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Purinergic Regulation of Basal and Arginine Vasopressin-Stimulated Hydraulic Conductivity in Rabbit Cortical Collecting Tubule

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Summary. An extracellular adenosine responsive site that stimulates adenylate cyclase activity has been identified in several tissues. There is limited information on the presence and physiologic significance of adenosine receptors in well-defined segments of the mammalian nephron. We therefore examined the effect of adenosine and selected analogues on basal hydraulic conductivity in rabbit cortical collecting tubules (CCT) perfused in vitro. Adenosine and analogues with an intact ribose moiety produced a significant, sustained increase in hydraulic conductivity. No increase in hydraulic conductivity was seen in either time control CCT's or CCT's exposed to an adenosine analogue with an altered ribose moiety. These experiments are compatible with the presence of a functional adenosine receptor which requires an intact ribose moiety and acts to increase hydraulic conductivity in the mammalian CCT.

An intracellular adenosine responsive site, termed the "*P* site," which inhibits adenylate cyclase activity, has also been described in several tissues. We therefore examined the effect of a *P* site agonist on hydraulic conductivity responses to arginine vasopressin, forskolin and cAMP. *P* site stimulation with 2′5′ dideoxyadenosine inhibited the effect of AVP and of forskolin but not of cAMP to increase hydraulic conductivity. These results are compatible with a functional *P* site in the rabbit CCT which acts at the catalytic subunit of adenylate cyclase to inhibit hydraulic conductivity. Together, these results demonstrate purinergic modulation of basal and arginine vasopressin-stimulated water flux in the mammalian collecting tubule.

Key Words vasopressin · adenosine · water transport

Introduction

Adenosine is produced by all cells and regulates numerous biological processes (Daly, 1982). One mechanism whereby adenosine regulates cell function is by its effect on 3′5′ cyclic adenosine monophosphate (cAMP) formation. Present evidence suggests three sites at which adenosine can modulate cellular cAMP formation (Londos & Wolff, 1977; Londos, Cooper & Wolff, 1980; Daly, 1982; Londos, Wolff & Cooper, 1983). A low affinity cell surface adenosine receptor, termed R_a, stimulates adenylate cyclase activity. Another cell surface receptor, termed R_i, is of higher affinity and inhibits adenylate cyclase activity. A third adenosine responsive site, termed the "*P* site," is located inside the cell, is of lower affinity, and also inhibits adenylate cyclase activity.

Adenosine stimulates cAMP formation in rat glomerular (Abboud & Dousa, 1983) and inner medullary tissue (Woodcock, Loxley, Leung & Johnston, 1984) and in vasopressin-responsive cultured pig kidney cells (Stivelman, Skorecki, Ausiello & Brenner, 1984). However, there is limited information on the presence and physiologic significance of adenosine receptors in well-defined segments of the mammalian nephron. Since osmotic water flux in the mammalian cortical collecting tubule (CCT) is a cAMP-dependent process, we examined the effect of adenosine and selected analogues on water transport in rabbit cortical collecting tubules perfused in vitro. We measured the effect of Ra and P site agonists on basal and arginine vasopressin (AVP) stimulated hydraulic conductivity. Our results provide evidence for an adenosine-mediated effect on water transport in the mammalian collecting tubule.

Materials and Methods

Tubule Perfusion

Isolation and perfusion of CCT was carried out by slight modifications of the method developed by Burg and have been described in detail (Burg, Grantham, Abramow & Orloff, 1966; Horster & Zink, 1982; Dillingham, Kim, Horster & Anderson, 1984). New Zealand white rabbits weighing 1–2 kg were maintained on rabbit chow (Purina, St. Louis, MO) and *ad libitum* water. The animals were killed by cervical dislocation and the left kidney was removed quickly. One- to two-mm slices were cut along the cortico-medullary axis and placed in a glass dish in a solution of NaCl 115, $MgSO_4$ 1.2, $CaCl_2$ 1.0, KCl 5.0, sodium acetate 10, NaH_2PO_4 1.2, $NaHCO_3$ 25, and dextrose 5.5 (all in mmol/liter) with 5% bovine serum albumin. This solution was maintained at 17°C (petri dish on crushed ice) and pH of 7.4

Table 1. Effect of adenosine (10^{-4} M), NECA (10^{-4} M), 2′5′ dideoxyadenosine (10^{-4} M) and AVP (50 μU/ml) on basal L_p and J_v

Group	n	Length (mm)	Net volume flux (nl/mm/min)		Hydraulic conductivity (cm/atm sec × 10^{-7})	
			Basal	Stimulated	Basal	Stimulated
Adenosine	7	1.00 ± 0.15	0.07 ± 0.05	0.50 ± 0.05[a]	5.1 ± 4.0	40.4 ± 4.9[a]
NECA	6	1.18 ± 0.11	0.14 ± 0.06	0.64 ± 0.09[a]	12.0 ± 5.3	61.8 ± 6.1[a]
2′5′ dideoxyadenosine	8	1.13 ± 0.10	0.25 ± 0.07	0.12 ± 0.03	22.2 ± 5.1	14.3 ± 2.7
AVP	6	1.28 ± 0.20	0.15 ± 0.06	0.60 ± 0.07[a]	12.0 ± 5.0	53.7 ± 6.6[a]
Time control	5	1.48 ± 0.18	0.22 ± 0.08	0.10 ± 0.02	19.0 ± 7.0	9.2 ± 2.0

[a] = $P < 0.05$ versus basal value.

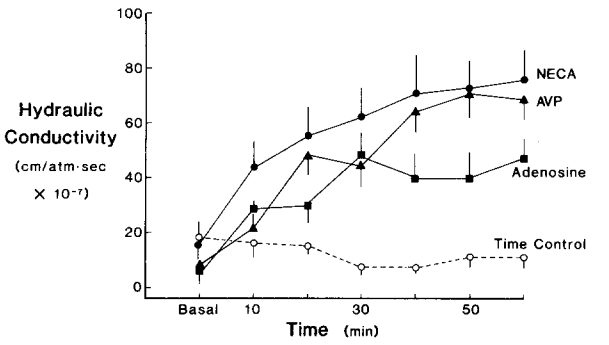

Fig. 1. Time course of effect of adenosine (10^{-4} M) NECA (10^{-4} M), and (AVP 50 μU/ml) added to bathing fluid on hydraulic conductivity. Tubules exposed to 2′5′ dideoxyadenosine (10^{-4} M) did not differ from time control tubules

during dissection. The cortex was separated from outer medulla and CCT dissected from medullary rays. Tubules were taken distal to the last branch. Tubules were transferred to a Lucite perfusion chamber (volume about 2.0 ml) set on the stage of an inverted phase contrast microscope (Olympus, Model CK, Tokyo, Japan).

Tubules were perfused by an assembly of concentric glass pipettes described previously (Dillingham et al., 1984). Tubules were usually perfused within 30 min of kidney removal. During perfusion, tubules were bathed in the same solution used for dissection except that no albumin was present. Bath fluid of pH 7.40 and temperature of 25°C was completely changed every 3–4 min (Hall & Grantham, 1980). Because perfusion rate affects hydraulic conductivity response to arginine vasopressin (Dixon & Anderson, 1985), special care was taken to maintain constant collection rates by slight adjustments in hydrostatic pressure of the fluid entering the fluid exchange pipette. Perfusion rates averaged between 10 and 12 nl/mm/min in all groups of tubules. Perfusion fluid composition was the same as bathing fluid except that the final concentration of NaCl was reduced to 50 mmol/liter. Perfusion fluid also contained a sufficient amount of ^{14}C inulin (New England Nuclear, Boston, MA) to result in collected fluid cpm at least 10- to 15-fold above background, which were usually 30–40 cpm. The ^{14}C inulin was dissolved in perfusion solution and passed through a 0.22 μm filter immediately prior to use. The tubule was visually inspected at 1–3 min intervals throughout the study. Tubular length was measured at the conclusion of each study by a calibrated microscope eyepiece reticle.

Collected samples were obtained as described previously (Dillingham et al., 1984) and were placed under water-equilibrated mineral oil on the bottom of a siliconized dish. Seventy nanoliter aliquots of these samples were taken for scintillation counting (10 min per sample; Packard Tricarb 460C).

Perfusion rate was calculated by the rate of appearance of the impermeant marker ^{14}C inulin in the collection pipette according to the algorithm:

$$\text{Perfusion rate} = \frac{[^{14}C]c}{[^{14}C]p} \times \text{collection rate}$$

where $[^{14}C]c$ = cpm of collected fluid

$[^{14}C]p$ = cpm of perfusate.

Collection rate was measured directly with a calibrated volumetric pipette and stopwatch. Net volume flux (J_v) was calculated by subtracting the collection rate from the perfusion rate. All studies with (−) basal J_v's were excluded.

Hydraulic conductivity was calculated from the formula (Al-Zahid, Schafer, Troutman & Andreoli, 1977):

$$L_p = \frac{V_o C_o}{\text{RTA}} \left[\frac{C_o - C_L}{C_o C_L C_b} + \frac{1}{(C_b)^2} \ln \frac{(C_L - C_b)C_o}{(C_o - C_b)C_L} \right]$$

where L_p is in cm/atm · sec × 10^{-7}, V_o is the perfusion rate in cm³/sec; C_o, C_L, and C_b are the osmotic concentrations of perfusate, collected fluid, and bathing medium, respectively; R is the gas constant, T is the absolute temperature, and A is the surface area (calculated from the length of the tubule and an assumed internal diameter of 20 μm). Collected fluid osmolality was calculated from the measured perfusate osmolality and the relative increase in the concentration of the volume marker measured in the collected fluid.

The following studies were performed:

Effect of Adenosine and Selected Analogues on Basal J_v and L_p

In these studies, the effects of adenosine and adenosine analogues on basal J_v and L_p were measured. After 3 hr equilibration at 25°C, 3–4 basal samples were obtained using a 90 nl collection pipette. Subsequently, 4–6 collections were obtained immediately following the addition of adenosine 10^{-5} and 10^{-4} M, N-ethylcarboxyamide adenosine (NECA, an agent with high affinity for the R_a site, 10^{-4} and 10^{-3} M), 2′5′ dideoxyadenosine (DDA, an agent without R_a site activity, 10^{-4} M) and arginine

Table 2. Effect of 2'5' dideoxyadenosine (DDA) on net volume flux response to AVP forskolin and ClPheScAMP

Group	*n*	Length (mm)	Net volume flux (nl/mm/min)	
			Basal	Stimulated
AVP	8	1.11 ± 0.19	0.20 ± 0.07	0.90 ± 0.14[a]
DDA + AVP	9	1.12 ± 0.10	0.15 ± 0.05	0.48 ± 0.08[a,b]
Forskolin	7	1.04 ± 0.15	0.25 ± 0.06	0.79 ± 0.11[a]
DDA + forskolin	5	1.53 ± 0.16[b]	0.20 ± 0.07	0.30 ± 0.05[b]
ClPheScAMP	8	1.12 ± 0.12	0.15 ± 0.08	0.88 ± 0.09[a]
DDA + ClPheScAMP	5	1.03 ± 0.11	0.18 ± 0.07	1.12 ± 0.10[a]

[a] $P < 0.05$ versus basal.
[b] $P < 0.05$ versus tubules not exposed to DDA.
Concentrations of agents were AVP (250 μU/ml), forskolin (5×10^{-5} M), ClPheScAMP (10^{-4} M) and DDA (10^{-4} M).

vasopressin (AVP, 50 μU/ml) to the bathing fluid. All agents were dissolved in bathing fluid. Collections were also obtained in a time control group of studies in which no agent was added to bathing fluid. For data analysis, the values of J_v and L_p were calculated for each 10-min collection period before and immediately after addition of the test agent. The values for individual collection periods for 2–3 periods before and all periods following the test agent were meaned to arrive at a single basal and stimulated J_v and L_p value for each tubule.

Effect of DDA on J_v and L_p Response to AVP, Forskolin and ClPheScAMP

In these studies, J_v and L_p were measured before and following addition of supramaximal concentrations of AVP (250 μU/ml) to the bathing fluid in a group of tubules exposed for 30 min to DDA (a "*P* site" agonist, 10^{-4} M) and in another group of tubules exposed for 30 min to bathing fluid alone (the carrier solution of DDA). Additional studies were carried out to examine the site of the observed effect of DDA to inhibit AVP-stimulated J_v and L_p. In these studies, J_v and L_p were measured before and following addition of forskolin (5×10^{-5} M) to the bathing fluid of either a group of tubules exposed to DDA (10^{-4} M) for 30 min or a group of control tubules exposed for 30 min to bathing fluid alone. In a final group of experiments, J_v and L_p were measured before and following addition of ClPheScAMP (10^{-4} M) to the bathing fluid of tubules exposed to either DDA (10^{-4} M) or to control tubules not exposed to DDA. The mean J_v and L_p before and following AVP, forskolin and ClPheScAMP were compared in control and in DDA-treated tubules.

Results shown are mean ± SEM. The level of statistical significance was calculated using Student's paired or unpaired *t* test, as required by the experimental design (Swinscow, 1980; Diem & Lentner, 1970).

Results

In preliminary experiments, adenosine at 10^{-6} and 10^{-5} M did not exert a consistent effect on basal L_p ($n = 5$). At 10^{-4} M, adenosine resulted in a sustained hydroosmotic response (Table 1, Fig. 1). Higher concentrations of adenosine did not increase L_p further. N-ethylcarboxyamideadenosine did not affect basal J_v and L_p at 10^{-6} M and only slightly increased J_v and L_p at 10^{-5} M ($n = 3$). At 10^{-4} M, NECA resulted in a sustained increase in J_v and L_p (Table 1, Fig. 1). Higher concentrations of NECA (10^{-3} M) did not further increase J_v and L_p. The effect of NECA to increase L_p was comparable in magnitude and time course that to that observed after 50 μU/ml AVP (Fig. 1). A second adenosine analogue with an intact ribose moiety (2-chloroadenosine, 10^{-4} M) also increased basal L_p (15.0 ± 5.0 to 64.0 ± 5.0, $P < 0.05$, $n = 4$). The increases in J_v and L_p following adenosine, NECA and 2-chloroadenosine were associated with accentuation of intercellular spaces and cellular swelling similar to that seen following AVP. When NECA and 2-chloroadenosine were removed from bathing fluid, a decrease in intercellular space and cell swelling as well as a prompt decline in L_p to basal values occurred (63.6 ± 13.6 to 10.0 ± 4.0, $P < 0.05$, $n = 4$). The compound 2'5' dideoxyadenosine (DDA) did not affect basal J_v and L_p and no increase in J_v and L_p was observed in time control tubules (Table 1, Fig. 1).

The effect of DDA on J_v and L_p response to supramaximal concentrations (250 μU/ml) of AVP are in Table 2 and Fig. 2. DDA decreased J_v and L_p response to AVP. To determine if DDA also inhibits the established phase of L_p response to AVP, studies were performed in which each tubule served as its own control. In these studies ($n = 4$), L_p following 250 μU/ml AVP was reduced in each tubule by addition of 10^{-4} M DDA to bathing fluid containing AVP (83.0 ± 11.1 to 59.3 ± 9.6, respectively, $P < 0.05$). To determine if adenosine affects response to AVP, 4 CCT's were bathed in 10^{-4} M adenosine for 30 min. Arginine vasopressin (50 μU/ml) resulted in a further increase in J_v and L_p in each of 4 tubules.

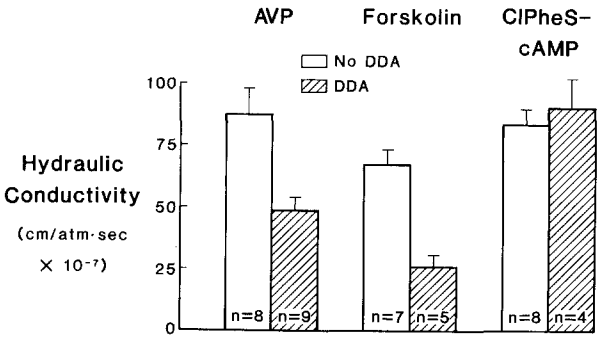

Fig. 2. Effect of 2'5' dideoxyadenosine (DDA) on hydraulic conductivity response to AVP, forskolin and ClPheScAMP

Measurements of water permeability in these tubules after combined adenosine and AVP did not differ significantly from the six tubules exposed to 50 μU/ml of AVP alone (Table 1).

Additional studies were undertaken to determine the site at which DDA inhibits the hydroosmotic effect of AVP. We first examined the effect of DDA on L_p response to forskolin, an agent which can act independent of both an AVP receptor and a functional stimulatory regulatory unit to activate adenylate cyclase and stimulate rabbit CCT J_v and L_p (Dillingham et al., 1984). DDA also resulted in a significant inhibition of J_v and L_p response to forskolin (Table 2, Fig. 2). By contrast, DDA did not significantly affect J_v and L_p responses to ClPheScAMP (Table 2, Fig. 2).

Discussion

Adenosine-responsive sites capable of regulating cellular cAMP formation are known to exist in several tissues (Londos & Wolff, 1977; Londos et al., 1980, 1983; Daly, 1982). Although many transport processes are cAMP-dependent, there is limited information on the presence and functional significance of adenosine responsive sites in mammalian transporting epithelial tissues. Recently, Dobbins and coworkers demonstrated that adenosine and some of its analogues increase short-circuit current, net chloride secretion and cAMP content in the rabbit ileum (Dobbins, Laurenson & Forrest, 1984). The stimulatory effects of adenosine analogues could be partially blocked by an adenosine receptor antagonist (Dobbins et al., 1984). These observations provide the first evidence for a functional adenosine receptor which stimulates cAMP-dependent ion transport in a mammalian epithelium (Dobbins, Laurenson & Forrest, 1984).

In the present studies, adenosine resulted in a sustained increase in L_p suggestive of an R_a effect. Since adenosine is rapidly transported into renal epithelial cells (Hir & Dubach, 1984; Trimble & Coulson, 1984) and, at the concentrations studied, can affect all three adenosine-responsive sites (Londos & Wolff, 1977; Londos et al., 1980, 1983; Daly, 1982), we also examined the effect of NECA on L_p. The compound NECA is an adenosine analogue with an intact ribose moiety and high affinity for the R_a receptor (Londos & Wolff, 1977; Londos et al., 1980, 1983; Daly, 1982). The increase in L_p following NECA was comparable to that observed with 50 μU/ml of AVP and was reversible upon removal of NECA. Another adenosine analogue with an intact ribose moiety, 2-chloroadenosine also increased L_p and J_v. An increase in L_p was not observed in either time control tubules or in tubules exposed to 2'5' dideoxyadenosine, an adenosine analogue with an altered ribose moiety and without R_a site activity (Londos & Wolff, 1977; Londos et al., 1980, 1983; Daly, 1982). Recent studies demonstrate an effect of adenosine and NECA to stimulate cAMP formation in AVP-responsive tissues such as rat renal medullary homogenates and pig kidney cells (Stivelman et al., 1984; Woodcock et al., 1984). Together these biochemical studies and the present physiologic observations support the presence of a functional R_a receptor which requires an intact ribose moiety and acts via cAMP to stimulate osmotic water flux in the rabbit cortical collecting tubule.

Our results also suggest the presence of a functional "*P* site" in the mammalian cortical collecting tubule. Thus, DDA, a known *P* site agonist (Londos & Wolff, 1977; Londos et al., 1980, 1983; Daly, 1982; Pohlman, Yates, Needleman & Klahr, 1983), inhibited the J_v and L_p response to AVP. Inhibition of AVP-stimulated J_v and L_p was observed when DDA was given both prior to and following AVP exposure. DDA also inhibited the hydroosmotic response to forskolin, an agent which can stimulate adenylate cyclase activity and increase J_v and L_p independent of the AVP receptor and of functional stimulatory guanine nucleotide regulatory proteins (Seamon, Padgett & Daly, 1981; Dillingham et al., 1984). However, DDA did not inhibit the J_v and L_p response to ChPheScAMP. Together, there observations are compatible with a functional *P* site which acts directly at the catalytic subunit of adenylate cyclase to inhibit AVP stimulated osmotic water flux in the rabbit cortical collecting tubule. Biochemical studies will be necessary to confirm these observations.

The presence of adenosine responsive sites that both increase basal water flux and inhibit AVP-stimulated water flux in the rabbit CCT is of interest and

has been found in other tissues (Daly, 1982). Moreover, biochemical studies in vasopressin-responsive LLC PK kidney cells also suggest the presence of a R_i receptor (Roy, 1984). Thus, it is clear that additional studies will be necessary to determine if a functional R_i receptor is present in rabbit CCT or if intracellular and extracellular adenosine is an endogenous regulator of AVP action.

In summary, the present studies demonstrate that adenosine and analogues with an intact ribose moiety increase J_v and L_p, supporting the presence of a functional R_a receptor in the rabbit CCT. Our results also support the presence of a functional P site in rabbit CCT which inhibits L_p response to AVP by preventing cAMP formation. Together, these observations provide the first evidence for purinergic regulation of basal and AVP-stimulated L_p in the mammalian nephron.

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