Luminal P2Y₂ Receptor-Mediated Inhibition of Na⁺ Absorption in Isolated Perfused Mouse CCD

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Abstract. Extracellular nucleotides regulate renal transport. A luminal P2Y₂ receptor in mouse cortical collecting duct (CCD) principal cells has been demonstrated elsewhere. Herein the effects of adenosine triphosphate (ATP) and uridine triphosphate (UTP) on electrogenic Na⁺ absorption in perfused CCD of mice kept on a low-NaCl diet were investigated. Simultaneously, transepithelial voltage (Vte), and equivalent short-circuit current (Isc), were measured. Baseline parameters were Vte = -16.5 ± 2.0 mV; Rte, 80.8 ± 0.7 Ω cm²; and equivalent short-circuit current (Isc), -261.0 ± 25.1 μA/cm² (n = 45). Amiloride (10 μM) almost completely inhibited Isc to -3.9 ± 3.8 μA/cm² (n = 10). Luminal ATP (100 μM) reduced Vte from -16.5 ± 2.1 to -12.5 ± 1.93 and increased Rte from 113.1 ± 16.2 to 123.8 ± 16.7 Ω cm², which resulted in a 31.7% inhibition of amiloride-sensitive Isc (n = 12). Similarly, luminal UTP reversibly reduced Vte from -22.0 ± 2.1 to -13.6 ± 2.1 mV and increased Rte from 48.4 ± 5.3 to 59.2 ± 7.1 Ω cm², which resulted in 49.1% inhibition of Na⁺ absorption (n = 6). In parallel, luminal ATP and UTP elevated [Ca²⁺] in CCD, increasing the fura-2 ratio by 2.7 ± 0.7 and 4.0 ± 1.2, respectively. Basolateral ATP and UTP (100 μM) also inhibited amiloride-sensitive Isc by 21.8 (n = 14) and 20.1% (n = 8), respectively. Inhibition of luminal nucleotide-induced [Ca²⁺], by Ca²⁺ store depletion with cyclopiazonic acid (3 μM) did not affect nucleotide-mediated inhibition of Na⁺ transport (n = 7). No evidence indicated the activation of a luminal Ca²⁺-activated Cl⁻ conductance, a phenomenon previously shown in M-1 CCD cells (J Physiol 524: 77–99, 2000). In essence, these data indicate that luminal ATP and UTP, most likely via P2Y₂ receptors, mediate inhibition of amiloride-sensitive Isc in perfused mouse CCD. This inhibition appears to occur independently of an increase of cytosolic Ca²⁺.

A ubiquitous and pronounced feature of nearly all transporting epithelial cells is the expression of luminal purinergic (P2) receptors. To name a few, luminal P2 receptor expression has been demonstrated in epithelial tissues like bronchus (1), colon (2), epididymis (3), sweat duct (4), or inner ear (5). Mammalian P2 receptors are subdivided into metabotropic (G-protein coupled) P2Y (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, and P2Y₁₂) and ionotropic (nonselective Ca²⁺-permeable cation channels) P2X (P2X₁₋₇) receptors. In the above-presented tissues, the luminal P2Y₂ receptor subtype appears to be the most relevant P2 receptor expressed. It is important to note that P2 receptor expression is also very frequently observed in the basolateral membrane and that each epithelial tissue seems to be equipped with an individual expression pattern of P2 receptors. Recent studies have indicated that, commonly, a variety of different P2Y and P2X receptors are expressed in the same epithelial tissue (7–9). In mouse gallbladder, for example, a luminal P2Y₆ receptor is equally important as the P2Y₂ receptor in the activation of luminal nucleotide-mediated Na⁺ secretion (10). Other epithelia, like the rat submandibular gland duct, do not show functional expression of luminal P2Y₂ receptors. They do, however, respond to luminal 2',3'-O-4-benzoylbenzoyl adenosine triphosphate (ATP), which suggests the presence of luminal ionotropic P2X receptors (11). The expression of luminal P2X receptors has also been proposed in rat pancreatic ducts (8,9).

Freshly isolated mammalian kidney tubules express a number of different P2 receptors, apparently along the entire nephron (12). Functional expression of basolateral P2Y receptors could be demonstrated, e.g., in mouse loop of Henle (13), rabbit cortical collecting duct (CCD) (14), or rat inner medullary collecting duct (15–17). In addition, in our earlier article (18), we identified a luminal P2Y₂ receptor coupled to an increase of [Ca²⁺], in the isolated perfused mouse CCD. Using a confocal microscope, we could localize the luminal ATP-induced [Ca²⁺] elevation to principal cells. The data indicated that the P2Y₂ receptor subtype was the only P2 receptor expressed in the luminal membrane of mouse principal cells. Our report is in close agreement with a recent immunohistochemistry study that demonstrated the expression of P2Y₂ receptors in the basolateral, which was even more pronounced in the luminal membrane of rat inner medullary collecting duct principal cells (15). Studies that have focused on functional
consequences of P2 receptor activation in intact kidney tubules are scarce. Basolateral ATP has been shown to inhibit the antidiuretic hormone (ADH)–induced increase in H₂O permeability in rat inner medullary collecting duct (16) and rabbit CCD (14). In rat inner medullary collecting duct, the inhibition of H₂O transport was attributed to a P2 receptor–mediated decrease of cyclic ATP (16). A number of investigators, including ourselves, have used different cell lines derived from renal tubular cells in an Ussing chamber set-up to investigate the effect of basolateral and luminal nucleotides on electrogenic ion transport. Two key findings were observed, namely that luminal and basolateral nucleotides activate Cl⁻ secretion (19–21) and inhibit amiloride-sensitive Na⁺ absorption (19,20,22). The Cl⁻/H⁺ cotransport, e.g., observed in M-1 CCD cells, is activated by an increase in [Ca²⁺]i (20), whereas the inhibition of Na⁺ transport appears to be regulated [Ca²⁺]r-independently (22,23).

In this follow-up work, we investigate the effect of luminal and basolateral ATP and uridine triphosphate (UTP) on electrogenic Na⁺ absorption in perfused mouse CCD. To this end, mice were treated with a low Na⁺ diet for 10 to 20 d. We were also interested in whether the intact CCD is equipped with luminal Ca²⁺-activated Cl⁻ channels. In M-1 CCD cells, this was reported in our study elsewhere (20).

Materials and Methods

Microdissection of Isolated CCD

Experiments were carried out in mouse CCD segments. To investigate electrogenic Na⁺ absorption, mice were fed a low-Na⁺ diet (Altromin, Lage, Germany: Na⁺, 136 mg/kg; Cl⁻, 178.5 mg/kg; K⁺, 7170 mg/kg) for 10 to 20 d before the experiments. Mice had free access to drinking water. Nephron segments of both genders of mice (30 to 40 g) were microdissected at 4°C from kidney slices in carbogen (98.5% O₂ and 1.5% CO₂) gassed dissection solution (see below) by use of ultrafine watchmaker forceps. The kidney tubules were transferred into a specialized perfusion chamber mounted on an inverted microscope.

Isolated Tubule Perfusion and Measurement of Transepithelial Electrical Parameters

Isolated tubules were perfused by use of a system of concentric glass pipettes previously used and developed by R. F. Greger and W. Hampel (24). To quantify electrogenic Na⁺ transport, Ohm’s law was applied:

\[ I_{te} = \frac{V_{te}}{R_{te}} (\mu A/cm^2) \]  

(1)

where \( I_{te} \) is the equivalent short-circuit current, \( V_{te} \) the transepithelial voltage, and \( R_{te} \) the transepithelial resistance. \( V_{te} \) was measured continuously on both ends of the perfused CCD. The system for recording was symmetric, so that no corrections due to superimposed liquid junction potentials were necessary when identical solutions were present on both sides of the epithelium. The determination of \( R_{te} \) was performed by use of the cable equation (equation 3). A detailed description of the quantitative estimation of electrogenic ion transport in kidney tubules is given in publications elsewhere (25–27). \( R_{te} \) (Ω cm²) was calculated from the input resistance \( R_{inp} \) (Ω), the length constant \( \lambda \) (μm), the length of the tubule segment \( L \) (μm), and the resistance \( \rho \) (Ω cm) of the perfusate. To obtain \( R_{inp} \) and \( \lambda \) rectangular current pulses (\( I_p \), 50 nA; pulse width 800 ms every 3 s), were injected through one channel of the perfusion pipette. The corresponding voltage deflections \( \Delta V_L \) at the perfusion side and \( \Delta V_L \) at the collection side were measured by two voltmeters (Keithley Instruments, Cleveland, OH). \( R_{inp} \) is defined as \( \Delta V_L/I_p \). \( \lambda \) was obtained from the exponential equation 2, as given by Helman et al. (25):

\[ L \lambda = \cos h^{-1} \left( \frac{\Delta V_L}{V_0} \right) \]  

(2)

The length and the inner radius \( r \) (μm) of the tubule were measured by a size-calibrated transmission image. Previously obtained resistivity values were used. For the calculation of \( R_{te} \), the following equation was used:

\[ R_{te} = 2 \int \pi \lambda^2 \frac{\Delta V_L}{I_p} \tan h \frac{L}{\lambda} \]  

(3)

A slightly different approach was taken for long tubule segments in which no accurate value of \( \Delta V_L \) could be obtained. Herein, equation 4 was used to determine \( \lambda \) (27):

\[ \frac{\Delta V_L}{I_p} = \frac{\lambda}{\rho} \]  

(4)

Digital Video Imaging

The setup consisted of an inverted microscope (Axiovert 100 TV; Zeiss, Jena, Germany) with a 40× objective (Fluar 40×, 1.3 oil; Zeiss, Jena, Germany), a monochromator (Till Photonics, Planegg, Germany), and a GEN 3 intensified CCD camera (ICCD 350; Videocope, Dulles, VA). Image acquisition and data analysis were performed with the software package Metamorph/Metafluor (Universal Imaging, West Chester, PA). Freshly dissected CCD were mounted into the perfusion system previously built and used in this laboratory (24). Measurement of [Ca²⁺]i was performed with the Ca²⁺-dye fura-2. Tubules were incubated in 20 μM basolateral fura-2/AM for 15 min at room temperature in Ringer’s solution to which 1.6 μM pluronic F127 had been added. Pluronic F127 is a surfactant polyol that helps solubilize water-insoluble dyes like fura-2/AM. As a measure of [Ca²⁺]i, the fluorescence emission ratio at 345/380 nm excitation was used. In each experiment, the fluorescence signal was recorded from the entire tubule. During the dye-loading period, the tubule was continuously perfused from the luminal side. The experiment was started 5 min after washout of extracellular dye. To demonstrate successful luminal agonists perfusion, we added 10 μM lucifer yellow (LY, excitation 430 nm and emission ≥500 nm) to the luminal perfusate. LY is not excited with the two fura-2 wavelengths (345 and 380 nm), and fura-2 is not excited with 430 nm light. Thus, the two fluorescence signals could be observed without significant cross-contamination. Images were recorded sequentially after 345, 380, and 430 nm excitation. One fura-2 ratio and the LY fluorescence were recorded every 2 s, respectively.

Solutions and Chemicals

Pluronic F127, LY, and fura-2/AM were obtained from Molecular Probes (Eugene, OR). All other chemicals were of the highest grade of purity available and were obtained from Sigma (Deisenhofen, Germany) and Merck (Darmstadt, Germany). The experiments of continuous basolateral and luminal perfusion of kidney tubules were performed with the following solutions (in mM): 145 NaCl, 1 MgCl₂, 1.3 Ca-gluconate, 5 D-glucose, 0.4 KH₂PO₄, and 1.6 K₂HPO₄. A few
experiments were also performed in a HCO₃⁻-buffered solution (in mM): 120 NaCl, 1 MgCl₂, 1.3 Ca-gluconate, 5 D-glucose, 0.4 KH₂PO₄, 1.6 K₂HPO₄, and 25 NaHCO₃ gassed with 95% O₂/5%CO₂. All solutions were titrated to pH 7.4. Kidney tubules were dissected in F12 (HAM) medium (Life Technologies, Karlsruhe, Germany) to which 5 mM glycin, 10 μM amiloride, and 1 mg/ml bovine serum albumin had been added.

Statistical Analyses
The data shown are either original traces or mean values ± SEM (n), where n refers to the number of experiments. A paired t test was used to compare mean values within one experimental series. A P < 0.05 was accepted to indicate statistical significance.

Results
Baseline Parameters in Mice Treated with a Low-Na⁺ Diet
This study investigates the effect of luminal and basolateral nucleotides on electrogenic Na⁺ absorption in mice. Mice and other rodents like rats kept on a normal diet commonly show very little electrogenic Na⁺ absorption (28). This is reflected in a small Vₑ of −3.8 ± 0.6 (n = 9) in mice kept on a regular diet. After 14.6 ± 0.6 d (n = 45) on a low-Na⁺ diet, the following baseline parameters were measured: Vₑ (lumen-negative), −16.5 ± 1.2 mV; Rₑ, 80.8 ± 7.1 Ω cm²; and calculated equivalent Iₑ, −261.0 ± 25.1 μA/cm² (n = 45). In a series of 10 CCD, we investigated the effect of 10 μM luminal amiloride (Figure 1). Luminal amiloride reversibly decreased lumen-negative Vₑ from −16.3 ± 2.5 to −0.3 ± 0.5 mV and increased Rₑ from 113.3 ± 19.3 to 141.2 ± 28.6 Ω cm², which resulted in nearly complete inhibition of Iₑ from −173.5 ± 36.2 to −3.9 ± 3.8 μA/cm². These results indicate that a low-Na⁺ diet substantially activates electrogenic Na⁺ absorption in CCD of mice, which is completely inhibitable by luminal amiloride.

Luminal ATP and UTP Inhibit Electrogenic Na⁺ Absorption in Isolated Perfused Mouse CCD
Subsequently, in perfused mouse CCD, we simultaneously investigated the effect of luminal ATP on transepithelial electrical parameters and [Ca²⁺]ₑ. Luminal perfusion of agonists was performed with a perfusion pump, which allowed for a continuous application of different luminal solutions without altering tubular geometry. Figure 2A shows an original experiment in which the upper panel displays the measurement of Vₑ and ΔVₑ induced by injection of a 50 nA current. The middle panel depicts the [Ca²⁺]ₑ signal of the entire tubule measured as the fura-2 345/380 nm fluorescence ratio. The bottom panel depicts the measurement of LY fluorescence intensity as indicator for luminal perfusion. In this CCD, resting Vₑ was close to −17 mV. The addition of luminal ATP (100 μM) slowly, within 5 to 6 min, reduced Vₑ to −9 mV. Subsequent addition of luminal amiloride (10 μM) rapidly reduced Vₑ further to 2.5 mV. A close inspection of the upper panel also reveals that during perfusion with luminal ATP the ΔVₑ deflections increase, reflecting an increase in Rₑ (from 123.3 to 179.0 Ω cm²). As has been previously demonstrated, luminal ATP also increased [Ca²⁺]ₑ, with an initial peak leveling off toward the baseline during the following 5 to 7 min of ATP administration. It is noteworthy that no close temporal correlation could be observed between the stimulated [Ca²⁺]ₑ transient and the reduction of Vₑ or the increase of Rₑ. The luminal amiloride solution did not contain LY, which explains the wash-out of the luminal “perfusion marker” during the effect of amiloride. Our previous study (18) strongly suggested that principal cells of mouse CCD express a luminal P2Y₂ receptor. Thus, it must be expected that luminal UTP, the other known ligand of this receptor, should have similar effects. A single experiment is depicted in Figure 2B. The addition of luminal UTP (100 μM)
reversibly reduced resting $V_{te}$ from $-29$ to $-17$ mV and increased $R_{te}$, as is seen from an increase in $\Delta V_{te}$ deflections (from $69.9$ to $91.6 \, \Omega \, \text{cm}^2$). Luminal UTP also elevated $[\text{Ca}^{2+}]_i$ in a biphasic manner, with an initial peak and a secondary plateau. Thus, luminal UTP induces similar effects as luminal ATP. Again, no close temporal correlation was observed between $[\text{Ca}^{2+}]_i$ elevation and the effect on $V_{te}$ and $R_{te}$. In Figure 3, the entire experimental series with luminal ATP is summarized. Luminal ATP reversibly reduced $V_{te}$ from $-16.5 \pm 2.1$ to $-12.5 \pm 1.9$ mV and increased $R_{te}$ from $113.1 \pm 16.2$ to $123.8 \pm 16.7 \, \Omega \, \text{cm}^2$ ($n = 12$). This led to a reduction of $I_{sc}$ from $-201.3 \pm 46.0$ to $-140.8 \pm 35.0 \, \mu A/cm^2$, $31.7\%$ inhibition of $\text{Na}^+$ absorption. Most experiments were performed as shown in Figure 2A, with direct administration of luminal amiloride following that of luminal ATP. Often, luminal washout lasted too long to demonstrate full reversibility of the ATP effect. In the UTP series, however, as also shown in Figure 2B, reversibility of the effect is shown unequivocally. This is also reflected in the mean values of the UTP experiments. Luminal UTP reduced $V_{te}$ from $-22.0 \pm 2.1$ to $-13.6 \pm 2.1$, and $V_{te}$ returned back to $-20.5 \pm 2.7$ mV after UTP washout. UTP also reversibly increased $R_{te}$ from a resting value of $48.4 \pm 5.3$ to $59.2 \pm 7.1 \, \Omega \, \text{cm}^2$. This resulted in a reduction of amiloride-sensitive short circuit current from $478.3 \pm 67.3$ to $-248.3 \pm 49.4$ and after washout of UTP back to $-426.8 \pm 82.1 \, \mu A/cm^2$ ($n = 6$). Altogether, UTP inhibited 49.1% of the entire $\text{Na}^+$ transport.
Basolateral ATP and UTP Inhibit Electrogenic Na\(^+\) Absorption in Isolated Perfused Mouse CCD

Principal cells of the distal nephron express P2 receptors also in their basolateral membrane. We therefore investigated the effect of basolateral ATP and UTP on Na\(^+\) absorption in the same mice kept on a low-Na\(^+\) diet. Similarly, basolateral nucleotides inhibit electrogenic Na\(^+\) absorption. An original experiment is shown in Figure 4. The upper panel shows the measurement of \(V_{te}\) and \(\Delta V_{te}\) and the lower panel that of \([Ca^{2+}]_i\). The addition of basolateral ATP (100 \(\mu M\)) reversibly reduced \(V_{te}\) from a resting value of \(-26\) to \(-24\) mV. Simultaneously, \(R_{te}\) increased, as reflected by an increase of \(\Delta V_{te}\) deflections (from 58.0 to 68.9 \(\Omega \text{ cm}^2\)). The summary of all data with 100 \(\mu M\) basolateral ATP is shown in Figure 5A. \(V_{te}\) was reversibly reduced from \(-13.6 \pm 2.5\) to \(-12.0 \pm 2.3\) mV, and \(R_{te}\) increased from a resting value of \(56.5 \pm 8.0\) to \(63.5 \pm 9.6\) \(\Omega \text{ cm}^2\). This resulted in small but significant reduction of amiloride-sensitive \(I_{sc}\) from \(-272.8 \pm 53.4\) to \(-210.6 \pm 42.1\) and back to \(-261.2 \pm 47.9\ \mu \text{A/cm}^2 (n = 14)\). Altogether, the basolateral ATP-mediated inhibition of Na\(^+\) absorption amounted to 21.8%. We also used basolateral UTP, which demonstrated effects similar to those described for basolateral ATP. Figure 5B summarizes the entire series of basolateral nucleotides on Na\(^+\) transport. Basolateral UTP and ATP inhibited \(I_{sc}\) with an inhibitory concentration of \(1.24\) and \(3.22\ \mu M\), respectively, with a similar maximal inhibition of Na\(^+\) absorption at 100 \(\mu M\).

Effect of Luminal ATP After Depletion of Intracellular Ca\(^{2+}\) Stores

In subsequent experiments, we set out to investigate the effects of ATP under the depletion of cytosolic Ca\(^{2+}\) stores. Depletion of Ca\(^{2+}\) stores was performed with cyclopiazonic

Figure 4. Original recording of simultaneously measured \(V_{te}\) and \([Ca^{2+}]_i\), in mouse CCD. Effect of basolateral ATP (100 \(\mu M\)). The upper panel shows the measurement of \(V_{te}\). Here the upper trace indicates the time course of \(V_{te}\) (arrow). Injection of small currents (50 nA) induced \(\Delta V_{te}\). According to Ohm’s law these voltage deflections report about \(R_{te}\). An increase of \(\Delta V_{te}\) deflection indicates an increase in \(R_{te}\). The lower panel shows the \([Ca^{2+}]_i\), recording.

Figure 5. (A) Summary of basolateral ATP (100 \(\mu M\)) induced effects on \(V_{te}\), \(R_{te}\), \(I_{sc}\), and cytosolic Ca\(^{2+}\) in mice kept for 15 d on a low-Na\(^+\) diet (\(n = 14\)). \(*\), Significant change between precontrol and agonist; \(\ddagger\), significant change between the agonist and postcontrol. (B) Concentration-response curve of basolateral ATP and UTP on inhibition of \(I_{sc}\) in isolated perfused mouse CCD. The inhibition of \(I_{sc}\) (in \(\mu A/cm^2\)) is plotted as a function of nucleotide concentration. UTP was slightly more potent to inhibit Na\(^+\) absorption.
acid (CPA, 3 μM). CPA is commonly used to reversibly inhibit the microsomal Ca\(^{2+}\)-ATPase, thereby depleting Ca\(^{2+}\) stores. An original experiment is shown in Figure 6. CPA led to a slow [Ca\(^{2+}\)]\(_i\) increase with a peak after 3 min (mean fura-2 ratio peak increase, 4.1 ± 1.5; \(n = 9\)), and, subsequently, in its continuous presence, [Ca\(^{2+}\)]\(_i\) leveled off toward an elevated plateau. In the whole series, CPA induced small decreases of \(V_{te}\) from a resting value of −15.6 ± 1.9 to −13.6 ± 1.9 mV (measured at maximum 4 min after CPA). During CPA, \(V_{te}\) returned to −15.0 ± 1.8 mV. Under CPA, no significant changes of \(R_e\) were observed (control, 80.3 ± 14.2; CPA, 82.6 ± 13.7; postcontrol, 78.0 ± 13.9). Therefore, CPA alone (4 min after application) inhibited \(I_{sc}\) from a resting value of −263.0 ± 62.4 to −205.6 ± 43.8 and during the continuous presence of CPA returned back to −226.6 ± 42.3 μA/cm\(^2\). Subsequently, luminal ATP (100 μM) was given in the presence of CPA. As seen in Figure 6, luminal ATP reduced \(V_{te}\) from −20 to −15 mV and increased \(\Delta V_{te}\) deflections, again indicating an increase in \(R_e\) (from 86.9 to 98.2 Ω cm\(^2\)). In summary, luminal ATP under CPA reduced \(V_{te}\) from −15.0 ± 1.8 to −10.2 ± 1.5 and back to −13.4 ± 3.5 mV, \(R_e\) increased from 78.0 ± 13.9 to 89.9 ± 17.8 Ω cm\(^2\), and \(I_{sc}\) decreased from −226.6 ± 42.3 to −134.6 ± 27.3 μA/cm\(^2\), amounting to 37% inhibition of Na\(^{+}\) absorption. Importantly, under these conditions, luminal ATP did not increase but rather reduced [Ca\(^{2+}\)]\(_i\) by 0.6 ± 0.3 fura-2 ratio units (\(n = 7\)). These results suggest that the observed inhibition of Na\(^{+}\) absorption possibly occurs independently from an increase of [Ca\(^{2+}\)]\(_i\). In an attempt to investigate the role of [Ca\(^{2+}\)]\(_i\) further, we also tried BAPTA-AM (50 or 100 μM for 30 min) to buffer the ATP-induced [Ca\(^{2+}\)]\(_i\) increase. Under BAPTA-AM, the initial ATP-induced (100 μM) [Ca\(^{2+}\)]\(_i\) peak was completely inhibited; however, a Ca\(^{2+}\) influx was stimulated, which led to a significant [Ca\(^{2+}\)]\(_i\) increase that extended the duration of either luminal or basolateral ATP application by some 5 min (fura-2 ratio increase 0.67 ± 0.48, \(n = 4\)). More importantly, BAPTA-AM incubation reduced \(V_{te}\) from −15.8 ± 6.8 mV to −4.8 ± 3.5 mV (\(n = 5\)), rendering the further investigation of Na\(^{+}\) transport almost impossible.

**Discussion**

In an article elsewhere (18), we presented evidence for a P2Y\(_2\) receptor in the luminal membrane of mouse CCD principal cells coupled to an increase of [Ca\(^{2+}\)]\(_i\). In this follow-up work, we investigated the functional effects of ATP and UTP on Na\(^{+}\) absorption in isolated perfused mouse CCD. Na\(^{+}\) absorption was determined in mice treated with a low-Na\(^{+}\) diet using transepithelial electrical measurements. We found that luminal ATP and UTP increase [Ca\(^{2+}\)]\(_i\) in perfused mouse CCD and inhibit electrogenic Na\(^{+}\) absorption. Similarly, basolateral ATP and UTP inhibit Na\(^{+}\) transport. No evidence suggested the functional expression of luminal Ca\(^{2+}\)-activated Cl\(^-\) channels in the intact nephron, as has been suggested elsewhere in M-1 CCD cells (20). This casts significant doubt on the use of culture models to assess tubular functions. The nucleotide-mediated inhibition of Na\(^{+}\) absorption appears to be independently regulated from an increase in [Ca\(^{2+}\)]\(_i\).

**Regulation of Na\(^{+}\) Transport in the CCD**

The collecting duct serves to finely tune the urinary Na\(^{+}\) excretion. The absorptive process is dependent on constitutively open epithelial sodium channel (ENaC) channels in the luminal membrane of principal cells. Na\(^{+}\) is transported transeptally, enters the cytosol via a steep electrochemical driving force over the apical membrane, and is subsequently pumped into the basolateral space via the Na\(^{+}\)/K\(^{+}\) ATPase. Principal cells are the target of aldosterone- and ADH-mediated activation of Na\(^{+}\) transport (29). Na\(^{+}\) absorption is furthermore under the modulation of a variety of different agonists (30). For example, a number of [Ca\(^{2+}\)]\(_i\) elevating agonists like PGE\(_2\) or acetylcholine were shown to inhibit electrogenic Na\(^{+}\) transport in rabbit CCD (31,32). More recently, a number of studies also identified different P2 in renal epithelia (16,17,33). A unique phenomenon of many epithelial cells, including the distal nephron, is the expression of P2 receptors in both the luminal and basolateral membrane (9,18).

**P2Y Receptor Identification in Mouse CCD**

Despite the fact that numerous P2 receptors have been described in transporting epithelia (34), the most prominent luminal subtype is the P2Y\(_2\) receptor, expressed, e.g., in bronchial or colonic mucosal tissue (1,2). Similarly, in our earlier article, we presented evidence that the luminal nucleotide-mediated increase of [Ca\(^{2+}\)]\(_i\) in mouse CCD principal cells occurs via a P2Y\(_2\) receptor. The conclusion was based on an

![Figure 6](image-url)
extensive pharmacologic screening with a large number of different P2 receptor agonists. We currently believe that the P2Y2 receptor is the only P2 receptor expressed in the luminal membrane of mouse CCD principal cells. A number of additional arguments strengthen this conclusion. Our own data in M-1 cells identifies specific mRNA for this receptor subtype and shows a typical pharmacology, with UTP being as potent as ATP (20). Even more conclusive is a recent study in rat inner medullary collecting duct that used a specific antibody that localized this receptor predominantly to the luminal membrane (15). It needs, however, to be considered that pharmacologic evidence with UTP and ATP being of similar potency would also allow the P2Y2 receptor to be a possible candidate (6). Yet no other evidence indicates that the latter receptor is involved.

**Mechanism of ATP-Mediated Inhibition of Na\(^+\) Transport**

In general, the simultaneous function of luminal ENaC channels, the basolateral Na\(^+\)/K\(^+\) ATPase, and a basolateral K\(^+\) conductance is required to permit the transcellular movement of Na\(^+\) in the distal nephron. Thus the inhibition of any of these transporting modules could account for the observed ATP effect. Our study does not allow us to conclude which of the relevant membrane transport molecules was the target of regulation. An interesting recent cell attached patch-clamp study investigated the effect of P2Y2 receptor stimulation on small-conductance K\(^+\) channels (renal outer medulla K\(^+\) channel [ROMK]–type) in the luminal membrane of mouse CCD principal cells. Luminal ATP reversibly inhibited \(\sim 90\%\) of constitutively open ROMK channels (35). An ATP-mediated closure of ROMK channels will inhibit electrogenic Na\(^+\) transport, because this would reduce the steep electrochemical gradient over the luminal membrane necessary for the entry of Na\(^+\).

However, if ATP as the sole mechanism would close luminal K\(^+\) channels, the transepithelial measurement should have increased to even more lumen-negative \(V_{te}\) values. The opposite was the case—namely, ATP decreased \(V_{te}\). Thus, because \(R_{te}\) increased, additional ion conductances must have been inhibited with luminal ATP. One attractive hypothesis is that ENaC channels are the target of ATP-mediated inhibition. In other words, luminal ATP would inhibit both ROMK and ENaC channels, with the latter being the dominant effect, to explain the \(V_{te}\) measurements. In this context, a single-channel patch-clamp study in A6 cells (36) suggested that luminal ATP via P2Y receptors inhibits ENaC channels. Also, our previous M-1 work (23) would suggest that ENaC is the target of extracellular ATP regulation.

**Is [Ca\(^{2+}\)]\(_i\) Required for the ATP-Mediated Inhibition of Na\(^+\) Absorption in CCD?**

Over the past two decades, numerous studies that have used microperfused collecting ducts have established that [Ca\(^{2+}\)]\(_i\) elevating agonists like PGE\(_2\) or acetylcholine inhibit Na\(^+\) transport (32,37,38). Downstream of the [Ca\(^{2+}\)]\(_i\) signal, protein kinase C (PKC) activation has been suggested to mediate the inhibitory effect on Na\(^+\) transport (39). Thus, in rabbit tissue, current evidence draws a homogeneous picture of these initial signaling events. In contrast, in rodents like rats, intracellular Ca\(^{2+}\) and PKC do not seem to be involved as inhibitory regulators of electrogenic Na\(^+\) absorption (40).

Three arguments would suggest that [Ca\(^{2+}\)]\(_i\) is not a crucial regulator of ATP-mediated Na\(^+\) transport inhibition in mice. (1) Under microosmolar Ca\(^{2+}\)-ATPase inhibition, the ATP-mediated [Ca\(^{2+}\)]\(_i\) increase was obliterated, and the nucleotide under these conditions even decreased [Ca\(^{2+}\)]. This, however, left the Na\(^+\) transport inhibition unaltered. However, because Ca\(^{2+}\) entry and basal [Ca\(^{2+}\)]\(_i\) were elevated under continuous CPA, this interpretation is somewhat weakened. (2) In M-1 cells, a much more robust system to investigate possible signaling mechanisms, more aggressive means to lower cytosolic Ca\(^{2+}\), did not alter the ATP-inhibited Na\(^+\) absorption. (3) The use of BAPTA-AM in cultured rabbit connecting tube cells (22) similarly did not effect ATP/UTP-inhibited Na\(^+\) and Ca\(^{2+}\) absorption. Thus, we suggest that, also in mice, an elevation of [Ca\(^{2+}\)]\(_i\) is not a necessary event to mediate the observed inhibition.

The previous work in mouse M-1 cells excluded the involvement of PKC (23). In the intact mouse CCD, we tried to use the PKC activator PMA (100 nM); however, we observed a rapid deterioration of tubule morphology and function. On the basis of the above-mentioned data from M-1 cells, we suggest that the activation of PKC may not be involved in the ATP-mediated inhibition of Na\(^+\) transport in the intact nephron.

Basolateral nucleotide–mediated inhibition of ADH-stimulated H\(_2\)O transport in rat inner medullary collecting duct has been suggested to occur via a P2Y receptor–mediated decrease of cAMP (16). As was mentioned above, Na\(^+\) absorption in rodents is activated by agonists like ADH, which is known to elevate cAMP (30). It thus may be the case that luminal P2Y2 receptor stimulation decreases cAMP and thereby inhibits Na\(^+\) transport. Our study has not addressed this question.

**Hypothetical Model for an Integrated Function of ATP as Signaling Molecule along the Nephron**

The source of extracellular ATP, especially on the luminal side of epithelia, is still largely obscure. Numerous articles have indicated that ATP can be released by epithelial cells after cellular swelling and therefore may serve as an autocrine-signaling molecule. Following the arguments presented from bile duct epithelial cells (41), we speculated that ATP could serve a role as stimulator of regulatory volume decrease in renal epithelial cells (18). This hypothesis was especially attractive because the kidney is an organ with an outstandingly high demand for efficient volume regulatory mechanisms. One argument in favor of such a hypothesis was that extracellular ATP in cultured renal and other epithelial cells activated CI\(^−\) and K\(^+\) channels, a key mechanism to reduce cellular volume. This hypothesis is substantially weakened by the current study. Obviously, in the intact CCD, an ATP-stimulated activation of...
Cl\textsuperscript{−} and K\textsuperscript{+} channels does not occur. What other functional relevance may be attributed to P2Y receptors in the nephron? One common denominator is that ATP, via P2Y receptors, inhibits transport. This includes, as we show, Na\textsuperscript{+} absorption, K\textsuperscript{+} secretion (35), and H\textsubscript{2}O transport (16). We therefore speculate that ATP could serve as a protective signal when ischemic episodes occur. Ischemia will eventually lead to cell swelling, a phenomenon known to release ATP from epithelial cells. Released ATP, in turn, would bind to P2 receptors and inhibit energy-consuming transepithelial transport. In addition, another interesting line of evidence suggests that extracellular ATP could mediate the stimulation of cell proliferation and regeneration in renal tubular cells (42).

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