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Pyrimidinoceptors-mediated activation of Ca^{2+}-dependent Cl^- conductance in mouse endometrial epithelial cells

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Abstract

Previous studies have demonstrated the activation of endometrial Cl^- secretion through P_{2Y2} (P_{2U}) purinoceptors by extracellular ATP. The present study further explored the presence of pyrimidine-sensitive receptors in the primary cultured mouse endometrial epithelial cells using the short-circuit current (I_{SC}) and whole-cell patch-clamp techniques. UDP induced a transient increase in I_{SC} in a concentration-dependent manner ($EC_{50} \approx 8.84$ μM). The UDP-induced I_{SC} was abolished after pretreating the epithelia with a calcium chelator, 1,2-bis-(2-aminophenoxy)-ethane-*N*,*N*,*N*′*N*′tetraacetic acid-acetomethyl ester (BAPTA-AM), suggesting the dependence of the I_{SC} on cytosolic free Ca^{2+}. The type of receptor involved was studied by cross-desensitization between ATP and UDP. ATP or UDP desensitized its subsequent I_{SC} response. However, when ATP was added after UDP, or vice versa, a second I_{SC} response was observed, indicating the activation of distinct receptors, possibly pyrimidine-sensitive receptors in addition to P_{2Y2} (P_{2U}) receptors. Similar results were observed in the patch-clamp experiments where UDP and ATP were shown to sequentially activate whole-cell current in the same cell. The UDP-activated whole-cell current exhibited outward rectification with delay activation and inactivation at depolarizing and hyperpolarizing voltages, respectively. In addition, the UDP-evoked whole-cell current reversed near the equilibrium potential of Cl^- in the presence of a Cl^- gradient across the membrane, and was sensitive to 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), indicating the activation of Ca^{2+}-activated Cl^- conductance. These characteristics were very similar to that of the ATP-activated whole-cell current. Taken together, our findings indicate the presence of distinct receptors, pyrimidinoceptors and P_{2Y2} (P_{2U}) receptors in mouse endometrial epithelial cells. These distinct receptors appear to converge on the same Ca^{2+}-dependent Cl^- channels.

Keywords: Endometrium; Mouse; Uridine diphosphate; Pyrimidinoceptor; Cross-desensitization

1. Introduction

It has long been recognized that extracellular adenosine nucleotides regulate a range of physiological responses by activation of cell surface receptors which have been shown to consist of a full cohort of members [1]. Accumulated evidence also suggests that extracellular uridine nucleotides regulate important physiological responses through specific pyrimidine-sensitive receptors, the existence of which have been postulated more than 20 years ago [2]. Although evidence for regulated release of uridine nucleotides is sparse, the high selectivity of several of the cloned receptors for UTP or UDP supports an important role of extracellular uridine nucleotides in regulating physiological functions. The first study

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showing activation of endogenously expressed pyrimidinoceptors by uridine nucleotides but not adenosine nucleotides in the rat glioma cells was reported in 1994 [3]. Since then, pyrimidinoceptors have been successfully cloned from human [4] and rat aortic smooth muscle cells [5]. Mechanically induced release of UTP has recently been demonstrated in 1321N1 human astrocytoma cells, further implicating a physiological role of uridine nucleotide receptors [6]. Recently, UDP, in addition to ATP, has been demonstrated to regulate Cl^- secretion through specific pyrimidinoceptors in airway epithelial cells [7] and equine sweat gland cells [8,9]. However, the type of ion channels involved in mediating the UDP-induced secretory response has not been elucidated.

The involvement of P_{2Y2} (P_{2U}) receptors in mediating extracellular ATP-induced Cl^- secretion has been reported in a number of secretory epithelia including the epididymis [10,11], CF pancreatic duct cells [12] and endometrial epithelium [13]. We undertook the present study to demonstrate the existence of pyrimidinoceptors in the endometrial epithelial cells by doing cross-desensitization experiments using both the short-circuit current and whole-cell patch-clamp techniques. We also characterized the Cl^- conductance activated by UDP and found that the UDP-linked pyrimidinoceptors and ATP-activated P_{2Y2} (P_{2U}) receptors converge on the same Ca^{2+}-dependent Cl^- channel. The existence of pyrimidinoceptors in the endometrial epithelial cells suggests a potential physiological role of UDP in regulating Cl^- secretion independently from the ATP-linked P_{2Y2} (P_{2U}) purinoceptors.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium with nutrient mixture F-12 (DMEM/F-12), phosphate-buffered saline (PBS), fetal bovine saline (FBS), non-essential amino acid and pancreatin were purchased from Gibco (Grand Island, New York, USA) while penicillin/streptomycin and trypsin (type II), adenosine 5′-triphosphate (ATP), 1,2-bis-(2-aminophenoxy)-ethane-*N*,*N*,*N*′*N*′-tetraacetic acid-acetomethyl ester (BAPTA-AM) and 4,-4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) were from Sigma (St. Louis, MO, USA). Uridine diphosphate (UDP) and hexokinase were obtained from Boehringer.

Since commercially available UDP was more or less contaminated with UTP, untreated UDP may assemble with some undesired UTP responses. The contaminant UTP could be removed by incubating the UTP-contaminated UDP with D-glucose (22 mM) in the presence of hexokinase (100 IU/ml UDP). After incubating at 37°C for 1 h, UTP will be hydrolyzed to UDP.

2.2. Cell isolation and culture

Endometrial epithelial cells were enzymatically isolated from the mouse uterus according to the method described by McCormack and Glasser [14] with slight modifications [15]. Uteri were obtained from 3.5- to 4-week-old immature ICR mice to avoid the complication of the estrous cycle. Uteri obtained were washed in sterile PBS (without Ca^{2+} and Mg^{2+}). After trimming off the fatty and connective tissues, the uteri were sliced longitudinally. The sliced uteri were transferred to a 15-ml centrifuge tube containing 10 ml of 7.5 mg/ml trypsin and 25 mg/ml pancreatin in PBS. Tissues were incubated at 0°C for 60 min and room temperature for another 45 min. The enzyme containing PBS was poured away carefully, 10% FBS supplemented DMEM/F-12 growth medium was added to stop activity of trypsin. The medium was replaced with PBS 5 min after. The tissue was then gently shaken for 30 s. Uterine tissue was removed and the crude cell suspension was centrifuged at $1000\times g$ for 3 min. The supernatant was discarded and the cell pellet was resuspended in 12 ml PBS. The cells were allowed to settle for 5 min and the top 2 ml was discarded. The cell suspension was centrifuged at $1000\times g$ for another 3 min. Then the cell pellet was resuspended in DMEM/F-12 containing 10% fetal bovine serum, 1% non-essential amino acids, 100 IU/ml penicillin and 100 μg/ml streptomycin. The isolated endometrial cells were seeded on a 0.45-cm^2 matrigel-coated nitrocellulose Millipore filters for short-circuit current measurement and plated onto 35-mm Petri dishes for patch clamp experiments. Cells were incubated at 37°C in 95% O_2/5% CO_2 and studied on the third day when the confluence of cell growth was reached.

2.3. Short-circuit current I_{SC} measurement

The measurement of I_{SC} has been described previously [16,17]. Monolayers grown on permeable supports were clamped vertically between two halves of the Ussing chamber. The monolayers were bathed on both sides with Krebs–Henseleit solution which was maintained at 37°C by a water jacket enclosing the reservoir. The Krebs–Henseleit solution had the following composition (in mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 24.8 $NaHCO_3$, 1.2 KH_2PO_4, 11.1 glucose. The solution was bubbled with 95% O_2/5% CO_2 to maintain the pH of the solution at 7.4. Drugs could be added directly to the apical or basolateral side of the epithelium. The epithelium exhibited a basal transepithelial potential difference for every monolayer examined, which was measured by the Ag/AgCl reference electrodes (World Precision Instruments) connected to a preamplifier which was connected in turn to a voltage clamp amplifier (DVC 1000; World Precision Instruments). The change in I_{SC} was defined as the maximal rise in I_{SC} following agonist stimulation and it was normalized as current change per unit area of epithelial monolayer (in $\mu A/cm^2$). In each experiment, a transepithelial potential difference of 0.1 mV was applied. The change in current in response to the applied potential was used to calculate the transepithelial resistance of the monolayer using Ohm's Law. Experiments were normally repeated in different batches of culture to ensure that data were reproducible.

2.4. Whole-cell patch-clamp measurement

After 3–4 days in culture, the cells formed colonies in dish. The cells were bathed in low Ca^{2+} (15 nM) containing isotonic NaCl solution for 15–20 min so as to isolate single cells for patch-clamp study. When the cells slightly detached from the Petri dish, the low Ca^{2+} solution was replaced with bath solution for patch clamp measurement.

The whole-cell patch-clamp technique as described by Hamill and coworkers [18] was employed. Current recordings were achieved by Axo patch amplifier (Axopatch-200, Axon Instruments, Forster City, CA, USA). Patch pipettes, made of borosilicate glass (Vitrex, Modulohm I/S, Herlev, Denmark), were prepared as previously described [19]. After formation of whole-cell configuration, the series resistance and cell capacitance were measured. The control of command voltages was carried out using a computer equipped with interface (TL-1-125, Axon Instruments) and utilizing the software *p*Clamp Version 5.5. The output current signals, after being filtered through the 8-pole Bessel filter (AI-2040, Axon Instruments) at a cutoff frequency of 10 kHz, were displayed on a chart recorder (Graphic, Yokohama, Japan).

The following pipette solution was used (in mM): 140 KCl, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10 HEPES, 16 glucose, 1 EGTA and 0.1 $CaCl_2$ (pH 7.2). The final concentration of Ca^{2+} of the pipette solution was 15 nM, calculated from a computer software EQCAL. The bath solution contained (in mM): 40 KCl, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10 HEPES, 16 glucose and 1 $CaCl_2$ (pH 7.4). Osmolarity of the pipette and bath solutions were adjusted to isotonic (300 mOsm) by the addition of mannitol and monitored using a vapor pressure osmometer (Wescor 5500, Logan, UT, USA).

2.5. Statistical analysis

Results are expressed as mean ± S.E.M. Comparisons between groups of data were made by Student's unpaired *t*-test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. UDP-induced I_{SC}

The primary cultured mouse endometrial epithelial monolayers responded to apical UDP (100 μM) with a prompt increase in the I_{SC} which usually returned to basal level in 5 min (Fig. 1A). The UDP-evoked I_{SC} was very similar to that induced by apical addition of ATP (10 μM) (Fig. 1B), which has previously been demonstrated to activate Ca^{2+}-dependent Cl^- secretion in the mouse endometrial epithelial cells [13]. About 56% of the UDP responses (38 out of 68) exhibited biphasic characteristic: a large, rapid and transient peak followed by a relatively small and sustained peak. In this study, only the first peak was used for analysis. The dose–response curve

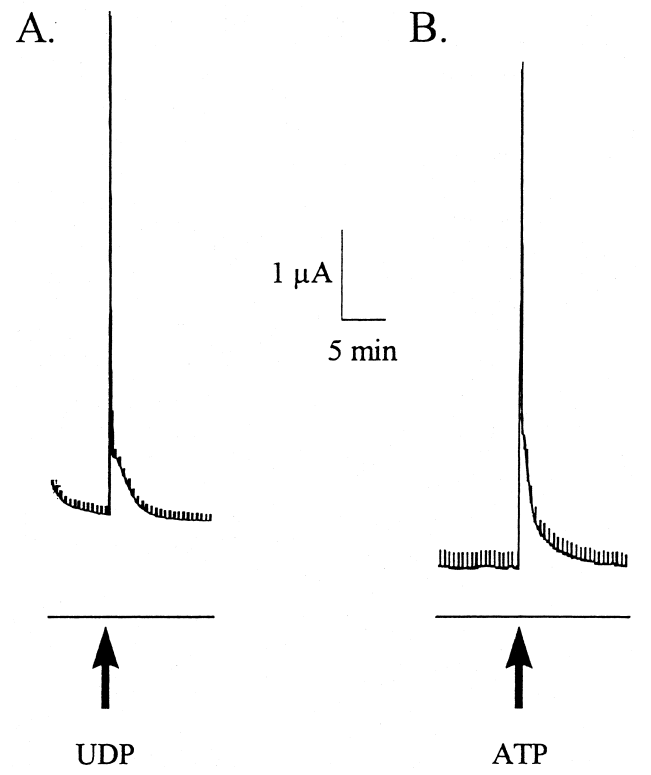

Fig. 1. UDP- and ATP-induced I_{SC} responses. Representative I_{SC} recordings showing transient increase in I_{SC} response to apical addition of (A) UDP (100 μM), and (B) ATP (10 μM). Note that the appearance of UDP- and ATP-induced I_{SC} is very similar. The horizontal bar indicates zero I_{SC}.

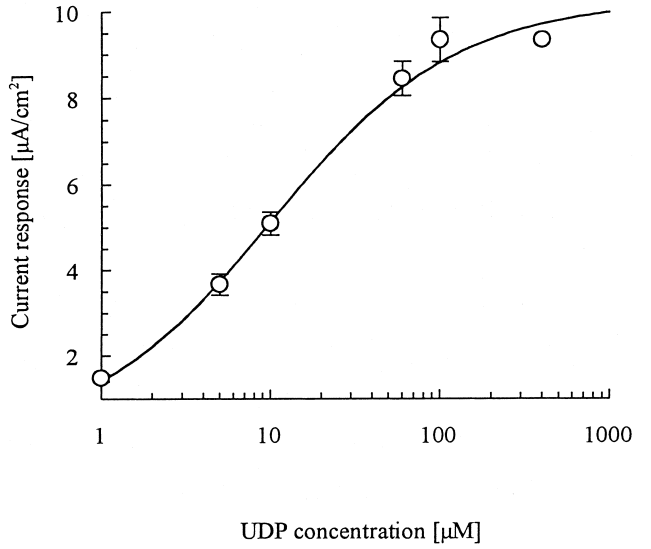

Fig. 2. Dose–response curve demonstrating the dependence of I_{SC} on the apical UDP concentrations. The increase in transient peak I_{SC} upon UDP stimulation is plotted against the logarithmic concentration of UDP. Data for each point were obtained from at least three independent experiments. Values are mean ± S.E.M. The EC_{50} is 8.84 μM.

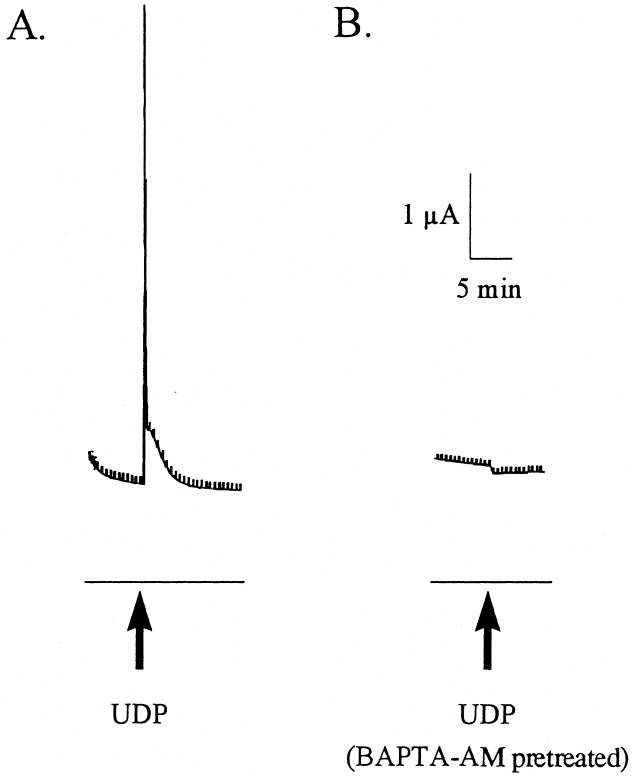

Fig. 3. Effect of Ca^{2+} chelator (BAPTA-AM) on the UDP response. I_{SC} recordings of (A) control and (B) BAPTA-AM-pretreated cells. The epithelia were apically and basolaterally pretreated with 50 μM BAPTA-AM for 20 min before the addition of UDP (100 μM, $n=3$). Note that the I_{SC} response to UDP is abolished when intracellular Ca^{2+} is chelated indicating the involvement of cytosolic free Ca^{2+}.

in Fig. 2 shows a concentration-dependent effect of UDP on the I_{SC} (EC_{50} at about 8.84 μM). The mean I_{SC} responded to apical 100 μM UDP is 9.35 ± 0.5 μA/cm^2 ($n=20$). Separate sets of experiments were performed with different recording scale such that current changes in response to voltage pulse could be recorded and used to calculate transepithelial resistance or conductance. No significant change in transepithelial resistance before and after UDP challenge was observed, 316 ± 74 and 297 ± 74 Ωcm^2 ($n=4$), respectively. This suggested that the possible change in conductance induced by UDP was not detectable using this technique (see Section 3.2).

3.2. Dependence of intracellular free Ca^{2+}

In order to see if the UDP-induced I_{SC} depends on

cytosolic free Ca^{2+}, the epithelia were pretreated with a Ca^{2+} chelator, BAPTA-AM (50 μM, apical and basolateral). As shown in Fig. 3, the UDP response was abolished when possible contribution of intracellular free Ca^{2+} was eliminated by increasing cytosolic Ca^{2+} buffering capacity with BAPTA-AM. This suggests that the UDP-induced I_{SC} in mouse endometrial epithelial cells is Ca^{2+}-dependent.

3.3. Involvement of pyrimidinoceptors

We undertook cross-desensitization experiments to see whether the same or distinct types of receptors were involved in mediating the UDP and ATP responses. The epithelia were repeatedly stimulated with ATP of the same concentration (100 μM) (Fig. 4A) followed by UDP (100 μM) stimulation. The epithelia showed desensitization to the second challenge of ATP but responded to the subsequent UDP stimulation although the I_{SC} response was greatly reduced. A similar result was obtained when the order of drug addition was reversed. However, UDP showed less cross-desensitization with ATP-activated receptors, presumably P_{2Y2} (P_{2U}) receptors, since UDP did not greatly suppress the subsequent I_{SC} activation by ATP (Fig. 4B). Since desensitization depends critically on the time duration between the first and the second challenge, separate experiments were conducted with second challenge of UDP or ATP given at the same time, 10 min after the first challenge by UDP. The response to ATP after UDP challenge (Fig. 4D) was much greater than the second response to UDP (Fig. 4C), suggesting that the two responses were mediated by distinct receptors subtypes, the pyrimidinoceptors by UDP, and P_{2Y2} (P_{2U}) purinoceptors by ATP, in mouse endometrial epithelial cells.

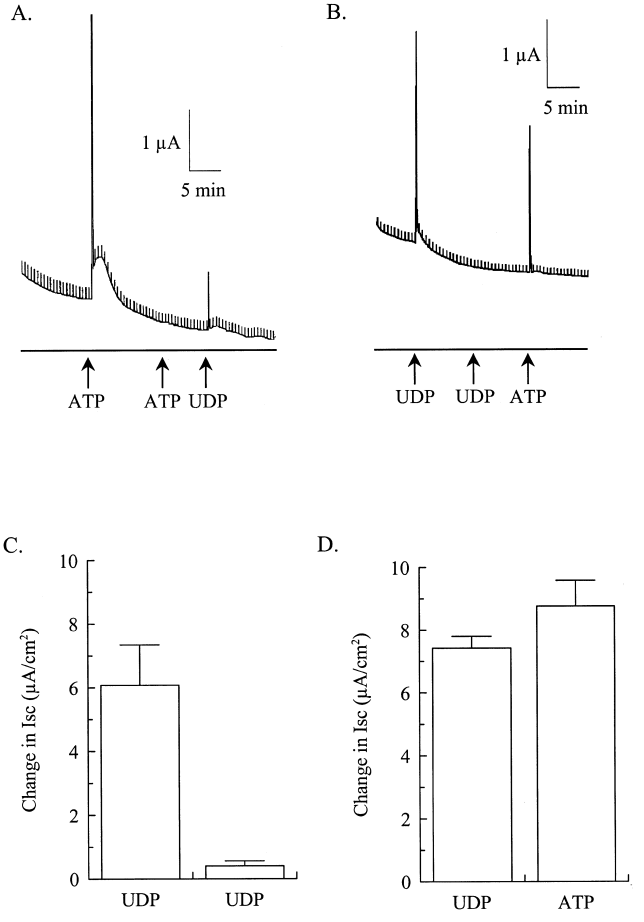

Fig. 4. Cross-desensitization experiments demonstrating the presence of distinct receptor subtypes. I_{SC} recordings showing the subsequent I_{SC} response to (A) UDP in the presence of ATP ($n=5$) and (B) ATP in the UDP-pretreated cells ($n=6$). Separate experiments were conducted to compare current responses to the second challenge of UDP (C, $n=6$); and of ATP (D, $n=4$), 10 min after the first challenge of UDP.

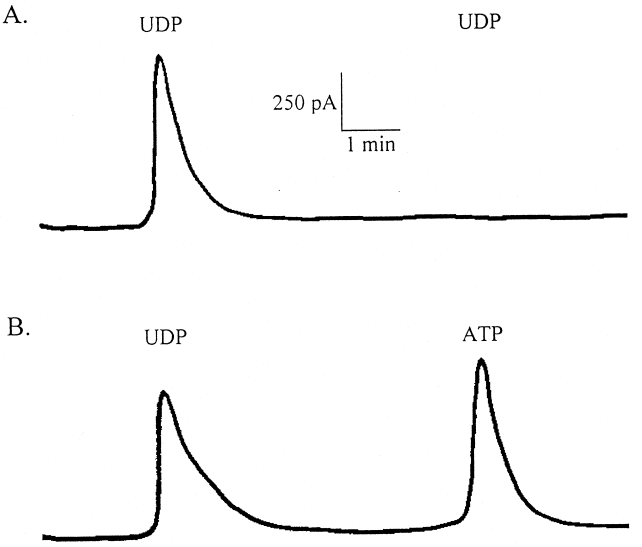

Fig. 5. Demonstration of the activation of distinct receptor subtypes using whole-cell patch-clamp technique. Whole-cell current recordings showing the sequential addition of (A) UDP and UDP ($n=3$), and (B) UDP and ATP ($n=3$) in the same patch-clamped cell. The tracings are the time-dependent change in whole-cell current elicited by a command voltage of 120 mV. Note that UDP and ATP sequentially activate whole-cell currents in the same patch-clamped cell further confirming the activation of distinct receptors.

The presence of UDP-selective pyrimidinoceptors was further demonstrated using the whole-cell patch-clamp technique. As illustrated in Fig. 5, UDP (100 μM) and ATP (100 μM) sequentially evoked whole-cell current in the same patch-clamped endometrial epithelial cells ($n=3$). The tracings were the time-dependent changes in whole-cell current elicited by a command voltage of 120 mV which was given at a 3.25-s interval. Repeating challenge of cells with the same nucleotides, UDP, did not give rise to second response (Fig. 5A). However, ATP activated a whole-cell current, after current activation by UDP (Fig. 5B), indicating little cross-desensitization between UDP and ATP. This could only be the case when UDP and ATP independently activated whole-cell currents through distinct receptors, presumably pyrimidinoceptors and P_{2Y2} (P_{2U}) receptors.

3.4. UDP-evoked whole-cell current

The I_{SC} experiments (see above) were not able to detect significant change in conductance upon UDP or ATP challenge since the intracellular conductance could be masked by the paracellular conductance using the technique. Patch-clamp technique was employed to resolve the problem since the observed current change reflects activation of membrane conductance. In addition, the type of ion channels involved in mediating the UDP response could be characterized by examining the whole-cell current profile elicited by UDP. Whole-cell current was generated under a series of command voltage pulses which ran from 0 mV to potentials between −120 and 120 mV with 20 mV increment. UDP (100 μM) evoked a 21-fold increase in peak whole-cell current at 120 mV (Fig. 6B). The UDP-induced whole-cell current exhibited delayed activation and inactivation at depolarizing and hyperpolarizing voltages, respectively, with outward rectifying current-voltage (I–V) relationship (Fig. 6C). In the presence of a Cl^- gradient (pipette 140 mM: bath 40 mM), the reversal potential 22.7 ± 2.9 mV ($n=7$), was close

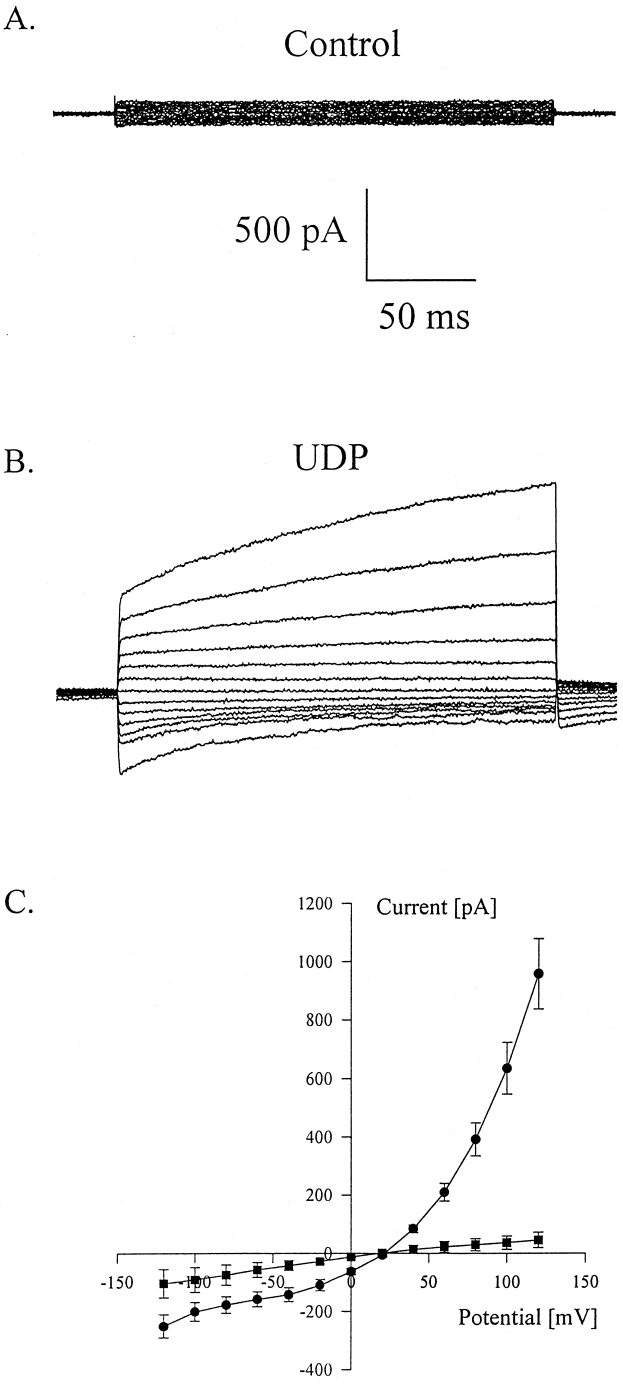

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Fig. 6. Characteristics of the UDP-evoked whole-cell current. Whole-cell current recordings (A) prior to and (B) after UDP stimulation. The whole-cell currents were elicited by a series of command voltages which ran from 0 mV to potentials between −120 and 120 mV with 20 mV increment. (C) Averaged I–V relationship curve of the UDP-induced whole-cell current at 220 ms ($n=7$). The UDP-activated whole-cell current reverses at 22.7 ± 2.9 mV. Note that the UDP-activated whole-cell current exhibits delayed activation and inactivation at depolarizing and hyperpolarizing voltages, respectively, with outward rectifying I–V relationship.

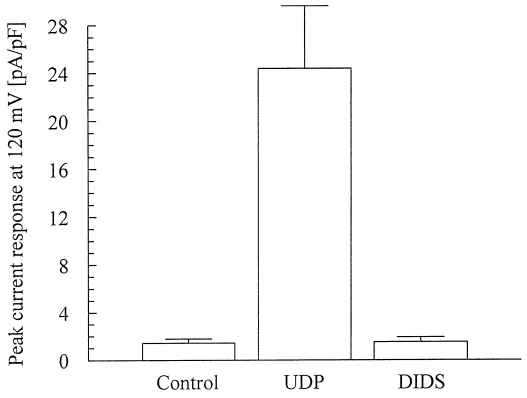

Fig. 7. Effect of DIDS on the UDP-activated whole-cell current. Statistical results showing the peak current magnitude at 120 mV before, after UDP stimulation and after DIDS inhibition. DIDS inhibits 99 ± 0.2% of the UDP-stimulated whole-cell current. The high sensitivity of the UDP-evoked whole-cell current to DIDS indicates the activation of Ca^{2+}-dependent Cl^- channels.

to the calculated equilibrium potential for Cl^-, 30 mV, indicating the activation of Cl^- conductance.

3.5. Sensitivity to Cl^- channel blocker DIDS

The Cl^- channel blocker, DIDS, was well documented to block Ca^{2+}-dependent Cl^- channels in many epithelial cells. At a concentration of 100 μM, DIDS inhibited most of the UDP-evoked whole-cell current ($n = 6$). As summing up in Fig. 7, 99.7 ± 0.2% of the UDP-activated peak currents at depolarizing voltage, 120 mV, were inhibited ($P < 0.001$) by DIDS. The high sensitivity of the UDP-evoked whole-cell current to DIDS indicated the activation of Ca^{2+}-dependent Cl^- channels.

3.6. Comparison to the ATP-activated whole-cell current

The UDP-induced whole-cell current profile was similar to that elicited by ATP (Fig. 8). Concentration of UDP and ATP used in the whole-cell patch-clamp experiments was 100 μM. Both UDP- and ATP-activated whole-cell current showed time and voltage dependency: delayed activation at depolarizing voltages and delayed inactivation at hyperpolarizing voltages. In addition, with an outwardly rectifying *I–V* relationship. The UDP- and ATP-induced whole-cell current reversed at 22.7 ± 2.9 mV

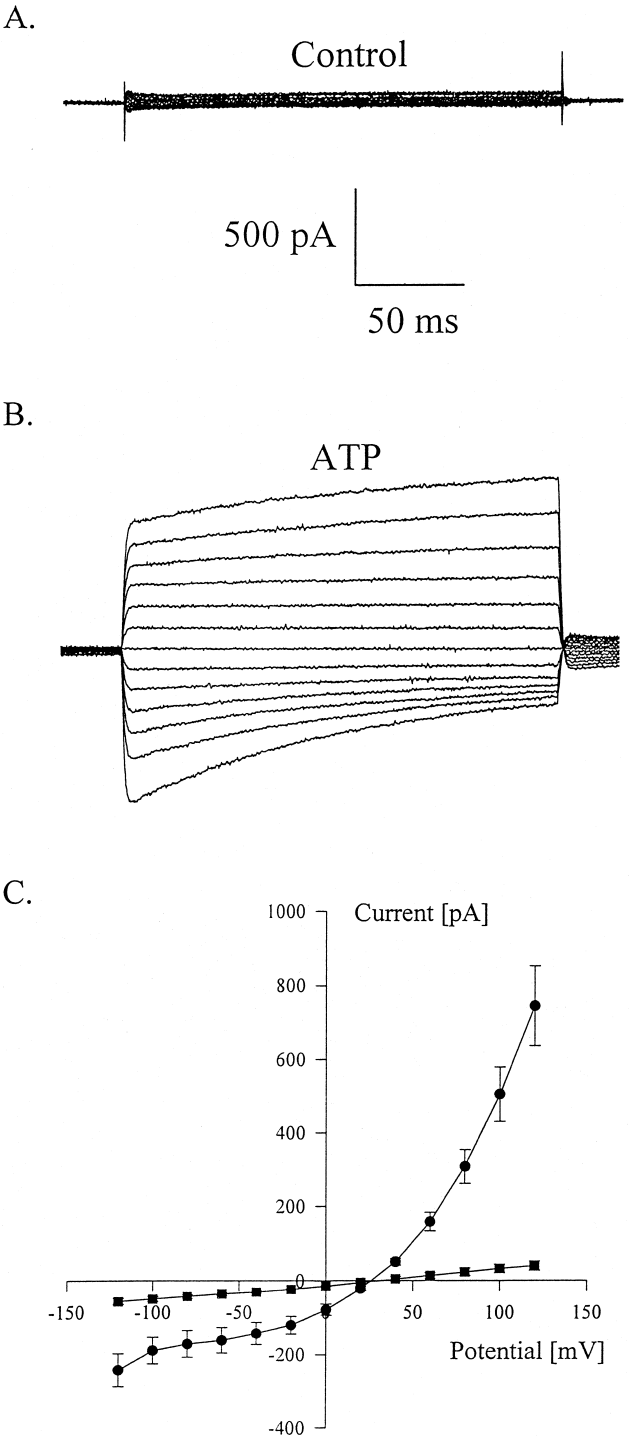

Fig. 8. Characteristics of the ATP-stimulated whole-cell current. Whole-cell current recordings (A) prior to and (B) after ATP stimulation. (C) Averaged *I–V* plot of the ATP-activated whole-cell current. The reversal potential of the ATP-induced whole-cell current is 26 ± 1.1 mV ($n = 8$). Note that the ATP-induced whole-cell current is similar to that evoked by UDP (Fig. 6) suggesting the activation of the same Ca^{2+}-activated Cl^- conductance.

($n=7$) and 26 ± 1.1 mV ($n=8$), respectively, close to the theoretical equilibrium potential of Cl^-. Together with the high sensitivity of the UDP- and ATP-evoked whole-cell current to DIDS, this study indicates that UDP and ATP converged on the Ca^{2+}-dependent Cl^- channels although they activated distinct receptors.

4. Discussion

The present study has demonstrated the presence of pyrimidinoceptors in the mouse endometrial epithelium which has been previously shown to respond to extracellular ATP via the activation of P_{2Y2} (P_{2U}) receptors [13]. Supporting evidence for the existence of pyrimidinoceptors came from the study of cross-desensitization between UDP and ATP. Since neither ATP nor UDP induced complete cross-desensitization, it indicates that UDP and ATP act on pharmacologically distinct receptors, presumably UDP for pyrimidinoceptors, and ATP for P_{2Y2} (P_{2U}) receptors. Cross-desensitization experiments using whole-cell patch-clamp technique also showed that UDP and ATP sequentially activated whole-cell current in the same patch-clamped cells indicating the presence of two distinct types of receptors in the same cell population. Taken together, our data suggest the existence of UDP-stimulated pyrimidinoceptors in the mouse endometrial epithelial cells in addition to the previously reported ATP-linked P_{2Y2} (P_{2U}) receptors [13]. However, it should be noted that the nucleotide concentrations used to activate I_{SC} are different from that used to activate whole-cell current under patch-clamped condition; i.e. an order of magnitude higher was used in patch-clamp experiments. This may be due to the difference in culture conditions leading to the difference in the amount of receptors and/or ion channels expressed. When cells are grown in Petri dishes, where they do not form polarized epithelium, fewer receptors/channels are expressed [20] as compared to when cells are grown in permeable supports (for I_{SC} experiments); therefore, higher concentrations are required to elicit a whole-cell current response. Similar observations have been made previously with other agonists, i.e. adrenaline and PGE_2, indicating a higher magnitude of concentration required for the patch-clamp experiments as compared to the concentration required for the I_{SC} experiments on endometrial epithelial cells [21] as well as for epididymal cells [22]. Alternatively, direct patching of the cell with dialysis of cytosolic components into pipette solution may also reduce the sensitivity to the agonist accounting for a higher concentration required.

Previous studies have demonstrated the P_{2Y2} (P_{2U})-mediated activation of Ca^{2+}-dependent Cl^- channel by extracellular ATP in the mouse endometrial epithelial cells [13]. The presently demonstrated presence of pyrimidinoceptors has posted a question as to whether these receptors use a signaling pathway distinct from that used by P_{2Y2} (P_{2U}) receptors. The present study has also suggested the involvement of Ca^{2+} in mediating the pyrimidinoceptor-activated secretory response in mouse endometrial epithelial cells since the UDP-activated I_{SC} was abolished in the presence of a Ca^{2+} chelator, BAPTA-AM. The similarities between the UDP-activated and ATP-activated I_{SC}, i.e. the I_{SC} current kinetics, biphasic nature, tend to suggest a similar signaling mechanism, at least at the cellular messenger level, for the two distinct receptors.

Although the presence of pyrimidinoceptors and their involvement in regulating Cl^- secretion have been demonstrated previously in the airway epithelial cells [7] and equine sweat gland cells [8,9], the type of ion channels involved has not been elucidated. The present study has further characterized the pyrimidinoceptor-mediated UDP-activated conductance using the whole-cell patch-clamp technique. UDP-activated whole-cell current exhibits characteristics of delayed activation at depolarizing voltages and inactivation at hyperpolarizing voltages, respectively, with outwardly rectifying I–V relationship. The UDP-activated whole-cell current reversed at the equilibrium potential of Cl^- in the presence of a Cl^- gradient across the membrane indicating the activation of Cl^- conductance. In addition, the UDP-activated whole-cell current was blocked by Ca^{2+}-dependent Cl^- channel blocker, DIDS, further suggesting the activation of Ca^{2+}-mediating Cl^- channel by UDP. These characteristics are consistent with those exhibited by the Ca^{2+}-dependent Cl^- channels in many epithelial cells including the airways [19,23] and epididymal epithelial cells [24]. In addition to the similarity between the UDP and ATP-activated

I_{SC}, the UDP-activated whole-cell current also exhibits the same characteristics as that activated by ATP, which have been previously shown to be associated with Ca^{2+}-activated Cl^- channels [13]. Taken together, our data suggest that distinct pyrimidinoceptors and P_{2Y2} (P_{2U}) receptors converge on the same final effector, the Ca^{2+}-activated Cl^- channels, to elicit secretory response in the mouse endometrial epithelial cells.

Although the reason for the presence of two distinct receptors converged on the same Ca^{2+}-dependent Cl^- channel in the same endometrial epithelial cell is not clear, it appears to be a general phenomenon observed in a variety of cells for P_{2Y} receptors to have a common signaling pathway [6,8]. Our previous studies on the mouse endometrial cells have also demonstrated the convergence of multiple neurohormonal regulatory pathways on another Cl^- conductance, i.e. activation of cAMP-dependent Cl^- channel, presumably cystic fibrosis transmembrane conductance regulator, by β-adrenoceptor and prostaglandin receptors (EP_2/EP_4) [20,25,26]. These findings suggest that either cAMP-activated or Ca^{2+}-activated Cl^- channels are important for mediating various neurohormonal responses in the mouse endometrium.

The physiological source of extracellular nucleotides remains to be determined. It is believed that ATP and UTP can be released from the damaged cells and exert their effect [7]. However, the effect of ATP and UTP could be reduced since they are rapidly hydrolyzed by ecyonucleotidases, which occur widely in every cell. The presence of pyrimidinoceptors may then serve as the target for the degradation product of UTP, i.e. UDP. Recently, mechanically induced release of uridine has been demonstrated, suggesting a possible physiological source of uridine [6]. Similar uridine release mechanism may be anticipated in the uterus during its contraction. On the other hand, sperm traveling through the uterus represents another possible source of nucleotides, particularly ATP. The fact that ATP highly cross-desensitizes pyrimidinoceptors, i.e. ATP greatly reduced subsequent I_{SC} activated by UDP, suggests that ATP may be the physiological agonist of both P_{2Y2} (P_{2U}) and pyrimidinoceptors in the uterus. On the other hand, UDP show less cross-desensitization with P_{2Y2} (P_{2U}) receptors, suggesting that UDP may selectively activates pyrimidinoceptors. Therefore, these distinct receptors may act coherently or independently depending on the physiological availability of specific types of nucleotides.

In summary, the present study has demonstrated the presence of pyrimidinoceptors in addition to P_{2Y2} (P_{2U}) receptors in the mouse endometrial epithelial cells. The present study has also demonstrated for the first time the pyrimidinoceptor-mediated activation of Ca^{2+}-dependent Cl^- channels. The results show that distinct receptors, P_{2Y2} (P_{2U}) and pyrimidinoceptors, converge on a Ca^{2+}-dependent pathway leading to the activation of the same type of Cl^- channels. The present study suggests a role of endogenous endometrial pyrimidinoceptors and P_{2Y2} (P_{2U}) receptors in the regulation of uterine fluid environment. However, the physiological significance of the presence of distinct receptors coupled to the same signaling pathway remains to be elucidated.

Acknowledgements

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