Electrophysiological Effects of Extracellular ATP on Necturus Gallbladder Epithelium

CALVIN U. COTTON and LUIS REUSS

From the Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77550

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 µM) caused a rapid hyperpolarization of both apical (Vmc) and basolateral (Vcs) cell membrane voltages (ΔVmc = 18 ± 1 mV), a fall in transepithelial resistance (Rt) from 142 ± 8 to 122 ± 7 Ω·cm², and a decrease in fractional apical membrane resistance (fRα) from 0.93 ± 0.02 to 0.83 ± 0.03. The rapid initial hyperpolarization of Vmc and Vcs was followed by a slower depolarization of cell membrane voltages and a lumen-negative change in transepithelial voltage (Vm). This phase also included an additional decrease in fRα. Removal of the ATP caused a further depolarization of membrane voltages followed by a hyperpolarization and then a return to control values. fRα 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 [K⁺] induced a larger depolarization of Vmc and Vcs during exposure to ATP than under control conditions. Reduction of mucosal solution [Cl⁻] induced a slow hyperpolarization of Vmc and Vcs before exposure to ATP and a rapid depolarization during exposure to ATP. We conclude that ATP 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.

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),...
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 P$_1$ and P$_2$ 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 Ca$^{2+}$-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 Ca$^{2+}$. 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 permeability 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 NaHCO$_3$, 2.5 KCl, 1.8 CaCl$_2$, 1.0 MgCl$_2$, and 0.5 NaH$_2$PO$_4$ and was equilibrated with 1% CO$_2$/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 CaCl$_2$ and MgCl$_2$ with NaCl or Na cyclamate plus sucrose to maintain osmolality. In high-Mg$^{2+}$ bathing solutions 10 mM MgCl$_2$ 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 MΩ 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 sodium 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 ($V_{b}$) 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×.

Transepithelial constant current pulses, $I_c$ ($50–100$ μA/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, 1986b), 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, 1986a, 1986b) was done to evaluate the changes in $R_t$ ($=R_a/(R_a + R_b)$) 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_m) 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_p) 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, 1988b; 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_p 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_m 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_m, V_m, V_cs, and V_m) 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 (Assyst; Macmillan Software Co., New York, NY). Means ± 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_m 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_m and V_c, followed by a slower depolarization. Finally, V_m and V_c slowly hyperpolarized to a value beyond the control membrane voltages measured before exposure to ATP. In some tissues the hyperpolarization exceeded
Effects of Extracellular ATP on Necturus Gallbladder Epithelium

**Figure 1.** Effects of ATP addition to the mucosal solution (100 μM) on voltages and resistances. \( V_{mc}, V_{ms}, \) and \( V_a \) are transepithelial, apical membrane, and basolateral membrane voltages, respectively. \( V_{ms} \) is referenced to the serosal solution and \( V_{mc} \) and \( V_a \) are referenced to the adjacent bathing solutions. The voltage spikes result from transepithelial current pulses (~100 μA/cm²) and are used to calculate transepithelial resistance \( (R_t = \Delta V/\Delta I) \) and fractional apical membrane resistance \( \left( fR_a = \frac{\Delta V_{mc}/\Delta V_{ms}}{R_a/(R_a + R_b)} \right) \). 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**

|        | \( V_{mc} \) | \( V_{ms} \) | \( V_a \) | \( fR_a \) | \( R_t \) |
|--------|--------------|--------------|-----------|-------------|----------|
| Pre-ATP| ± 0.4        | -69 ± 1      | -69 ± 1   | 0.93 ± 0.02 | 142 ± 8  |
| ATP    | 0.5 ± 0.5    | -88 ± 2*     | -87 ± 2*  | 0.83 ± 0.03*| 122 ± 7* |
|        | -5.4 ± 1.1*  | -68 ± 2*     | -73 ± 2*  | 0.55 ± 0.05*| 115 ± 9  |
| Post-ATP| -2.9 ± 0.8*  | -61 ± 2      | -64 ± 2*  | 0.33 ± 0.08*| 139 ± 6* |
|        | -1.0 ± 0.5*  | -88 ± 5*     | -89 ± 5*  | 0.84 ± 0.04*| 148 ± 7* |

Values are means ± SEM (n = 8). \( V_{ms} \), \( V_{mc} \), and \( V_a \) 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 μM). 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 $f_{Ra}$. During the initial hyperpolarization of $V_{mc}$ and $V_{cs}$, $f_{Ra}$ decreased slightly and continued to fall during the repolarization phase. Upon removal of the ATP, $f_{Ra}$ 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, $f_{Ra}$ 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 $f_{Ra}$.

The dose–response relationships for the initial hyperpolarizations elicited by ATP, ADP, and adenosine are illustrated in Fig. 3. The concentrations are expressed as calculated free [ATP$^{4-}$] and free [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 $V_{o}$ 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
Effects of Extracellular ATP on Necturus Gallbladder Epithelium

The magnitude of the initial hyperpolarization of apical membrane voltage \( (\Delta V_{ma}) \) was determined for each free concentration. The free [ATP\(^{4-}\)] and [ADP\(^{3-}\)] were calculated according to Fabiato (1988). Total [ATP]'s were 0.1, 1, 10, 100, and 1,000 \( \mu \text{M} \). Total [ADP]'s were 1, 10, 35, 100, 333, and 1,000 \( \mu \text{M} \). Each point is the mean \( \pm \) SEM, \( n = 8 \) for [ATP] (except 0.1 \( \mu \text{M} \) ATP, \( n = 4 \)), \( n = 4-5 \) for each [ADP], and \( n = 3 \) for 1,000 \( \mu \text{M} \) adenosine. Curves are least-square fits of the Michaelis-Menten equation to the data. ATP: \( \Delta V_{ma} = 18.3 \text{ mV}, K_m = 2.7 \times 10^{-2} \text{ M} \); ADP: \( \Delta V_{ma} = 19.7 \text{ mV}, K_m = 18 \text{ M} \).

Cable Analysis

The dominant electrodiffusive permeabilities in Necturus gallbladder epithelium bathed in \( \text{HCO}_3^-/\text{CO}_2 \)-buffered media are an apical \( G_K \) and basolateral \( G_K \) and \( G_{Cl} \) (Stoddard and Reuss, 1988a, 1989a). Therefore, the initial fall in \( fR_a \) 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 to 10 mM \( \text{Mg}^{2+} \) Ringer solution without ATP induced only small (1–2 mV) changes in membrane voltages, expected from intraepithelial current flow arising from small transepithelial bionic 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 \( \text{HCO}_3^-/\text{CO}_2 \)-buffered media are an apical \( G_K \) and basolateral \( G_K \) and \( G_{Cl} \) (Stoddard and Reuss, 1988a, 1989a). Therefore, the initial fall in \( fR_a \) 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 to 10 mM \( \text{Mg}^{2+} \) Ringer solution without ATP induced only small (1–2 mV) changes in membrane voltages, expected from intraepithelial current flow arising from small transepithelial bionic 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 \( \text{HCO}_3^-/\text{CO}_2 \)-buffered media are an apical \( G_K \) and basolateral \( G_K \) and \( G_{Cl} \) (Stoddard and Reuss, 1988a, 1989a). Therefore, the initial fall in \( fR_a \) 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 to 10 mM \( \text{Mg}^{2+} \) Ringer solution without ATP induced only small (1–2 mV) changes in membrane voltages, expected from intraepithelial current flow arising from small transepithelial bionic 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.
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, 1988b, 1989b). Fig. 5 illustrates the procedure (see legend for details). The first pulse ($\Delta V_3$) is the result of current injection into a distant impalement site. The second pulse ($\Delta V_x$) is the result of current injection via the recording electrode and provides an estimate of the input resistance of the epithelium. A decrease in $\Delta V_x$ 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, $\Delta V_x$ is a sensitive measure of changes in cell–cell coupling. The effects of 10 and 100 $\mu$M 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 $\mu$M ATP there was a rapid, transient fall in $\Delta V_x$ followed by an increase during exposure. Upon removal of the ATP, $\Delta V_x$ increased reversibly.

Throughout the exposure there were only small changes in $\Delta V_i$. The large fall in $\Delta V_x$ in the absence of changes in $\Delta V_i$ suggests that $R_x$ ($= R_a R_b/[R_a + R_b]$) is initially decreased by ATP. This result, in conjunction with the small fall in $R_a$, 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 $\mu$M ATP (mucosal side). All ion substitutions were isomolar. Na’ concentration was decreased from 100 to 10 mM (replaced with TMA’), [K+] was increased from 2.5 to 25 mM (K’ replaced Na’), or [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 [Na\(^+\)] is not significantly altered by exposure to 10 \(\mu\)M ATP (Fig. 7 A). The lumen-positive change in \(V_{m}\) results from the bionic 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, 1975a, b). Similarly, the voltage changes that occur during Na\(^+\) replacement in the presence of 100 \(\mu\)M ATP can be entirely explained by the paracellular bionic potential. The large depolarization of \(V_{cs}\) is explained by the fall of \(R_{s}\) in 100 \(\mu\)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 \(\mu\)M ATP.

Elevation of mucosal solution [K\(^+\)] caused a depolarization of \(V_{mc}\) and \(V_{cs}\) and a 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, 1975a, b; Van Os and Slegers, 1975). Elevation of [K\(^+\)] during exposure to 10 or 100 \(\mu\)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 [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

![Figure 6. Effects of ATP on intraepithelial current spread (\(\Delta V_{x}\)) and input resistance (\(\Delta V_{i}\)). All values were normalized to \(\Delta V_{x}\) and \(\Delta V_{i}\) recorded immediately before the addition of ATP. \(\Delta V_{x}(\text{exp})/\Delta V_{x}(\text{cont})\) is expressed as a ratio, whereas \(\Delta V_{i}\) is the difference, \(\Delta V_{i}(\text{exp}) - \Delta V_{i}(\text{cont})\). The values are mean ± SEM for three tissues. (A) 10 \(\mu\)M ATP. (B) 100 \(\mu\)M ATP. The two values of \(\Delta V_{x}\) immediately after ATP were significantly less than control at both 10 and 100 \(\mu\)M.](image-url)
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 ($E_{c1}^{cl}$) caused by the reduction in intracellular Cl$^-$ activity during luminal Cl$^-$ replacement (Stoddard and Reuss, 1989b). During exposure to 10 μM ATP, transient reduction of mucosal solution [Cl$^-$] induced large and rapid hyperpolarizations of $V_{mc}$ and $V_{cs}$ (Fig. 9 A). 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 [Cl$^-$] and [HCO$^-$] 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 [Na$^+$] from 100 to 10 mM before, during, and after exposure to mucosal ATP. Abbreviations as in Fig. 1. Mucosal solution [Na$^+$] was reduced (isomolar replacement of NaCl with TMACl) for ~40 s. Exposures to ATP lasted 3 min. (A) 10 μM ATP. (B) 100 μM ATP.

---

driving force on the apical membrane Cl$^-$/HCO$^-$ exchanger remains constant and intracellular Cl$^-$ and pH do not change (Reuss, 1987). If [Cl$^-$] and [HCO$^-$] are simultaneously reduced 10-fold in the presence of 10 μM ATP, the hyperpolarization is still observed (data not shown). Further, the rate of fall of intracellular Cl$^-$ ($aCl^-$) upon reduction of mucosal solution [Cl$^-$] is the same with or without 10 μM ATP. Finally, there is no change in $aCl^-$ when mucosal solution [Cl$^-$] and [HCO$^-$] are simultaneously reduced 10-fold in the presence of 10 μM ATP. Therefore, the large, rapid hyperpolarization of $V_{mc}$ and $V_{cs}$ induced by 9.8 mM Cl$^-$ in the presence of 10 μM ATP is not due to a change in $aCl^-$.
Effects of Extracellular ATP on Necturus Gallbladder Epithelium

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 μM ATP. (B) 100 μM 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 Mg²⁺ and/or Ca²⁺ would be expected to shift the equilibrium between bound and free ATP⁴⁻, raising free ATP⁴⁻ levels. Since 10 μM 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⁺]

|       | ΔVᵐᶜ | ΔVᵐᵃ | ΔVᵐᵇ |
|-------|------|------|------|
| 10 μM ATP |
| Before | -2.1 ± 0.1 | 8.8 ± 1.3 | 6.7 ± 1.3 |
| During | -7.5 ± 1.7* | 25.3 ± 4.4* | 17.8 ± 3.9* |
| After  | -1.9 ± 0.1 | 8.3 ± 1.2 | 6.3 ± 1.2 |
| 100 μM ATP |
| Before | -1.9 ± 0.1 | 8.0 ± 1.3 | 6.0 ± 1.3 |
| During | -7.4 ± 1.7* | 31.8 ± 2.3* | 24.4 ± 2.2* |
| After  | -1.9 ± 0.1 | 5.8 ± 1.9 | 4.2 ± 1.7 |

Values are means ± SEM; 10 μM ATP, n = 6; 100 μM 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. ΔVᵐᶜ, ΔVᵐᵃ, and ΔVᵐᵇ 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 \(\mu\)M) resulted in a small depolarization of \(V_{\text{mc}}\) and \(V_{\text{cs}}\), followed by a slower hyperpolarization (Fig. 9 B). Upon return to 98 mM Cl\(^-\) solution a small hyperpolarization of \(V_{\text{mc}}\) and \(V_{\text{cs}}\) was observed. Second, a similar response is observed if the 9.8 mM Cl\(^-\) and the 10 \(\mu\)M 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 \(\mu\)M ATP/divalent cation free or 100 \(\mu\)M ATP) resulted in a small depolarization of \(V_{\text{mc}}\) and \(V_{\text{cs}}\). The return to 98 mM Cl\(^-\) induced a small hyperpolarization. These results suggest that ATP\(^+\) concentrations in excess of \(\sim 0.2 \mu\)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 [Cl\(^-\)] from 98 to 9.8 mM before, during, and after exposure to mucosal solution ATP. Abbreviations as in Fig. 1. Mucosal solution [Cl\(^-\)] was reduced (isomolar replacement of NaCl with Na cyclamate) for \(\sim 40\) s. Exposure to ATP lasted 3 min. (A) 10 \(\mu\)M ATP. (B) 100 \(\mu\)M ATP.

**ATP Induces an Increase in Mucosal Solution [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 [K\(^+\)] after 3 min, by \(0.4 \pm 0.1\) mM. K\(^+\) secretion was not detected with 100 \(\mu\)M ATP. This is probably due to the relatively poor sensitivity of this technique; i.e., luminal solution volume is \(\sim 20\) times larger than the intracellular volume.
Intracellular Cl\(^-\) activity was measured in three tissues before, during, and after exposure to 10 or 100 \(\mu\)M mucosal ATP. During a 2-min exposure to 10 \(\mu\)M ATP, aCl\(^-\) was not significantly changed (20.2 \(\pm\) 1.4 vs. 19.1 \(\pm\) 1.7 mM). However, after 1-min exposure to 100 \(\mu\)M ATP, aCl\(^-\) fell from 16.6 \(\pm\) 0.7 to 10.9 \(\pm\) 1.9 mM. An example of the decline in aCl\(^-\) 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 \(\mu\)M).

![Graph](image)

**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 \(\sim\) 40 s from 98 to 9.8 mM simultaneously with omission of divalent cations (0 Mg\(^{2+}\), 0 Ca\(^{2+}\), 9.8 Cl\(^-\) Ringer). Subsequently, the tissue was exposed to 10 \(\mu\)M ATP in divalent cation-free Ringer (0 Mg\(^{2+}\), 0 Ca\(^{2+}\), 10 \(\mu\)M ATP Ringer) for \(\sim\) 3 min. During the exposure to ATP, mucosal solution [Cl\(^-\)] was again reduced to 9.8 mM (divalent cation free) for \(\sim\) 40 s. (B) The tissue was exposed for \(\sim\) 40 s to mucosal 0 Mg\(^{2+}\), 0 Ca\(^{2+}\) Ringer. Subsequently, the tissue was exposed to 10 \(\mu\)M ATP in divalent cation-free Ringer for \(\sim\) 3 min. During the ATP exposure, mucosal solution [Cl\(^-\)] was again reduced to 9.8 mM (divalent cation-free) for \(\sim\) 40 s. After removal of the ATP, the tissue was again exposed to 9.8 mM Cl\(^-\) (divalent cation-free) for \(\sim\) 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 [HCO\(_3\)] were simultaneously reduced 10-fold before, during, and after exposure to ATP. In the absence of ATP, aCl\(^-\) did not change; however, ATP induced a fall in aCl\(^-\) that continued during exposure to reduced [Cl\(^-\)] and [HCO\(_3\)]. 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\(^+\) is \(\sim\) 100 mM), small changes in its chemical activity are
**TABLE II**

*Effects of Addition of ATP to the Mucosal Bathing Solution on the Response to Reduction of Mucosal Solution [Cl−]*

| ATP Concentration | Before | During | After |
|-------------------|--------|--------|-------|
| 10 μM ATP/Mg²⁺- and Ca²⁺-free | -1.8 ± 0.5 | 0.9 ± 0.5 | -0.9 ± 0.5 |
|                  | -0.8 ± 0.5 | 3.5 ± 0.9* | 2.8 ± 0.9* |
| 100 μM ATP       | -1.6 ± 0.5 | 0.6 ± 0.4 | -1.0 ± 0.4 |
|                  | -1.2 ± 0.6 | 3.3 ± 0.7* | 1.8 ± 1.0* |
|                  | -1.7 ± 0.5 | 4.0 ± 0.6* | 2.2 ± 1.0* |

Values are means ± SEM; 10 μM ATP, n = 4; 100 μM ATP, n = 6. The 10 μM ATP solution and also the 9.8 CI 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 Vᵢ and Vᵢ (since Vᵢ is recorded as the difference Vᵢ − Vᵢ) 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](image-url)

**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 ~100 μm above the tissue (see Methods). Both barrels were referenced to the serosal compartment. Vᵢ − Vᵢ 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 ~600 μm; volume ~30 μl) covered the tissue.

(A) The apical surface was rapidly superfused (10–15 s) with ~200 μl 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 ~200 μl 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 μM ATP caused a significant cell shrinkage of 18 ± 2%.

**Effects of Ouabain on the Responses to ATP**

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

![Figure 12](https://example.com/figure12.png)

**FIGURE 12.** Effects of ATP on intracellular Cl⁻ activity (aCl⁻). aCl⁻ was measured with a double-barrel microelectrode (see Methods). \( V_{cl} - V_{cn} \) 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 μM ATP. Note the falls in aCl⁻ 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 [HCO₃⁻] was reduced from 10 to 1 mM (both anions replaced with cyclamate). Note that aCl⁻ did not change appreciably during the period of exposure to 9.8 Cl⁻, 1 HCO₃⁻. However, 100 μM ATP caused a large rapid fall in aCl⁻ that continued during the period of exposure to 9.8 Cl⁻, 1 HCO₃⁻.

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 \( fR_f \) during exposure to ATP were similar with and without ouabain. In contrast, the hyperpolarization and the increase in \( fR_f \) 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 HCO₃⁻ 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 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), HCO₃⁻ (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 Ca²⁺ levels by exposure to the Ca²⁺ ionophore A23187

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

where \( V(0) \) = initial cell water volume, \( V(t) \) = cell water volume at time \( t \), \( a\text{TMA}^+(0) \) = initial intracellular TMA⁺ activity, \( a\text{TMA}^+(t) \) = intracellular TMA⁺ activity at time \( t \).
or to cyanide. These agents elicit monotonic hyperpolarization of the cell membranes and $fR_c$ 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 [Ca$^{2+}$] to < 1 μM did not alter the response to 100 μM 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 $R_c$ and $fR_c$. 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; 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.

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 $V_m$ and $V_c$. At maximal doses of ATP (> 100 µM) the membrane voltages hyperpolarize to $-88 \pm 2$ 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 $R_z$ 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 $R_a$ 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 ($< 1$ mV) lumen-positive change in $V_m$ or no change.

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

|            | $V_m$ (mV) | $V_c$ (mV) | $R_a$ (Ω cm$^{-2}$) | $R_z$ (Ω cm$^{-2}$) |
|------------|------------|------------|---------------------|---------------------|
| Control    |            |            |                     |                     |
| Pre-ATP    | 0.0 ± 0.4  | -69 ± 3    | 0.89 ± 0.01         | 142 ± 16            |
| ATP hyperpolarization | -5.5 ± 0.5 | -89 ± 5    | 0.77 ± 0.04         | 129 ± 13            |
| Post-ATP   | -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 ± 4*   | 0.75 ± 0.04         | 132 ± 15            |
| Post-ATP   | -0.5 ± 0.3 | -59 ± 4*   | 0.63 ± 0.03*        | 164 ± 20            |

Values are means ± 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 µM). Measurements were made ~1 min before addition of ATP (pre-ATP), at the peak hyperpolarization after addition of ATP (ATP hyperpolarization), and ~1 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 $E_k$ precedes the change in $E_a$ (where $E_a$ and $E_k$ are the zero-current voltages of apical and basolateral membranes, respectively).

Repolarization of $V_m$ and $V_c$

The slow, partial repolarization of membrane voltages after the peak hyperpolarization could result from a fall in $E_k$, a decrease in $G_{cl}$, 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 $[K^+]_o$ does increase in the unstirred fluid layer (Cotton and Reuss, 1989) adjacent to the epithelium. Elevation of basolateral solution $[K^+]_o$ is less likely since the basolateral
Na⁺,K⁺-ATPase would tend to reduce accumulation. $E_K$ could also decrease if $aK^+$ fell. If the depolarization of $V_{mc}$ and $V_{cl}$ resulted only from a fall in $aK^+$, the observed depolarization would require a large fall in $aK^+$ (i.e., ~50% decrease in $aK^+$ to account for an 18-mV depolarization). The results of preliminary measurements of $aK^+$ (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 $V_{cl}$ 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 $V_{cl}$ 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 $aCl^-$ 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 $aK^+$ and $aCl^-$ 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). Ca²⁺-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 Ca²⁺ 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 $V_c$ 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 $R_a$ 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 $V_c$ began a slow hyperpolarization. The peak hyperpolarization ($V_c - 88 \pm 5$ 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 $\mu$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 $V_c$, 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 $V_c$ (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 $\mu$M ATP. In three tissues we observed a slight increase in aNa$^+$, which started after ~1 min of exposure to ATP and reached a plateau ~4 min later (aNa$^+$ 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$^-$, probably interacts with an apical membrane purinergic receptor ($P_o$) to increase apical membrane $G_K$ and $G_{cl}$ and
basolateral membrane \( G_K \). Although we have not measured intracellular \( \text{Ca}^{2+} \) activity in these cells, it is likely that extracellular ATP causes an increase in \( \text{aCa}_{\text{i}}^{2+} \), 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 \( \text{K}^+ \) secretion, a fall in \( \text{aCl}^- \), 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 \( \text{Necturus} \) 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 (\( \text{Na}^+/\text{H}^+ \) and \( \text{Cl}^-/\text{HCO}_3^- \) 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.

We thank Guillermo A. Altenberg and Steven A. Weinman for comments on a preliminary version of the manuscript, Jennifer Chilton for software development, Bernice Perry for technical assistance, and Lynette Morgan for secretarial help.

This work was supported by National Institutes of Health grant DK-38588.

Original version received 30 July 1990 and accepted version received 19 November 1990.

REFERENCES

Altenberg, G., J. Copello, C. Cotton, K. Dawson, Y. Segal, F. Wehner, and L. Reuss. 1990. Electrophysiological methods for studying ion and water transport in \( \text{Necturus} \) gallbladder epithelium. Methods in Enzymology. 192:650–683.

Bello-Reuss, E., T. P. Grady, and L. Reuss. 1981. Mechanism of the effect of cyanide on cell membrane potentials in \( \text{Necturus} \) of gallbladder epithelium. Journal of Physiology. 314:343–357.

Benham, C. D., and R. W. Tsien. 1987. A novel receptor-operated \( \text{Ca}^{2+} \)-permeable channel activated by ATP in smooth muscle. Nature. 328:275–278.

Burnstock, G. 1978. A basis for distinguishing two types of purinergic receptors. In Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. R. W. Straub and L. Bolis, editors. Raven Press, New York. 107–110.

Chapal, J., and M. M. Loubatières-Mariani. 1983. Evidence for purinergic receptors in vascular smooth muscle in rat pancreas. European Journal of Pharmacology. 87:423–430.

Cockcroft, S., and B. D. Gomperts. 1979. Activation and inhibition of calcium-dependent histamine secretion by ATP ions applied to rat mast cells. Journal of Physiology. 296:229–243.

Cotton, C. U., and L. Reuss. 1989. Measurement of the effective thickness of the mucosal unstirred layer in \( \text{Necturus} \) gallbladder epithelium. Journal of General Physiology. 93:651–647.

Cotton, C. U., and L. Reuss. 1991. Effects of changes in mucosal solution Cl^- or K^- concentration on cell water volume of \( \text{Necturus} \) gallbladder epithelium. Journal of General Physiology. 97:667–686.

Cotton, C. U., A. M. Weinstein, and L. Reuss. 1989. Osmotic water permeability of \( \text{Necturus} \) gallbladder. Journal of General Physiology. 93:649–679.
DeMey, J. G., and P. M. Vanhoutte. 1981. Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *Journal of Physiology.* 316:437–455.

Démêze, J., and J. C. Hervé. 1983. Effect of several uncouplers of cell-to-cell communication on gap junction morphology in mammalian heart. *Journal of Membrane Biology.* 74:203–215.

De Weer, P., D. C. Gadsby, and R. F. Rakowski. 1988. Voltage dependence of the Na-K pump. *Annual Review of Physiology.* 50:225–242.

Dubya, G. R., and M. B. DeYoung. 1985. Intracellular Ca2+ mobilization activated by extracellular ATP in Ehrlich ascites tumor cells. *Journal of Biological Chemistry.* 260:10653–10661.

Eisenberg, R. S., and E. A. Johnson. 1970. Three-dimensional electrical field problems in physiology. *Progress in Biophysics and Molecular Biology.* 20:1–65.

Fabio, A. 1988. Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods in Enzymology.* 157:378–417.

Friedrich, F., H. Weiss, M. Paulmichl, and F. Lang. 1989. Activation of potassium channels in renal epithelioid cells (MDCK). *American Journal of Physiology.* 256:C1016–C1021.

Gallacher, D. V. 1982. Are there purinergic receptors on parotid acinar cells? *Nature.* 296:83–86.

Gordon, J. L. 1986. Extracellular ATP: effects, sources and fate. *Biochemical Journal.* 233:309–319.

Heppel, L. A., G. A. Weisman, and I. Friedberg. 1985. Permeabilization of transformed cells in culture by external ATP. *Journal of Membrane Biology.* 86:189–196.

Jungwirth, A., F. Lang, and M. Paulmichl. 1989. Effect of extracellular adenosine triphosphate on electrical properties of subconfluent Madin-Darby canine kidney cells. *Journal of Physiology.* 408:333–344.

Lang, F., B. Plockinger, D. Haussinger, and M. Paulmichl. 1988. Effects of extracellular nucleotides on electrical properties of subconfluent Madin Darby canine kidney cells. *Biochimica et Biophysica Acta.* 943:471–476.

Paulmichl, M., and F. Lang. 1988. Enhancement of intracellular calcium concentration by extracellular ATP and UTP in Madin Darby canine kidney cells. *Biochemical and Biophysical Research Communications.* 156:1139–1143.

Petersen, K.-U., and L. Reuss. 1983. Cyclic AMP-induced chloride permeability in the apical membrane of Necturus gallbladder epithelium. *Journal of General Physiology.* 81:705–729.

Petersen, K.-U., and L. Reuss. 1985. Electrophysiologic effects of propionate and bicarbonate on gallbladder epithelium. *American Journal of Physiology.* 248:C58–C69.

Petersen, O. H. 1988. The control of ion channels and pumps in exocrine acinar cells. *Comparative Biochemistry and Physiology.* 90A:717–720.

Reuss, L. 1979. Electrical properties of the cellular transepithelial pathway in Necturus gallbladder. III. Ionic permeability of the basolateral cell membrane. *Journal of Membrane Biology.* 47:239–250.

Reuss, L. 1985. Changes in cell volume measured with an electrophysiologic technique. *Proceedings of the National Academy of Sciences, USA.* 82:6014–6018.

Reuss, L. 1987. Cyclic AMP inhibits Cl–/HCO3– exchange at the apical membrane of Necturus gallbladder epithelium. *Journal of General Physiology.* 89:172–196.

Reuss, L. 1989a. Ion transport across gallbladder epithelium. *Physiological Reviews.* 69:503–545.

Reuss, L. 1989b. Regulation of transepithelial chloride transport by amphibian gallbladder epithelium. *Annals of the New York Academy of Sciences.* 574:370–384.

Reuss, L., and A. L. Finn. 1975a. Electrical properties of the cellular transepithelial pathway in Necturus gallbladder. I. Circuit analysis and steady-state effects of mucosal solution ionic substitutions. *Journal of Membrane Biology.* 25:115–139.
Reuss, L., and A. L. Finn. 1975b. Electrical properties of the cellular transepithelial pathway in Necturus gallbladder. II. Ionic permeability of the apical cell membrane. Journal of Membrane Biology, 25:141–161.

Reuss, L., S. A. Lewis, N. K. Wills, S. I. Helman, T. C. Cox, W. F. Boron, A. W. Siebens, W. B. Guggino, G. Giebisch, and S. G. Schultz. 1984. Ion transport processes in basolateral membranes of epithelia. Federation Proceedings. 43:2488–2502.

Reuss, L., and J. S. Stoddard. 1987. Role of $\text{H}^+$ and $\text{HCO}_3^-$ in salt transport in gallbladder epithelium. Annual Review of Physiology. 49:35–49.

Segal, Y., and L. Reuss. 1990. Maxi K+ channels and their relationship to apical membrane conductance in Necturus gallbladder epithelium. Journal of General Physiology. 95:791–818.

Soltoff, S. P., M. K. McMillian, E. J. Cragoe, Jr., L. C. Cantley, and B. R. Talamo. 1990. Effects of extracellular ATP on ion transport systems and $[\text{Ca}^{2+}]$, in rat parotid acinar cells. Comparison with the muscarinic agonist carbachol. Journal of General Physiology. 95:319–346.

Stoddard, J. S., and L. Reuss. 1988a. Dependence of cell membrane conductances on bathing solution $\text{HCO}_3^-/\text{CO}_2$ in Necturus gallbladder. Journal of Membrane Biology. 102:165–174.

Stoddard, J. S., and L. Reuss. 1988b. Voltage- and time-dependence of apical membrane conductance during current clamp in Necturus gallbladder epithelium. Journal of Membrane Biology. 103:191–204.

Stoddard, J. S., and L. Reuss. 1989a. $\text{pH}$ effects on basolateral membrane ion conductances in gallbladder epithelium. American Journal of Physiology. 256:C1184–C1195.

Stoddard, J. S., and L. Reuss. 1989b. Electrophysiologic effects of mucosal Cl⁻ removal in Necturus gallbladder epithelium. American Journal of Physiology. 257:C568–C578.

Van Os, C. H., and J. F. G. Siegers. 1975. The electrical potential profile of gallbladder epithelium. Journal of Membrane Biology. 24:341–363.