Adenosine stimulates the basolateral 50 pS K channels in the thick ascending limb of the rat kidney

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Gu R, Wang J, Zhang Y, Li W, Xu Y, Shan H, Wang W-H, Yang B-F. Adenosine stimulates the basolateral 50 pS K channels in the thick ascending limb of the rat kidney. Am J Physiol Renal Physiol 293: F299–F305, 2007. First published May 2, 2007; doi:10.1152/ajprenal.00008.2007.—We used the patch-clamp technique to examine the effect of adenosine on the basolateral K channels in the thick ascending limb (TAL) of the rat kidney. A 50-pS inwardly rectifying K channel was detected in the basolateral membrane, and the channel activity was decreased by hyperpolarization. Application of adenosine (10 μM) increased the activity of basolateral 50 pS K channels, defined by NPo, from 0.21 to 0.41. The effect of adenosine on the 50 pS K channels was mimicked by cyclohexyladenosine (CHA), which increased channel activity by a dose-dependent manner. However, inhibition of the A1 adenosine receptor with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) failed to block the effect of CHA. In contrast, application of 8-(3-chlorostoryl) caffeine (CSC), an A2 adenosine antagonist, abolished the stimulatory effect of CHA. The possibility that the effect of adenosine and adenosine analog on the basolateral 50 pS K channel was the result of activation of the A2 adenosine receptor was also supported by the observation that application of CGS-21680, a selected A2A adenosine receptor agonist, increased the channel activity. Also, inhibition of PKA with N-[2-(methylamino)ethyl]-5-isouquinoline sulfonamide-2HC1 abolished the stimulatory effect of CHA on the basolateral 50 pS K channel. Moreover, addition of the membrane-permeable cAMP analog increased the activity of 50 pS K channels. We conclude that adenosine activates the 50 pS K channel in the basolateral membrane of the TAL and the stimulatory effect is mainly mediated by a PKA-dependent pathway via the A2 adenosine receptor in the TAL.

METHODS

Preparation of the TAL. Pathogen-free Sprague-Dawley rats of either sex (<90 g) were purchased from the animal facility of the Second Affiliated Hospital of Harbin Medical University. The animals were kept on a normal rat chow and had free access to water for 7 days before experiments. Rats were killed by cervical dislocation, and the kidneys were removed immediately. Thin coronal sections were cut with a razor blade, and kidney slices were incubated in a HEPES buffer solution containing collagenase type 1A (1 mg/ml; Sigma, St. Louis, MO) at 37°C for 45–60 min. After the collagenase treatment, the kidney slices were rinsed with a solution containing (in mM): 140 NaCl, 5 KCl, 1.5 MgCl2, 1.8 CaCl2, and 10 HEPES (pH 7.4). We used an Axon 200A patch-clamp amplifier to record channel current. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
current was low-pass filtered at 0.5 KHz and digitized by an Axon interface (Digidata 1200). Data were collected by an IBM-compatible Pentium computer at a rate of 2 KHz and analyzed by using pClamp software system 6.04 (Axon Instruments, Burlingame, CA). Channel activities were defined as $N P_o$, a product of channel open probability ($P_o$) and channel number ($N$). The $N P_o$ was calculated from data samples of 60-s duration in the steady state as follows:

$$ NP_o = \sum (1t_1 + 2t_2 + \cdots + it_i) $$

where $t_i$ is the fractional open time spent at each of the observed current levels. Because the onset time of the adenosine effect varied and depended on adenosine concentrations, data selected for analysis were not always at the same time point. Thus, we selected a 60s-long trace at the steady state after the onset of adenosine effect to calculate the channel activity. Because the 50 pS K channel activity is very stable without stimulation by adenosine, the selection of the data at different time points should not affect the interpretation of the results. The channel conductance was determined by measuring K current at several different holding potentials.

Chemicals and statistics. Adenosine, cyclohexyladenosine (CHA), the selected $A_2A$ adenosine receptor agonist CGS-21680, 8-(3-chlorostyryl) caffeine (CSC), 8-Cyclopentyl-1, 3-dipropylxanthine (DPCPX), and dibutyryl cAMP (db-cAMP) were purchased from Sigma. N-[2-(methylamino)ethyl]-5-isoquinoline sulfonamide-2HCl (H8) was obtained from Biomol. Data are shown as means (SD). We used Student’s $t$-tests to determine the significance between the control and experimental periods. If the $P$ value is <0.05, the difference was considered to be significant.

RESULTS

We confirmed our previous finding that a 50 pS K channel is highly expressed in the basolateral membrane of the TAL. Fig. 1 is a typical recording showing the activity of a 50 pS K channel in the basolateral membrane of the TAL with 140 mM KCl in the pipette and Na Ringer in the bath. As shown before (8), the channel activity decreased when the membrane potential was hyperpolarized. The mean $NP_o$ was 0.26 ± 0.08 under control conditions (spontaneous cell membrane potential) and decreased to 0.08 ± 0.01 by a 60-mV hyperpolarization ($n = 4$).

Fig. 1. A recording showing the activity of the basolateral K channel in a cell-attached patch. The holding potential (equivalent to the cell membrane potential) is indicated on the top of each trace, and the channel-closed level is indicated by a dotted line and C.

We next examined the effect of adenosine on the basolateral 50-pS K channel in cell-attached patches. Fig. 2 is a recording demonstrating that application of adenosine (10 μM) increased the channel activity ($NP_o$) from 0.21 ± 0.06 to 0.41 ± 0.15 ($n = 8$, $P < 0.01$). Because adenosine could stimulate both A1 and A2 adenosine receptors, which have been shown to be expressed in the TAL (28), we then used CHA, an analog of

Fig. 2. The effect of adenosine (10 μM) on the 50 pS K channel activity in the basolateral membrane of the thick ascending limb (TAL). The experiment was performed in a cell-attached patch. The top trace shows the time course of the experiment. Two parts of the trace, indicated by numbers, are extended to show the fast time resolution. The holding potential was 0 mV, and the channel closed current is indicated by “C.”

Fig. 3. The effect of cyclohexyladenosine (CHA; 5 μM) on the 50 pS K channel in the basolateral membrane of the TAL. The experiment was performed in a cell-attached patch with 140 mM K solution in the pipette and the Ringer solution in the bath. The top trace shows the experimental time course. Two parts of the trace, indicated by numbers, are extended to show the fast time resolution. The holding potential was 0 mV, and the channel closed currents is indicated by “C.”
adenosine, which has a higher affinity to the A1 adenosine receptor than to the A2 adenosine receptor, to test whether CHA could stimulate the 50 pS K channels in the TAL. Fig. 3 is a representative recording showing that CHA activated the 50 pS K channel in a cell-attached patch. Application of 5 μM CHA increased channel activity from 0.21 ± 0.05 to 0.36 ± 0.13 (n = 8, P < 0.01) within 5–8 min. The stimulatory effect of CHA on the 50 pS K channel was dose-dependent. Fig. 4 is a recording from five such experiments showing the effect of 0.5 μM and 10 μM CHA on the 50 pS K channels. It is apparent that application of 0.5 μM CHA had no significant effect on the 50 pS K channels (control, 0.24 ± 0.06 and 0.5 μM CHA, 0.27 ± 0.09) but 10 μM CHA significantly increased NPo to 0.48 ± 0.1 (P < 0.01) within 3–5 min.

After demonstrating that CHA mimicked the effect of adenosine, we examined the effect of CGS21680, a selective A2A adenosine receptor agonist, on the 50 pS K channel activity. Fig. 5 is a representative recording from seven such experiments demonstrating the effect of CGS21680 on the 50 pS K channels. Similar to adenosine and CHA, application of CGS21680 (1 μM) increased the channel activities from 0.26 to 0.56 in this particular patch. Fig. 6 is a dose-response curve showing the effect of CGS21680 on the 50 pS K channels and demonstrates that further increasing CGS21680 concentrations to 10 and 20 μM raises NPo to 0.76 ± 0.1 (P < 0.05, n = 4) and 0.83 ± 0.2 (P < 0.05, n = 3), respectively. Because application of CHA and CGS21680 has the same stimulatory effect on the basolateral 50 pS K channels, we speculated that the effect of CHA on the 50 pS K channel was also induced by stimulation of the A2 adenosine receptor rather than the A1 adenosine receptor. It has been shown that high concentrations of CHA could activate the A2 adenosine receptor and increase cAMP production in nTAL cells (1, 25). To test this hypothesis, we studied the effect of CHA on the basolateral 50 pS K channels at different doses. Decreasing CHA concentrations...
below to 2 μM had no significant effect on channel activity whereas increasing CHA concentrations progressively augmented the channel activity (Fig. 6). Increased CHA concentrations to 10, 20, and 40 μM significantly raised the mean NPo from 0.21 ± 0.05 (control) to 0.48 ± 0.1, 0.65 ± 0.1 and 0.79 ± 0.1, respectively.

To further test the hypothesis that the effect of adenosine and adenosine analog on the basolateral 50 pS K channel was the result of stimulation of the A2 adenosine receptor rather than the A1 adenosine receptor, we examined the effect of CHA in the presence of DPCPX, an antagonist of the A1 adenosine receptor (17). Fig. 7 is a recording showing the effect of 10 μM CHA on the 50 pS K channels in the presence of DPCPX (1 μM). Inhibition of A1 adenosine receptor not only had no significant effect on the channel activity but also failed to block the effect of CHA on K channels because the addition of 10 μM CHA increased the channel activity to 0.45 ± 0.06 (n = 4), a value not significantly different from the corresponding control value without DPCPX (0.48 ± 0.1). Data summarized in Fig. 8 show that addition of CHA (5 μM) still increased channel activity from 0.22 ± 0.06 to 0.39 ± 0.10 (n = 5, P < 0.05) in the presence of DPCPX. In contrast, the stimulatory effect of CHA on K channels was absent in the presence of CSC, an agent which blocks A2A (13) and to some extent also the A2B adenosine receptor (5). Fig. 9 is a representative recording showing that inhibition of the A2 adenosine receptor with 1 μM CSC abolished the effect of CHA. Fig. 8 summarizes results from five such experiments showing that addition of CHA did not alter channel activity in the presence of CSC (control NPo, 0.28 ± 0.13; CHA, 0.32 ± 0.10). Thus, our results suggest that the effect of CHA was the result of stimulation of A2 adenosine receptor.

Stimulation of A2 adenosine receptor has been shown to activate adenylate cyclase and increase cAMP production. We hypothesize that the effect of adenosine on the basolateral 50 pS K channels was mediated by PKA. Thus, we first examined whether stimulation of PKA increases the K channel activity in the basolateral membrane of the TAL. Fig. 10 is a recording showing that addition of 100 μM db-cAMP, a membrane-permeable cAMP analog, increased the channel activity from 0.21 ± 0.06 to 0.47 ± 0.08 (P < 0.01, n = 5). We then...
examine the effect of CHA in the TAL which has been treated by H8 (1 \mu M), an inhibitor of PKA (Fig. 11). Indeed, inhibition of PKA completely abolished the effect of CHA on the basolateral 50 pS K channels. Data summarized in Fig. 8 demonstrate that in the presence of H8, NPo before CHA and after CHA was 0.21 ± 0.04 and 0.25 ± 0.03 (n = 5), respectively. Thus, inhibition of PKA abolished the effect of adenosine on the basolateral K channels in the TAL.

DISCUSSION

The main findings of the present study are that adenosine increases the basolateral 50 pS K channel activity in the TAL and that the stimulatory effect of adenosine is mediated by stimulation of the cAMP-PKA pathway. This view is supported by the observation that inhibition of PKA abolished the stimulatory effect of adenosine or adenosine analog on the basolateral K channels. Adenosine plays an important role in regulating renal blood flow, glomerular filtration rate (9, 17), renin secretion (14), tubuloglomerular feedback, and epithelial transport (2, 20, 27). It has been reported that adenosine increases bicarbonate exit across the basolateral membrane (27), inhibited the Na/H exchanger 3 (NHE3) in the proximal tubule cells (24) and regulated Na transport in A6 cells (16).

We have previously demonstrated that adenosine stimulated the apical 70 pS K channels in the TAL and inhibited epithelial Na channels (ENaC) in the cortical collecting duct (26). The TAL is responsible for the reabsorption of 20–25% of filtered Na load and plays a key role in mediating urinary concentrating ability. We have previously shown that adenosine stimulates the apical 70 pS K channels (15), which play an important role in K recycling across the apical membrane. Because K recycling is essential for maintaining the normal function of the Na/K/Cl cotransporter, adenosine should play a role in the regulation of Na transport in the TAL. Two functions are served by basolateral K channels: 1) they are responsible for K recycling across the basolateral membrane; and 2) they are involved in generating the cell membrane potential. Because Cl exit across the basolateral membrane is through Cl channels and is electrogenic, the Cl current is expected to be affected by alteration in basolateral membrane such that hyperpolarization increases, while depolarization decreases the driving force for Cl exit. Therefore, the basolateral membrane potential determines the driving force for Cl exit across the basolateral membrane. Thus, adenosine-induced increase in basolateral 50 pS K channel activity is expected to stimulate Cl exit and thus NaCl absorption in the TAL.

Although adenosine could stimulate Na absorption in the TAL through activation of apical and basolateral K channels, it inhibits ENaC in the cortical collecting duct (CCD) (29). This seeming paradox regarding the effect of adenosine on Na transport may have a physiological significance. High Na intake has been shown to increase adenosine concentrations in the renal interstitial tissue (23). Thus, adenosine may play a role in suppressing the Na absorption during high-salt intake. In contrast, adenosine may play a role in high Na delivery-induced stimulation of Na transport in the TAL. An increase in Na delivery to the TAL is expected to stimulate Na transport and increase ATP consumption. As a consequence of an increase in adenosine concentrations, adenosine stimulates the apical K channels and K recycling, which enhances the activity of the Na/K/Cl cotransporter and basolateral K channels. Thus, Cl exit across the basolateral membrane is facilitated due to hyperpolarization induced by high basolateral K channel activity. Therefore, adenosine can serve as a positive feedback mediator for Na transport during tubuloglomerular feedback. Also, the effect of adenosine on ENaC is through the activation of the A1 adenosine receptor. It has been reported that salt intake alters the expression of adenosine receptors in the kidney (23). It is possible that A2 and A1 adenosine receptors in the TAL and CCD are differently regulated. Thus, the net effect of adenosine on Na transport depends on salt intake and...
the conflicting effect of adenosine on Na transport in the kidney would not occur in the same physiological setting.

Three types of adenosine receptors, A1, A2A, and A2B, have been shown to be expressed in the kidney (12, 18). Stimulation of the A1 receptor has been shown to decrease cAMP concentrations, activate PKC, and stimulate phospholipase A2. Activation of A2A or A2B adenosine receptors has been reported to increase cAMP production and stimulate PKA (21). Both A1 and A2A receptors are expressed in the renal vascular structure: stimulation of the A2A adenosine receptor causes vasodilation in the kidney (9), while activation of the A1 adenosine receptor is responsible for vasoconstriction of the afferent arteriole of the glomerulus. Using RT-PCR technique, it has been reported that the A1 adenosine receptor is heavily expressed in the CCD and, to a lesser extent, in the TAL, whereas the A2B adenosine receptor is highly expressed in the TAL (28). Although the expression of the A2A adenosine receptor has not been detected in the TAL, functional assay has demonstrated the presence of the A2 adenosine receptor because incubation of TAL cells with adenosine analogs increases cAMP production (1).

In the present study, we have observed that both CHA, a presumably A1 adenosine receptor agonist, and CGS21680, an agonist of the A2 adenosine receptor, have the same stimulatory effect on the basolateral 50 pS K channel. Two lines of evidence indicate that the effect of CHA on the basolateral K channels was mediated by stimulation of the A2 adenosine receptor rather than A1 adenosine receptor: 1) the effect of CHA was completely abolished by CSC, an agent which blocks A2 adenosine receptors, but not by inhibition of the A1 adenosine receptor; 2) the effect of CHA on the basolateral 50 pS K channels was absent after inhibition of PKA. It is possible that neither raising cellular Ca2+ nor decreasing intracellular cAMP affects the basal activity of the 50 pS K channel. Thus, even if CHA could activate the A1 adenosine receptor and decrease cAMP, stimulation of the A1 adenosine receptor is not expected to alter the channel activity. This speculation is also supported by the finding that inhibition of PKA did not decrease the basal level of 50 pS K channel activity. Thus, it is conceivable that high concentrations of CHA increased cAMP via A2A/A2B adenosine receptors. Consequently, CHA mimicked the effect of CGS21680 and stimulated the 50 pS K channels. Although CGS21680 is a very specific A2A adenosine receptor agonist, it could also activate A2B adenosine receptor with a low affinity (4). Thus, we could not determine whether A2A or A2B adenosine receptor is involved in mediating the effect of adenosine. However, the observation that DPCPX, an agent which has been reported to block both A1 and A2B adenosine receptors (3), did not abolish the stimulatory effect of CHA suggests the possibility that A2A adenosine receptor may be responsible for the effect of adenosine on the basolateral 50 pS K channels. We conclude that adenosine activates the 50 pS K channel in the basolateral membrane of the TAL and that the stimulatory effect of adenosine is mediated by a cAMP-dependent pathway via the A2 adenosine receptor in the TAL.

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