that binds exclusively to collecting tubule cells when assayed by indirect immunofluorescence on rabbit kidney cryotome sections (14). Both kidneys were removed from 4–5-wk-old rabbits and the cortical tissue was separated and minced into a brei. The tissue was digested in 0.1% collagenase for 40 min with agitation every 10 min. Following centrifugation to remove the collagenase, red blood cells were lysed with hypotonic saline and the suspension was filtered through 250-μm Gelman filter holders. The cells were centrifuged and washed in 10% BSA to remove cellular debris. The resulting cell suspension was placed on the antibody-treated dishes and after 2–3 min was washed off with 10–15 ml/plate of phosphate-buffered saline (PBS, composition in mM: 151 NaCl, 45 KH_2PO_4, and 2.5 NaOH). Freshly isolated RCCT cells were grown to confluency (4–5 d) in 100-mm Costar culture dishes in DME with 10% FBS, 2 mM glutamine, and 1 μM dexamethasone in a water-saturated, 7% CO_2 environment at 37°C. Confluent RCCT cells were detached from the dishes by treatment with trypsin (0.1%, with 0.05% EDTA in PBS, pH 7.4), transferred into 24-well culture dishes (1×10^4 cells/well), and cultured under the conditions described above.

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; CHA, N^6-cyclohexyladenosine; DME, Dulbecco's modified Eagle's medium; DPX, 1,3-diethyl-8-phenylxanthine; IBMX, isobutylmethylxanthine; NECA, 5'-N-ethylcarboxamideadenosine; PIA, R-N^6-phenylisopropyladenosine; RCCT, rabbit cortical collecting tubule.

Determination of total cAMP production. Treatments were done in triplicate using first passage cells grown for 5 d in 24-well dishes. Culture medium was removed and the cells were washed once with Krebs buffer (composition in mM: 118 NaCl, 25 $NaHCO_3$, 14 glucose, 4.7 KCl, 2.5 $CaCl_2$, 1.8 $MgSO_4$, and 1.8 KH_2PO_4, pH 7.4). The cells were pretreated for 1 h with 0.1 mM R0 20-1724, a phosphodiesterase inhibitor that is not an adenosine receptor antagonist (15, 16), and 1.5 U adenosine deaminase/ml Krebs at 37°C. After pretreatment, the buffer was aspirated and the cells were treated with the same buffer containing the various hormones and effectors at 37°C. When necessary, dimethyl sulfoxide was used as solvent and was included in the control wells. Treatment was terminated by adding an equal volume of 5% (wt/vol) trichloroacetic acid. The cells were frozen, thawed, and allowed to stand at 4°C for 60 min. The samples were extracted three times with 5 vol of water-saturated ether, the residual ether was evaporated, and the samples were dried under vacuum with vortexing. The samples were reconstituted with distilled water, and total cAMP was determined by radioimmunoassay as described by Frandsen and Krishna (17).

Determination of PGE_2 production. Treatments were done in triplicate using first passage cells grown for 5 d in 24-well dishes. The culture medium was removed and the cells were washed with Krebs buffer. The cells were incubated with adenosine analog in Krebs for 30 min at 37°C. At the end of the incubation period, the effector solution was removed and assayed for PGE_2 concentration by radioimmunoassay as described previously (18).

Protein determination. Following removal of the media for cAMP assay, the remaining liquid was aspirated and the cells were solubilized with a solution of 2% Na_2CO_3, 0.4% NaOH, 0.16% sodium tartrate, and 1% sodium dodecyl sulfate. After 24 h at 24°C, samples were assayed for protein using a modification of the Lowry method (19).

Statistical methods. Data were analyzed for statistical significance using two-way analysis of variance with the multiple comparisons test of Student–Newman–Kuels. Significance is defined as $P < 0.05$.

Results

To determine the incubation times optimal for measuring the effects of adenosine analogs, time courses of cAMP accumulation by RCCT cells were performed. For all of the adenosine analogs, maximal increases in total cAMP levels occurred between 10 and 30 min of incubation. Inhibition of cAMP accumulation by adenosine analogs was maximal by 10 min and remained unchanged for up to 60 min. Therefore, a 30-min incubation period was used for most experiments. Each of the three adenosine analogs studied, NECA, CHA, and PIA, produced both a dose-dependent inhibition and a dose-dependent stimulation of cAMP accumulation (Fig. 1). The decreases in cAMP of 30–50% from basal levels were consistently observed at analog concentrations of 10–100 nM. The rank order of potency for stimulation of cAMP accumulation was NECA > PIA > CHA. The opposite order of potency was observed for the inhibition of cAMP accumulation by adenosine analogs, i.e., CHA > PIA > NECA.

Further evidence for the existence of specific adenosine receptors in RCCT cell populations was obtained using the adenosine receptor antagonists isobutylmethylxanthine (IBMX) and DPX. Both IBMX and DPX produced dose-dependent attenuation of the adenosine analog–induced increases and decreases in cAMP levels. DPX was a more potent inhibitor of the effects of the adenosine analogs than IBMX. As little as 0.1 μM DPX produced 50% inhibition of the analog-induced stimulation of cAMP accumulation; the concentration of IBMX required to inhibit stimulation was 100 μM. Neither IBMX nor DPX affected basal cAMP levels, presumably because these incubations already contained the phosphodiesterase inhibitor R0 20-1724.

To investigate the potential role of adenosine in modulating hormonal regulation of tubular function, the effects of adenosine analogs on AVP- and isoproterenol-induced stimulation of cAMP accumulation by RCCT cells were studied. Treatment of RCCT cells with 50 nM CHA and 1 μM AVP attenuated the AVP-induced stimulation of cAMP accumulation (Table I). Higher concentrations of CHA (100 μM), and PIA or NECA at low (50 nM) or high (100 μM) concentrations were much less effective in modulating AVP-induced cAMP accumulation although they produced slight inhibition. The response of RCCT cells to isoproterenol (1 μM) was also attenuated by 50 nM CHA (Table I) and, to a lesser extent, 100 μM CHA.

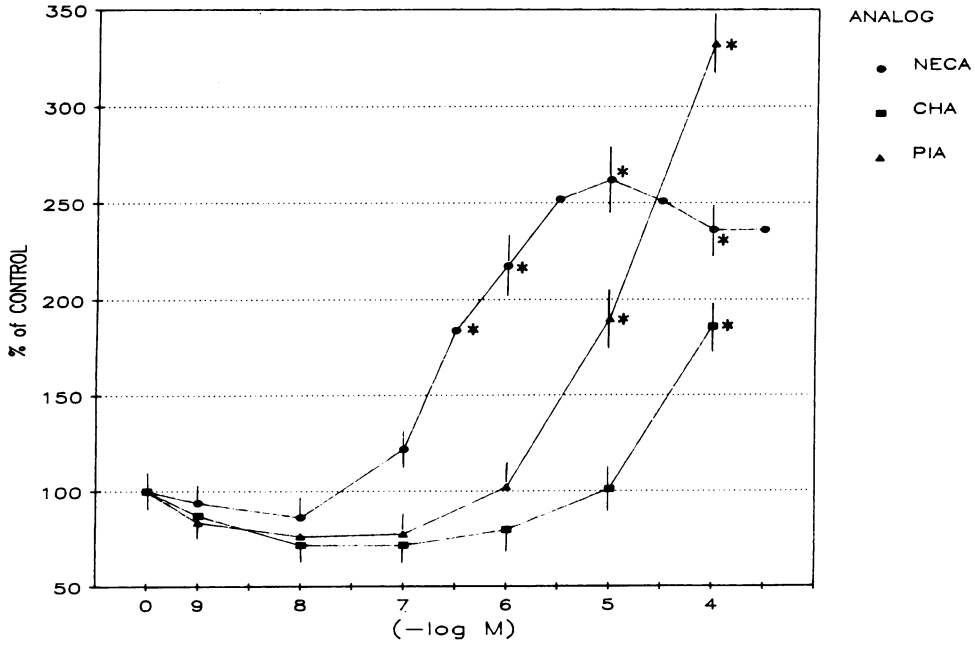

Figure 1. Effect of adenosine analogs on total RCCT cell cAMP production, expressed as percent of control (basal). Mean control values for each analog were: NECA, 341; CHA, 367; PIA, 455 pmol cAMP/mg prot/30 min. Each point is the mean of three samples from at least three different isolations.

Table I. Effects of Adenosine Analogs on Hormone-stimulated cAMP Production in RCCT Cells

Adenosine analog		Hormone-stimulated cAMP production (pmol cAMP/mg protein) None	Vasopressin	Isoproterenol
			1 μM	*1 μM*
CHA	0	358±66	2570±722*	3274±247*
	50 nM	126±15†	651±77†	1295±353†
	100 μM	675±45†	1650±60	2547±493
PIA	0	219±13	1059±72*	
	50 nM	113±14†	629±45†	
	100 μM	912±295†	807±87	
NECA	0	441±31	2199±167*	
	50 nM	433±22	1979±160	
	100 μM	1525±76†	1607±216	

First passage RCCT cells were incubated for 30 min with RO 20-1724 and adenosine deaminase as described in Methods and then treated for 20 min with either Krebs, adenosine analog, or hormone alone or with adenosine analog and hormone together. All incubations were performed at 37°C under a 7% CO_2 atmosphere. cAMP production was determined as described in Methods.
* $P < 0.05$ when compared with respective sample without hormone.
† $P < 0.05$ when compared with respective sample without adenosine analog.

Products of the cyclooxygenase pathway can inhibit AVP's stimulation of cAMP production in canine cortical collecting tubule cells (20). Therefore, we tested the hypothesis that CHA stimulated the formation of prostaglandins that, in turn, mediate the attenuation of the AVP response. Treatment of RCCT cells with CHA (100 μM) failed to cause a significant increase in PGE_2 production (control, 115±14 vs. treated, 150±11 fmol PGE_2/μg protein, NS).

To test the possibility that CHA attenuates AVP-induced increases in cAMP levels in RCCT cells via the inhibitory guanine nucleotide-binding protein, N_i, the effect of pertussis toxin was studied. Pretreatment of RCCT cells (12 h) with 1 μg of pertussis toxin per ml of culture medium completely prevented the CHA-induced inhibition of cAMP accumulation from both basal and AVP-stimulated states (Fig. 2). Pertussis toxin had no effect on the ability of AVP alone to increase cAMP concentrations, however, despite an increase in basal cAMP production seen in pertussis toxin–treated cells, stimulation of cAMP accumulation by higher concentrations of CHA (10–100 μM) was enhanced (Table II).

Discussion

Interest in the renal actions of adenosine stems from its possible involvement in the regulation of renal function (21, 22). Previous work has defined the effects of adenosine on renal hemodynamics (1–3), renin release (3–5), and salt and water excretion (1, 5, 7–9). Whereas these actions of adenosine have been well documented, the mechanism(s) by which adenosine produces these effects in the kidney remains unclear. A primary impediment to the study of the cellular actions of adenosine in the kidney is the heterogeneous nature of renal tissue. With the advent of the technique of immunodissection, used originally by Wysocki and Sato (23) for selective isolation of lymphocytes and modified by Garcia-Perez and Smith (24) for use with renal tissue, large populations of homogeneous cells can be isolated and cultured for study. In the present study, RCCT cells were isolated as described by Spielman et al. (14) for use in investigating the cellular mechanism of action of adenosine on renal collecting tubule epithelium.

Previous work on adenosine receptors and adenosine post-receptor mechanisms in other tissues has indicated that there are at least two types of adenosine receptors (reviewed in reference 25), both of which interact with the cAMP second messenger system. Several previous reports have identified the presence of both inhibitory (A_1) and stimulatory (A_2) receptors in the kidney. Stimulatory receptors have been identified in bovine renal medulla (26), cultures of rat (27) and toad (10) kidney cortex, and isolated rat glomeruli (28), whereas inhibitory re-

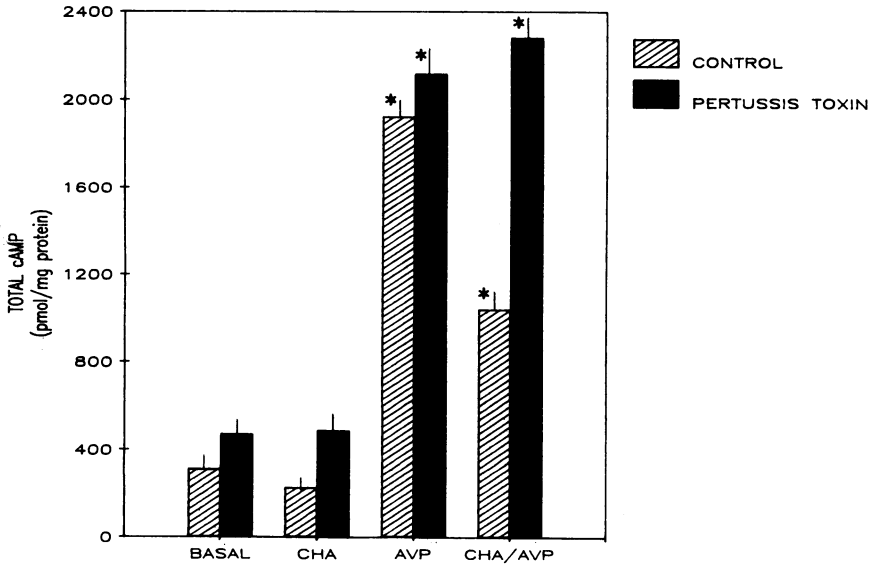

Figure 2. Effect of pretreatment with pertussis toxin on the modulation by CHA (50 nM) of 1 μM AVP-stimulated cAMP production in RCCT cells. The cells were pretreated for 12 h with 1 μg/ml pertussis toxin. Each bar is the mean of three samples from at least three different isolations.

Table II. Effect of Pertussis Toxin on Stimulation of cAMP Production by N^6-cyclohexyladenosine (CHA) in RCCT Cells

	cAMP production	
	pmol/mg protein	% change from control
No treatment		
CHA 0	406±38	
10 μM	369±47	−9
30 μM	412±42	+1
100 μM	686±86*	+70
Pertussis toxin		
CHA 0	974±107	
10 μM	912±129	−6
30 μM	1208±104*	+24
100 μM	1654±162*	+70

First passage RCCT cells were treated with 1 μg pertussis toxin/ml culture medium for 12 h and the effects of CHA on cAMP accumulation were determined. Experiments were performed as described for Table I and cAMP production was determined as detailed in Methods.
* $P < 0.05$ when compared with respective sample with no CHA.

ceptors have been reported to be present in rat renal cortex (29, 30).

The present study provides evidence that RCCT cell populations contain both A_1 and A_2 adenosine receptors. Accordingly CHA and PIA produce an inhibition of cAMP production, while NECA produces only slight inhibition. Moreover, the rank order of potency for stimulation (NECA > PIA > CHA) and inhibition (CHA > PIA > NECA) of cAMP production seen with RCCT cells is similar to those reported for other cell types (15, 31–34). In addition, pretreatment of the cells with pertussis toxin completely eliminated the inhibitory action of the adenosine analogs on cAMP accumulation while enhancing the stimulatory action, suggesting the presence of both receptors. Particularly relevant is the observation that 30 μM CHA, which did not increase the cAMP content of untreated RCCT cells, caused a significant increase in cAMP accumulation in cells pretreated with pertussis toxin. In this instance, pertussis toxin appears to have prevented A_1 receptor-mediated inhibition of cAMP production, thereby unmasking the stimulatory effect of CHA acting via the A_2 receptor.

Inhibition by methylxanthines of the effects on cAMP accumulation provides additional evidence that these receptors are extracellular, as opposed to the intracellular P site receptor for adenosine, which is not affected by methylxanthines. The finding of both A_1 and A_2 receptors in cultured RCCT cells that contain both principal and intercalated cell types (14) raises the question of whether both receptor types coexist on the same cell type or if the receptor types are partitioned between subpopulations of the cultured cells. Investigation of the distribution of adenosine receptors on RCCT cells awaits separation of cultured RCCT cells into homogeneous cultures of principal and intercalated cells.

RCCT cells responded to AVP treatment as reported earlier (14), with an increase in cAMP production. CHA caused a significant reduction in the stimulatory effect of AVP. This modulation by CHA of AVP-stimulated cAMP accumulation is in accord with a preliminary study by Berl and Teitelbaum (35) in which they report inhibition of AVP-mediated increases in papillary cell cAMP levels by 0.5 μM adenosine. In RCCT cells, inhibition of AVP-stimulated cAMP accumulation was observed with all three adenosine analogs at stimulatory and inhibitory concentrations. Attenuation by stimulatory concentrations of the analogs was apparently mediated by A_1 receptors, because those concentrations (100 μM) are well above that required for A_1 activation. There does not appear to be a specific interaction between adenosine and AVP because CHA also inhibited isoproterenol-induced stimulation of cAMP production. These results indicate that adenosine can influence tubular transport processes by acting alone or by modulating the actions of other hormones.

Prostaglandin E_2 inhibits AVP-induced increases in cAMP by canine collecting tubule cells (20), and could therefore provide a mechanism for the CHA-induced inhibition of the AVP response in RCCT cells. However, PGE_2 production was unaffected by 100 μM CHA, suggesting that prostaglandins do not mediate the adenosine inhibition of hormone-induced cAMP accumulation.

Hormones that act through receptors to inhibit cAMP accumulation typically activate the inhibitory GTP-binding protein of the adenylate cyclase system (36). This protein, N_i, is a substrate for ADP-ribosylation by pertussis toxin (37), and ADP-ribosylation prevents N_i from exerting its inhibitory effect on adenylate cyclase. Pertussis toxin pretreatment of RCCT cells completely abolished the action of CHA to inhibit basal as well as AVP-stimulated cAMP production. This suggests that N_i is involved in the modulation of the AVP response by CHA. However, it has been reported recently that the rise in intracellular free calcium levels produced by some hormones through the breakdown of membrane phospholipids is also mediated through a pertussis toxin–sensitive N protein (38–40). Therefore, adenosine analogs may cause an increase in intracellular free calcium levels that subsequently result in inhibition of the increased cAMP produced by AVP. An interaction of calcium with AVP-stimulated cAMP has previously been reported by Teitelbaum and Berl (41), where inhibition of AVP-induced cAMP stimulation in rat papillary collecting tubule cells was produced by treatment with calcium ionophore A23187. That adenosine and adenosine analogs may act through intracellular calcium is suggested by the actions of adenosine to inhibit neurotransmitter release from nerve terminals and glucose oxidation in adipose tissue, both of which are thought to be mediated by elevation of intracellular calcium concentrations (6, 42).

The results of the present study provide evidence for stimulatory (A_2) and inhibitory (A_1) adenosine receptors on rabbit cortical collecting tubule cells and suggest a role for adenosine in the regulation of tubular function by a direct action on cAMP production, and by modulation of hormone-induced cAMP production.

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References

1. Osswald, H., W. S. Spielman, and F. G. Knox. 1978. Mechanisms of adenosine-mediated decreases in glomerular filtration rate in dogs. *Circ. Res.* 43:465–469.

2. Spielman, W. S., S. L. Britton, and M. J. Fiksen-Olsen. 1980. Effect of adenosine on the distribution of renal blood flow in dogs. *Circ. Res.* 46:449–456.

3. Tagawa, H., and A. J. Vander. 1970. Effects of adenosine compounds on renal function and renin secretion in dogs. *Circ. Res.* 26: 327–338.

4. Osswald, H., H. J. Schmitz, and R. Kemper. 1978. Renal action of adenosine: effect on renin secretion in the rat. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* 303:95–99.

5. Spielman, W. S. 1984. Antagonistic effect of theophylline on the adenosine-induced decrease in renin release. *Am. J. Physiol.* 247:F246–F251.

6. Hedqvist, P., B. B. Fredholm, and S. Olundh. 1978. Antagonistic effects of theophylline and adenosine on adrenergic neuroeffector transmission in the rabbit kidney. *Circ. Res.* 43:592–598.

7. Churchill, P. C. 1982. Renal effects of 2-chloroadenosine and their antagonism by aminophylline in anesthetized rats. *J. Pharmacol. Exp. Ther.* 222:319–323.

8. Coulson, R., and S. J. Scheinman. 1985. Evidence for renal tubular adenosine receptors. *Kidney Int.* 27:255. (Abstr.)

9. Osswald, H. 1975. Renal effects of adenosine and their inhibition by theophylline. *Naunyn-Schmiedebergs Arch. Pharmakol.* 288:79–86.

10. Lang, M. A., A. S. Preston, J. S. Handler, and J. N. Forrest, Jr. 1985. Adenosine stimulated sodium transport in kidney A6 epithelia in culture. *Am. J. Physiol.* 249:C330–C336.

11. Forrest, J. N., Jr., E. Poeschla, and D. Rieck. 1982. Ribose-specific adenosine receptors mediate cyclic AMP accumulation and chloride transport in the shark rectal gland. *Kidney Int.* 21:253. (Abstr.)

12. Dobbins, J. W., J. P. Laurenson, and J. N. Forrest, Jr. 1984. Adenosine and adenosine analogs stimulate adenosine cyclic 3′,5′-monophosphate-dependent chloride secretion in the mammalian ileum. *J. Clin. Invest.* 74:929–935.

13. Dillingham, M. A., and R. J. Anderson. 1985. Purinergic regulation of basal and arginine vasopressin-stimulated hydraulic conductivity in rabbit cortical collecting tubule. *J. Membr. Biol.* 88:277–281.

14. Spielman, W. S., W. K. Sonnenburg, M. L. Allen, L. J. Arend, K. Gerozissis, and W. L. Smith. 1986. Immunodissection and culture of rabbit cortical collecting tubule cells. *Am. J. Physiol.* 251:F348–F357.

15. Londos, C., D. M. F. Cooper, W. Schlegel, and M. Rodbell. 1978. Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis. *Proc. Natl. Acad. Sci. USA.* 75: 5362–5366.

16. Huang, M., and G. I. Drummond. 1976. Effect of adenosine on cyclic AMP accumulation in ventricular myocardium. *Biochem. Pharmacol.* 25:2713–2719.

17. Frandsen, E. K., and G. Krishna. 1976. A simple ultrasensitive method for the assay of cyclic AMP and cyclic GMP in tissues. *Life Sci.* 18:529–541.

18. Grenier, F. C., T. E. Rollins, and W. L. Smith. 1981. Kinin-induced prostaglandin synthesis by renal papillary collecting tubule cells in culture. *Am. J. Physiol.* 241:F94–F104.

19. Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* 87: 206–210.

20. Garcia-Perez, A., and W. L. Smith. 1984. Apical-basolateral membrane asymmetry in canine cortical collecting tubule cells: bradykinin, AVP, PGE_2 interrelationships. *J. Clin. Invest.* 74:63–74.

21. Spielman, W. S., and C. I. Thompson. 1982. A proposed role for adenosine in the regulation of renal hemodynamics and renin release. *Am. J. Physiol.* 242:F423–F435.

22. Osswald, H., G. Nabakowski, and H. Hermes. 1980. Adenosine as a possible mediator of metabolic control of glomerular filtration rate. *Int. J. Biochem.* 12:263–267.

23. Wysocki, L. J., and G. L. Sato. 1978. "Panning" for lymphocytes: a method of cell selection. *Proc. Natl. Acad. Sci. USA.* 75:2844–2848.

24. Garcia-Perez, A., and W. L. Smith. 1983. Use of monoclonal antibodies to isolate cortical collecting tubule cells: AVP induces PGE release. *Am. J. Physiol.* 244:C211–C220.

25. Daly, J. W. 1982. Adenosine receptors: targets for future drugs. *J. Med. Chem.* 25:197–207.

26. Birnbaumer, L., T. Nakahara, and P. C. Yang. 1974. Studies on receptor-mediated activation of adenylyl cyclases. *J. Biol. Chem.* 259: 7557–7566.

27. Newman, M. E., and A. Levitski. 1983. Desensitization of normal rat kidney cells to adenosine. *Biochem. Pharmacol.* 32:137–140.

28. Abboud, H. E., and T. P. Dousa. 1983. Action of adenosine on cyclic 3′5′-nucleotides in glomeruli. *Am. J. Physiol.* 244:F633–F638.

29. Coulson, R., and W. W. Harrington. 1979. Renal metabolism of N6,02′-dibutyryl adenosine 3′,5′-monophosphate. *Am. J. Physiol.* 237: F75–F84.

30. McKenzie, S. G., and H. P. Bar. 1973. On the mechanism of adenyl cyclase inhibition by adenosine. *Can. J. Physiol. Pharmacol.* 51: 190–196.

31. Londos, C., D. M. F. Cooper, and J. Wolff. 1980. Subclasses of external adenosine receptors. *Proc. Natl. Acad. Sci. USA.* 77:2551–2554.

32. Daly, J. W., R. F. Bruns, and S. H. Snyder. 1981. Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. *Life Sci.* 28:2083–2097.

33. Fain, J. N., and C. C. Malbon. 1979. Regulation of adenylate cyclase by adenosine. *Mol. Cell. Biochem.* 25:143–169.

34. Bruns, R. F. 1980. Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. *Can. J. Physiol. Pharmacol.* 58:673–691.

35. Berl, T., and I. Teitelbaum. 1985. Control of prostaglandin synthesis by rat inner medullary collecting tubule cells: role of cAMP. *Kidney Int.* 27:253. (Abstr.)

36. Rodbell, M. 1980. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature (Lond.).* 284:17–22.

37. Katada, T., G. M. Bokoch, J. K. Northrup, M. Ui, and A. G. Gilman. 1984. The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Properties and function of the purified protein. *J. Biol. Chem.* 259:3568–3577.

38. Molski, T. F. P., P. H. Naccache, M. L. Marsh, J. Kermode, E. L. Becker, and R. I. Sha'afi. 1984. Pertussis toxin inhibits the rise in the intracellular concentration of free calcium that is induced by chemotactic factors in rabbit neutrophils: possible role of "G proteins" in calcium mobilization. *Biochem. Biophys. Res. Commun.* 124:644–650.

39. Nakamura, T., and M. Ui. 1985. Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. *J. Biol. Chem.* 260:3584–3593.

40. Smith, C. D., C. C. Cox, and R. Snyderman. 1986. Receptor coupled activation of phosphoinositide-specific phospholipase C by an N protein. *Science (Wash. DC).* 232:97–100.

41. Teitelbaum, I., and T. Berl. 1986. Effects of calcium on vasopressin-mediated cyclic adenosine monophosphate formation in cultured rat inner medullary collecting tubule cells. Evidence for the role of intracellular calcium. *J. Clin. Invest.* 77:1574–1583.

42. Souness, J. E., and V. Chagoya de Sanchez. 1981. The stimulation of [1-14C]glucose oxidation in isolated fat cells by N6-methyladenosine: an effect independent of cAMP. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 125:249–252.