

Electrophysiological Effects of Extracellular ATP on *Necturus* Gallbladder Epithelium

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ABSTRACT The effects of addition of ATP to the mucosal bathing solution on transepithelial, apical, and basolateral membrane voltages and resistances in *Necturus* gallbladder epithelium were determined. Mucosal ATP (100 μ M) caused a rapid hyperpolarization of both apical (V_{mc}) and basolateral (V_{cs}) cell membrane voltages ($\Delta V_m = 18 \pm 1$ mV), a fall in transepithelial resistance (R_t) from 142 ± 8 to 122 ± 7 $\Omega \cdot \text{cm}^2$, and a decrease in fractional apical membrane resistance (fR_a) from 0.93 ± 0.02 to 0.83 ± 0.03 . The rapid initial hyperpolarization of V_{mc} and V_{cs} was followed by a slower depolarization of cell membrane voltages and a lumen-negative change in transepithelial voltage (V_{ms}). This phase also included an additional decrease in fR_a . Removal of the ATP caused a further depolarization of membrane voltages followed by a hyperpolarization and then a return to control values. fR_a fell to a minimum after removal of ATP and then returned to control values as the cell membrane voltages repolarized. Similar responses could be elicited by ADP but not by adenosine. The results of two-point cable experiments revealed that ATP induced an initial increase in cell membrane conductance followed by a decrease. Transient elevations of mucosal solution $[\text{K}^+]$ induced a larger depolarization of V_{mc} and V_{cs} during exposure to ATP than under control conditions. Reduction of mucosal solution $[\text{Cl}^-]$ induced a slow hyperpolarization of V_{mc} and V_{cs} before exposure to ATP and a rapid depolarization during exposure to ATP. We conclude that ATP^{4-} is the active agent and that it causes a concentration-dependent increase in apical and basolateral membrane K^+ permeability. In addition, an apical membrane electrodiffusive Cl^- permeability is activated by ATP^{4-} .

INTRODUCTION

Biologic responses to extracellular ATP have been identified in a number of cells including endothelial cells (DeMey and Vanhoutte, 1981), smooth muscle cells (Chapal and Loubatières-Mariani, 1983), mast cells (Cockcroft and Gomperts, 1979),

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and epithelial cells (Lang et al., 1988; Jungwirth et al., 1989; Soltoff et al., 1990). The effects are thought in most cases to be mediated by specific ATP receptors. Such "purinergic" receptors have been divided into P₁ and P₂ subtypes, activated by adenosine and ATP, respectively (Burnstock, 1978).

A prominent effect of ATP in several cell types is a change in membrane permeability (Gordon, 1986). A nonselective cation-conductive pathway can be reversibly induced in many cell lines (Heppel et al., 1985) and in mast cells (Cockcroft and Gomperts, 1979). A selective increase in K⁺ permeability, probably via activation of Ca²⁺-dependent K⁺ channels, has also been observed (Gallacher, 1982). Recently, Benham and Tsien (1987) reported that extracellular ATP directly gated a channel in smooth muscle cells that was permeable to both Na⁺ and Ca²⁺. Soltoff et al. (1990) have recently suggested that extracellular ATP may function as a neurotransmitter and modulate fluid secretion by activation of calcium-sensitive Cl⁻ and K⁺ permeabilities in rat parotid acini. An ATP-induced increase in K⁺ permeability due to activation of K⁺ channels has been observed (Friedrich et al., 1989) in subconfluent MDCK cells (a renal cell line). In these epithelial cell preparations the authors could not distinguish apical membrane from basolateral membrane effects.

The epithelium of *Necturus* gallbladder is a useful model system for the study of near-isosmotic salt and water absorption, and its native transport pathways are well characterized (Reuss, 1989a). The experiments reported here used intracellular microelectrode techniques (Altenberg et al., 1990) to examine the effects of mucosal exposure to extracellular ATP on membrane voltages and resistances. The apical membrane permselectivity was evaluated with ion substitution experiments. Our results indicate that mucosal exposure to ATP induces changes in both apical and basolateral membrane K⁺ conductance and apical Cl⁻ conductance. Furthermore, ATP either directly or indirectly stimulates the basolateral membrane Na⁺, K⁺-ATPase.

METHODS

Preparation, Solutions, and Chemicals

Mudpuppies (*Necturus maculosus*) maintained in aquaria at 5–10°C were anesthetized with tricaine methanesulfonate. Gallbladders were removed, opened, rinsed free of bile, and mounted in a chamber, apical side up. The upper part of the chamber was open, had a volume of 0.2 ml, and was exchanged at a rate of 15–20 ml/min. The lower compartment was closed, had a volume of ~0.8 ml, and was perfused at a rate of 10–15 ml/min (Cotton and Reuss, 1989).

The control bathing solution (NaCl Ringer's solution) contained (in mM): 90 NaCl, 10 NaHCO₃, 2.5 KCl, 1.8 CaCl₂, 1.0 MgCl₂, and 0.5 NaH₂PO₄ and was equilibrated with 1% CO₂/99% air. The pH was ~7.65 and the osmolality was ~200 mosmol/kg. ATP-, ADP-, and adenosine-containing solutions were prepared fresh for each experiment. Divalent cation-free solutions were prepared by replacement of CaCl₂ and MgCl₂ with NaCl or Na cyclamate plus sucrose to maintain osmolality. In high-Mg²⁺ bathing solutions 10 mM MgCl₂ replaced 15 mM NaCl. ATP (disodium salt; grade I), ADP (sodium salt; grade III), and adenosine (free base) were purchased from Sigma Chemical Co. (St. Louis, MO).

Microelectrodes

Single-barrel microelectrodes were prepared from borosilicate glass with inner fiber (1.0 mm o.d., 0.5 mm i.d.; Omega dot; Friedrich and Dimmock, Inc., Milville, NJ). The electrodes were

filled with 3 M KCl and had resistances between 20 and 60 M Ω when immersed in Ringer's solution. Double-barrel Cl⁻, Na⁺, and K⁺-sensitive microelectrodes were prepared from fused borosilicate glass with inner fiber (each barrel 1.0 mm o.d., 0.43 mm i.d.; Hilgenberg, Malsfeld, Germany). The fused doublet was heated, twisted 360°, allowed to cool, and then pulled (PD-5 microelectrode puller; Narishige Scientific Laboratory, Tokyo, Japan). The reference barrel was partially filled with deionized water and the other barrel was partially filled with hexamethyldisilazane (Sigma Chemical Co.). The electrodes were placed on a hot plate under a stream of hot air until the silane and then the water evaporated. Extracellular K⁺ electrodes were prepared by placing a drop of resin (potassium tetrakis [*p*-chlorophenylborate], 5 mg in 0.1 ml of 3-nitro-O-xylene) in the silanized barrel and allowing the tip to fill. The reference barrel was filled with Ringer's solution and the ion-selective barrel was back-filled with Ringer's solution. Double-barrel electrodes that were used to measure intracellular Cl⁻ were prepared in the same manner except that Corning Cl⁻ exchanger 477913 (Corning Medical, Medfield, MA) was the resin and the reference barrel was filled with a 1 M Na formate/10 mM KCl solution. Na⁺-sensitive microelectrodes were prepared in the same manner except that the resin was solumion ionophore I-cocktail A (Fluka Chemical Co., Ronkonkoma, NY) and the reference barrel was filled with 1 M KCl. Double-barrel tetramethylammonium-sensitive (TMA⁺) microelectrodes were prepared from borosilicate glass with inner fiber (reference barrel 1.0 mm o.d., 0.5 mm i.d.; ion-sensitive barrel 1.0 mm o.d., 0.75 mm i.d.; Omega dot EN-1 glass; Friedrich and Dimmock). The doublet was heated, twisted 360°, allowed to cool, and then pulled (PD-5 microelectrode puller; Narishige Scientific Laboratory). The tip of the thin-wall barrel was filled with hexamethyldisilazane (by capillarity from the rear) and placed on a hot plate for 10 min. This barrel was filled with K⁺ resin (see above) and backfilled with Ringer's solution. The reference barrel was filled with a 1 M Na formate/10 mM KCl solution. Electrical connections and calibration of the microelectrodes were as previously described (Altenberg et al., 1990).

Electrical Measurements

The transepithelial voltage (V_{ms}) was referred to the serosal fluid compartment. The lower compartment electrode was an Ag-AgCl pellet in series with a Ringer's/agar bridge. The upper compartment electrode was a calomel half-cell in series with a flowing saturated KCl macroelectrode constructed from a fiber-filled glass pipette (Ultrawick; World Precision Instruments, New Haven, CT) pulled to a tip diameter of ~ 1 mm, which was placed in the upper compartment next to the suction (outflow) pipette. At the superfusion rates used, the KCl leakage into the mucosal solution compartment did not elicit measurable elevations in K⁺ activity. In experiments in which a static mucosal bathing solution was used, the flowing junction macroelectrode was removed and V_{ms} was measured with the Ringer-filled reference barrel of the double-barrel microelectrode.

Apical (V_{mc}) and basolateral (V_{cs}) membrane voltages were referred to the adjacent bathing solution. Ion-sensitive microelectrodes were connected to a high input-impedance electrometer (model FD-223; World Precision Instruments). The Ag-AgCl pellet in the lower compartment served as ground. Cell impalements were carried out with hydraulic micromanipulators (model MO-103; Narishige Scientific Laboratory). The tissue was observed with a microscope (Diavert; E. Leitz, Inc., Rockleigh, NJ) equipped with Hoffman modulation-contrast optics at 300 \times .

Transepithelial constant current pulses, I_c , (50–100 μ A/cm², 2–3 s duration) were passed between an Ag-AgCl pellet in the lower compartment and an Ag-AgCl wire in the upper compartment. The resulting voltage deflections, measured between 600 and 800 ms after the onset of the pulse (Stoddard and Reuss, 1988b), were corrected for series solution resistances and used to calculate the transepithelial resistance, R_t ($=\Delta V_{ms}/I_c$) and the fractional resistance of the apical membrane, fR_a ($=\Delta V_{mc}/\Delta V_{ms} = R_a/[R_a + R_b]$).

A two-point cable analysis (Bello-Reuss et al., 1981; Petersen and Reuss, 1985; Stoddard and Reuss, 1988b, 1989b) was done to evaluate the changes in R_z ($= R_a \cdot R_b/[R_a + R_b]$) induced by

exposure to ATP. Briefly, two cells (separated by 100–150 μm) were impaled and negative current pulses were injected (10–15 nA) through one electrode and the resultant cell membrane voltage deflections (ΔV_x) were measured in the second cell. Smaller current pulses (1 nA) were injected through the second electrode and the voltage deflections in that cell (ΔV_i) were also recorded. Both current pulses were 1 s in duration and of cell-negative polarity to prevent activation of the apical membrane K^+ conductance (Stoddard and Reuss, 1988*b*; Segal and Reuss, 1990). This procedure was carried out before, during, and after exposure to ATP. Measurements of input resistance with a single microelectrode are difficult to perform and interpret because of series resistance and other problems (Eisenberg and Johnson, 1970). We have tested this method in *Necturus* gallbladder epithelial cells by exposing the apical surface to *n*-heptanol, an agent known to block intercellular coupling via gap junctions (Délèze and Hervé, 1983). In four experiments in which the intracellular current was adjusted to elicit a voltage change of 5–10 mV, 3 mM *n*-heptanol elevated ΔV_i 18–64 mV within 30 s. This effect was fully reversible. When two cells were impaled to carry out two-point cable analysis, heptanol reduced ΔV_x to <20% of control. These observations qualitatively validate the measurements of input resistance and the two-point cable analysis method.

Changes in cell water volume were measured with an electrophysiological technique (Reuss, 1985). Briefly, the apical cell membrane was permeabilized by exposure to nystatin (a pore-forming antibiotic). Permeabilization during exposure to a tetramethylammonium (TMA^+)-sulfate Ringer solution (TMA^+ replacing Na^+) allowed the normally impermeant TMA^+ into the cells. The nystatin was removed and the cell membrane resealed, trapping TMA^+ in the cell interior. After returning to control Ringer's solution, the intracellular concentration of TMA^+ was measured with a double-barrel microelectrode. Inasmuch as TMA^+ is not transported by the cell membranes, changes in its intracellular activity can be used to calculate changes in cell water volume (Reuss, 1985; Cotton et al., 1989; Cotton and Reuss, 1991).

Data Analysis

All voltages (V_{ms} , V_{mc} , V_{cs} , and V_{ion}) were low-pass filtered and digitized. Data were sampled at 10 Hz with a data acquisition system and stored on a personal computer for subsequent analysis (Asyst; Macmillan Software Co., New York, NY). Means \pm SE are presented throughout. Student's *t* test for paired or unpaired data was used as appropriate. *P* values of <0.05 were considered significant.

RESULTS

Effects of ATP on Voltages and Resistances

The effects of a 2-min exposure of *Necturus* gallbladder epithelium to mucosal solution ATP (100 μM) are illustrated in Fig. 1 and summarized in Table I. There was a brief, small lumen-positive change in V_{ms} followed by a larger, sustained lumen-negative change. The transepithelial voltage returned slowly toward zero during the period of exposure and continued to recover after ATP removal. The initial effect of ATP on cell membrane voltages was a large, rapid hyperpolarization followed by a slower, partial repolarization. In Fig. 1 the voltages reached a plateau and then continued to depolarize; however, in some tissues the membrane voltages oscillated during continued exposure to ATP. Upon removal of the ATP there was a rapid initial depolarization of V_{mc} and V_{cs} followed by a slower depolarization. Finally, V_{mc} and V_{cs} slowly hyperpolarized to a value beyond the control membrane voltages measured before exposure to ATP. In some tissues the hyperpolarization exceeded

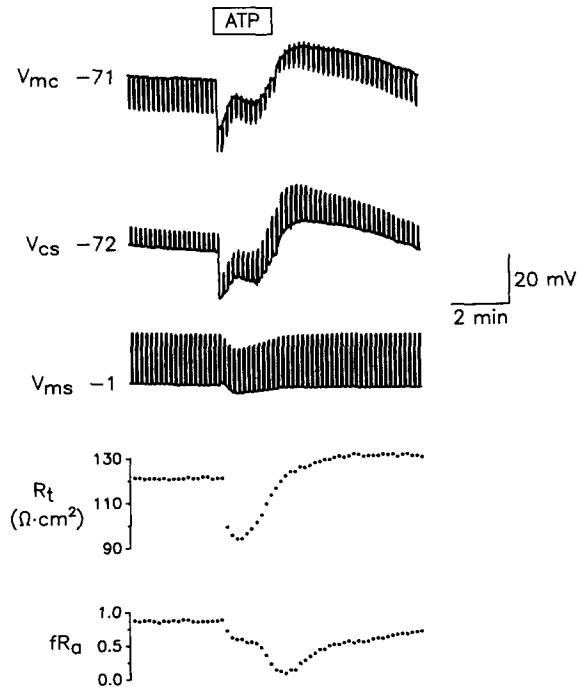


FIGURE 1. Effects of ATP addition to the mucosal solution ($100 \mu\text{M}$) on voltages and resistances. V_{ms} , V_{mc} , and V_{cs} are transepithelial, apical membrane, and basolateral membrane voltages, respectively. V_{ms} is referenced to the serosal solution and V_{mc} and V_{cs} are referenced to the adjacent bathing solutions. The voltage spikes result from transepithelial current pulses ($\sim 100 \mu\text{A}/\text{cm}^2$) and are used to calculate transepithelial resistance ($R_t = \Delta V/I$) and fractional apical membrane resistance ($fR_a = \Delta V_{mc}/\Delta V_{ms} = R_a/(R_a + R_b)$). In all figures, upward deflections denote positive changes in voltage.

that obtained immediately after exposure to ATP (see Fig. 2). Within 10–15 min after removal of the ATP the voltages returned to control values.

The transepithelial resistance (R_t) was reduced during exposure to ATP and recovered within 5–10 min after ATP removal. ATP also caused large and complex

TABLE I
Effects of Addition of $100 \mu\text{M}$ ATP to the Mucosal Bathing Solution on Voltages and Resistances

	V_{ms}	V_{mc}	V_{cs}	fR_a	R_t
	<i>mV</i>	<i>mV</i>	<i>mV</i>		Ωcm^2
Pre-ATP	0.1 ± 0.4	-69 ± 1	-69 ± 1	0.93 ± 0.02	142 ± 8
ATP					
a	0.5 ± 0.5	$-88 \pm 2^*$	$-87 \pm 2^*$	$0.83 \pm 0.03^*$	$122 \pm 7^*$
b	$-5.4 \pm 1.1^*$	$-68 \pm 2^*$	$-73 \pm 2^*$	$0.55 \pm 0.05^*$	115 ± 9
Post-ATP					
c	$-2.9 \pm 0.8^*$	-61 ± 2	$-64 \pm 2^*$	$0.33 \pm 0.08^*$	$139 \pm 6^*$
d	$-1.0 \pm 0.5^*$	$-88 \pm 5^*$	$-89 \pm 5^*$	$0.84 \pm 0.04^*$	$148 \pm 7^*$

Values are means \pm SEM ($n = 8$). V_{ms} , V_{mc} , and V_{cs} are transepithelial voltage, apical membrane voltage, and basolateral membrane voltage, respectively. fR_a and R_t are fractional apical membrane resistance and transepithelial resistance, respectively. Values are from continuous impalements before, during, and after a 1–2 min exposure to mucosal ATP ($100 \mu\text{M}$). The values were recorded at the peak hyperpolarization (a), the plateau before ATP removal (b), the peak depolarization after ATP removal (c), and the late hyperpolarization that usually occurred 2–3 min after ATP removal (d). *Significantly different from preceding condition, $P < 0.05$.

changes in fR_a . During the initial hyperpolarization of V_{mc} and V_{cs} , fR_a decreased slightly and continued to fall during the repolarization phase. Upon removal of the ATP, fR_a fell further and then began a slow recovery as V_{mc} and V_{cs} hyperpolarized. Within 10–15 min after the removal of the ATP, fR_a returned to control values.

The changes in V_{mc} in response to several ATP concentrations ranging between 1 and 1,000 μM are illustrated in Fig. 2. There are both qualitative and quantitative differences in the changes in membrane voltage with different concentrations of ATP. The most variable aspects of the response are the rate and extent of the repolarization of V_{mc} during continued exposure to ATP, the extent of the depolarization and hyperpolarization after ATP removal, and the changes in fR_a .

The dose–response relationships for the initial hyperpolarizations elicited by ATP,

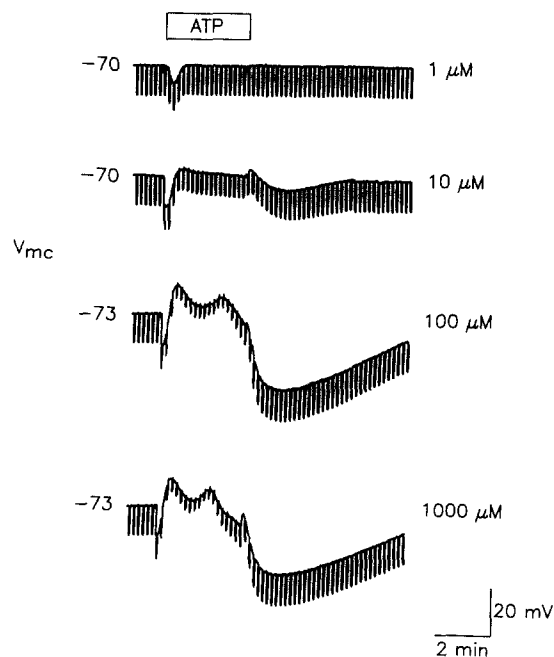


FIGURE 2. Concentration dependence of the effect of ATP on apical membrane voltage (V_{mc}). The traces depict sequential additions of ATP (~ 3 -min exposure) during a continuous impalement. The tissue was allowed to fully recover (10–15 min) between exposures to ATP.

ADP, and adenosine are illustrated in Fig. 3. The concentrations are expressed as calculated free $[\text{ATP}^{4-}]$ and free $[\text{ADP}^{3-}]$ according to Fabiato (1988). The free ATP^{4-} , rather than MgATP^{2-} , appears to be the active form (see below). The dose–response relationship for ADP^{3-} is shifted to the right by ~ 100 -fold relative to ATP^{4-} . Adenosine (1 mM) was without effect.

The effect of divalent cations on the ATP-induced hyperpolarization was examined. Tissues were exposed sequentially to 10 μM ATP in 10 mM Mg^{2+} Ringer, control Ringer, and Ca^{2+} -, Mg^{2+} -free Ringer. The effect of each solution is illustrated in Fig. 4. In five tissues, exposure to 10 μM ATP hyperpolarized V_{cs} by 12 ± 2 mV in high- Mg^{2+} Ringer (10 mM Mg^{2+}), 19 ± 1 mV in control Ringer, and 24 ± 2 mV in Ringer solution nominally free of divalent cations. Exposure to the latter solution or

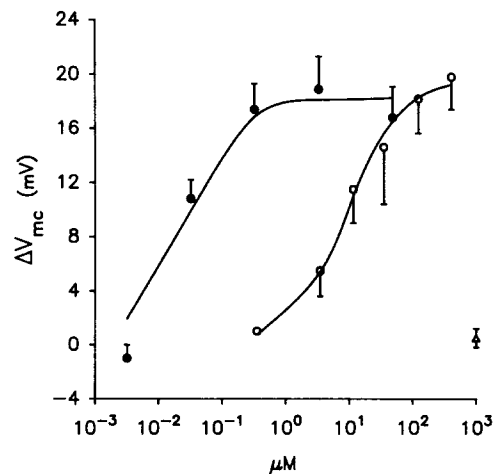


FIGURE 3. Dose-response relationships for ATP (filled circles), ADP (open circles), and adenosine (triangle). The magnitude of the initial hyperpolarization of apical membrane voltage (ΔV_{mc}) was determined for each free concentration. The free $[ATP^{4-}]$ and $[ADP^{3-}]$ were calculated according to Fabiato (1988). Total $[ATP]_t$'s were 0.1, 1, 10, 100, and 1,000 μM . Total $[ADP]_t$'s were 1, 10, 33, 100, 333, and 1,000 μM . Each point is the mean \pm SEM, $n = 8$ for $[ATP]$ (except 0.1 μM ATP, $n = 4$), $n = 4-5$ for each $[ADP]$, and $n = 3$ for 1,000 μM adenosine. Curves are least-square fits of the Michaelis-Menten equation to the data. ATP: $\Delta V_{max} = 18.3$ mV, $K_m = 2.7 \cdot 10^{-2}$ μM ; ADP: $\Delta V_{max} = 19.7$ mV, $K_m = 18$ μM .

to 10 mM Mg^{2+} Ringer solution without ATP induced only small (1–2 mV) changes in membrane voltages, expected from intraepithelial current flow arising from small transepithelial biionic potentials. Other phases of the response to ATP are also affected by divalent cation concentration (see Fig. 4), e.g., the hyperpolarization after ATP removal.

Cable Analysis

The dominant electrodiffusive permeabilities in *Necturus* gallbladder epithelium bathed in HCO_3^-/CO_2 -buffered media are an apical G_K and basolateral G_K and G_{Cl} (Stoddard and Reuss, 1988a, 1989a). Therefore, the initial fall in iR_a and the hyperpolarization could in principle be accounted for by an increase in apical G_K , a decrease in basolateral G_{Cl} , or both. The rapid changes in resistance during exposure

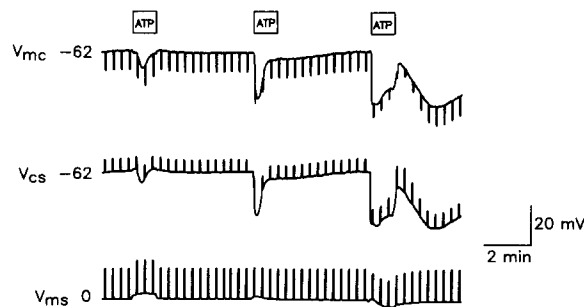


FIGURE 4. Effects of divalent cations on the response to ATP. Abbreviations as in Fig. 1. During a continuous impalement, the tissue was successively exposed on the mucosal side, for ~ 1 min in each case, to 10 μM ATP–10 mM Mg^{2+} Ringer, 10 μM ATP Ringer, and then 10 μM ATP–0 Ca^{2+} , 0 Mg^{2+} Ringer. Throughout the trace

the serosal bathing solution was control Ringer. The calculated free $[ATP]_f$'s are: 0.035 μM for the 10 ATP–10 mM Mg^{2+} Ringer, 0.35 μM for the 10 ATP–Ringer, and 10 μM for the 10 ATP–0 Ca^{2+} , 0 Mg^{2+} Ringer. Solution osmolality was maintained constant.

to ATP precluded a complete cable analysis. Therefore, to decide among these possibilities we did a two-point cable analysis (Petersen and Reuss, 1985; Stoddard and Reuss, 1988*b*, 1989*b*). Fig. 5 illustrates the procedure (see legend for details). The first pulse (ΔV_x) is the result of current injection into a distant impalement site. The second pulse (ΔV_i) is the result of current injection via the recording electrode and provides an estimate of the input resistance of the epithelium. A decrease in ΔV_x implies a decrease in intraepithelial current spread due to either a decrease in cell-cell coupling (gap junctional coupling) or a decrease in cell membrane resistance (R_a and R_b in parallel). Since most of the current injected into the cell spreads to adjacent cells via gap junctions, ΔV_i is a sensitive measure of changes in cell-cell coupling. The effects of 10 and 100 μM ATP were determined. An example is presented in Fig. 5 and the results obtained from three tissues are summarized in Fig. 6. With either 10 or 100 μM ATP there was a rapid, transient fall in ΔV_x followed by an increase during exposure. Upon removal of the ATP, ΔV_x increased reversibly.

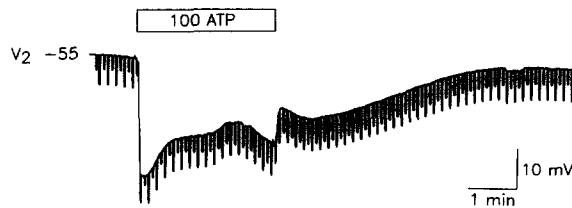


FIGURE 5. Effects of mucosal ATP (100 μM) on input resistance (ΔV_i) and intraepithelial current spread (ΔV_x). Two cells separated by $\sim 120 \mu\text{m}$ were impaled with microelectrodes. Both electrodes were referenced to the serosal bathing

solution. Negative current pulses of 10 nA, 1-s duration, and a frequency of 0.1 Hz were injected into one cell and the resultant voltage deflections (ΔV_x) were monitored in the other cell. Smaller negative current pulses of 1 nA, 1-s duration, and a frequency of 0.1 Hz were injected into the second cell and the resultant voltage deflections (ΔV_i) in the same cell were recorded. The pulses were timed so that they alternated. ΔV_x results from current spread within the epithelial sheet, whereas ΔV_i is indicative of the input resistance of the epithelium. The trace is the voltage recorded from the second cell. The first pulse in the series is ΔV_x and the second pulse is ΔV_i . The bridge circuit in the second electrode was balanced to compensate for microelectrode resistance before the cell was impaled.

Throughout the exposure there were only small changes in ΔV_i . The large fall in ΔV_x in the absence of changes in ΔV_i suggests that $R_z (= R_a \cdot R_b / [R_a + R_b])$ is initially decreased by ATP. This result, in conjunction with the small fall in fR_a , suggests that the initial, rapid hyperpolarization results from an increase in G_K at both apical and basolateral cell membranes.

Effects of ATP on the Voltage Changes Elicited by Mucosal Solution Ion Substitutions

Tissues were exposed briefly (~ 40 s) to mucosal bathing solutions of altered ion composition before, during, and after exposure to either 10 or 100 μM ATP (mucosal side). All ion substitutions were isomolar. Na^+ concentration was decreased from 100 to 10 mM (replaced with TMA⁺), $[\text{K}^+]$ was increased from 2.5 to 25 mM (K^+ replaced Na^+), or $[\text{Cl}^-]$ was reduced from 98 to 9.8 mM (replaced with cyclamate). The electrophysiologic effects of each substitution are illustrated in Figs. 7–10 and the results from the K^+ and Cl^- experiments are summarized in Tables II and III.

The response to a reduction of mucosal solution $[\text{Na}^+]$ is not significantly altered by exposure to $10 \mu\text{M}$ ATP (Fig. 7 *A*). The lumen-positive change in V_{ms} results from the biionic potential (Na^+/TMA^+) arising across the paracellular pathway, and the changes in V_{mc} and V_{cs} are largely a consequence of changes in intraepithelial current flow (Reuss and Finn, 1975*a, b*). Similarly, the voltage changes that occur during Na^+ replacement in the presence of $100 \mu\text{M}$ ATP can be entirely explained by the paracellular biionic potential. The large depolarization of V_{cs} is explained by the fall of fR_{a} in $100 \mu\text{M}$ ATP; most of the IR drop now occurs across the basolateral membrane rather than across the apical membrane as observed without ATP and with $10 \mu\text{M}$ ATP.

Elevation of mucosal solution $[\text{K}^+]$ caused a depolarization of V_{mc} and V_{cs} and a

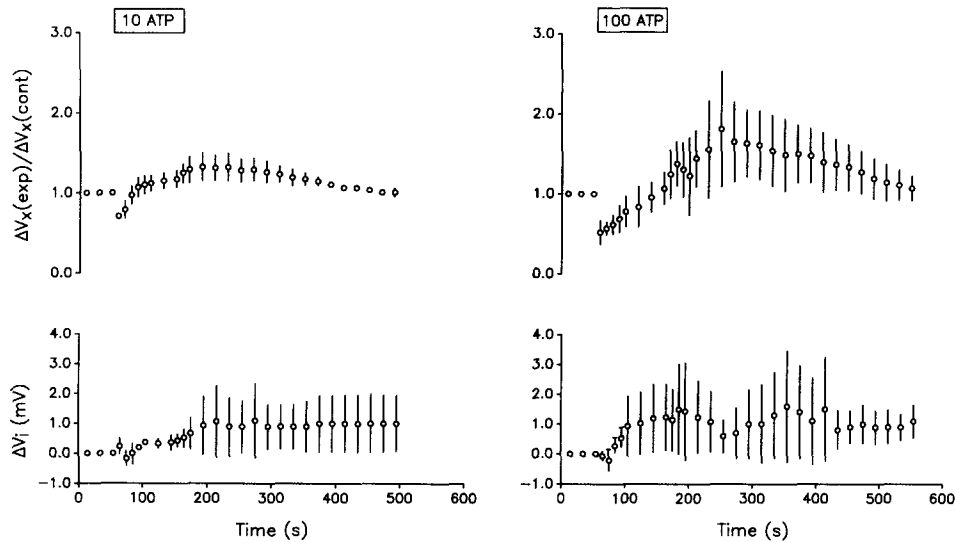


FIGURE 6. Effects of ATP on intraepithelial current spread (ΔV_x) and input resistance (ΔV_i). All values were normalized to ΔV_x and ΔV_i recorded immediately before the addition of ATP. $\Delta V_x(\text{exp})/\Delta V_x(\text{cont})$ is expressed as a ratio, whereas ΔV_i is the difference, $\Delta V_i(\text{exp}) - \Delta V_i(\text{cont})$. The values are mean \pm SEM for three tissues. (*A*) $10 \mu\text{M}$ ATP. (*B*) $100 \mu\text{M}$ ATP. The two values of ΔV_x immediately after ATP were significantly less than control at both 10 and $100 \mu\text{M}$.

lumen-negative change in V_{ms} (Fig. 8, *A* and *B*). These voltage changes are expected because of the dominant K^+ conductance of the apical cell membrane (Reuss and Finn, 1975*a, b*; Van Os and Slegers, 1975). Elevation of $[\text{K}^+]$ during exposure to 10 or $100 \mu\text{M}$ ATP resulted in a significantly larger depolarization of V_{mc} and V_{cs} and a greater hyperpolarization of V_{ms} . These results are consistent with an ATP-induced increase in P_{K} . Upon removal of the ATP, the response to an elevation of mucosal solution $[\text{K}^+]$ was not different from the control response. These results are summarized in Table II.

Partial replacement of mucosal solution Cl^- with cyclamate caused small transient changes in V_{ms} and a slow hyperpolarization of V_{mc} and V_{cs} (Fig. 9, *A* and *B*). The changes in V_{ms} can be explained by liquid junction potentials at the mucosal

macroelectrode. The hyperpolarization of V_{mc} and V_{cs} is due to both a decrease in basolateral membrane Cl^- conductance and a fall in the Cl^- equilibrium potential across the basolateral membrane ($E_b^{\text{Cl}^-}$) caused by the reduction in intracellular Cl^- activity during luminal Cl^- replacement (Stoddard and Reuss, 1989b). During exposure to $10 \mu\text{M}$ ATP, transient reduction of mucosal solution $[\text{Cl}^-]$ induced large and rapid hyperpolarizations of V_{mc} and V_{cs} (Fig. 9A). A similar response was seen in tissues in which Cl^- was replaced by gluconate, isethionate, or sulfate, ruling out the possibility that the effect is specific for cyclamate. Under control conditions (i.e., without ATP), if mucosal solution $[\text{Cl}^-]$ and $[\text{HCO}_3^-]$ are simultaneously reduced 10-fold, the slow hyperpolarization of V_{mc} and V_{cs} is not observed because the net

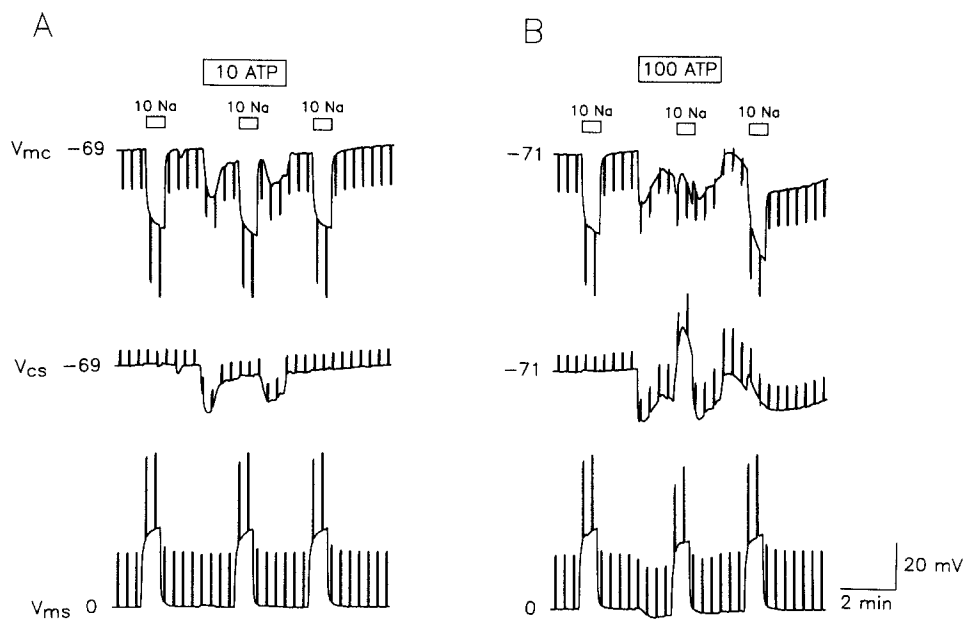


FIGURE 7. Effects of reduction of mucosal solution $[\text{Na}^+]$ from 100 to 10 mM before, during, and after exposure to mucosal ATP. Abbreviations as in Fig. 1. Mucosal solution $[\text{Na}^+]$ was reduced (isomolar replacement of NaCl with TMACl) for ~ 40 s. Exposures to ATP lasted 3 min. (A) $10 \mu\text{M}$ ATP. (B) $100 \mu\text{M}$ ATP.

driving force on the apical membrane $\text{Cl}^-/\text{HCO}_3^-$ exchanger remains constant and intracellular Cl^- and pH do not change (Reuss, 1987). If $[\text{Cl}^-]$ and $[\text{HCO}_3^-]$ are simultaneously reduced 10-fold in the presence of $10 \mu\text{M}$ ATP, the hyperpolarization is still observed (data not shown). Further, the rate of fall of intracellular Cl^- ($a\text{Cl}_i^-$) upon reduction of mucosal solution $[\text{Cl}^-]$ is the same with or without $10 \mu\text{M}$ ATP. Finally, there is no change in $a\text{Cl}_i^-$ when mucosal solution $[\text{Cl}^-]$ and $[\text{HCO}_3^-]$ are simultaneously reduced 10-fold in the presence of $10 \mu\text{M}$ ATP. Therefore, the large, rapid hyperpolarization of V_{mc} and V_{cs} induced by 9.8 mM Cl^- in the presence of $10 \mu\text{M}$ ATP is not due to a change in $a\text{Cl}_i^-$. An alternative explanation for the rapid hyperpolarization in the presence of ATP is that the replacement anions (cyclamate,

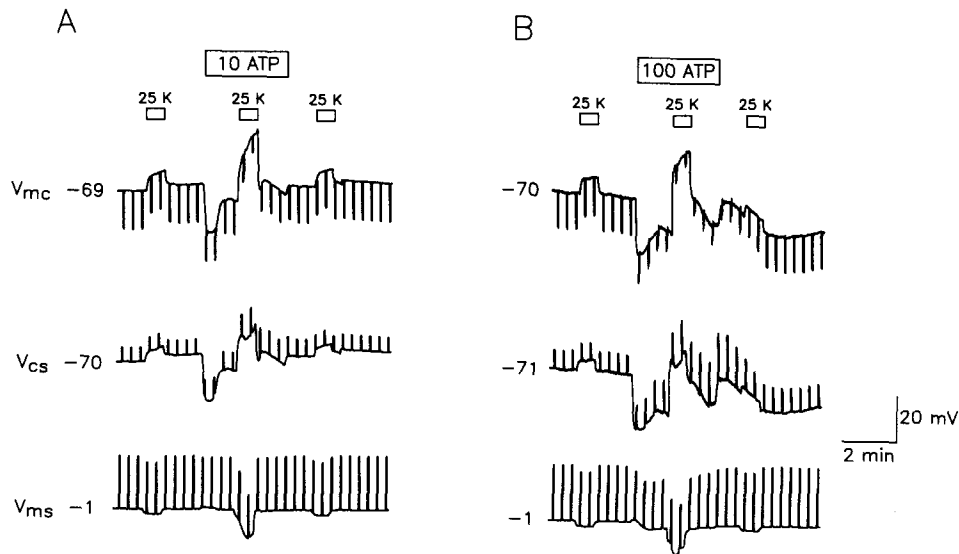


FIGURE 8. Effects of elevation of mucosal solution $[K^+]$ from 2.5 to 25 mM before, during, and after exposure to mucosal ATP. Abbreviations as in Fig. 1. Mucosal solution $[K^+]$ was increased (isomolar replacement of NaCl with KCl) for ~ 40 s. Exposure to ATP lasted 3 min. (A) 10 μ M ATP. (B) 100 μ M ATP.

gluconate, isethionate, or sulfate) reduce the free concentration of divalent cations (a well-known effect of gluconate). A decrease in the concentration of free Mg^{2+} and/or Ca^{2+} would be expected to shift the equilibrium between bound and free ATP^{4-} , raising free ATP^{4-} levels. Since 10 μ M ATP is a submaximal concentration, an increase in the concentration of the active form of ATP could explain the hyperpo-

TABLE II
Effects of Addition of ATP to the Mucosal Bathing Solution on the Response to Elevation of Mucosal Solution $[K^+]$

	ΔV_{ms}	ΔV_{mc}	ΔV_{cs}
	<i>mV</i>		
10 μ M ATP			
Before	-2.1 ± 0.1	8.8 ± 1.3	6.7 ± 1.3
During	$-7.5 \pm 1.7^*$	$25.3 \pm 4.4^*$	$17.8 \pm 3.9^*$
After	-1.9 ± 0.1	8.3 ± 1.2	6.3 ± 1.2
100 μ M ATP			
Before	-1.9 ± 0.1	8.0 ± 1.3	6.0 ± 1.3
During	$-7.4 \pm 1.7^*$	$31.8 \pm 2.3^*$	$24.4 \pm 2.2^*$
After	-1.9 ± 0.1	5.8 ± 1.9	4.2 ± 1.7

Values are means \pm SEM; 10 μ M ATP, $n = 6$; 100 μ M ATP, $n = 5$. Mucosal $[K^+]$ was increased from 2.5 to 25 mM for ~ 40 s. The $[K^+]$ steps were begun ~ 2 min before addition of the ATP, 1 min after the exposure to ATP began, and ~ 1 min after the exposure to ATP was terminated. The total period of exposure to ATP was ~ 3 min. ΔV_{ms} , ΔV_{mc} , and ΔV_{cs} are the changes in transepithelial, apical membrane, and basolateral membrane voltages, respectively, measured 10 s after the substitution. *Significantly different from the response before ATP, $P < 0.05$.

larization. Several lines of evidence support this interpretation. First, replacement of Cl^- by cyclamate during exposure to a higher concentration of ATP (100 μM) resulted in a small depolarization of V_{mc} and V_{cs} , followed by a slower hyperpolarization (Fig. 9 B). Upon return to 98 mM Cl^- solution a small hyperpolarization of V_{mc} and V_{cs} was observed. Second, a similar response is observed if the 9.8 mM Cl^- and the 10 μM ATP solutions are free of divalent cations (Fig. 10, A and B, and Table III).

Replacement of mucosal Cl^- with cyclamate 1 min after removal of the ATP (10 μM ATP/divalent cation free or 100 μM ATP) resulted in a small depolarization of V_{mc} and V_{cs} . The return to 98 mM Cl^- induced a small hyperpolarization. These results suggest that ATP^{4-} concentrations in excess of $\sim 0.2 \mu\text{M}$ induce or activate an apical membrane Cl^- conductance that persists for several minutes after the removal of ATP.

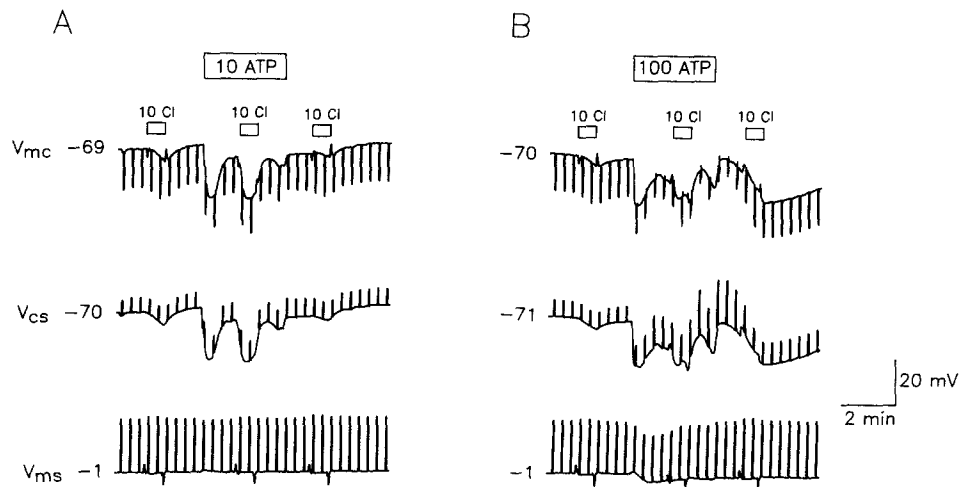


FIGURE 9. Effects of reduction of mucosal solution $[\text{Cl}^-]$ from 98 to 9.8 mM before, during, and after exposure to mucosal solution ATP. Abbreviations as in Fig. 1. Mucosal solution $[\text{Cl}^-]$ was reduced (isomolar replacement of NaCl with Na cyclamate) for ~ 40 s. Exposure to ATP lasted 3 min. (A) 10 μM ATP. (B) 100 μM ATP.

ATP Induces an Increase in Mucosal Solution $[\text{K}^+]$

The effect of 1 mM ATP on the K^+ activity of a static mucosal bathing solution is illustrated in Fig. 11. In the upper trace the mucosal superfusion was halted and the K^+ activity was recorded with an extracellular double-barrel microelectrode. In the lower trace, the static mucosal bathing solution contained 1 mM ATP. The difference between the two traces represents the ATP-induced net K^+ secretion. In four tissues, ATP significantly increased mucosal $[\text{K}^+]$ after 3 min, by 0.4 ± 0.1 mM. K^+ secretion was not detected with 100 μM ATP. This is probably due to the relatively poor sensitivity of this technique; i.e., luminal solution volume is ~ 20 times larger than the intracellular volume.

Effect of ATP on Intracellular Cl⁻ Activity

Intracellular Cl⁻ activity was measured in three tissues before, during, and after exposure to 10 or 100 μM mucosal ATP. During a 2-min exposure to 10 μM ATP, aCl_i⁻ was not significantly changed (20.2 ± 1.4 vs. 19.1 ± 1.7 mM). However, after 1-min exposure to 100 μM ATP, aCl_i⁻ fell from 16.6 ± 0.7 to 10.9 ± 1.9 mM. An example of the decline in aCl_i⁻ is illustrated in Fig. 12. In *A*, mucosal solution Cl⁻ was lowered from 98 to 9.8 mM before, during, and after exposure to ATP (100 μM).

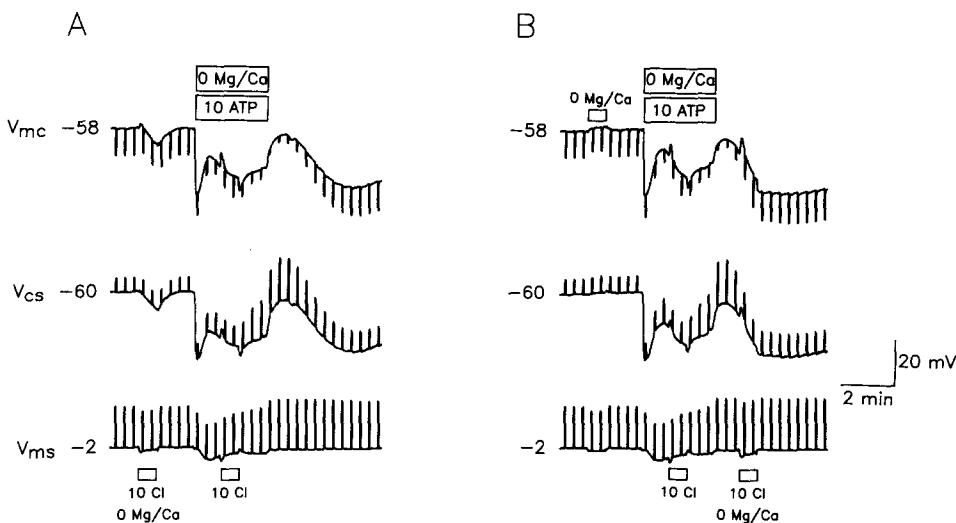


FIGURE 10. Effects of removal of divalent cations on the response to a reduction of mucosal solution [Cl⁻] from 98 to 9.8 mM. Abbreviations as in Fig. 1. (*A*) Mucosal solution [Cl⁻] was reduced for ~40 s from 98 to 9.8 mM simultaneously with omission of divalent cations (0 Mg²⁺, 0 Ca²⁺, 9.8 Cl⁻ Ringer). Subsequently, the tissue was exposed to 10 μM ATP in divalent cation-free Ringer (0 Mg²⁺, 0 Ca²⁺, 10 μM ATP Ringer) for ~3 min. During the exposure to ATP, mucosal solution [Cl⁻] was again reduced to 9.8 mM (divalent cation free) for ~40 s. (*B*) The tissue was exposed for ~40 s to mucosal 0 Mg²⁺, 0 Ca²⁺ Ringer. Subsequently, the tissue was exposed to 10 μM ATP in divalent cation-free Ringer for ~3 min. During the ATP exposure, mucosal solution [Cl⁻] was again reduced to 9.8 mM (divalent cation-free) for ~40 s. After removal of the ATP, the tissue was again exposed to 9.8 mM Cl⁻ (divalent cation-free) for ~40 s.

Intracellular Cl⁻ activity fell upon lowering mucosal solution [Cl⁻] and also in response to mucosal ATP at constant [Cl⁻]. In the experiment depicted in *B*, [Cl⁻] and [HCO₃⁻] were simultaneously reduced 10-fold before, during, and after exposure to ATP. In the absence of ATP, aCl_i⁻ did not change; however, ATP induced a fall in aCl_i⁻ that continued during exposure to reduced [Cl⁻] and [HCO₃⁻]. Intracellular Cl⁻ activity slowly returned to control values after removal of the ATP. The intracellular K⁺ activity was not measured in these studies. Inasmuch as K⁺ is the most abundant intracellular cation (aK_i⁺ is ~100 mM), small changes in its chemical activity are

TABLE 111
Effects of Addition of ATP to the Mucosal Bathing Solution on the Response to
Reduction of Mucosal Solution $[Cl^-]$

	ΔV_{ms}	ΔV_{mc}	ΔV_{cs}
	<i>mV</i>		
10 μM ATP/Mg ²⁺ - and Ca ²⁺ -free			
Before	-1.8 \pm 0.5	0.9 \pm 0.5	-0.9 \pm 0.5
During	-0.8 \pm 0.5	3.5 \pm 0.9*	2.8 \pm 0.9*
After	-1.5 \pm 0.5	4.3 \pm 1.3*	2.8 \pm 0.8*
100 μM ATP			
Before	-1.6 \pm 0.5	0.6 \pm 0.4	-1.0 \pm 0.4
During	-1.2 \pm 0.6	3.3 \pm 0.7*	1.8 \pm 1.0*
After	-1.7 \pm 0.5	4.0 \pm 0.6*	2.2 \pm 1.0*

Values are means \pm SEM; 10 μM ATP, $n = 4$; 100 μM ATP, $n = 6$. The 10 μM ATP solution and also the 9.8 Cl solutions before, during, and after 10 μM ATP were nominally Mg²⁺- and Ca²⁺-free (replaced with Na⁺). Mucosal $[Cl^-]$ was reduced from 98 to 9.8 mM for ~ 40 s. The rapid, initial changes in V_{ms} and V_{mc} (since V_{mc} is recorded as the difference $V_{cs} - V_{ms}$) result from mucosal solution liquid junction potentials and were ignored. See Table II for additional details. *Significantly different from the response before ATP, $P < 0.05$.

difficult to detect. Further, isosmotic loss of salt and water from the cells (see below) minimizes the expected change in aK_+^+ .

Effects of ATP on Cell Water Volume

Inasmuch as exposure to mucosal solution ATP induces net K⁺ secretion and a fall in aCl_+^- , it is likely that these ion fluxes are accompanied by changes in cell water volume. The effect of 100 μM ATP is illustrated in Fig. 13. The cells started to shrink

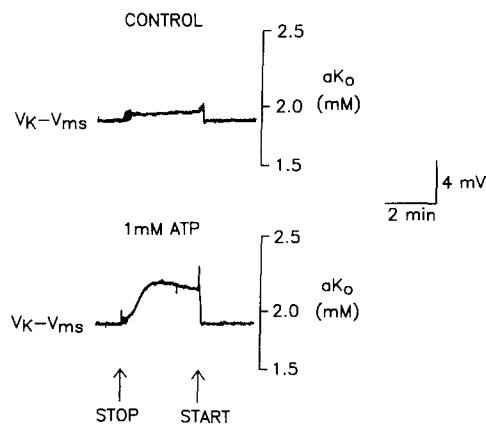


FIGURE 11. Effects of ATP on mucosal solution K⁺ activity. K⁺ activity was measured with a double-barrel microelectrode placed in the mucosal bathing solution $\sim 100 \mu m$ above the tissue (see Methods). Both barrels were referenced to the serosal compartment. $V_K - V_{ms}$ is the difference between the voltage outputs of the K⁺-sensitive and conventional barrels of the microelectrode. The suction pipettes were positioned so that when superfusion was halted a thin layer of bathing solution (height $\sim 600 \mu m$; volume $\sim 30 \mu l$) covered the tissue.

(A) The apical surface was rapidly superfused (10–15 s) with $\sim 200 \mu l$ of Ringer's solution and superfusion was halted. After ~ 3 min superfusion was resumed. (B) The apical surface was rapidly superfused (10–15 s) with $\sim 200 \mu l$ of Ringer's solution that contained 1 mM ATP and superfusion was halted. After ~ 3 min superfusion was resumed.

~20 s after exposure to ATP and cell water volume reached a minimum ~30 s later. The cell volume recovered after removal of the ATP. In three experiments, 100 μ M ATP caused a significant cell shrinkage of $18 \pm 2\%$.

Effects of Ouabain on the Responses to ATP

In four tissues the response to a 3-min exposure to 100 μ M ATP was determined before and during exposure to serosal ouabain (1 mM) as illustrated in Fig. 14 and

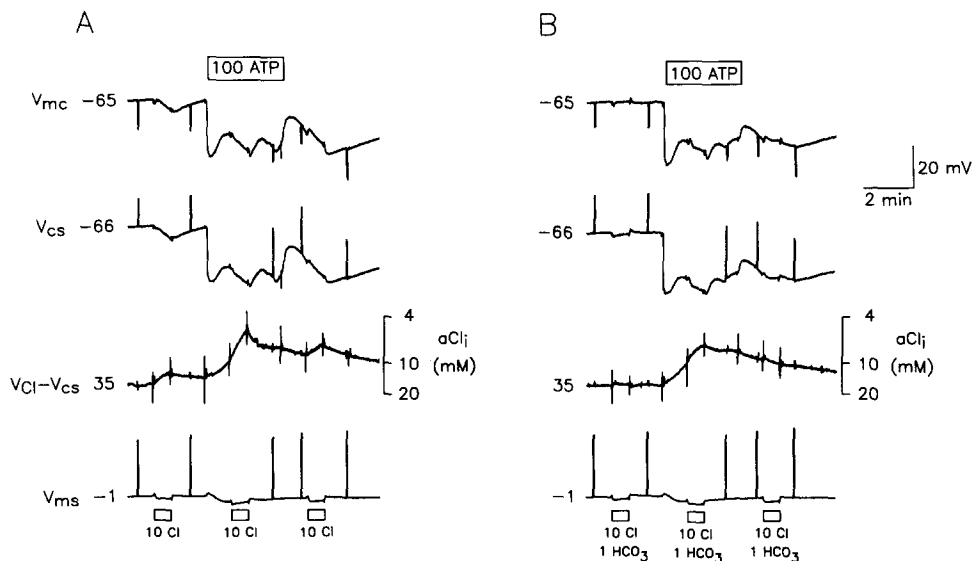


FIGURE 12. Effects of ATP on intracellular Cl^- activity (aCl^-_i). aCl^-_i was measured with a double-barrel microelectrode (see Methods). $V_{Cl} - V_{cs}$ is the differential voltage output of the ion-selective and reference barrels. Other abbreviations as in Fig. 1. (A) Mucosal solution $[Cl^-]$ was reduced from 98 to 9.8 mM (replaced by cyclamate) for 40-s periods before, during, and after exposure to 100 μ M ATP. Note the falls in aCl^-_i caused by lowering mucosal solution $[Cl^-]$ and by exposure to ATP before the reduction of $[Cl^-]$. (B) Mucosal solution $[Cl^-]$ was reduced from 98 to 9.8 mM and simultaneously $[HCO_3^-]$ was reduced from 10 to 1 mM (both anions replaced with cyclamate). Note that aCl^-_i did not change appreciably during the period of exposure to 9.8 Cl^- , 1 HCO_3^- . However, 100 μ M ATP caused a large rapid fall in aCl^-_i that continued during the period of exposure to 9.8 Cl^- , 1 HCO_3^- .

summarized in Table IV. The initial hyperpolarizations of V_{mc} and V_{cs} induced by ATP were unaffected by ouabain. Furthermore, the changes in R_i and fR_a during exposure to ATP were similar with and without ouabain. In contrast, the hyperpolarization and the increase in fR_a measured 1 min after removal of the ATP were significantly attenuated by exposure to ouabain. These results suggest that the basolateral Na^+, K^+ -ATPase is responsible, either directly or indirectly, for the hyperpolarization observed after ATP removal.

DISCUSSION

The native ion transport pathways across the apical and the basolateral cell membranes of *Necturus* gallbladder epithelium are well characterized (Reuss, 1989a). In tissues bathed in HEPES-buffered Ringer solution (nominally HCO_3^- free) the only sizable conductive pathways are K^+ conductances at both apical and basolateral cell membranes (Reuss and Finn, 1975a, b; Van Os and Slegers, 1975; Reuss, 1979). However, tissues bathed in bicarbonate-buffered Ringer solution also express a significant basolateral Cl^- conductance (Stoddard and Reuss, 1988a, 1989a, b). In

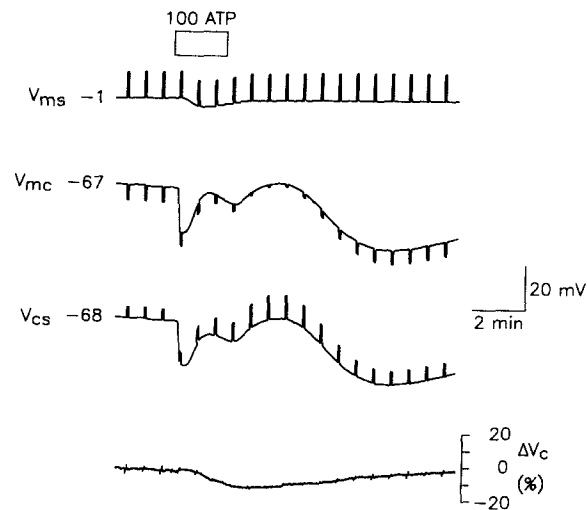


FIGURE 13. Effect of 100 μM ATP on cell water volume. The tissue was loaded with 15 mM TMA^+ as described in Methods. Abbreviations as in Fig. 1. The differential voltage output of the ion-selective (V_{TMA}) and reference (V_{cs}) barrels was recorded ($V_{\text{TMA}} - V_{\text{cs}}$), and the intracellular TMA^+ activity was determined for each data point. The percent change in cell water volume was calculated from the following equation:

$$\frac{V(t) - V(0)}{V(0)} \times 100 = \left(\frac{a\text{TMA}_i^+(0)}{a\text{TMA}_i^+(t)} - 1 \right) \times 100$$

where $V(0)$ = initial cell water volume, $V(t)$ = cell water volume at time t , $a\text{TMA}_i^+(0)$ = initial intracellular TMA^+ activity, $a\text{TMA}_i^+(t)$ = intracellular TMA^+ activity at time t .

addition, elevations of intracellular cAMP levels activate an apical membrane Cl^- conductance; with maximal stimulation, the apical membrane becomes Cl^- permselective (Petersen and Reuss, 1983). Several agents, including propionate (Petersen and Reuss, 1985), HCO_3^- (Petersen and Reuss, 1985), and cyanide (Bello-Reuss et al., 1981), have been shown to cause large changes in membrane voltages due to increases in the K^+ permeability of both apical and basolateral cell membranes. It was suggested that these agents act via pathways involving the elevation of intracellular calcium (Petersen and Reuss, 1985). The effects of ATP are different from those of elevation of intracellular free Ca^{2+} levels by exposure to the Ca^{2+} ionophore A23187

or to cyanide. These agents elicit monotonic hyperpolarization of the cell membranes and fR_a rises instead of falling (Bello-Reuss et al., 1981). Prolonged exposure to Ca^{2+} -free media on both sides produces irreversible tissue damage and hence cannot be used to test for a role of Ca^{2+} entry in the effects of ATP. However, reducing mucosal bathing solution $[\text{Ca}^{2+}]$ to $< 1 \mu\text{M}$ did not alter the response to $100 \mu\text{M}$ ATP (data not shown). We have observed that acetylcholine (unpublished observations) and ATP (this report) induce a rapid hyperpolarization of membrane voltages and a fall in R_i and fR_a . Recent studies in dispersed rat parotid acini (Soltoff et al., 1990) and in nonconfluent cultures of a renal epithelial cell line (MDCK) (Lang et al., 1988;

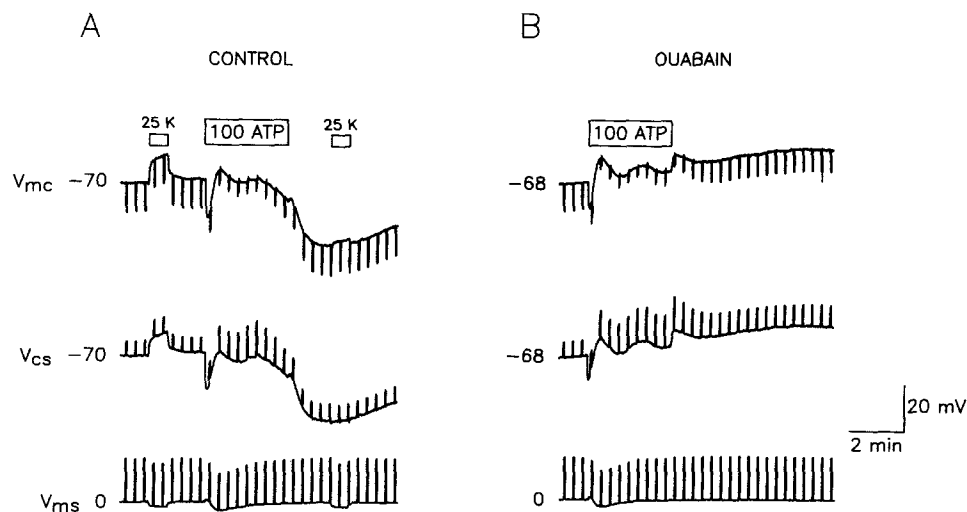


FIGURE 14. Effects of ouabain addition to the serosal solution on the response to mucosal solution ATP. Abbreviations as in Fig. 1. (A) The tissue was exposed for 3 min to mucosal ATP ($100 \mu\text{M}$). The response to an elevation of mucosal solution $[\text{K}^+]$ from 2.5 to 25 mM was assessed before and after exposure to ATP. At ~ 1 min after removal of the ATP, V_{mc} and V_{cs} were hyperpolarized by ~ 30 mV. (B) Continuation of the records in A. 1 mM ouabain was added to the serosal bathing solution 5 min before the beginning of this trace. Note that V_{mc} and V_{cs} have depolarized ~ 2 –3 mV and that the initial hyperpolarization upon exposure to ATP is essentially unchanged by ouabain. However, ~ 1 min after removal of the ATP V_{mc} and V_{cs} , instead of hyperpolarizing, depolarized by ~ 5 mV.

Friedrich et al., 1989; Jungwirth et al., 1989) have demonstrated that ATP induces an increase in K^+ permeability (MDCK cells), a net loss of intracellular K^+ and Cl^- (parotid acini), and an increase in intracellular Ca^{2+} (parotid acini).

In these preparations it was not possible to ascertain whether the "ATP receptor" was located in the apical or the basolateral membrane. Furthermore, the site of the change in permeability and the location of K^+ and Cl^- efflux pathways could not be determined. In light of these observations we have examined the effect of mucosal exposure to ATP on voltages, resistances, and apical membrane ion permeability of *Necturus* gallbladder epithelium.

Initial Hyperpolarization

The initial effect of mucosal ATP is a hyperpolarization of V_{mc} and V_{cs} . At maximal doses of ATP ($> 100 \mu\text{M}$) the membrane voltages hyperpolarize to $-88 \pm 2 \text{ mV}$, a value close to E_K (approximately -95 mV) (Reuss and Stoddard, 1987). This response is consistent with an increase in apical or basolateral membrane G_K , a decrease in basolateral G_{Cl} , or a combination of these. The fall in R_t induced by ATP (Figs. 5 and 6) suggests that the dominant effect is an increase in G_K rather than a fall in G_{Cl} . Furthermore, the small change in fR_a suggests that the G_K of both the apical and basolateral cell membranes increases. The results of the ion substitution experiments, as well as the measurements of K^+ secretion, indicate that at least apical membrane P_K increases and remains elevated during exposure to ATP. However, other permeability changes cannot be excluded. During the initial hyperpolarization (3–5 s) there is either a small ($< 1 \text{ mV}$) lumen-positive change in V_{ms} or no change.

TABLE IV
Effects of Ouabain on the Changes in Voltages and Resistances in Response to Addition of ATP to the Mucosal Solution

	V_{ms} mV	V_{mc} mV	fR_a	R_t Ωcm^2
Control				
Pre-ATP	0.0 ± 0.4	-69 ± 3	0.89 ± 0.01	142 ± 16
ATP hyperpolarization	-3.5 ± 0.5	-89 ± 3	0.77 ± 0.04	129 ± 13
Post-ATP hyperpolarization	-1.0 ± 0.4	-88 ± 5	0.80 ± 0.03	164 ± 19
Ouabain				
Pre-ATP	-0.3 ± 0.5	-68 ± 3	0.88 ± 0.02	152 ± 17
ATP hyperpolarization	-2.5 ± 0.7	$-84 \pm 4^*$	0.75 ± 0.04	132 ± 15
Post-ATP hyperpolarization	-0.5 ± 0.3	$-59 \pm 4^*$	$0.63 \pm 0.03^*$	164 ± 20

Values are means \pm SEM ($n = 4$). Ouabain was added to the serosal perfusate to a final concentration of 1 mM 5 min before exposure to mucosal ATP ($100 \mu\text{M}$). Measurements were made $\sim 1 \text{ min}$ before addition of ATP (pre-ATP), at the peak hyperpolarization after addition of ATP (ATP hyperpolarization), and $\sim 1 \text{ min}$ after the removal of ATP (post-ATP hyperpolarization). *Value in ouabain significantly different from control, $P < 0.05$.

An initial lumen-positive change suggests that the effect of ATP on E_a precedes the change in E_b (where E_a and E_b are the zero-current voltages of apical and basolateral membranes, respectively).

Repolarization of V_{mc} and V_{cs}

The slow, partial repolarization of membrane voltages after the peak hyperpolarization could result from a fall in E_K , a decrease in G_K , an increase in G_X (X denotes any ion with an E_X more positive than V_m), or a combination of these. We have observations to support each possibility. E_K may decrease due to loss of intracellular K^+ or accumulation of extracellular K^+ . Although our measurements of mucosal solution K^+ were made with a static luminal solution, it is likely that $[\text{K}^+]$ does increase in the unstirred fluid layer (Cotton and Reuss, 1989) adjacent to the epithelium. Elevation of basolateral solution $[\text{K}^+]$ is less likely since the basolateral

Na^+, K^+ -ATPase would tend to reduce accumulation. E_K could also decrease if $a\text{K}_i^+$ fell. If the depolarization of V_{mc} and V_{cs} resulted only from a fall in $a\text{K}_i^+$, the observed depolarization would require a large fall in $a\text{K}_i^+$ (i.e., ~50% decrease in $a\text{K}_i^+$ to account for an 18-mV depolarization). The results of preliminary measurements of $a\text{K}_i^+$ (data not shown) suggest that intracellular K^+ activity does not change significantly during the early period of exposure to ATP (i.e., within 30–60 s) when V_{mc} and V_{cs} depolarize. The membrane voltages would depolarize if G_x increased. The results of the Na^+ substitution experiments indicate that ATP does not appreciably increase apical membrane G_{Na} . However, the Cl^- substitution experiments with 100 μM ATP or 10 μM ATP in divalent cation-free solution did reveal an electrodiffusive apical membrane Cl^- permeability. E_{Cl} is approximately -40 mV in untreated tissues (Reuss and Stoddard, 1987; Reuss, 1989b), and therefore activation of G_{Cl} would be expected to depolarize membrane voltages. In these experiments the ion substitutions were done after V_{mc} and V_{cs} had depolarized; therefore, it is possible that the depolarization activated G_{Cl} , rather than the activation of G_{Cl} causing the depolarization. However, it is unlikely that the depolarization of V_{mc} activated G_{Cl} , since V_{mc} is still hyperpolarized relative to the voltage before exposure to ATP. Furthermore, the fall in $a\text{Cl}_i^-$ during the repolarization phase, significant with 100 μM ATP (see Results), is consistent with activation of an apical membrane G_{Cl} .

The rapid decrease in cell water volume during exposure to extracellular ATP can be explained by activation of a Cl^- conductance. Both $a\text{K}_i^+$ and $a\text{Cl}_i^-$ are maintained above electrochemical equilibrium in *Necturus* gallbladder epithelial cells. Since in the native tissue both apical and basolateral cell membranes are K^+ conductive (Reuss and Finn, 1975a, b; Van Os and Slegers, 1975; Reuss, 1979), the activation of a parallel anion conductance would be expected to depolarize the cell membranes and thereby accelerate K^+ and Cl^- efflux. The net effect is therefore cell shrinkage due to loss of KCl and water since the osmotic water permeability of both cell membranes is high (Cotton et al., 1989). Activation of apical membrane Cl^- conductance by the elevation of intracellular cAMP has been recently demonstrated to cause cell shrinkage in this tissue (Cotton and Reuss, 1991). Soltoff et al. (1990) reported that both extracellular ATP and carbachol stimulated K^+ and Cl^- efflux and caused cell shrinkage in parotid acinar cells.

There are several mechanisms by which an apical membrane Cl^- conductance might be activated by ATP. Direct channel activation (e.g., an ATP-gated Cl^- channel) is unlikely since the increase in Cl^- conductance is slow (~10–15-s delay) and the decrease in Cl^- conductance after ATP removal is also slow (2–3 min). Ca^{2+} -activated Cl^- conductance has been proposed in several secretory epithelia including pancreatic (Petersen, 1988) and salivary (Soltoff et al., 1990) acinar cells. It is likely, but unproven, that the effect of ATP on P_K is due to an increase in intracellular Ca^{2+} activity. Elevation of intracellular cAMP (with theophylline or forskolin) is known to induce an apical membrane Cl^- conductance in *Necturus* gallbladder cells (Petersen and Reuss, 1983). It is therefore possible that extracellular ATP causes an increase in intracellular cAMP sufficient to activate apical Cl^- channels. Additional experiments will be required to determine the mode of activation and the properties of the channels that underlie the ATP-induced increase in apical membrane Cl^- conductance.

ATP Removal

The depolarization of V_{mc} and V_{cs} upon ATP removal suggests a rapid decrease in G_K or an increase in G_X . Since R_z was increased by removal of the ATP it is likely that G_K is reduced. The fall in fR_z immediately after removal of the ATP suggests that the dominant change in G_K occurs at the basolateral cell membrane. The depolarization of V_{mc} would normally be expected to activate the voltage-dependent apical K^+ conductance. However, this conductance is Ca^{2+} dependent and may be maximally activated before the depolarization (see below). The results of the experiment in which mucosal solution K^+ was elevated suggest that the increase in apical membrane G_K induced by ATP is rapidly lost, since the magnitude of the depolarization caused by elevating K^+ returns to a control value within 1 min after ATP removal. In contrast, the apical membrane G_{Cl} persisted for at least 2 min after removal of ATP.

Hyperpolarization after ATP Removal

Within 30–60 s after removal of ATP, V_{mc} and V_{cs} began a slow hyperpolarization. The peak hyperpolarization ($V_{cs} -88 \pm 5$ mV) was usually attained within 3 min; thereafter, the membrane voltages returned toward control values over the next 5–10 min. The magnitude and duration of the hyperpolarization appear to depend on the ATP concentration and the duration of the period of exposure. Exposure to 100 or 1,000 μ M ATP for 2–3 min frequently yielded a hyperpolarization to a voltage greater than the initial hyperpolarization with ATP, in some cases exceeding the expected value of E_K . Since serosal ouabain (1 mM) completely abolished the late hyperpolarization, with almost no effect on the other phases of the response to ATP, it is likely that the Na^+ pump is responsible either directly or indirectly for the hyperpolarization. In a number of epithelial (Reuss et al., 1984) and nonepithelial cells (De Weer et al., 1988) the Na^+ pump is electrogenic and therefore stimulation of pump activity would be expected to result in a hyperpolarization of V_{mc} . Stoddard and Reuss (1989b) suggested that a portion of the hyperpolarization of V_{mc} and V_{cs} observed when *Necturus* gallbladder was exposed to Cl^- -free Ringer on the mucosal side was due to pump activation.

Alternatively, an increase in pump activity might be expected to deplete $[K^+]$ adjacent to the basolateral membrane, thus increasing E_K and causing V_{cs} (and V_{mc}) to hyperpolarize. Either of these mechanisms would be inhibited by ouabain. In three tissues we measured intracellular Na^+ activity during a 3-min exposure to 100 or 1,000 μ M ATP. In three tissues we observed a slight increase in aNa_i^+ , which started after ~ 1 min of exposure to ATP and reached a plateau ~ 4 min later (aNa_i^+ increased from 10 ± 2 to 13 ± 1 mM; $n = 3$). It is possible that this small increase in intracellular Na^+ is sufficient to stimulate the Na^+ pump and hyperpolarize the cell. However, this observation does not allow us to conclude that the hyperpolarization is due to stimulation of an electrogenic pump.

Conclusions

The electrophysiological effects of exposure of *Necturus* gallbladder to mucosal solution ATP are complex. The active form, ATP^{4-} , probably interacts with an apical membrane purinergic receptor (P_2) to increase apical membrane G_K and G_{Cl} and

basolateral membrane G_K . Although we have not measured intracellular Ca^{2+} activity in these cells, it is likely that extracellular ATP causes an increase in aCa_i^{2+} , as has been demonstrated in other cell types (Dubyak and DeYoung, 1985; Paulmichl and Lang, 1988; Soltoff et al., 1990). The ATP-induced changes in membrane ionic permeability underlie net K^+ secretion, a fall in aCl_i^- , and cell shrinkage. In contrast to observations in other systems (Cockcroft and Gomperts, 1979; Heppel et al., 1985), ATP does not produce nonselective permeabilization of *Necturus* gallbladder epithelial cells. This conclusion is based on experiments in which preparations were exposed to a high-TMA⁺ mucosal solution plus 1 mM ATP. There was no measurable TMA⁺ influx, in contrast with the effects of nystatin, which under similar circumstances can be used to load cells with TMA⁺ (Reuss, 1985; Cotton et al., 1989). We do not know to what extent ATP may alter the electroneutral transport mechanisms (Na^+/H^+ and Cl^-/HCO_3^- exchange and KCl cotransport) that are important for isosmotic NaCl and water absorption by the gallbladder (Reuss, 1989a). Further, we do not know if extracellular ATP plays any role in regulation of transport *in vivo*; nevertheless, it may be a useful tool to study the mechanisms of salt and water transport in leaky epithelia.

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