P$_{2U}$ purinergic receptor inhibits apical I$_{sk}$/KvLQT1 channel via protein kinase C in vestibular dark cells

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Abstract: The transepithelial short-circuit current (I$_{sc}$) has been shown to be accounted for by electrogenic K$^+$ secretion (18, 19) and has recently been found to be reduced in the presence of apically perfused extracellular nucleotides (13). The mediator of this effect was determined to be a purinergic receptor of the P$_{2U}$ (P2Y$_2$) subtype in the apical membrane. The present experiments were designed to test the hypothesis that activation of the apical P$_{2U}$ receptors results in inhibition of the K$^+$ secretory flux (I$_{sk}$) by inhibition of the current through the apical I$_{sk}$/KvLQT1 channels and to determine the signal pathway between these two events. In other systems, P$_{2U}$ receptors are coupled to phospholipase C (PLC), which activates parallel signal pathways, resulting in elevation of cytosolic Ca$^{2+}$ via inositol 1,4,5-trisphosphate (InsP$_3$) and in phosphorylation of effectors proteins via protein kinase C (PKC) (4, 6). The present results are consistent with the phosphorylation of the I$_{sk}$/KvLQT1 channel by PKC as the signal mechanism of the apical P$_{2U}$ receptor, rather than elevation of cytosolic Ca$^{2+}$.

METHODS

Tissue preparations. Gerbils were anesthetized with pentobarbital sodium (50 mg/kg, ip) and decapitated. The temporal bone was removed, and VDC epithelia was dissected without enzymatic treatment at 4°C in solution 2 (Table 1) as described previously (31). The tissue was either transferred to a recording chamber continuously perfused at 37°C or frozen in liquid nitrogen within 10 min of death for reverse transcription-polymerase chain reaction (RT-PCR). The heart was cut and frozen on dry ice or liquid nitrogen within 5 min of death.

Solutions and chemicals. The compositions of solutions are listed in Table 1. Nystatin (200 μg/ml; Sigma, St. Louis, MO) was dissolved with sonication in the pipette solution (solution 4, Table 1) just before use. ATP (Sigma) was dissolved directly in pipette and bath solution, whereas U-73122, U-73343 (Biomedical, Plymouth Meeting, PA), A-23187, phorbol-12-myristate-13-acetate (PMA), 4α-phorbol 12,13-didecanoate (4α-PMA) (Calbiochem, La Jolla, CA), and GF109203X (Tocris Cookson, St. Louis, MO) were predissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 0.1%. All other chemicals for the electrophysiological experiments were purchased from Sigma or Fluka (Ronkonkoma, NY).

Self-referencing K$^+$-selective probe. The self-referencing probe techniques used were nearly identical to those previously described (14, 18). Signals were sampled from the probe amplifier with a 16-bit analog-to-digital convertor (CIO-DAS1602/16, Computer Boards, Mansfield, MA), and the probe was moved on manipulators (Applicable Electronics, Forest Dale, MA) by a 486-based computer and specialized probe software (ASET version 1.0, Science Wares, East Falmouth, Massachusetts).
Extraction of total RNA. The total RNA was extracted using TRIzol reagent according to the manufacturer’s procedure. Total RNA was precipitated by isopropanol and dissolved in ribonuclease (RNase)-free water (diethyl pyrocatechol-treated water). The nucleic acid concentration was determined spectrophotometrically, the integrity of the RNA was determined by the presence of 28S and 18S ribosomal RNA bands by horizontal agarose (1%) gel electrophoresis, and the final nucleic acid concentration was adjusted to ~1–2 µg/µl. RNA samples were stored at ~70°C.

Vestibular labyrinth from gerbil was isolated by microdissection, and tissues were frozen in liquid nitrogen within 12 min of death of each animal. Tissues from eight ears were pooled in TRIzol reagent and were triturated through a 25-gauge needle. Total RNA was then extracted according to the manufacturer’s procedure. Total RNA from 100 µl of blood was also isolated using 1 ml of TRIzol reagent. The final nucleotide concentration of the RNA samples from vestibular labyrinth and blood was ~0.3 and 0.25 µg/µl, respectively. Directly before the RT-PCR procedure, residual genomic DNA in RNA samples from heart, vestibular labyrinth, and blood was removed by treatment with amplification-grade RNase-free deoxyribonuclease I (GIBCO BRL, Life Technologies) for 30 min at room temperature followed by heat inactivation in the presence of EDTA, according to the protocol specified by the manufacturer.

dCAS synthesis and PCR amplification. Total RNA was reverse transcribed into cDNA in a 10-µl reaction. The reaction contained 0.1–0.5 µg total RNA, 10 units RNasin (Promega), 1 mM dNTP (GIBCO BRL, Life Technologies), 25 units Moloney murine leukemia virus MMLV) reverse transcriptase (Perkin-Elmer), 2.5 mM MgCl2 (GIBCO BRL, Life Technologies), 25 pmol oligo(dT), 20 mM tris(hydroxymethyl)-amino- thane (Tris)-HCl, and 50 mM KCl. Tris-HCl and KCl were added from a 10× PCR buffer (GIBCO BRL, Life Technologies). The RT reaction was incubated at room temperature for 10 min, at 42°C for 50 min, at 99°C for 5 min, and at 5°C for 5 min.

The 50-µl PCR mixture contained the 10 µl RT reaction mixture in addition to 25 pmol each of antisense and sense primers for 1K, and 1.25 units Taq DNA polymerase (GIBCO BRL, Life Technologies). The final concentrations of MgCl2, KCl, and Tris-HCl were adjusted to 2.5, 50, and 20 mM, respectively. The PCR mixture was incubated as follows: 1 denaturation cycle for 3 min at 95°C; 45 amplification cycles consisting of denaturation for 1 min at 95°C, annealing for 1 min at 63°C, and extension for 1 min at 72°C; and one extension cycle for 5 min at 72°C in an Perkin-Elmer thermocycler 480. PCR products were analyzed by horizontal electrophoresis in 2.5% agarose gels and visualized by ethidium bromide.

Cloning and sequencing of amplified cDNA fragments. Amplified cDNA fragments were extracted from the agarose gels using the QIAquick gel extraction kit (Qiagen) and cloned into a pCR2.1 vector with a TA cloning kit (Invitrogen). Recombinant plasmids were isolated from the colonies using the standard alkaline lysis procedure, purified by phenol/chloroform extraction, and precipitated and washed with ethanol. Insertion of the PCR product into the plasmid was confirmed by restriction endonuclease digestion with EcoRI and subsequent horizontal gel electrophoresis. The recombinant plasmid served as a template for cycle sequencing using M13 forward and reverse primers and fluorescence-based dideoxy nucleotides (PRISM Ready Reaction dye deoxy terminator cycle sequencing, Perkin Elmer). The sequence was then determined using the ABI model 373 DNA sequencer (Applied Biosystems, CReighton Molecular Biology Core Facility).
Micro-Ussing chamber. The methods were described previously (16). Briefly, tissue was placed in a micro-Ussing chamber, and the seal to the aperture (80 µm diameter) between two hemichambers was made with the apical side of the VDC epithelium. The apical and basolateral sides of the tissue were perfused independently, and exchange of solution (24 or 37°C) on each side was complete within 1 s. Transepithelial voltage (Vt) was measured between calomel electrodes connected to the hemichambers via flowing 1 M KCl bridges. Transepithelial resistance (Rt) was obtained from the voltage change induced by current pulses (50 nA for 34 ms at 0.3 Hz). Sample and hold circuitry was used to obtain a signal proportional to Rt. The equivalent IS was derived from Vt and Rt (Isc = Vt/Rt). IS and Rt were normalized for the area defined by the aperture of the micro-Ussing chamber.

Macropatch clamp. The macropatch technique was described previously (17). Pipettes (3–6 µm ID) were made from Corning 7052 glass capillary with a two-stage puller and a microforge. Pipette tips were coated with a 2:1 mixture of α-tocopherol acetate and heavy mineral oil and filled with NaCl pipette solution (solution 3, Table 1). Tissues were folded into a loop with the apical membrane facing outside and mounted in the recording chamber. High-resistance seals (2–8 GΩ) were made to the apical membrane of VDC, and currents were recorded in the cell-attached configuration. The recording technique was identical to that previously reported (17).

The patch-pipette perfusion technique was described previously (25). In brief, an internal perfusion pipette was connected to either of two solution vials via a valve and the internal pipette positioned within the macropatch pipette. Positive pressure applied to the vials drove the selected solution through the patch pipette. The arrival of new solution was delayed by ∼2 min due to diffusion over ∼300 µm between the outlet of the internal perfusion pipette and the membrane patch.

Perforated-patch whole cell clamp. The perforated-patch whole cell clamp technique was chosen over the conventional whole cell technique to avoid rundown of channel activity (17). Perforated-patch experiments were conducted in the absence of Cl⁻ to reduce the contribution of the basolateral Cl⁻ conductance to the whole cell conductance and thereby to increase the ratio between the cell membrane resistance and the access resistance. This was a necessary condition to effectively clamp the membrane potential in these cells. An unusually high Cl⁻ conductance in the basolateral membrane of these cells is responsible for an extremely low resistance of the cell membrane, typically <20 MΩ/cell in the presence of 150 mM Cl⁻ in the bathing medium (32). K⁺ secretion by the apical conductive pathway was supplied under whole cell conditions by the pipette electrolyte diffusing through the nystatin perforations.

The tissues were continuously perfused with Cl⁻-free bath solution (solution 2, Table 1). The internal diameter of the tip of the patch pipette was 1–2 µm with a resistance of 8–12 MΩ in the Cl⁻-free bath solution. The tip of the patch pipette was backfilled with a 1-cm column of K⁺-rich, Cl⁻-free solution (solution 4, Table 1) containing 200 µg/ml nystatin. The rest of the pipette was backfilled with 15 mM Cl⁻ solution (solution 5) to stabilize the Ag/AgCl junction. Gigaohm seals were made to the apical membrane, after which the membrane patch was perforated by insertion of the nystatin. After a stable whole cell configuration was established, the access resistance (31 ± 1 MΩ, n = 23) and the membrane capacitance (53 ± 23 pF, n = 23) were measured with the circuitry in the patch amplifier (Dagan 3900 and Axon Instruments 200A). Capacitance was nearly fully compensated and resistance compensated by typically 65%.

The seemingly large capacitance observed in the present study is consistent with the large membrane area due to extensive basolateral infoldings. Currents from individual VDC can be recorded in the native epithelium, since no gap junctions have been found among them (9).

Voltage protocols. Voltages are expressed with the usual convention of the intracellular compartment with respect to the extracellular side. In on-cell experiments, voltages are not corrected for the cell membrane potential. The membrane potential is about −18 mV in vitro with NaCl physiological saline bathing both sides of the epithelium (32).

The whole cell voltage protocol has been described in detail previously (27), and the protocol was repeated every 30 s to record the time course of changes during experimental treatments. For each instance of the protocol, the cell was first held at 0 mV; a tail-current I-V plot was produced from four pulses of 10–20 ms each. The IsK channel current (IIsK) was then deactivated by holding the cell at −50 mV for 4 s (17), and a tail-current I-V plot was produced of the “leak” current. The IsK currents were sampled for the I-V plots after decay of capacitive transients, and the active currents were corrected for the leak currents. The interval between protocols was chosen to allow ample time for recovery of channel activity at the activating holding potential (0 mV). Measurements of currents under both control and experimental conditions in each cell allowed the application of paired statistics.

The voltage protocol used for on-cell macropatch recordings was similar, but repeated every 15 s. This interval was found to be adequate for full recovery of activation under these conditions. The deactivating voltage was −40 mV (the cell membrane voltage hyperpolarized the membrane an additional amount of −18 mV). Currents at two voltages (−40 and 0 mV) were used to estimate the leak conductance. Parameters derived from these recordings include the current and conductance of the apical IsK channels (IIsK and gIsK, respectively), the reversal voltage without correction for leak (Vr), and the time constant of deactivation (τr)."
however, that the response of VDC to apical ATP involves a decrease in transepithelial $I_K$.

Inhibition of $I_{SK}$ by extracellular ATP. On-cell macropatch-clamp recordings were made of $I_{SK}$, $g_{SK}$, $V_r$, and $\tau_{off}$ from VDC as described in METHODS. Data were obtained by averaging the last three values of the control period and of the experimental period for each experiment. Perfusion of ATP (10 µM) for 4 min through the patch pipette led to a decrease in $I_{SK}$ by 83.4 ± 7.4% and in $g_{SK}$ by 48.6 ± 9.3% and to a depolarization of $V_r$ by 24.6 ± 5.6 mV (n = 5; Fig. 2, Table 2). The $\tau_{off}$ could not be measured for the entire duration of inhibition by ATP but did not change significantly during the first 90 s, when the current had already decreased by 58 ± 18%.

Inhibition of PLC reduces effect of apical ATP. Addition of U-73122 at 4 µM to the apical perfusate had no significant effect on $I_{SC}$ (n = 5). However, apical ATP (1 µM) caused less of a decrease of $I_{SC}$ in the presence of U-73122 than in its absence (Fig. 3A, Table 2). ATP decreased $I_{SC}$ by 24.1 ± 3.9% in the presence of U-73122 and by 32.7 ± 4.1% in its absence corresponding to a fractional response to ATP of 73 ± 4% in the presence of U-73122 compared with that in its absence. Raising the concentration of U-73122 to 10 µM resulted in a further reduction of the effect of ATP by 52.0 ± 8.0% without U-73122 vs. 30.0 ± 6.4% (n = 6) in the presence of U-73122, although there was also a small decrease of $I_{SC}$ from the U-73122 itself at this concentration (17.6 ± 4.3%). The fractional response to ATP in the presence of 10 µM U-73122, 56 ± 7% of that in the absence of U-73122, was less than that at 4 µM U-73122. The inactive analog, U-73343 (4 µM), had no effect on $I_{SC}$ and ATP (1 µM) caused the same magnitude decrease in the absence (30.3 ± 4.2%) and presence (32.0 ± 3.9%, n = 6; Fig. 3B, Table 2) of U-73343.

| Table 2. Transepithelial voltage, transepithelial resistance, and short-circuit current in the absence or presence of ATP or drug |
|---|---|---|
| | Before Addition of ATP or Drug | In the Presence of ATP or the Added Drug |
| | $V_t$, mV | $R_t$, Ω·cm² | $I_{SC}$, µA/cm² | $V_t$, mV | $R_t$, Ω·cm² | $I_{SC}$, µA/cm² |
| ATP (100 µM), 24°C (n = 6) | 5.3 ± 6 | 13.0 ± 1.9 | 428 ± 47 | 3.0 ± 0.3* | 12.9 ± 2.0 (NS) | 257 ± 41* |
| ATP (100 µM), 37°C (n = 9) | 13.4 ± 1.1 | 25.4 ± 2.1 | 535 ± 28 | 3.1 ± 0.5* | 15.9 ± 11.1* | 192 ± 26* |
| U-73122 (4 µM) (n = 5) | 4.0 ± 0.3 | 5.2 ± 0.7 | 815 ± 97 | 4.0 ± 0.3 (NS) | 5.4 ± 0.7 (NS) | 793 ± 96 (NS) |
| ΔATP (1 µM) = U-73122 (4 µM) | −1.8 ± 0.1 | −0.2 ± 0.1 | −243 ± 17 | −0.7 ± 0.1* | 0.0 ± 0.1 (NS) | −174 ± 16* |
| U-73122 (10 µM) (n = 6) | 5.8 ± 0.6 | 8.8 ± 1.3 | 674 ± 98 | 3.6 ± 0.6* | 6.2 ± 0.6* | 577 ± 109 (NS) |
| ΔATP (1 µM) = U-73122 (10 µM) | −3.4 ± 0.5 | −0.2 ± 0.1 | −310 ± 20 | −0.8 ± 0.2* | 0.2 ± 0.2 (NS) | −122 ± 19* |
| U-73343 (10 µM) (n = 6) | 4.1 ± 0.3 | 6.9 ± 0.3 | 564 ± 20 | 4.1 ± 0.3 (NS) | 7.0 ± 0.5 (NS) | 539 ± 25 (NS) |
| ΔATP (1 µM) = U-73343 (10 µM) | −1.5 ± 0.3 | −0.6 ± 0.4 | −172 ± 23 | −1.1 ± 0.2 (NS) | −0.2 ± 0.5 (NS) | −172 ± 13 (NS) |
| GF109203X (10 µM) (n = 7) | 5.5 ± 0.4 | 7.2 ± 0.5 | 715 ± 46 | 5.2 ± 0.3 (NS) | 7.0 ± 0.4 (NS) | 714 ± 45 (NS) |
| ΔATP (1 µM) = GF109203X (10 µM) | −2.4 ± 0.3 | −0.3 ± 0.4 | −273 ± 25 | −1.1 ± 0.1* | −0.3 ± 0.2 (NS) | −146 ± 13* |

Values are means ± SE. Values for 100 µM ATP at 37°C are from a previous study (13). Numbers for ΔATP represent change in values due to apical perfusion of ATP, either in the absence or presence of drug. $V_t$, transepithelial voltage; $R_t$, transepithelial resistance; $I_{SC}$, short-circuit current. *P < 0.05; NS, not significant.
Elevation of cytosolic Ca\(^{2+}\) increases \(I_{\text{IsK}}\). The effect on the \(I_{\text{IsK}}\) of raising cytosolic Ca\(^{2+}\) was tested in whole cell patch-clamp recordings by addition of the Ca\(^{2+}\) ionophore A-23187 (5 µM) to the bathing solution. Data were obtained from the average of the last two values of the control and experimental periods in each experiment.

Bath perfusion of VDC with A-23187 for 3 min led to an increase in \(I_{\text{IsK}}\) by 43 \(\pm\) 11% of the original current (\(n = 11\)) and an increase in \(g_{\text{IsK}}\) by 34 \(\pm\) 9% of the original conductance and to a hyperpolarization of \(V_t\) by 4.8 \(\pm\) 2.0 mV (Fig. 4, Table 3). The \(\tau_{\text{off}}\) did not change significantly.

Activation of PKC reduces \(I_{\text{IsK}}\). The effect of stimulating PKC on the \(I_{\text{IsK}}\) was tested in whole cell patch-clamp recordings by addition of the phorbol ester PMA to the bathing solution. Data were obtained from the average of the last 2 values of the control and experimental periods in each experiment.

Bath perfusion of VDC with PMA (20 nM) for 3 min led to a decrease in \(I_{\text{IsK}}\) by 79.1 \(\pm\) 2.8% of the original current (\(n = 6\)) and in \(g_{\text{IsK}}\) by 58.1 \(\pm\) 3.5% of the original conductance and to a depolarization of \(V_t\) by 25.5 \(\pm\) 3.3 mV (Fig. 5A, Table 3). There was no significant change in \(\tau_{\text{off}}\). None of these parameters changed significantly after 3-min perfusion of 20 nM 4\(\alpha\)-PMA, an inactive analog of PMA (\(n = 6\); Fig. 5B, Table 3).

Sequence of gerbil cDNA at putative PKC phosphorylation site. RT-PCR of total RNA from a tissue known to express the isk gene (heart) resulted in a product of the expected size based on the gene-specific primers used during amplification (Fig. 6). Furthermore, an RT-PCR product of the same expected size was also amplified from the vestibular labyrinth RNA and thus indicates the presence of an isk gene transcript in this tissue. The \(I_{\text{IsK}}\) identity of the 170-bp DNA fragments from the heart and vestibular labyrinth was confirmed by cloning and sequencing. There is no evidence of genomic DNA contamination as seen by the absence of the RT-PCR product in RNA samples obtained from the RT-PCR in the absence of the reverse transcriptase (MMLV). In addition, the isk gene transcript was not a result of blood contamination of the tissues, since the same RNA isolation procedure and RT-PCR of the isolated RNA from blood did not result in the amplification of a 170-bp product. The sequence is shown in Fig. 7 (GenBank no. AF029765) and is distinguished by its high homology to the same region of rat isk cDNA (89% sequence identity between the primers) and predicted amino acid composition (91%). In particular, the PKC consensus sequence in rat is preserved in the gerbil. The serine at position 102 in the rat isk amino acid sequence, which mediates the species-specific response to PKC, was found to be present at the analogous position in the gerbil isk sequence.

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<th>Table 3. (I_{\text{IsK}}, g_{\text{IsK}}, V_t,) and (\tau_{\text{off}}) in the absence or presence of ATP or drug</th>
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<td><strong>Before Addition of ATP or Drug</strong></td>
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<tr>
<td>(I_{\text{IsK}},) pA</td>
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<td>ATP (10 µM) ((n = 5))</td>
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<td>PMA (20 nM) ((n = 6))</td>
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<td>4(\alpha)-PMA (20 nM) ((n = 6))</td>
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<td>A-23187 (5 µM) ((n = 11))</td>
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Values are means \(\pm\) SE, \(I_{\text{IsK}},\) slowly activating K\(^{+}\) channel current; \(g_{\text{IsK}},\) slowly activating K\(^{+}\) channel conductance; \(V_t,\) reversal voltage; \(\tau_{\text{off}},\) time constant of deactivation; PMA, phorbol 12-myristate 13-acetate. * \(P < 0.05;\) NS, not significant.
Inhibition of PKC reduces response to ATP. It would be expected that if PKC is a mediating signal element in the effect of extracellular ATP on the $I_{\text{IsK}}$ then interference with PKC activation would reduce the effect of ATP. We tested this hypothesis by addition of the PKC inhibitor GF109203X to the apical perfusate.

Addition of GF109203X at 10 µM to the apical perfusate had no significant effect on $I_{sc}$ ($n = 7$). However, apical ATP (1 µM) caused less of a decrease of $I_{sc}$ in the presence of GF109203X than in its absence (Fig. 8, Table 2). ATP decreased $I_{sc}$ by $38.5 \pm 3.2\%$ in the absence of GF109203X and by $20.7 \pm 1.6\%$ in its presence. The fractional response to ATP in the presence of 10 µM GF109203X was $54 \pm 2\%$.

**DISCUSSION**

Purinergic ($P_2$) receptors responsive to UTP have been referred to as $P_{2U}$ subtypes. More recently, $P_{2U}$...
IsK protein. The IsK protein in rat is known to have a decrease in current, as found here for gerbil, whereas rat. The result of molecular cloning and sequencing of a segment of the gerbil IsK cDNA from the vestibular labyrinth confirms that the isk gene is expressed in this tissue and that it contains the putative PKC phosphorylation site, as predicted. On the basis of these results and the presence of the same PKC phosphorylation site in human isk (21), it can be expected that the secretion of endolymph in the human vestibular system is controlled in the same way as in our animal model.

It is conceivable that extracellular nucleotides inhibit K⁺ secretion indirectly via cAMP, rather than via direct phosphorylation of the IsK channel. In DDT1 MF-2 smooth muscle cells, activation of the P₂U receptor activated PLC but also activated a distinct, pertussis toxin-sensitive G protein pathway, which caused a large sustained decrease of the level of cytosolic cAMP (26). We have recently shown that there is constitutive production of cAMP and that elevation of cytosolic cAMP stimulates the IsK in VDC (27), so it is likely that reduction of cAMP would lead to a decrease in IsK. If the P₂U receptors in the apical membrane of VDC are coupled to G₁ as well as to PLC, it is conceivable that at least part of the effect of apical perfusion of ATP was due to a reduced cAMP level, although no such coupling has yet been demonstrated in VDC.

The increase of IsK observed in VDC under conditions expected to elevate \([\text{Ca}^{2+}]_c\), argues against a major role of the InsP₃ pathway, with its subsequent increase of \([\text{Ca}^{2+}]_c\) in the response of K⁺ secretion to activation of the apical P₂U receptor. Although Ca²⁺ is classically known to increase PKC activity, isoforms have been identified that are Ca²⁺ independent (PKC-δ) (8). PKC-δ has been identified in stria vascularis (1), a tissue in the cochlea that contains cells homologous to VDC in many other respects (29). It is not yet clear what process is directly affected by addition of A-23187, although it is most likely due to an increase in cytosolic Ca²⁺. Although A-23187 is a divalent cation/H⁺ exchanger, it is not likely that the effect was due to a change in cytosolic pH, since an influx of Ca²⁺ would be expected to lead to alkalization and, by our current understanding, a concomitant decrease in K⁺ secretion (30).

Although the sources of agonist and the physiological functions of the apical purinergic receptors are not yet clear, several hypotheses can be advanced. The constitutive level of ATP in the luminal fluid of the cochlea has been estimated at 13 nM (20), a concentration that causes a small but significant decrease in \(I_{\text{sc}}\) of VDC (13). If one assumes a similar level of ATP in the vestibular labyrinth, the receptor would be poised to be either stimulated further by additional agonists or inhibited by antagonists. Functionally, it may be extremely important for these epithelial cells to receive signals from neighboring VDC, since they are not coupled to each other by gap junctions. In addition, there are many other epithelial cell types lining the vestibular lumen that may communicate via apical release of ATP. Conditions stimulating this hypothetical release of ATP remain to be discovered.

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