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Extracellular ATP Stimulates Na^+ and Cl^- Transport through the Activation of Multiple Purinergic Receptors on the Apical and Basolateral Membranes in M-1 Mouse Cortical Collecting Duct Cells

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The mammalian cortical collecting duct (CCD) plays a major role in regulating renal NaCl reabsorption, which is important in Na^+ and Cl^- homeostasis. The M-1 cell line, derived from the mouse cortical collecting duct, has been used as a mammalian model of the study on the electrolytes transport in CCD. M-1 cells were grown on collagen-coated permeable support and short circuit current (I_{sc}) was measured. M-1 cells developed amiloride-sensitive current 5～7 days after seeding. Apical and basolateral addition of ATP induced increase in I_{sc} in M-1 cells, which was partly retained in Na^+-free or Cl^--free solution, indicating that ATP increased Na^+ absorption and Cl^- secretion in M-1 cells. Cl^- secretion was mediated by the activation of apical cystic fibrosis transmembrane regulator (CFTR) chloride channels and Ca^{2+}-activated chloride channels, but Na^+ absorption was not mediated by activation of epithelal sodium channel (ENaC). ATP increased cAMP content in M-1 cells. The RT-PCR analysis demonstrated that M-1 cells express $P2Y_2$, $P2X_3$ and $P2Y_4$ receptors. These results showed that ATP regulates Na^+ and Cl^- transports via multiple P2 purinoceptors on the apical and basolateral membranes in M-1 cells.

Key Words: Cortical collecting duct cells, ATP, Ion transport, Purinergic receptors, Expression

INTRODUCTION

Nucleotides in the extracellular fluid act as autocrine and paracrine hormones to regulate a variety of physiological processes (Abbracchio et al, 1994; Barnard et al, 1994; Harden et al, 1995). ATP may be released from nerve endings, immune cells, and epithelial cells under physiological and pathophysiological conditions (Schwiebert et al, 1999). Once released from cells, nucleotides interact with specific plasma membrane-resident purinoreceptors that act as ion channels or couple with phospholipases and adenylyl cyclase-driven signal transduction pathways (Abbracchio et al, 1994; Barnard et al, 1994; Harden et al, 1995). Purinoceptors are divided into two classes: P1 (activated by adenosine) and P2 (activated by ATP and ADP). P2 purinoceptors have been subdivided into two families: P2X and P2Y (Abbracchio et al, 1994; Barnard et al, 1994; Harden et al, 1995). The P2X purinoceptor family contains at least eight distinct subtypes, each of which is a two transmembrane-spanning, ion channel-forming proteins and form Ca^{2+}-permeable, nonselective cation channels (Abbracchio et al, 1994; Barnard et al, 1994; Harden et al, 1995). P2Y purinoceptors are G protein-coupled metabotropic receptors containing at least six distinct receptor subtypes (Abbracchio et al, 1994; Barnard et al, 1994; Boarder et al, 1995).

Purinoceptors, mostly $P2Y_2$, have been identified along the length of the nephron, including the cortical collecting duct (CCD), of which activation by extra-

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cellular ATP modulates ion transport in various renal epithelial cell lines. In A6 cells, an amphibian distal renal cell line, ATP activates Cl^-, K^+ and non-selective cation channels (Middleton et al, 1993; Nilius et al, 1995; Mori et al, 1996; Atia et al, 1999). In Madin-Darby canine kidney (MDCK) cells, which display characteristics of distal nephron epithelial cells, ATP stimulates K^+ channels (Jungwirth et al, 1989), basolateral capacitive Ca^{2+} entry (Gordjani et al, 1997) and electrogenic chloride secretion (Simmons et al, 1981; Woo et al, 1998). In the collecting duct, ATP has been shown to inhibit apical small conductance K^+ channels (Lu et al, 2000) and Ca^{2+} transport (Rubera et al, 2000). Thus, it has been speculated that extracellular ATP may be a regulator of renal tubular transport (Chan et al, 1998).

M-1 cells were derived from the outer CCD of SV40 transgenic mouse, which retain properties typical of CCD principal cells in vivo including the amiloride-sensitive epithelial sodium channel (ENaC) (Stoos et al, 1991; Korbmacher et al, 1993; Letz et al, 1995). In this paper, we examined the effects of extracellular ATP on electrogenic transepithelial ion transport of M-1 cells using Ussing-type chambers. In addition, PCR experiments were performed to further characterise the purinoceptor subtype mediating the effect of ATP on transepithelial ion transport in M-1 cells. Most of this work have been reported as abstracts (Jung et al, 1999; Park et al, 2000). During preparation of the manuscript, a similar result was published by Cuffe et al (2000).

METHODS

Cell culture

The M-1 cell line was obtained from ATCC (2038-CRL, American Type Culture Collection, Rockville, MD, USA). Cells were cultured in tissue culture dishes containing DMEM/F12, 10% fetal bovine serum (Life Technologies, Rockville, MD, USA) and 1 μM dexamethasone. For transepithelial studies cells were seeded onto 12 mm diameter Transwell Costar culture plate inserts coated with type I collagen and used 5～7 days after seeding.

Electrophysiological experiments

Short circuit current (I_{sc}) measurement was performed in a modified Ussing chamber designed to accept Transwell filter, as previously described (Hwang et al, 1996). Transepithelial potential (V_{te}) difference was short-circuited with a voltage clamp (Model DVC-1000, World Precision Instrument, Sarasota, FL, USA) connected to apical and basolateral chambers via Ag/AgCl electrodes. Transepithelial resistance (R_{te}) was evaluated by determining I_{sc} induced with a 10 mV voltage pulse. Conventionally a lumen negative V_{te} corresponds to a positive I_{SC}, which may be due to electrogenic cation absorption or electrogenic anion secretion or a combination of both. A standard bath solution (containing (mM): 140 Na^+, 4 K^+, 1 Ca^{2+}, 1 Mg^{2+}, 120 Cl^-, 2 SO_4^{2-}, 24 HCO_3^-, 5 glucose) was used on both the apical and basolateral side of the epithelial monolayer. Both solutions were maintained at 37°C and gassed with 95% O_2 and 5% CO_2 maintaining pH at 7.4. Chloride-free solutions were achieved by replacing NaCl and KCl with their gluconate salts. Sodium-free solutions were achieved by replacing NaCl with NMG Cl and $NaHCO_3$ with 10 mM Hepes and 100% O_2 was supplied.

Reverse transcription polymerase chain reaction (RT-PCR)

Confluent M-1 cells were collected with a cell scraper and total RNA was isolated by RNAzol B (Tel Test. Inc, Friendswood, TX, USA). Total RNA was then reverse transcribed using Superscript reverse transcriptase (Life Technologies, Rockville, MD, USA) and oligo dT primer (Promega, Madison, WI, USA). The first strand cDNA was amplified by 35 cycles (94°C, 1 min; 50°C, 1 min; 72°C, 1 min) of polymerase chain reaction using 20 pmoles of specific primers or 100 pmoles of degenerative primers. On completion of the PCR reaction, products were examined on 2% agarose gel. β-actin PCR amplification was performed to make sure that the equal amount of total RNA was used for each sample. Negative controls were performed without reverse transcriptase and without RNA. The obtained PCR product was subcloned into the pGMEM-T easy vector system (Promega, Madison, WI, USA) and the plasmids were sequenced using the T7 or SP6 primers (DNA Sequencing Facility, Pusan National University, Korea). The resulting DNA sequence was screened with the basic local alignment research tool (BLAST algorithm).

Measurement of intracellular cAMP content

Cells were grown on Transwell filters and subjected to the same procedure as in measurement of I_{sc}. After 5 min exposure to agonists, Transwells were rapidly removed from the Ussing chambers. A radioimmunoassay kit (Amersham, Arlington Heights, IL, USA) was used to measure the cAMP content. Protein concentration was determined using the Bio-Rad protein assay kit with gamma-globulin as a standard.

Reagents

Amiloride hydrochloride, adenosine 5'-triphosphate (ATP) disodium salt, adenosine 5'-diphosphate (ADP) sodium salt, adenosine 5'-monophosphate (AMP) sodium salt, uridine 5'-triphosphate (UTP) sodium salt, α,β-methylthioATP (MeSATP) tetrasodium salt, β, χ-MeSATP tetrasodium salt, 2-MeSATP tetrasodium salt, arginine vasopressin, 4,4'-diisothiocyanato-stilbene-2,2'-disulphonic acid disodium salt (DIDS), niflumic acid and glybenclamide were purchased from Sigma-Aldrich (St. Louis, MO, USA), diphenylamine-2-carboxylic acid (DPC) was obtained from Fluka (St. Louis, MO, USA). Amiloride was added from a 100 mM methanol stock solution to give a final concentration of 100 μM. DPC (100 mM) was dissolved in DMSO and stock solutions were freshly prepared on the day of the experiment. All chemicals used were of the highest grade commercially available.

Data analysis

Data are expressed as means $\pm$ standard error of the mean. Differences beween means in unpaired experiments were compared by repreated-measures ANOVA followed by Bonferoni's multiple comparisons test. Differences between means in paired experiments were compared by Student's test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of ATP on I_{sc} in M-1 cells

After an equilibration period of about 30 min after transfer into the Ussing-type chambers, confluent monolayers of M-1 cells displayed mean V_{te}, R_{te}, and I_{sc} values of 12.3 ± 1.3 mV (lumen negative), $1{,}509 \pm 169$ $\Omega.cm^2$, and 8.2 ± 0.9 $\mu A/cm^2$, respectively. The apical or basolateral addition of 10 μM ATP to M-1 monolayers elicited increase in I_{sc}. The response to the basolateral addition of 10 μM ATP was characterised by a short-lived peak increase in I_{sc} (Fig. 1A). The response to its apical addition showed slower response and broader peak than that to its basolateral addition.

We investigated the concentration dependence of the ATP effect in M-1 cells (Fig. 1B). The threshold concentration was 1 μM with a maximal I_{sc} peak occurring at 10 μM in both apical and basolateral addition of ATP. Therefore, 10 μM ATP was used throughout this study. Basolateral application of ATP elicited bigger I_{sc} responses than that observed with apical application of ATP.

Na^+ depedency of ATP effect

A positive I_{sc} may be due to either electrogenic cation absorption or anion secretion or a combination of both. To examine if the effect of ATP was mediated by increase in Na^+ absorption, we first determined the effect of amiloride on the ATP effect. Apical application of 100 μM amiloride decreased I_{sc} in M-1 cells (Fig. 2A). This represents that the pre-

Table 1. RT-PCR primers

Name	Sequences (S: sense, AS: antisense)	Base pairs (bp)
β-actin	S: 5'-TTG TAA CCA ACT GGG ACG ATA TGG-3'	757
	AS: 5'-GAT CTT GAT CTT CAT GGT GCT AGG-3'	
P2XR	S: 5'-TTC ACC MTY YTC ATC AAR AAC AGC ATC-3'	330
	AS: 5'-TGG CAA AYC TGA AGT TGW AGC C-3'	
$P2Y_2$	S: 5'-CGT AAT CCT GGT CTG TTA CGT GCT-3'	499
	AS: 5'-CTA TAG CCG AAT GTC CTT AGT C-3'	

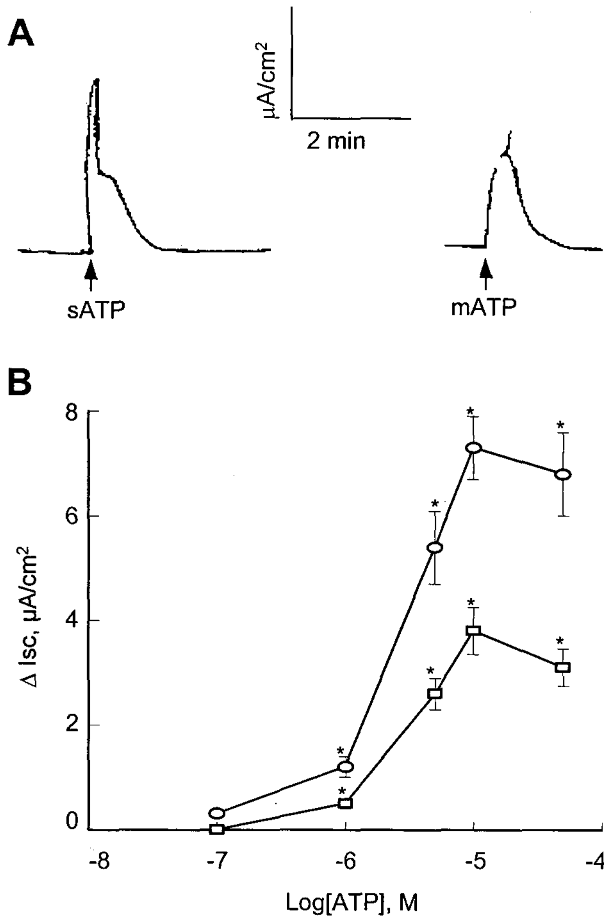

Fig. 1. Effect of the addition of ATP to the apical and basolateral sides of epithelium on I_{sc} in M-1 cells. A: representative current traces illustrating stimulation of I_{sc} by basolateral ATP (sATP, 10 μM) and apical ATP (mATP, 10 μM). Current and time scale bars provide raw current traces. B: Dose-response curves for sATP (○) or mATP (□) stimulation of peak I_{sc}. Maximal increases in I_{sc} are presented as mean ± SE (n=4).

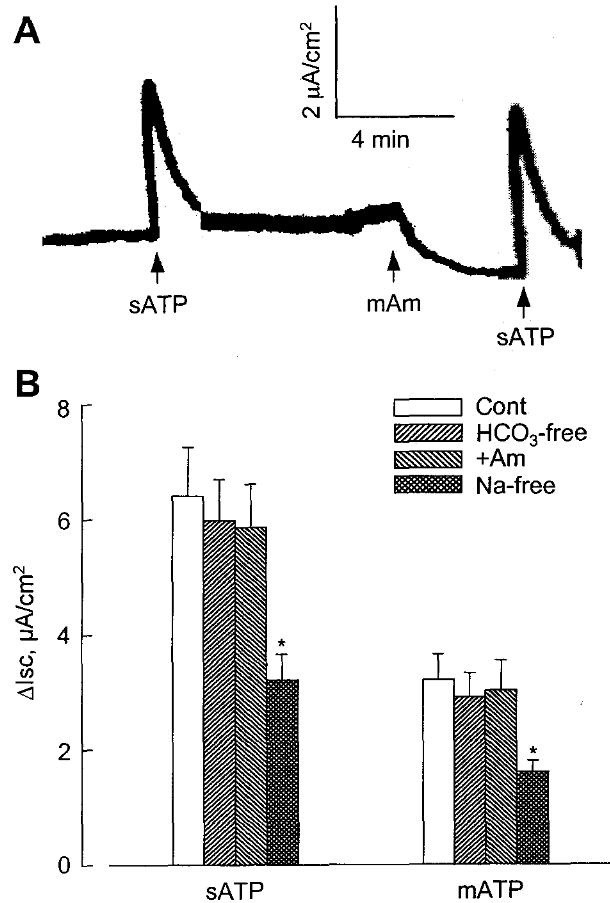

Fig. 2. Stimulation of Na^+ transport by the apical or basolateral addition of ATP in M-1 cells. A: Representative tracing of the effect of amiloride on ATP-induced response. Amiloride (100 μM) was added to the apical side of a monolayer and, after an additional 5 min, 10 μM ATP (sATP) was applied to the basolateral side. B: Statistical summary of the effects of amiloride or Na^+ depletion on the ATP responces applied to apical (mATP) or basolateral side (sATP). For depletion of Na^+, NaCl was replaced by NMG chloride, $NaHCO_3$ was replaced with 10 mM Hepes/Tris and 100% O_2 was supplied. Amiloride (100 μM) was added 5 min before the addition of ATP (10 μM) to the apical bathing solution. To determine the effect of ATP on Na^+ transport, the apical bathing solution was replaced by Na^+-free solution. Maximal increases in I_{sc} are presented as mean ± SE (n=4). *$p < 0.05$ compared with the control values.

dominant electrogenic ion transport across M-1 monolayers is sodium absorption via ENaC known to be expressed in M-1 cells (Korbmacher et al, 1993; Letz et al, 1995). Application of 10 μM ATP to the basolateral bathing solution in the presence of 100 μM amiloride in the apical bath induced almost identical increase in I_{sc} compared to that in the absence of amiloride. Similar pattern was observed in I_{sc} increase induced by apical ATP (Fig. 2B). To further examine the effect of ATP on sodium absorption in M-1 cells, the effect of sodium removal from the apical bathing solution on ATP-induced I_{sc} response was examined. In this experiment sodium bicarbonate was replaced by 10 mM Hepes-Tris for complete removal of extracellular sodium. Bicarbonate removal from the bathing solution itself did not have effect on basal I_{sc} (data not shown) and ATP-induced I_{sc} response in M-1 cells. Na^+ removal in the apical bathing solution

reduced the peak response to apical and basolateral ATP by 45% and 47%, respectively (Fig. 2A & B).

Cl^- dependency of ATP effect

To test whether the ATP-induced increase in I_{sc} was due to Cl^- secretion, we examined the ATP response in Cl^--free solution. Extracellular Cl^- removal significantly reduced the peak response to apical and basolateral ATP by 47% and 58%, respectively (Fig. 3A).

To confirm thė involvement of apical Cl^- channels, we determined the effects of apically administered Cl^- channel inhibitors on the I_{sc} peak response to ATP. In the presence of apical DPC (1 mM) the peak I_{sc} responses elicited by apical and basolateral ATP (10 μM) were significantly reduced by 55% and 54%, respectively. However, apical DIDS (500 μM) did not affect the I_{sc} peak responses to apical and basolateral ATP (10 μM). We also tested the effect of glybenclamine, a known inhibitor of CFTR Cl^- channels (Sheppard et al, 1992; Schultz et al, 1996) and niflumic acid, a Ca^{2+}-activated chloride channel inhibitor (White & Aylwin, 1990). In the presence of apical glybenclamide (100 μM) the peak increases in I_{sc} induced by apical and basolateral ATP were reduced by 48% and 49%, respectively. Apical niflumic acid (100 μM) also inhibited them, although the effect was lower than that of glybenclamide (22% vs 48% in apical ATP; 19% vs 49% in basolateral ATP) (Fig. 3B).

Signaling pathway of the ATP effect

To determine whether the observed effect of ATP is dependent on increase in intracellular cAMP, the effect of pretreatment with forskolin on the ATP-induced response was examined. Preliminary studies showed that 5 μM forskolin induced the maximal response in M-1 cells. Forskolin (5 μM) was initially added to the basolateral side of a monolayer and, after an additional 5 min, 10 μM ATP was added to the basolateral side. The response of 10 μM ATP was decreased by 61% in the presence of 5 μM forskolin (Fig. 4). In contrast, when the vasopressin (10 nM), of which action is mediated by increase in intracellular cAMP (Nagy et al, 1994), was added to M-1 cells in the presence of forskolin, no additional increase of I_{sc} was observed. The pretreatment of forskolin also had similar effect on the I_{sc} response

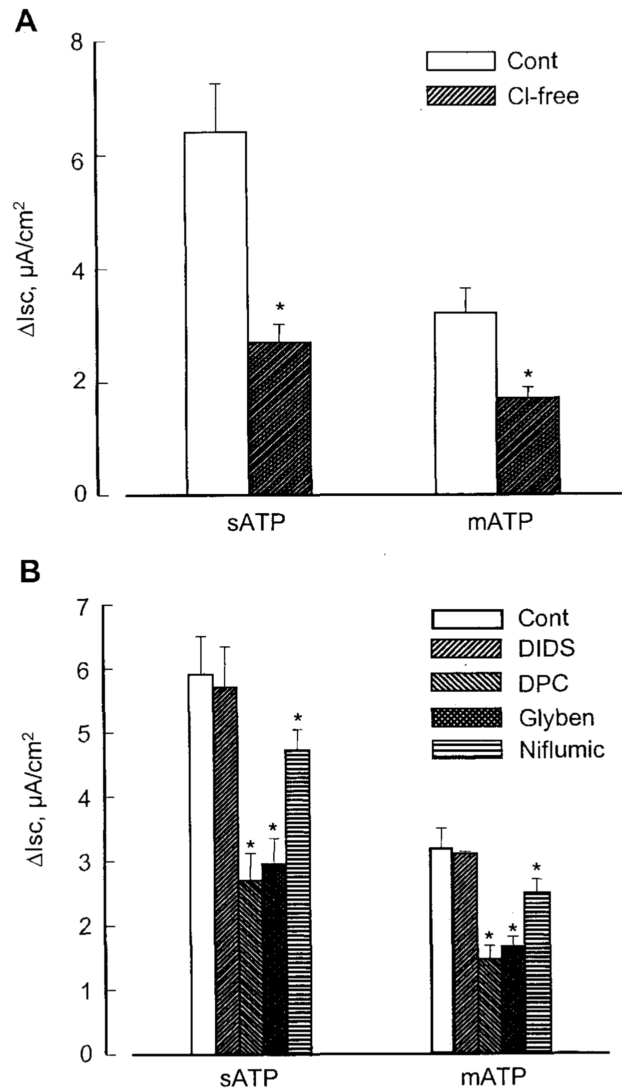

Fig. 3. Stimulation of Cl^- transport by the apical or basolateral addition of ATP in M-1 cells. A. Effect of Cl^- depletion on the mATP or sATP responses. For depletion of Cl^-, NaCl and KCl were replaced by Na^+ gluconate and K^+ gluconate. Maximal increases in I_{sc} are presented as mean±SE (n=5). *$p<0.05$ compared with the control values. B. Effect of Cl^- channel-blocking drugs on ATP-stimulated Cl^- secretion. DIDS (500 μM), DPC (100 μM), glybenclamide (Glyben, 100 μM) or niflumic acid (Niflumic, 100 μM) were added to the apical bathing solution for 5 min pretreatment before addition of 10 μM ATP. Maximal increases in I_{sc} are presented as mean±SE (n=4). *$p<0.05$ compared with the control values.

by apical administration of ATP (data not shown). To confirm whether ATP response was mediated by changes in intracellular cAMP levels, change in cAMP content elicited by ATP was measured (Table

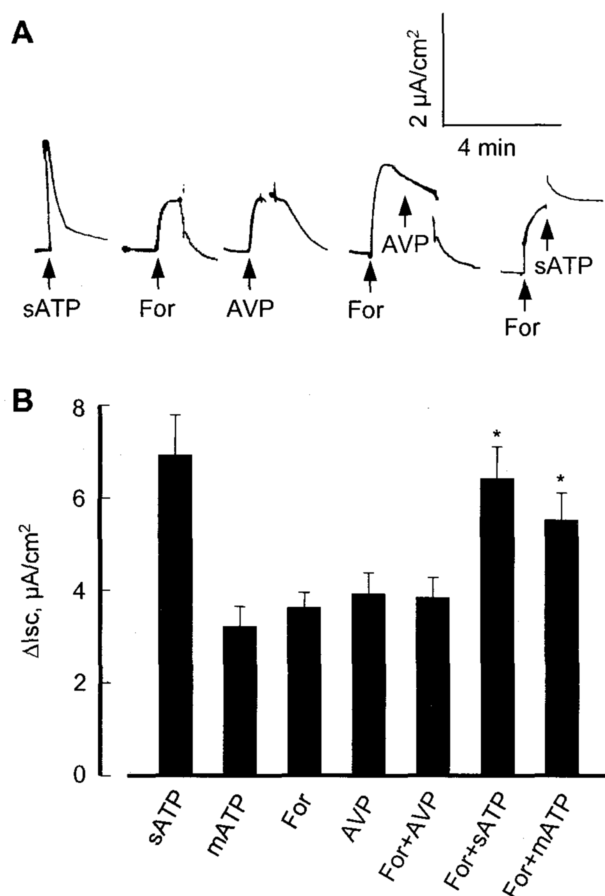

Fig. 4. Effect of the pretreatment of forskolin on ATP-induced increase in I_{sc}. After administration of forskolin (For, 10 μM), ATP (sATP, 10 μM) or arginine vasopressin (AVP, 10 nM) was added to the basolateral solution. A: Representative tracing of the effect of forskolin pretreatment. B: Statistical summary of I_{sc} values induced by agonists in the absence or presence of forskolin. Maximal increases in I_{sc} are presented as mean ± SE (n=4). *$p<0.05$ compared with the value in the presence of forskolin alone.

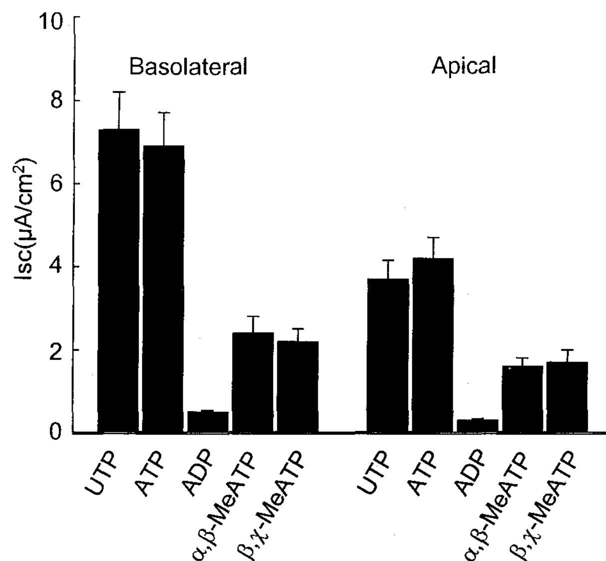

Fig. 5. Effect of various nucleotides on I_{sc}. M-1 cells grown on permeable filters for 5 to 7 days were stimulated with nucleotides. The concentrations of nucleotides were 100 μM. Maximal increases in I_{sc} are presented as mean ± SE (n=4).

Table 2. Changes in intracellular cAMP content in response to apical and basolateral ATP

	cAMP content (pmole/mg protein)
Basal	32.3±4.2
sATP (10 mM)	146.8±23.6
mATP (10 mM)	98.4±8.4
Forskolin (5 mM)	432.4±48.3

M-1 cell monolayers were stimulated for 5 min with 10 μM of reagents in the presence of 0.1 mM isobutylmethylxanthine from the apical or basolateral side. Data are presented as mean ± SE (n=4). *$p<0.01$ vs. basal level.

2). Apical and basolateral ATP increased the intracellular cAMP concentration by 3.0 and 4.5 fold, respectively.

Characterization of the underlying purinoceptor subtypes

We compared the effects of different nucleotides on I_{sc} to define the receptor subtype mediating the ATP response. The basolateral application of ATP and UTP (10 μM) had a similar effect with an average peak response. ADP did not elicit a significant response. Application of AMP (n=9) and 2-MeSATP, an agent specific for $P2Y_1$ receptors, had no effect (data not shown). Application of 100 μM α,β-MeSATP and 100 μM β,χ-MeSATP, P2X receptor agonists, induced I_{sc} increase in M-1 monolayers (Fig. 5). The apical application of different nucleotides showed similar effects. Taken together, these data indicate that the ATP-induced increase in I_{sc} is mediated by the same receptor subtype located on both membranes, possibly $P2Y_2$ and P2X receptors.

To identify which P2X and P2Y receptors are expressed in M-1 cells, we determined mRNA expres-

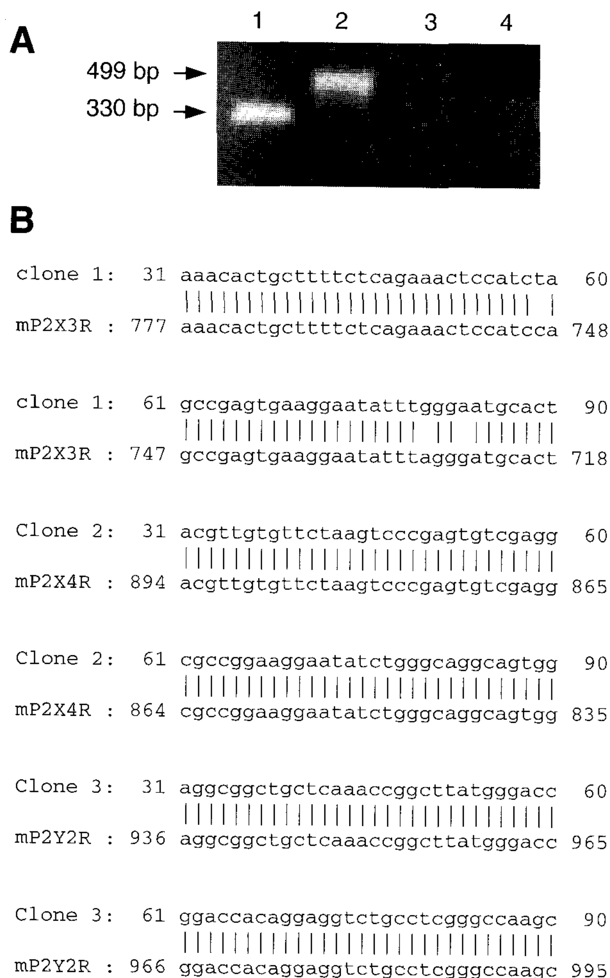

Fig. 6. Expression of P2X and $P2Y_2$ receptors in M-1 cells. RT-PCR was done with degenerative primers of P2X receptors and the specific primers of $P2Y_2$ receptors. A. Photograph of PCR products. Lane 1 and 2: Reverse-transcribed cDNA was amplified with P2X degenerative primers (330 bp product) or $P2Y_2$ primers (499 bp product), respectively. Lane 3 and 4: Total RNA was directly amplified with the same primers as a negative control. B. PCR products were subcloned to PGEM-T easy vector and the plasmids were sequenced by a DNA sequence analyzer. Two different subtypes of P2X and $P2Y_2$ receptors were identified.

sion of P2X and P2Y receptor subtypes by RT-PCR. Because the data in Fig. 5 indicated the presence of $P2Y_2$ receptors in M-1 cells, the specific primers for $P2Y_2$ were used for PCR amplification. However, degenerative primers were used for amplification of P2X receptors, because P2X receptors have eight distinct subtypes and their agonists do not have specificity to subtypes. RT-PCR products of the expected size for P2X receptors and for $P2Y_2$ receptors were identified in M-1 cells (data not shown). To identify subtypes of P2X receptors in M-1 cells, 11 different clones containing P2X PCR products were sequenced. Six of them were matched to the mouse $P2X_3$ cDNA sequence and five of them to that of mouse $P2X_4$. Representative sequence alignments for our $P2Y_2$, $P2X_3$ and $P2X_4$ PCR product with cloned cDNA sequences are shown in Fig. 6. These findings were consistent with the conclusion that the ATP effect in M-1 cells is mediated by $P2Y_2$ and P2X receptors.

DISCUSSION

Recently, several reports showed that extracellular ATP induces I_{sc} increase in collecting duct cells (McCoy et al, 1999; Cuffe et al, 2000; Woo et al, 2000). However, the sidedness of ATP responses is not consistent. In mouse inner medullar collecting duct (IMCD) cells ATP increased I_{sc} on the application to the apical bathing solution only (McCoy et al, 1999), whereas both the apical and basolateral application of ATP in rabbit IMCD cells induced I_{sc} increase (Woo et al, 2000). The present study showed that extracellular ATP induces increase in I_{sc} in M-1 cells, which is mediated through the activation of independent apical or basolateral purinergic receptors in M-1 mouse CCD cells. In the apical and basolateral membranes of M-1 cells the effects of ATP and UTP were equipotent, while ADP, AMP and 2-MeSATP had no effect. These data were consistent with the presence of $P2Y_2$ receptors. The apical and basolateral application of P2X receptor agonists also increased I_{sc} in M-1 cells. RT-PCR data further supported the presence of $P2Y_2$, $P2X_3$ and $P2X_4$ receptors in M-1 cells. These results indicated that the response to extracellular ATP is mediated by the activation of $P2Y_2$ and P2X receptors in the apical and basolateral membranes of M-1 cells. Expression of $P2Y_2$ receptors in the kidney has been reported in proximal cells (Jin & Hopfer, 1997; Takeda et al, 1998), MDCK cells (Post et al, 1996; Woo et al, 1998), and CCD (Rouse et al, 1994; Koster et al, 1996), and IMCD (Ecelbarger et al, 1994; Kishore et al, 1995). Recently, expression of P2X receptors was reported in LLC-PK1 cells (Filipovic et al, 1998) and mouse IMCD cells (McCoy et al, 1999).

Amiloride-sensitive Na^+ absorption and K^+ secretion are the predominant electrogenic transport fea-

tures of CCD principal cells (O'Neil, 1990). However, there are considerable evidences that various types of apical Cl^- channels are present in collecting cells that may mediate Cl^- secretion under favorable transepithelial electrochemical gradients (Christine et al, 1991; Superdock et al, 1993; Ling et al, 1994; Letz & Korbmacher, 1997). Moreover, it has been found that the intracellular Cl^- activity for rabbit CCD principal cells is significantly elevated above electrochemical equilibrium (Sauer et al, 1989; Simmons et al, 1993), favouring Cl^- secretion. In this study the apical or basolateral application of ATP induced I_{sc} increase in the absence of Na^+ and the ATP response was decreased by the removal of Cl^- from the bathing solution and the addition of apical Cl^- channel inhibitors. During preparation of manuscript McCoy et al (1999) & Cuffe et al (2000) reported ATP-induced chloride secretion in mouse collecting duct cell lines. These results support that ATP stimulates chloride secretion in the renal collecting duct cells.

Different classes of Cl^- channels have been identified in M-1 cells, including cystic fibrosis transmembrane conductance regulator (CFTR) (Todd-Turla et al, 1996; Letz & Korbmacher, 1997), ATP-dependent swelling-activated Cl^- channels, and calcium-activated Cl^- channels (Meyer & Korbmacher, 1996). A recent study in A6 cells concludes from current fluctuation analysis that the apical chloride channel activated by ATP is identical to the cAMP-activated Cl^- channel and possibly corresponds to CFTR (Atia et al, 1999). In this study, the pretreatment of forskolin decreased I_{sc} increase induced by the apical and basolateral application of ATP, suggesting that the ATP-induced change in I_{sc} may be mediated by the activation of cAMP-mediated activation of CFTR. In fact, extracellular ATP induced increase in intracellular cAMP in M-1 cells. In contrast to observations in some other cell systems in which ATP via $P2Y_2$ receptors reduces intracellular cAMP levels (Ralevic & Burnstock, 1998), extracellular ATP has been shown to stimulate intracellular cAMP production in MDCK (Post et al, 1996) and rabbit inner medullary collecting duct cells (Woo et al, 2000). The involvement of CFTR in the ATP-induced response in M-1 cells was further supported by inhibition of the ATP response by glybenclamide, a known CFTR inhibitor (Sheppard et al, 1992; Schultz et al, 1996). Stimulation of $P2Y_2$ receptors mediates an increase in $[Ca^{2+}]_i$ in renal epithelial cells (Ecelbarger et al, 1994; Cha et al, 1998). An ATP-induced rise in $[Ca^{2+}]_i$ with a concomitant chloride secretory response has previously been reported in various epithelial tissues including, for example, airway epithelium (Mason et al, 1991), pancreatic duct epithelium (Chan et al, 1996), and rat distal colon (Leipziger et al, 1997). DIDS inhibits swelling-activated and Ca^{2+}-activated chloride channel, but not cAMP-activated chloride channels (Cunningham et al, 1995; Rubera et al, 1997; Winpenny et al, 1998). In this study niflumic acid, a Ca^{2+}-activated chloride channel inhibitor (White & Aylwin, 1990) partly inhibited the ATP-induced I_{sc} response, whereas DIDS failed to inhibit ATP-induced I_{sc} response. These results may be related to the differences in inhibitory potencies to Ca^{2+}-activated chloride channel between DIDS and niflumic acid. Niflumic acid has stronger effect for inhibition of Ca^{2+}-activated chloride channel than DIDS (Winpenny et al, 1998). Taken together, these results indicate that the ATP-mediated increase in chloride secretion in M-1 cells is mostly mediated by the activation of apical CFTR Cl^- channel, although activation of Ca^{2+}-activated chloride channel is also involved.

The data in this study showed that the ATP-induced response was observed in the absence of Cl^- in the bathing solution and was decreased by removal of Na^+ from the apical bathing solution, indicating that ATP also increase transepithelial Na^+ absorption in M-1 cells. Because the apical administration of amiloride did not affect the ATP response, the involvement of ENaC in ATP-induced Na^+ absorption can be excluded. Experiments using various nucleotides and RT-PCR analysis in this study demonstrated that ATP-induced response in M-1 cells is partly mediated by activation of P2X receptors. The P2X purinoceptor family is a two transmembrane-spanning, ion channel-forming proteins and form nonselective cation channels (Abbracchio et al, 1994; Barnard et al, 1994; Harden et al, 1995). It has been reported that P2X receptors are also permeable to Na^+ (Housley et al, 1998), suggesting that P2X receptors may involve increase in Na^+ absorption by ATP in M-1 cells.

Studies using mouse collecting duct cells showed that ATP inhibited amiloride-sensitive Na^+ absroption (MacCoy et al, 1999; Cuffe et al, 2000). However, we could not observe inhibition of Na^+ absorption. We used 10 μM ATP, whereas MacCoy et al (1999) & Cuffe et al (2000) used 100 μM ATP. Differences of ATP concentration to be used for experiments may

explain the discrepancy of the results. When we applied 100 μM ATP to M-1 cells, we frequently observed irreversible changes in transepithelial resistance, especially on its apical application.

In summary, our findings in M-1 cells suggest that extracellular ATP stimulates Na^+ absorption and Cl^- secretion in the CCD. This effect is mediated through activation of $P2Y_2$ and P2X receptors located in the apical and basolateral membrane.

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