Korean J Physiol Pharmacol
Vol 4: 311−318, August, 2000

Asymmetrical Distribution of P2Y Nucleotide Receptors in Rabbit Inner Medullary Collecting Duct Cells

Jae Suk Woo, Jin Sup Jung, and Yong Keun Kim

Department of Physiology, College of Medicine, Pusan National University, Pusan 602−739, Korea

We cultured the rabbit inner medullary collecting duct (IMCD) cells as monolayers on collagen-coated membrane filters, and investigated distribution of the P2Y receptors by analyzing nucleotide-induced short circuit current (I_{sc}) responses. Exposure to different nucleotides of either the apical or basolateral surface of cell monolayers stimulated I_{sc}. Dose-response relationship and cross-desensitization studies suggested that at least 3 distinct P2Y receptors are expressed asymmetrically on the apical and basolateral membranes. A $P2Y_2$-like receptor, which responds to UTP and ATP, is expressed on both the apical and basolateral membranes. In addition, a uracil nucleotide receptor, which responds to UDP and UTP, but not ATP, is expressed predominantly on the apical membrane. In contrast, a $P2Y_1$-like receptor, which responds to ADP and 2-methylthio-ATP, is expressed predominantly on the basolateral membrane. These nucleotides stimulated intracellular cAMP production with an asymmetrical profile, which was comparable to that in the stimulation of I_{sc}. Our results suggest that the adenine and uracil nucleotides can interact with different P2Y nucleotide receptors that are expressed asymmetrically on the apical and basolateral membranes of the rabbit IMCD cells, and that both cAMP- and Ca^{2+}-dependent signaling mechanisms underlie the stimulation of I_{sc}.

Key Words: IMCD cells, Nucleotide, P2Y receptor, Chloride secretion, Short circuit current

INTRODUCTION

It is now well established that extracellular nucleotides act as agonists to regulate a broad range of physiological processes by interacting with the P2 receptors (Dubyak & El-Moatassim, 1993; North & Barnard, 1997; Williams & Burnstock, 1997). The IUPHAR Purine Nomenclature Subcommittee grouped the P2 receptors into 2 families (Fredholm et al, 1997), P2X and P2Y. The P2X is a ligand-gated ion channel receptor family, seven of which (P2X1-7) have been cloned to date (Surprenant et al, 1996). The P2Y is a G protein-coupled receptor family. Recent cloning of a new P2Y receptor (Communi et al, 1997) added the 11th member to this family. Among the P2Y receptors, the $P2Y_1$ and $P2Y_2$ have been studied most widely in relation with their expression and functions in various tissues. These receptors can be discriminated pharmacologically by the agonist selectivity; 2-methylthio-ATP (2MeSATP) $>$ ATP $>$ ADP $>>$ UTP for $P2Y_1$, and UTP=ATP $>$ ATPS $>>$ 2MeSATP=ADP for $P2Y_2$ (Williams & Burnstock, 1997). They have been known as P_{2y} and P_{2u}, according to the classical nomenclature (Fredholm et al, 1997; Williams & Burnstock, 1997). A peculiar characteristic of the $P2Y_2$ receptor is that it is responsive to UTP, a uracil nucleotide, as well as adenine nucleotides. The $P2Y_4$ and $P2Y_6$ are selective for UDP and UTP, and do not respond to adenine nucleotides (Communi & Boeynaems, 1997; Heilbronn et al, 1997).

It was reported that the P2Y receptors are expressed in a polarized manner in cells derived from the airway (Hwang et al, 1996), intestine (Inoue et al, 1997) and kidney (Woo et al, 1998). Recent studies suggested that the $P2Y_2$ receptor is expressed in the inner medullary collecting cells of the mouse (McCoy

Corresponding to: Jae Suk Woo, Department of Physiology, College of Medicine, Pusan National University, 1-10 Ami-dong, Suh-gu, Pusan 602-739, Korea. (Tel) 82-51-240-7713, (Fax) 82-51-246-6001, (E-mail) jswoo@hyowon.pusan.ac.kr

et al, 1999) and rat (Kishore et al, 2000), and is involved in regulation of ion transport across the epithleium. However, the exact nature of the distribution and function of the receptors in renal tubular cells are still unknown. The purpose of this study was to investigate distribution of the nucleotide receptors in the rabbit inner medullary collecting duct (IMCD) cells. Toward this end, IMCD cells were isolated from the rabbit kidney inner medulla and grown as monolayers on collagen-coated polycarbonate membrane filters and assayed for nucleotide-stimulated Cl^- secretion by measuring short circuit current (I_{sc}) responses. The results suggest that the uracil as well as adenine nucleotides can interact with the P2Y nucleotide receptors, which are expressed asymmetrically on the apical and basolateral membrane of the rabbit IMCD cells, and that both cAMP- as well as Ca^{2+}-dependent signaling mechanisms underlie the stimulation of Cl^- secretion.

METHODS

Cell preparation

IMCD cells were isolated from 1.5~2.0 kg New Zealand white rabbits of both sexes by use of the hypotonic lysis isolation method described previously by others (Grenier & Smith, 1978). In brief, rabbits were anesthetized by *i.v.* injection of phentobarbital sodium. After dissection of the abdominal wall, the kidneys were perfused through a cannula placed into the aorta with Dulbeccos phosphate-buffered saline (PBS). The kidneys were then removed and rinsed with Hanks balanced salt buffer solution (HBSS) containing 50 IU/ml penicillin G and 50 μg/ml streptomycin. The inner medulla was dissected, minced, suspended in HBSS containing 1 mg/ml collagenase, and incubated at 37°C for 2~3 h. IMCD cells were isolated by a further incubation for 5 min in the above solution made hypotonic (1/3 of HBSS) with two volumes of hypotonic water containing 10 μg/ml deoxyribonuclease. Cells were retrieved by centrifugation at 500 g for 5 min and washed twice with 1 : 1 mixture of Dulbecco's modified Eagles medium and Hams F-12 (DMEM/F12) with 10% fetal bovine serum (FBS) and antibiotics. Cells were then suspended in DMEM/F12 supplemented with 10% FBS, 10 μg/ml transferrin, 100 nM hydrocortisone, 10 μg/ml insulin, 10 nM sodium selenite, 5 pM triiodothyronine, 50 IU/ml penicillin G and 50 μg/ml streptomycin.

All the experiments in this study were performed using cells grown as monolayers on permeable filters. Cells were plated on 12-mm polycarbonate membrane filters (Snapwell; Costar, Cambridge, MA, USA). Usually material from one kidney was plated to 4 to 6 Snapwell filters. Before plating, the filters were coated with 1% Vitrogen 100 (Collagen, Palo Alto, CA, USA) and 1 mg/ml human fibronectin (Collaborative Research, Bedford, MA, USA) dissolved in DMEM/F12 supplemented with 1% FBS. After plating, one-third of medium was replaced with serum-free supplemented DMEM/F12 every 24 h. In this way, the serum was diluted gradually.

Cells were evaluated for formation of tight junctions by measuring transepithelial resistance using transepithelial voltohmeter (EVOM, World Precision Instruments, Sarasota, FL, USA). Usually, transepithelial resistance (R_{te}) reached its peak (627 ± 43 ohm · cm^2) 5 to 6 days after plating. Experiments were carried out on the 6th to 8th day after plating.

I_{sc} measurement

Short circuit current (I_{sc}) measurement was performed in a modified Ussing chamber designed to accept Snapwell filter (World Precision Instrument, Sarasota, FL, USA). Transepithelial potential 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. Experiments were carried out in bicarbonate-free Ringer solution that was composed of (in mM): 140 NaCl, 2.3 K_2HPO_4, 0.4 KH_2PO_4, 1.5 $CaCl_2$, 1.5 $MgCl_2$, 10 HEPES and 5 glucose (pH 7.4). Both the apical and basolateral bathing solution was maintained at 37°C, oxygenated with 100% O_2, and subject to constant circulation. Prior to stimulation with agonists, cell monolayers were equilibrated in Ringer solution for 30 min. After basal I_{sc} was stabilized, R_{te} was calculated by Ohms law by determining I_{sc} induced with a 10 mV voltage pulse. All the experiments were carried out in the presence of 50 μM amiloride to rule out the possible involvement of Na^+ current in the nucleotide-stimulated I_{sc},.

Measurement of intracellular cAMP content

Cells were grown on Snapwell filters and subjected

to the same procedure as in measurement of I_{sc}. After 3-min exposure to agonists, Snapwells were rapidly removed from the Ussing chambers and immersed in ice-cold ethanol-HCl solution (ethanol containing 20 mM HCl). Membrane filters were cut off the Snapwell support using sharp tuberculin needle. Cells attached to the membrane filters in ethanol-HCl solution were then transferred to microcentrifuge tubes and sonicated to disrupt the cell membrane and complete extraction of intracellular cAMP. The cell suspension was then centrifuged (12,000 g) for 10 min at 4°C to precipitate the protein, and the supernatant was collected. The supernatant was freeze-dried and dissolved in an adequate volume of 50 mM Tris/1 mM EDTA (pH 7.5). cAMP content was determined by radioimmunoassay using [^{3}H]cAMP assay kit from Amersham (Arlington Heights, IL, USA). Protein concentration was determined using the Bio-Rad protein assay kit with bovine serum albumin as a standard.

Reagents

ATP, ADP, UTP, UDP, and 2-methylthio-ATP (2MeSATP) were obtained from Research Biochemicals International (Natick, MA, USA). H-89 and BAPTA/AM were purchased from Calbiochem (La Jolla, CA, USA). Fura-2/AM was purchased from Molecular Probes (Eugene, OR, USA). Media and reagents for cell preparation and culture were obtained form GIBCO-BRL (Grand Island, NY, USA).

Data analysis

Nucleotide-stimulated I_{sc} was calculated by subtracting the basal I_{sc} from the peak I_{sc} responses, and were presented as means $\pm$ SE. When necessary, data were analyzed by one-way analysis of variance followed by Duncan's multiple comparison test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Functional integrity of cell monolayer

To test the development of functional integrity of cell monolayers, transepithelial potential difference (V_{te}), transepithelial resistance (R_{te}), and basal I_{sc} were monitored during culture days. The results are summarized in Fig. 1. Measurable V_{te} and R_{te} developed by day 3 after plating (-0.91 ± 0.24 mV and 174.4 ± 31.2 ohm · cm^2), indicating that functional tight junction had been formed. V_{te} and R_{te} increased gradually until day 5 to 6. Afterward, they remained at steady levels to day 10. At day 6, V_{te}, R_{te} and basal I_{sc} were -3.74 ± 0.72 mV, 627.8 ± 43.3 ohm · cm^2, and 4.2 ± 0.8 μA/cm^2, respectively. After day 10, R_{te} declined gradually, indicating that functional integrity of the monolayers might be deteriorating. Results in this study are from experiments carried out on the 6th to 8th day of culture after plating.

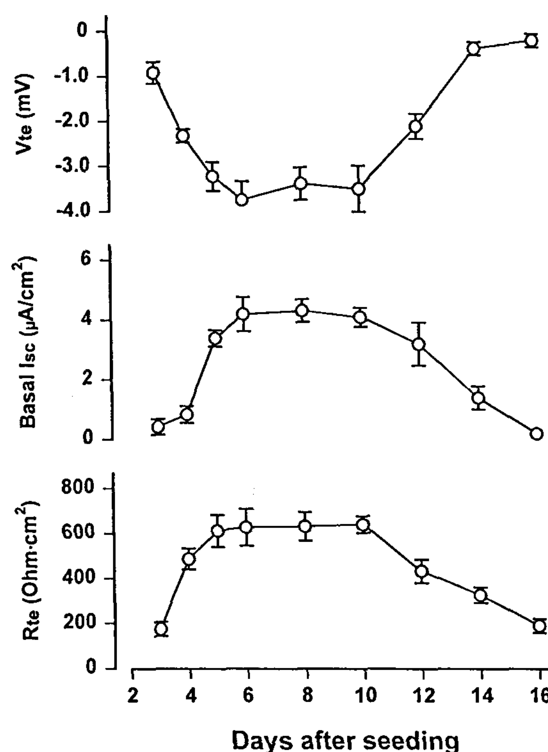

Fig. 1. Changes in transepithelial potential difference (V_{te}), basal short circuit current (basal I_{sc}), and transepithelial electrical resistance (R_{te}) at various times after cell seeding on permeable membrane supports. IMCD cells were seeded on membrane filters at a cell density of 1.2×10^6 cells/cm^2. At different time points, cell monolayers were mounted in Ussing chambers and equilibrated with Ringer solution for 20 min. Before stimulation with agonist, V_{te} and basal I_{sc} were measured. R_{te} was calculated by Ohm's law by determining I_{sc} induced with a 10 mV voltage pulse. Data are expressed as means $\pm$ SE from 17 to 24 separate cell preparations.

Stimulation of Isc by nucleotides

Dose-response relationship of the changes in I_{sc} in response to applications of ATP, UTP, UDP, ADP and 2MeSATP was studied. The apical or basolateral surface of the monolayers was exposed to single doses of the nucleotides at concentrations ranging from 10 nM to 200 μM, and the results are summarized in Fig. 2. UTP or ATP in either the apical or basolateral solution stimulated I_{sc}. The observation that UTP or ATP can stimulate I_{sc} from both sides of the epithelium indicates that the $P2Y_2$ (or P_{2U})-like receptors exist on both the apical and basolateral membranes. In contrast to the effects of ATP or UTP, the action of UDP was predominant on the apical side. It is worthwhile to note that, in the lower concentration range, the magnitude of stimulation by UTP is comparable to the sum of those by ATP and UDP. From these results, it is suggested that a uracil nucleotide receptor that responds to UDP and UTP, but not ATP, is expressed asymmetrically on the apical membrane. On the other hand, ADP and 2MeSATP elicited responses predominantly when applied to the basolateral membrane, suggesting that an additional population of nucleotide receptor is present. In the experiments summarized in Fig. 3, we determined I_{sc} responses to the nucleotides in monolayers of MDCK cells, a cell line established from the canine distal nephron. As shown in Fig. 3, each nucleotide in the apical and basolateral bathing solutions elicited a similar pattern of responses, which is comparable to that in the rabbit IMCD cells. These results lead us to speculate that the pattern of receptor loclaization observed in this study might be a generalized characteristic of distal nephron cells.

Cross-desensitization of nucleotide-stimulated Isc responses

Desensitization to the same or different kinds of nucleotides provides an indirect means of assessing receptor heterogeneity. Thus we carried out desensitization experiments to define whether the receptor which responds to UDP and UTP on the apical mem-

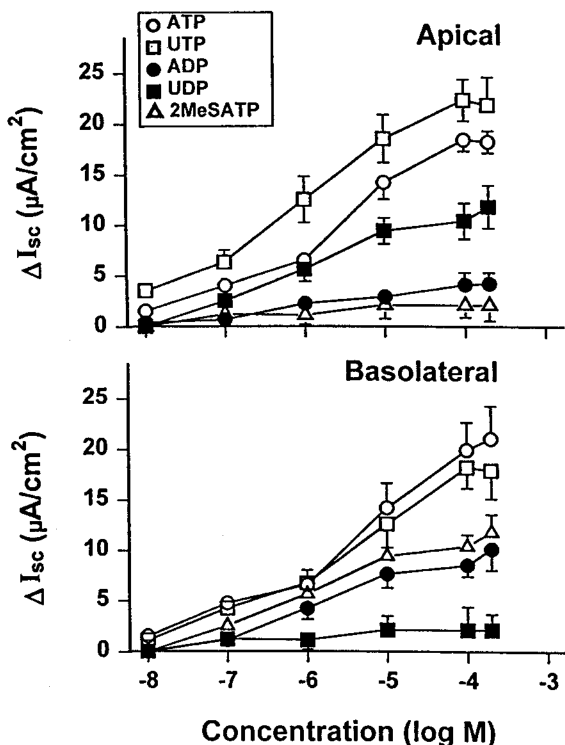

Fig. 2. Concentration-dependent effects of extracellular nucleotides on I_{sc}. IMCD cells grown on permeable membranes for 6 to 8 days were stimulated with indicated concentrations of each nucleotide from the apical or basolateral side. Maximum increases in I_{sc} are presented as means ± SE (n=5).

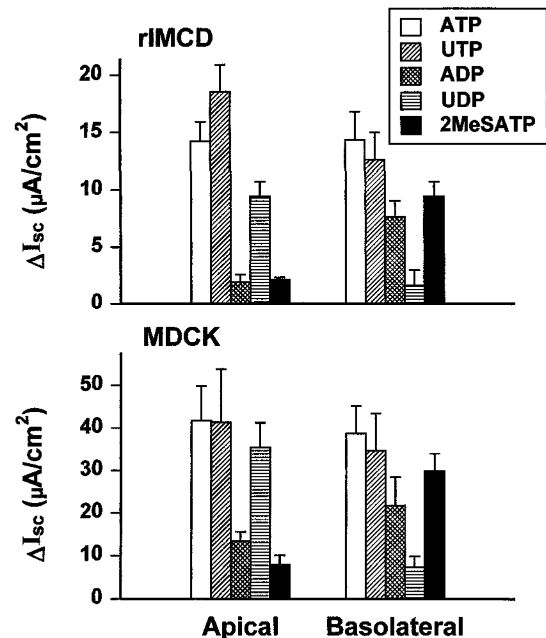

Fig. 3. Extracellular nucleotide-induced I_{sc} responses in rabbit IMCD cells (rIMCD) and canine distal nephron cells (MDCK). Cells grown on permeable membranes for 6 to 8 days were stimulated with 10 μM of each nucleotide from the apical or basolateral side. Maximum increases in I_{sc} are presented as means ± SE (n=4).

brane is distinct from the $P2Y_2$ receptor. As shown in Fig. 4A, when cells were pre-incubated (i.e., desensitized) with UDP (100 μM) for 40 min in the apical bathing solution, the subsequent challenge with UDP failed to stimulate I_{sc}. However, after this treatment, the cells still responded to ATP or UTP, although the response was less than that observed in cells not desensitized with UDP. This is in sharp contrast with the result from the cells pretreated with UTP, in which the response to ATP as well as UDP is desensitized completely. This result is consistent with the conclusion that there exists a receptor for UDP and UTP, but not ATP, which is distinguished from the $P2Y_2$, on the apical membrane. Similar desensitization experiments were carried out to define whether the responses to 2MeSATP and ADP in the basolateral solution were mediated via the same or separate receptors. As shown in Fig. 4B, preincubation with 2MeSATP (100 μM) in the basolateral solution resulted in a complete abolishment of a subsequent response to 2MeSATP (100 μM) as well as ADP (100 μM). The cells preincubated with ADP (100 μM) also did not respond to a subsequent challenge by ADP or 2MeSATP. These results suggest that 2MeSATP and ADP stimulate the same receptor subtype, which is distinct from the $P2Y_2$ receptor activated by ATP as well as UTP. Together, we conclude that three different subtypes of P2Y receptors are located asymmetrically in the apical and basolateral membranes of rabbit IMCD cells.

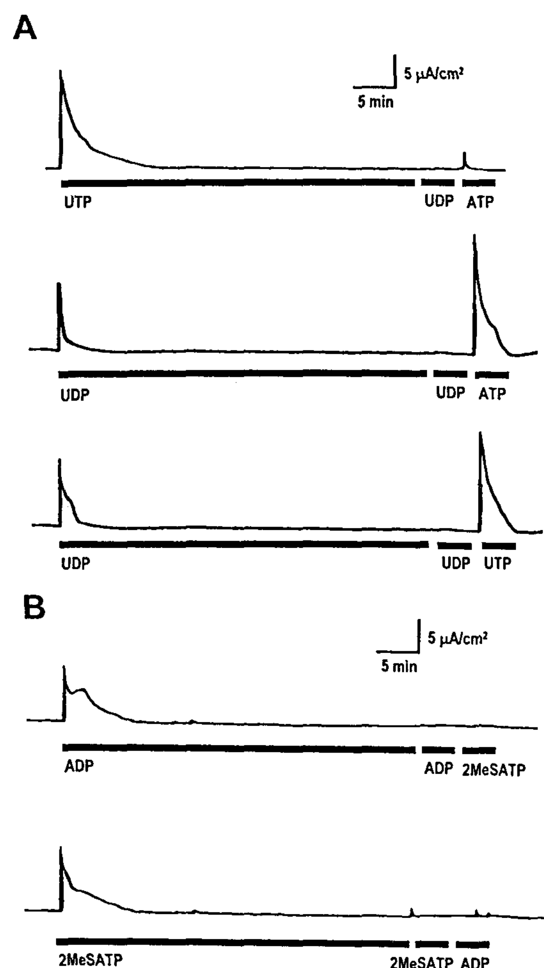

Fig. 4. A. Desensitization of I_{sc} responses on the apical membrane. Cell monolayers were preincubated for 40 min with 100 μM of UDP or UTP in the apical bathing solution followed by a 1-min wash, and stimulated again with UDP. After the second wash, cell monolayers were stimulated with 100 μM ATP or UTP. B. Desensitization of I_{sc} responses on the basolateral membrane. Cell monolayers were preincubated for 40 min with 100 μM of ADP or 2-methylthio-ATP (2MeSATP) in the basolateral bathing solution followed by a 1-min wash, and stimulated again with the same nucleotides. After the second wash, cell monolayers were stimulated with 100 μM 2MeSATP or ADP. Typical tracing of changes in I_{sc} observed after sequential addition of nucleotides is shown.

cAMP- as well as Ca^{2+}-dependent mechanism is involved in the ATP-stimulated Isc

G protein-dependent activation of phospholipase C-β (PLC_β) and subsequent increase in intracellular Ca^{2+} has been believed to be a major signaling mechanism to evoke cellular responses upon activation of P2Y receptors (Dubyak & El-Moatassim, 1993; Harden et al, 1995). In addition, there is accumulating evidence that adenylyl cyclase is also involved in the signaling mechanism of the P2Y receptor-mediated responses in a certain type of cells. As shown in Fig. 5, significant inhibition of the ATP-stimulated I_{sc} responses by pretreatment of cells with H-89 (20 μM), a specific protein kinase A inhibitor (Chijiwa et al, 1990), suggests that the stimulation of I_{sc} is dependent on protein kinase A. The result also shows that pretreatment with BAPTA/AM (10 μM), a cytosolic Ca^{2+} chelator (Harrison & Bers, 1987), inhibits significantly as well the stimulation of I_{sc} by ATP. These results may be a demonstration in support of the hypothesis that ATP lead to increase in intracellular cAMP as well as Ca^{2+} upon stimulation of I_{sc}.

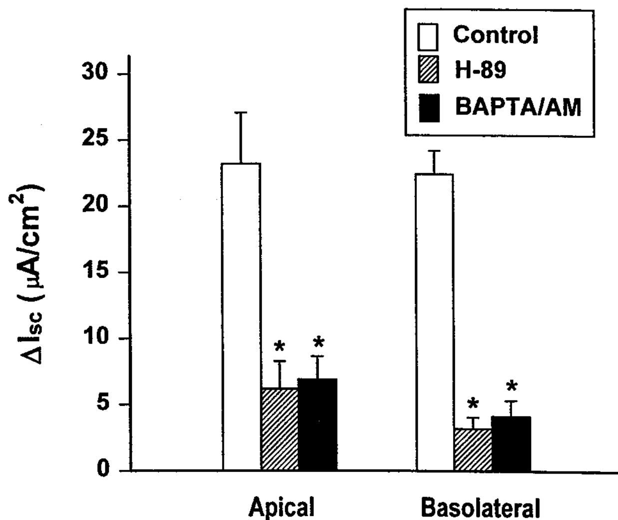

Fig. 5. Effects of the protein kinase A inhibitor and intracellular Ca^{2+} chelator on ATP-stimulated I_{sc}. Cell monolayers were preincubated for 30 min with H-89 (20 μM) or BAPTA/AM (10 μM), and then stimulated with 10 μM ATP from the apical and basolateral sides. Maximum increases in I_{sc} are presented as means ± SE (n=4). *P < 0.01 vs. control.

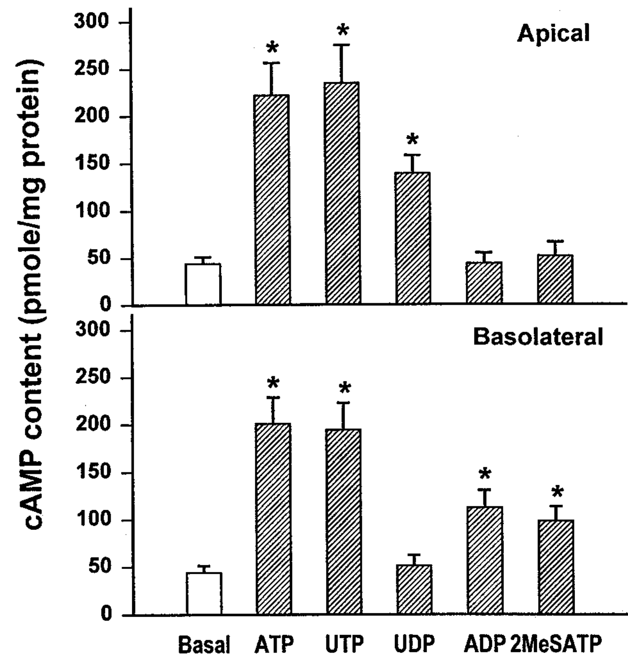

Fig. 6. Changes in intracellular cAMP content in response to different nucleotides. IMCD cell monolayers were stimulated for 5 min with 10 μM of each nucleotide in the presence of 0.1 mM isobutylmethylxanthine from the apical or basolateral side. Data are presented as means ± SE (n=4). *P < 0.01 vs. basal level.

Changes of intracellular cAMP concentrations in response to nucleotides

Measurement of intracellular cAMP content was carried out in cells prepared in parallel with those used to record I_{sc} in Ussing chambers. The results are summarized in Fig. 6. Exposure for 3 min to single doses of ATP (10 μM) in the apical or basolateral solution increased intracellular cAMP content by 4.8 and 4.5 fold, respectively. UTP was equipotent with ATP to stimulate cAMP production in either the apical or basolateral solution. UDP was effective in the apical side only to stimulate cAMP production. In contrast, ADP and 2MeSATP were effective in the basolateral side only. The magnitudes of cAMP production stimulated by UDP, ADP or 2MeSATP were smaller than those by ATP or UTP as was in those of I_{sc}.

DISCUSSION

Epithelial cells, when grown on a permeable support, form a polarized monolayer that is useful for studying the differentiated epithelial functions, including vectorial transport of inorganic and organic compounds. Increase of transepithelial resistance and potential difference are hallmarks of the formation of tight junctions and functional monolayer as described in the results summarized in Fig. 1. In polarized epithelial cell monolayers, responses of I_{sc} to various agonists including nucleotides have been well described to be accounted for by net basolateral to apical Cl^- secretion (Simmons, 1991). It is based on radioisotope flux studies, anion and cation dependence of the agonist-stimulated I_{sc}, and the action of inhibitors at the apical and basolateral surfaces.

In this study, exposure to different nucleotides of either the apical or basolateral surface of the cell monolayers stimulated I_{sc}. The dose-response relationship and cross-desensitization studies suggest that at least 3 distinct P2Y receptors are expressed asymmetrically on the apical and basolateral membrane. Two distinct receptors that are responsive to uracil nucleotides were found to be expressed in rabbit IMCD cells. Expression of a $P2Y_2$-like receptor on both the apical and basolateral membrane was not surprising, because this receptor is known to be expressed widely in a variety of mammalian tissues. Recent studies have shown that the $P2Y_2$ receptor is expressed in the

inner medullary collecting cells of the mouse (McCoy et al, 1999) and rat (Kishore et al, 2000). In contrast to symmetrical expression of the $P2Y_2$-like receptor, another uracil nucleotide receptor was present predominantly on the apical membrane. Several investigators have also reported similar pattern of asymmetrical expression of the uracil nucleotide receptors in other types of epithelial cells (Communi et al, 1997; Inoue et al, 1997). UDP and UTP, but not ATP activated the uracil nucleotide receptor expressed on the apical membrane. This agonist profile is consistent with those of cloned uracil nucleotide receptors (i.e., $P2Y_4$ or $P2Y_6$).

We also identified an ADP- and 2MeSATP-sensitive receptor. The most prominent feature of this receptor is its distinct location in the basolateral membrane. As we observed that the response to ADP and 2MeSATP were always parallel, we hypothesized that 2MeSATP and ADP stimulated the same receptor and consequently cross-desensitization studies were conducted. As shown in Fig. 4B, 2MeSATP and ADP cross-desensitized each other, suggesting that these two agonists interact with the same receptor. This receptor is certainly different from the $P2Y_2$ receptor because cells that had been desensitized with 2-MeSATP or ADP still responded to ATP or UTP. The agonist selectivity leads us to conclude that this ADP- and 2MeSATP-sensitive receptor is a $P2Y_1$-like receptor.

An interesting finding in this study is that the nucleotides stimulated intracellular cAMP production with an asymmetrical profile, which was comparable to that in the stimulation of I_{sc}. G protein-dependent activation of PLC and subsequent increase in intracellular Ca^{2+} has been believed to be a major signaling mechanism to evoke cellular responses upon activation of P2Y receptors responses (Dubyak & El-Moatassim, 1993; Harden et al, 1995). However, there is accumulating evidence that adenylyl cyclase is also involved in the signaling mechanism of the P2Y receptor-mediated responses in a certain type of cells. Recently, Post et al (1998) and Woo et al (1998) demonstrated that the P2Y receptor agonists enhance cAMP production in canine distal nephron cells. In addition, cloning of the $P2Y_{11}$ receptor coupled to adenylyl cyclase as well as PLC provided an evidence that cAMP- as well as Ca^{2+}-dependent mechanism might be an important mediator of the P2Y receptor-induced responses (Communi et al, 1997). In this study, inhibition of ATP-stimulated I_{sc} by the protein kinase A inhibitor H-89 indicates the role of cAMP and protein kinase A in ATP-stimulated signaling (Fig. 5). Determination of intracellular cAMP production provided direct evidence that UDP, ADP, and 2MeSATP as well as ATP and UTP stimulate cAMP production. The stimulation of cAMP production by these nucleotides showed the same profile of sidedness as in stimulation of I_{sc} indicating that the nucleotides stimulate cAMP production by interacting with the specific receptors located asymmetrically on the apical and basolateral membranes (Fig. 6). The molecular mechanism that links the adenylyl cyclase and the nucleotide receptors remains to be clarified. The characteristic localization of the nucleotide receptors rasies a question of how these nucleotides reach the luminal or serosal extracellular space. There is accumulating knowledge about a specific and controlled release of adenine nucleotides, such as exocytosis from nerve ending and conductive release through CFTR (Reisin et al, 1994; Schwiebert et al, 1995), into the extracellular space. In contrast, only limited information is available concerning the occurrence of uracil nucleotides in extracellular fluid. Uracil nucleotides are stored in granules of platelets and may be released upon stimulation (Goetz et al, 1971). Uracil nucleotides are also known to be present at concentration up to 0.7 mole/g wt. in liver, kidney, and brain (Keppler et al, 1970). By analogy with adenine nucleotides, uracil nucleotides may be released from cells under a variety of physiological and pathological conditions such as trauma, hypoxia and inflammation (Gordon, 1986). What a mechanism, these nucleotides released into extracellular space may play a role as an autocrine and paracrine regulator of cell function.

In conclusion, our results suggest that uracil as well as adenine nucleotides can interact with different P2Y nucleotide receptors which are expressed asymmetrically on the apical and basolateral membrane of the rabbit IMCD cells, and that both cAMP- and Ca^{2+}-dependent signaling mechanisms underlie the stimulation of Cl^- secretion.

ACKNOWLEDGEMENT

This work was supported by Pusan National University Research Grant, 1995.

REFERENCES

Chijiwa T, Mishima A, Hagiwara M, Sano M, Hayashi K, Inoue T, Naito K, Toshioka T, Hidaka H. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. *J Biol Chem* 265: 5267–5272, 1990

Communi D, Boeynaems JM. Receptors responsive to extracellular pyrimidine nucleotides. *Trends Pharmacol Sci* 18: 83-86, 1997

Communi D, Govaerts C, Parmentier M, Boeynaems JM. Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. *J Biol Chem* 272: 31969-31973, 1997

Dubyak GR, El-Moatassim C. Signal transduction via P_2-purinergic receptors for extracellular ATP and other nucleotides. *Am J Physiol* 265: C577-C606, 1993

Fredholm BB, Abbracchio MP, Burnstock G, Dubyak GR, Harden TK, Jacobson KA, Schwabe U, Williams M. Towards a revised nomenclature for P_1 and P_2 receptors. *Trends Pharmacol Sci* 18: 79-82, 1997

Goetz U, Da Prada M, Pletscher A. Adenine-, guanine- and uridine-5'-phosphonucleotides in blood platelets and storage organelles of various species. *J Pharmacol Exp Ther* 178: 210-215, 1971

Gordon JL. Extracellular ATP: effects, sources and fate. *Biochem J* 233: 309-319, 1986

Grenier FC, Smith WL. Formation of 6-keto-PGF_1 by collecting tubule cells isolated from rabbit renal papillae. *Prostaglansdins* 16: 759-772, 1978

Harden TK, Boyer JL, Nicholas RA. P_2-purinergic receptors: subtype-associated signaling responses and structure. *Annu Rev Pharmacol Toxicol* 35: 541-579, 1995

Harrison SM, Bers DM. The effect of temperature and ionic strength on the apparent Ca-affinity of EGTA and the analogous Ca-chelators BAPTA and dibromo-BAPTA. *Biochim Biophys Acta* 925: 133-143, 1987

Heilbronn E, Knoblauch BH, Muller CE. Uridine nucleotide receptors and their ligands: structural, physiological, and pathophysiological aspects, with special emphasis on the nervous system. *Neurochem Res* 22: 1041-1050, 1997

Hwang TH, Schwiebert EM, Guggino WB. Apical and basolateral ATP stimulates tracheal epithelial chloride secretion via multiple purinergic receptors. *Am J Physiol* 270: C1611-C1623, 1996

Inoue CN, Woo JS, Schwiebert EM, Morita T, Hanaoka K, Guggino SE, Guggino WB. Role of purinergic receptors in chloride secretion in Caco-2 cells. *Am J Physiol* 272: C1862-C1869, 1997

Keppler D, Rudigier J, Decker K. Enzymic determination of uracil nucleotides in tissues. *Anal Biochem* 38: 105-114, 1970

Kishore BK, Ginns SM, Krane CM, Nielsen S, Knepper MA. Cellular localization of $P2Y_2$ purinoceptor in rat renal inner medulla and lung. *Am J Physiol* 278: F43-F51, 2000

McCoy DE, Taylor AL, Kudlow BA, Karlson K, Slattery MJ, Schwiebert LM, Schwiebert EM, Stanton BA. Nucleotides regulate NaCl transport in mIMCD-K2 cells via P2X and P2Y purinergic receptors. *Am J Physiol* 277: F552-559, 1999

North RA, Barnard EA. Nucleotide receptors. *Curr Opin Neurobiol* 7: 346-357, 1997

Post SR, Rump LC, Zambon A, Hughes RJ, Buda MD, Jacobson JP, Kao CC, Insel PA. ATP activates cAMP production via multiple purinergic receptors in MDCK-D_1 epithelial cells. Blockade of an autocrine/paracrine pathway to define receptor preference of an agonist. *J Biol Chem* 273: 23093-23097, 1998

Reisin IL, Prat AG, Abraham EH, Amara JF, Gregory RJ, Ausiello DA, Cantiello HF. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J Biol Chem* 269: 20584-20591, 1994

Schwiebert EM, Egan ME, Hwang TH, Fulmer SB, Allen SS, Cutting GR, Guggino WB. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* 81: 1063-1073, 1995

Simmons NL. Chloride secretion stimulated by prostaglandin E_1 and by forskolin in a canine renal epithelial cell line. *J Physiol (Lond)* 432: 459-472, 1991

Surprenant A, Rassendren F, Kawashima E, North RA, Buell G. The cytolytic P_{2Z} receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). *Science* 272: 735-738, 1996

Williams M, Burnstock G. Purinergic neurotransmission and neuromodulation: a historical perspective. In: Jacobson KA, Jarvis MF ed, *Purinergic Approaches in Experimental Therapeutics*. Wiley-Liss, Inc., New York, p3-26, 1997

Woo JS, Inoue CN, Hanaoka K, Schwiebert EM, Guggino SE, Guggino WB. Adenylyl cyclase is involved in desensitization and recovery of ATP-stimulated Cl^- secretion in MDCK cells. *Am J Physiol* 274: C371-C378, 1998