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Triaminopyrimidinium (TAP^+) Blocks Luminal Membrane K Conductance in *Necturus* Gallbladder Epithelium

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Summary. The effect of triaminopyrimidinium (TAP^+) on the apical membrane of *Necturus* gallbladder epithelial cells was investigated with intracellular microelectrode techniques. TAP^+, added to the mucosal bathing solution only, produced the following effects (all rapid and reversible): (i) cell depolarization, (ii) increase of apical membrane resistance, and (iii) decrease of the apical membrane potential change produced by K for Na substitution on the mucosal side. These results can be explained by a decrease of apical membrane K conductance. The paracellular effects of TAP^+ were similar to the ones previously described by Moreno (J.H. Moreno, 1974; *Nature (London)* **251**:150; J.H. Moreno, 1975, *J. Gen. Physiol.* **66**:97). These results indicate that the change of transepithelial potential produced by TAP^+ cannot be ascribed solely to its effect on the paracellular pathway.

Moreno (1974, 1975) showed that the protonated form of triaminopyrimidine (triaminopyrimidinium or TAP^+) blocks reversibly and specifically Na conductance (gNa) at the junctional complexes of gallbladder epithelia from several species and that it exerts similar effects on other leaky epithelia (Moreno, 1974). If TAP^+ acted only at this site, it would be a very valuable experimental tool for the study of the function of leaky epithelia. Krejs, Seelig and Fordtran (1977) studied the action of this substance on dog ileum in vivo and, in addition to the blockage of paracellular Na conductance, found reduction of the rate of absorption of Na, Cl, and sugars. Furthermore, it has been found that TAP^+ exerts an amiloride-like effect (i.e., blocks Na conductance) at the outer membrane of the frog skin (Zeiske, 1975; Balaban & Mandel, 1978) and the rabbit urinary bladder (Lewis & Diamond, 1976). The studies reported here were designed to examine the possibility of actions of TAP^+ at the luminal membrane of *Necturus* gallbladder epithelium, one of the leaky epithelia in which Moreno (1974) found an effect on paracellular gNa. For this purpose, intracellular microelectrode techniques were employed. The results indicate that TAP^+, in addition to its effect on

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the junctional complexes, blocks apical membrane K conductance. Whereas exposure of the luminal membrane to low pH (necessary for the action of TAP) results in stable and moderate changes of electrical properties, acidification of the serosal medium causes larger and more complex alterations, which are presently under investigation. Therefore, in this paper the observations have been restricted to the action of TAP from the apical side.

Materials and Methods

Necturi, obtained from Mogul-Ed Co., Oshkosh, Wisc., were kept at 4–8 °C and fed live fish. The gallbladders were removed from anesthetized animals, opened longitudinally, washed with Ringer solution and mounted on a modified Ussing chamber, mucosal side up. The control bathing medium (Ringer A) had the following composition (mM): NaCl, 109.2; KCl, 2.5; $CaCl_2$, 1.0; Na_2HPO_4, 2.0; NaH_2PO_4, 0.13. The pH was 8.0. Modifications included the following: (i) reduction of the pH to 6.0, by changing the concentration of phosphate salts, keeping Na concentration constant (Ringer B); (ii) isosmotic substitution at pH 6.0 of 20 mmol/liter of NaCl with sucrose (Ringer C) or TAP^+ (TAP base titrated to pH 6.0 with HCl); (iii) addition of 2 to 20 mmol/liter of TAP to Ringer B (pH 6.0); (iv) substitution of all NaCl with KCl in any of the above media; (v) reduction of NaCl concentration to 50% (in any of the Ringer solutions), with isosmotic replacement with sucrose. TAP was obtained from Sigma Chemical Co. (St. Louis, Mo.), Aldrich Chemical Co. (Milwaukee, Wisc.), ICN Pharmaceuticals (Plainview, N.Y.) or Eastman Kodak Co. (Rochester, N.Y.). No differences in the effect of TAP obtained from these sources were noted. At pH 6.0, 85% of TAP is in the protonated form TAP^+. In a few experiments TAP was added in the range of 2 to 20 mmol/liter, to determine the concentration-dependence of its effects. All other observations were done with 20 mM TAP (17 mM TAP^+). All experiments were performed at room temperature (24 ± 1 °C). The serosal side was always exposed to Ringer A, pH 8.0.

Electrical Measurements

Potentials and resistances were measured as previously described (Reuss & Finn, 1975*a*, *b*; Reuss, 1978). Glass microelectrodes with inner fiber were filled with 3 M KCl or 4 M K acetate, and sometimes beveled by the technique described by Ogden, Citron and Pierantoni (1978). Electrodes with tip resistances higher than 20 MΩ were employed. Extracellular potential measuring electrodes were Ringer-agar or 3 M KCl-agar bridges connected to calomel half cells or Ag-AgCl pellets. Extracellular current-passing electrodes were connected to the bathing media with Na-Ringer-agar bridges. All potential measurements during substitutions were corrected for the respective liquid junction potentials as described previously (Reuss, 1978).

Cell impalements were performed with motorized, remote control micromanipulators (Stoelting, Chicago, Ill.), under microscopic observation at 200–400 ×. Intracellular (apical membrane: V_{mc}, basolateral membrane: V_{cs}) and transepithelial (V_{ms}) potentials (*see* Fig. 1) were measured with high impedance ($> 10^{12}$ Ω) electrometers, displayed on a storage oscilloscope (Tektronix, Beaverton, Ore.) and on a three-channel pen recorder (Brush 2400, Gould Inc., St. Louis, Mo.). For cable analysis experiments, a signal averager (model

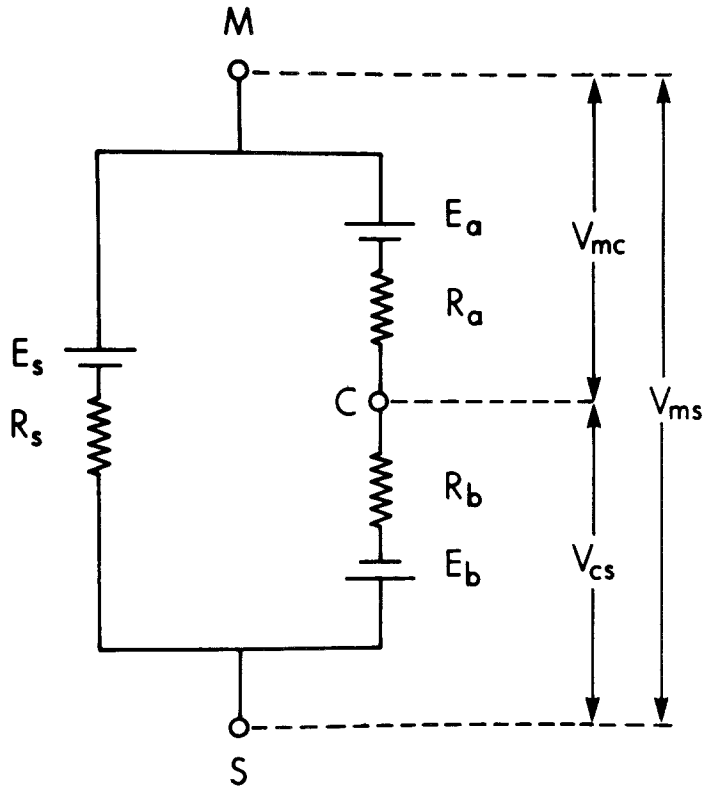

Fig. 1. Electrical equivalent circuit for *Necturus* gallbladder epithelium. Each element is represented by a Thevenin equivalent, i.e., an equivalent electromotive force (E) in series with an equivalent resistance (R). The subscripts a, b, and s refer to the apical (luminal) cell membrane, the basolateral (serosal) cell membrane, and the paracellular (shunt) pathway, respectively. On the right, the measured potentials (transepithelial, V_{ms}; apical, V_{mc}; and basolateral, V_{cs}) are defined

1070, Nicolet Instrument Corp., Madison, Wisc.) was employed. The reference was the serosal solution.

The transepithelial resistance (R_t) and the ratio of cell membrane resistances (R_a/R_b) were calculated from the deflections produced in V_{ms}, V_{mc}, and V_{cs} by calibrated transepithelial current pulses, usually 50 $\mu A \cdot cm^{-2}$, after appropriate corrections for the voltage drops in the solutions.

Experimental Procedure

Experiments were started at least 30 min after mounting the tissue, with Ringer A on both sides. Both bathing solutions were exchanged continuously. After an acceptable impalement was obtained, changes in the mucosal medium were performed by gravity superfusion while keeping the microelectrode in a cell. V_{ms}, V_{mc}, V_{cs}, R_t, and R_a/R_b were measured before, during, and after the substitution. Only fully reversible records were accepted.

Circuit Analysis

The direct data obtained in all experiments were the cell membrane and transepithelial potentials, the transepithelial resistance, and the ratio of cell membrane resistances. All these measurements were performed in two or more experimental conditions in each tissue. In every case, control values in the same cell were obtained before and after the experimental perturbation. In some conditions it was necessary to estimate absolute values of resistances. This was done, in general, as described before (Reuss & Finn, 1975*a*, *b*; Reuss, 1978). Flat sheet cable analysis (Frömter, 1972; Reuss & Finn, 1975*a*) was performed in four tissues bathed with Ringer A on both sides. During each impalement, the mucosal solution was changed to Ringer B (pH 6) and back to Ringer A. The substitution resulted in cell depolarization (*see Results*) and in a change of V_x (the voltage deflection elicited by intracellular current application, as a function of the interelectrode distance, x). The records were accepted only when upon returning to Ringer A the cell potential reversed to ± 1 mV of the control value, and V_x to $\pm 5\%$ of the control value. Similar experiments were performed during changes from Ringer B to Ringer B+TAP^+ in three preparations. In all these conditions, paired mean values of R_z [the resistance for current flow out of the cells, *see* Appendix, Eqs. (3) and (6)] were calculated. These mean values were employed for the computation of R_a, R_b, and R_s in all tissues in the same experimental condition. In addition to R_z, the mean values of R_t and R_a/R_b in a larger number of preparations were used for the calculations, according to Eqs. (6)–(8) (*Appendix*). Intracellular current pulses were 2 to 5 nA, 1–2 sec duration, always cell-negative (hyperpolarizing). Four to eight pulses were applied and averaged in each condition.

Equivalent electromotive forces of the cell membranes and the shunt pathway could not be calculated in these experiments because in the presence of asymmetric bathing media there is a diffusion potential across the paracellular pathway which makes $E_s \neq 0$. In this situation, the number of unknowns in the equivalent circuit is higher than the number of independent measurements (Reuss & Finn, 1975*a*, *b*).

Statistics

All results obtained directly are expressed as means $\pm$SE. Absolute resistances were calculated from means of the different experimental groups. Statistical comparisons were made by conventional paired data analysis.

Results

Effects of TAP^+ on Transepithelial and Cell Membrane Potentials

Lowering the pH of the mucosal solution from 8 to 6 resulted in reduction of cell potentials by about 10–20 mV and in a small change of V_{ms}. The change from Ringer A to Ringer B resulted in a V_{ms} increase from -0.7 ± 0.1 to -0.9 ± 0.1 mV, $P < 0.025$; the change from Ringer A to Ringer C resulted in a V_{ms} change from -0.5 ± 0.1 to 1.1 ± 0.2 mV, $P < 0.01$. The latter change is at least in part due to the larger NaCl

Table 1. Effects of TAP^+ on transepithelial and cell membrane potentials

	Condition	Transepithelial potential (V_{ms}, mV)	Apical membrane potential (V_{mc}, mV)	Basolateral membrane potential (V_{cs}, mV)
Ringer B (11)	Control	-0.9 ± 0.1	-52.7 ± 3.2	-53.6 ± 3.2
	TAP^+	-0.1 ± 0.3	-43.5 ± 3.5	-43.6 ± 3.5
	P	<0.001	<0.001	<0.001
Ringer C (8)	Control	1.1 ± 0.2	-57.3 ± 2.5	-56.2 ± 2.5
	TAP^+	0 ± 0.2	-51.1 ± 2.4	-51.2 ± 2.5
	P	<0.001	<0.001	<0.001

Polarity convention: V_{ms}: $V_m - V_s$; V_{mc}: $V_c - V_m$; V_{cs}: $V_c - V_s$. Number of experiments is indicated in parenthesis

concentration in the serosal medium (since across the shunt $P_{Na} > P_{Cl}$, this results in a mucosa-positive paracellular diffusion potential).

The effects of addition of TAP^+ to Ringer B or isosmotic substitution of TAP^+ for sucrose in Ringer C are summarized in Table 1. In the range of 1.7 to 17 mM, TAP^+ produced a decrease of V_{ms} (the mucosal medium became less negative) and a decrease of V_{mc} (the cell became less negative to the external solution). Within this range both changes were proportional to the logarithm of the TAP^+ concentration and significant at 3.4 mM TAP^+. At 17 mM TAP^+ the effects were not yet maximal. The upper half of Table 1 shows the effects of TAP^+ addition to Ringer B on transepithelial and cell membrane potentials. The reductions of all three potentials were highly significant. Addition of TAP to the control solution increases the osmolality of the mucosal bathing medium. This effect, by itself, can result in changes of V_{ms} (Wright *et al.* 1972; Reuss & Finn, 1977) and cell potentials (Reuss & Finn, 1977) of the direction observed. That an osmolality change is not the only mechanism by which TAP^+ affects the cell membrane potentials is shown in the lower half of the table. When TAP^+ was added at constant osmolality, the cells still depolarized significantly. The transepithelial potential changed in a direction opposite to the one observed in the previous experiment because of the initial salt concentration gradient across the epithelium.

Figure 2 shows a typical record of cell membrane and transepithelial potentials before, during, and after exposure to TAP^+. The effects are quite rapid and completely reversible. Full reversibility was also observed after prolonged exposure to TAP^+.

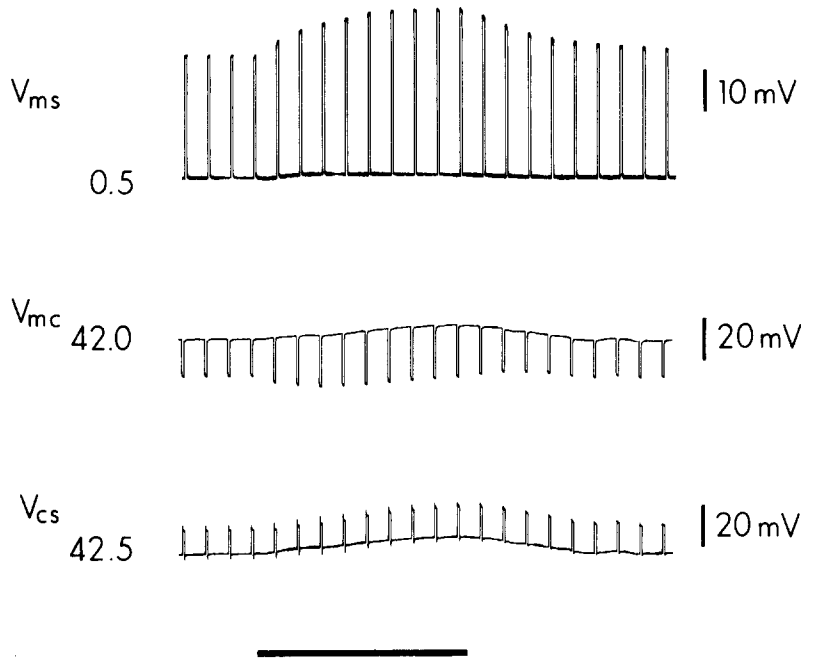

Fig. 2. Typical record of V_{ms}, V_{mc}, and V_{cs} before, during, and after a brief exposure to TAP^+ (17 mM) on the mucosal side. Records start with microelectrode in the cell. Baseline potential values are indicated at the start of each record (cell negative to both media, mucosal medium negative to the serosal medium). Brief deflections (ΔV_{ms}, ΔV_{mc}, ΔV_{cs}) are caused by transepithelial square current pulses of constant density at 10-sec intervals. During exposure to Ringer B+TAP (bar), V_{ms} changes in the mucosa-positive direction, and V_{mc} and V_{cs} decrease (cell becomes less negative to the respective bathing medium). The changes in ΔV_{ms}, ΔV_{mc}, and ΔV_{cs} indicate that both R_t and R_a/R_b increase. Effects on potentials and resistances are fully reversible

Effects of TAP^+ on Resistances

Exposure of the gallbladder to Ringer B (pH 6) on the mucosal side caused no significant change of R_a/R_b. R_t increased by about 13% ($P<0.01$). TAP^+ increased both R_t and R_a/R_b. In the range of 1.7 to 17 mM TAP^+, both effects were proportional to the logarithm of the TAP^+ concentration and did not saturate at the highest concentration. Table 2 shows the effects of 17 mM TAP^+ on resistances in the two incubation media. The increase of R_t was greater in Ringer B. The higher osmolality of the TAP^+-containing solution, in this condition, may explain the difference (Reuss & Finn, 1977). The changes of R_a/R_b were similar in the two series of experiments. The rapidity and reversibility of the effect of TAP^+ on R_t and R_a/R_b are illustrated in Fig. 2.

Table 2. Effects of TAP^+ on resistances

	Condition	Transepithelial resistance (R_t, Ωcm^2)	Ratio of cell membrane resistances (R_a/R_b)
Ringer B (11)	Control	185 ± 10	2.19 ± 0.18
	TAP^+	305 ± 20	3.26 ± 0.30
	P	<0.001	<0.005
Ringer C (8)	Control	200 ± 15	2.42 ± 0.23
	TAP^+	265 ± 15	3.47 ± 0.32
	P	<0.001	<0.001

Number of experiments is indicated in parenthesis.

The change of R_a/R_b during exposure to TAP^+ suggests an effect at the luminal membrane. To estimate absolute membrane and shunt resistances under control conditions and during exposure to TAP^+, cable analysis experiments were performed as described above. Exposure to Ringer B produced an increase of R_z from 1930 Ωcm^2 to 3040 Ωcm^2 ($n=4$). The addition of TAP^+ to Ringer B caused an increase of R_z from 2570 to 3510 Ωcm^2 ($n=3$ experiments). If these values and the means in the upper half of Table 2, are employed to calculate absolute resistances, the results are (in Ωcm^2 of tissue): R_a: 8200 (control), 14950 (TAP); R_b: 3740 (control), 4590 (TAP); R_s: 190 (control), 310 (TAP). This is, however, a rough estimate based on mean values obtained in different groups of tissues. Qualitatively similar results were obtained in tissues bathed in Ringer C: in every case (15 impalements in 6 tissues), V_x increased reversibly during exposure to TAP^+. Both in Ringer B and in Ringer C, the fractional increase of V_x produced by TAP^+ was directly proportional to the interelectrode distance. This indicates that TAP^+ produces an increase of the length constant for current spread within the epithelial sheet, as expected if R_z increases (*see Appendix*, Eq. (3), and *Discussion*).

Effects of Mucosal K-for-Na Substitutions on Potentials and Resistances in the Presence and Absence of TAP

Typical records of mucosal K for Na substitutions in the presence and absence of TAP are shown in Fig. 3. Results from several tissues incubated in Ringer B or C are summarized in Table 3. In both series, TAP^+

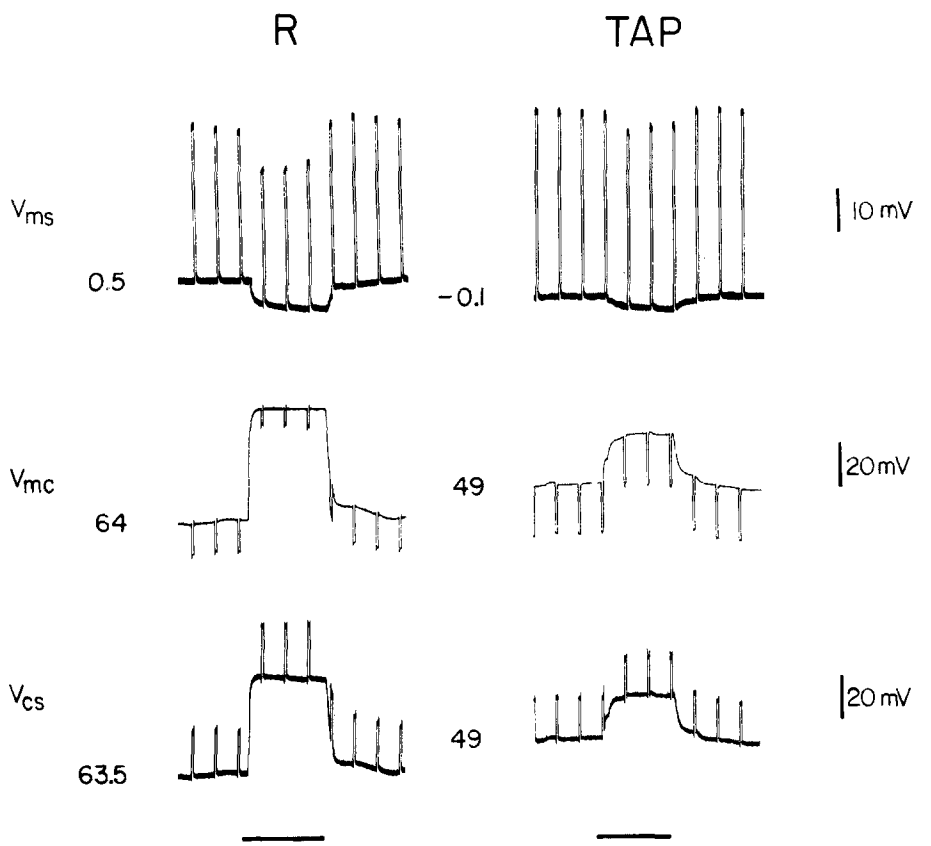

Fig. 3. Effect of TAP^+ on the dependence of potentials and resistances on external K concentration. Mucosal medium: Ringer B in the control condition (R), Ringer B+TAP (17 mM TAP^+) in the experimental condition (TAP). In both panels, records start with microelectrodes in the cell. Baseline potentials are indicated as in Fig. 2 (in TAP, V_{ms} was 0.1 mV, mucosa positive). During the periods indicated by the bars, Ringer B in the mucosal medium was replaced with K-Ringer B (109.2 mM KCl instead of 109.2 mM NaCl). In both conditions, exposure to K-Ringer produces depolarization of both cell membranes, mucosa-negative change of V_{ms}, transient drop of $R_t(\Delta V_{ms})$ and reduction of R_a/R_b. Note that all of these effects are smaller in TAP. *See also* legend to Fig. 2, Table 4, and text

produced significant decreases of the changes of V_{mc}, V_{cs}, and V_{ms}, caused by exposure of the tissue to K-Ringer on the mucosal side. The V_{mc} changes were smaller, in presence of TAP^+, by 12.6 and 15.3 mV, in Ringer B and Ringer C, respectively. These differences cannot be explained by an effect of TAP^+ on the paracellular K:Na diffusion potential, since the apical membrane IR drop produced by this mechanism cannot be greater than the change of E_s (which approximates ΔV_{ms}).

Table 3. Effects of TAP^+ on the changes of potentials and resistances produced by mucosal K for Na substitutions

Condition		Trans-epithelial potential (V_{ms}, mV)	Apical membrane potential (V_{mc}, mV)	Basolateral membrane potential (V_{cs}, mV)	Trans-epithelial resistance (R_t, Ωcm^2)	Ratio of cell membrane resistances (R_a/R_b)
A) *Ringer B* (7)						
Control	Na	-0.8 ± 0.2	-54.4 ± 4.2	-55.3 ± 4.2	225 ± 25	2.28 ± 0.25
	K	-7.2 ± 0.9	-14.3 ± 5.0	-21.5 ± 4.5	200 ± 15	0.57 ± 0.10
	Δ	6.4 ± 0.9	-40.2 ± 3.7	-33.8 ± 3.5	25 ± 15	1.71 ± 0.28
TAP^+	Na	0.0 ± 0.3	-46.0 ± 4.1	-46.0 ± 4.1	355 ± 30	3.42 ± 0.24
	K	-4.1 ± 0.9	-18.3 ± 4.3	-22.5 ± 3.8	310 ± 25	1.56 ± 0.39
	Δ	4.1 ± 0.8[a]	-27.6 ± 3.1[a]	-23.5 ± 2.8[a]	45 ± 10	1.86 ± 0.45
B) *Ringer C* (6)						
Control	Na	1.1 ± 0.4	-59.7 ± 6.4	-58.6 ± 2.3	200 ± 20	2.23 ± 0.24
	K	-4.8 ± 1.3	-15.1 ± 3.6	-19.9 ± 3.9	185 ± 20	0.81 ± 0.19
	Δ	5.9 ± 1.4	-44.5 ± 4.4	-38.6 ± 4.3	15 ± 5	1.42 ± 0.32
TAP^+	Na	0.4 ± 0.3	-45.6 ± 2.5	-45.2 ± 2.4	265 ± 30	3.42 ± 0.30
	K	-2.8 ± 0.5	-16.5 ± 2.7	-19.3 ± 2.4	235 ± 35	1.42 ± 0.27
	Δ	3.2 ± 0.5	-29.2 ± 3.7[a]	-26.0 ± 3.4[a]	30 ± 5	2.01 ± 0.41

Number of experiments is indicated in parentheses.
[a] Δ in TAP^+ was significantly different from Δ control ($P < 0.02$ or better).

The K-induced V_{ms} changes were reduced by TAP^+ only by 2.3 and 2.7 mV in Ringer B and Ringer C, respectively.

TAP^+, as shown before, causes increases of R_t and R_a/R_b, in Na-Ringer solutions, as compared to the control values. The changes of R_t and R_a/R_b produced by exposure to K-Ringer, although on the average larger in the presence of TAP, were not significantly different from the respective control values. In K-Ringer, R_t and R_a/R_b were larger in the presence than in the absence of TAP.

These results indicate that in the presence of TAP^+ the K selectivity of the apical membrane is reduced.

Effects of TAP^+ on Paracellular Na:Cl Selectivity

The effect of TAP^+ on the magnitude of the 1:2 transepithelial dilution potential (ΔV^{dil}) is shown in Table 4. ΔV^{dil} was reduced significantly, but the change was smaller than those reported by Moreno (1975) for

Table 4. Effect of TAP^+ on transepithelial NaCl 1:2 dilution potential

Condition	ΔV_{ms}(dil) (mV)
Ringer B	10.9 ± 0.8
TAP^+	5.7 ± 0.8
Δ	5.2 ± 0.4
P	<0.001

$n=8$ experiments.

ΔV_{ms}(dil): change in transepithelial potential produced by isosmotic replacement of sucrose for half of the NaCl present in the mucosal bathing medium, corrected for liquid junction potential. In both conditions, the change is in the mucosa positive direction.

frog and rabbit gallbladder. However, in Moreno's experiments TAP was added to both bathing media, and it was shown that the effects on paracellular Na conductance obtained from mucosal and serosal addition were additive.

Discussion

Our results confirm the observations of Moreno (1974, 1975) concerning the effects of TAP^+ on the paracellular pathway. We have shown that TAP^+ acts, in addition, at the luminal membrane of the epithelial cells, reducing K conductance. As we will show, demonstration of the latter effect renders untenable the argument for an electrogenic Na transport mechanism based on the effect of TAP^+ on fluid transport and transepithelial potential.

Paracellular Effects of TAP^+

TAP^+, added to the mucosal solution alone, reduced significantly the magnitude of the change in transepithelial potential produced by exposure of the mucosal side of the tissue to a reduced NaCl concentration (dilution potential). Considering that TAP was added only to the mucosal solution, the magnitude of the effect on the transepithelial NaCl dilution potential was comparable to the ones described by Moreno (1974, 1975) in several leaky epithelia.

When K was substituted for Na in the mucosal solution, the resulting change of transepithelial potential was significantly smaller in TAP^+

as compared to control conditions (Table 3). At least in part, this observation can be explained by the smaller luminal membrane depolarization produced by K in presence of TAP (Table 3). This effect, by itself, should result in a smaller change of V_{ms} during mucosal exposure to K-Ringer in the presence of TAP^+. Therefore, since in TAP^+ exposure to K-Ringer produces a V_{ms} change in the mucosa-negative direction, $P_K > P_{Na}$ at the junctional complexes. This conclusion is supported by the observation of a lower value of R_t in K-Ringer than in Na-Ringer (in the presence of TAP^+), as shown also in Table 3. Finally, R_t was greater, in the presence of TAP^+, both during exposure to Na-Ringer or to K-Ringer (Tables 2 und 4), indicating that both gNa and gK at the paracellular pathway are blocked by TAP^+. This observation supports the assumption of Van Driessche and Gogelein (1978) of a blocking effect of TAP^+ on paracellular gK.

Effects of TAP^+ at the Luminal Membrane

Our results show that TAP^+ blocks apical membrane K conductance. This conclusion is supported by three observations upon addition of TAP^+ to the mucosal bathing medium:

1) The cell depolarizes. In principle, this could result from changes in the apical membrane equivalent emf (E_a), the basolateral membrane equivalent emf (E_b), or both. A change of E_s alone can be ruled out immediately because in TAP^+ V_{mc} depolarized by 9.2 mV in Ringer B and 6.2 mV in Ringer C, whereas the respective changes of V_{ms} were only 0.8 and 1.1 mV. A change of E_s alone should result in a larger change of $V_{ms}(\Delta V_{ms})$ than of $V_{mc}(\Delta V_{mc})$ (*see* Fig. 1):

$$\Delta V_{ms} = \Delta E_s (R_a + R_b)/(R_a + R_b + R_s)$$

$$\Delta V_{mc} = \Delta E_s \, R_a/(R_a + R_b + R_s)$$

$$\text{Since } (R_a + R_b) > R_a, \; \Delta V_{ms} > \Delta V_{mc}.$$

The fact that TAP had a rapid effect when added only to the mucosal medium is a circumstantial argument in favor of an effect on E_a (and not on E_b), but the measurements of membrane potentials alone do not allow us to make this conclusion.

2) R_a/R_b increases. This indicates that TAP^+ produces an increase of R_a, a decrease of R_b, or a combined effect such that $\Delta R_a > \Delta R_b$. To distinguish between these possibilities, cable analysis experiments were

performed as described above; it was found that TAP^+ increases the length constant (λ) for intraepithelial current spread. This result indicates that R_z/R_i increases (where R_z is the resistance for current flow out of the cells, i.e., R_a and R_b in parallel, and R_i is the resistance to current flow within the epithelial sheet, *see Appendix*). The constant A was increased by exposure to TAP in two experiments and decreased in one, suggesting no large change of R_i [*see Appendix*, Eqs. (3)–(5)]. Therefore, the increase of λ appears to result from an increase of R_z. The possibility of a smaller change of R_i in either direction cannot be ruled out because of the small number of experiments. In conclusion, TAP^+ produces increases of both R_a/R_b and R_z. These two effects can be accounted for by an increase of R_a of about 82% (*see* above). A decrease of apical membrane gK would be necessary to explain such a change, because Na and Cl together account only for about 40% of the apical membrane conductance (Reuss & Finn, 1975*b*).

3) The K-selectivity of the luminal membrane decreases. Two sets of experiments, with low and normal NaCl concentration in the control mucosal bathing medium, gave essentially the same result: in the presence of TAP^+, the depolarization of the luminal membrane produced by a mucosal substitution of K for Na (ΔV_{mc}) is reduced, as compared to control conditions.

The magnitude of the effect of TAP^+ is such that shunt phenomena cannot account for the result. In TAP, as shown in Table 3, ΔV_{mc} was 29.2 mV, whereas in the absence of TAP, ΔV_{mc} was 44.5 mV. The difference is significant. Part of the reduction of the apical membrane depolarization could be explained by a smaller change of E_s in the presence of TAP, but this mechanism would account for no more than ca. 3 mV of ΔV_{mc}.

In conclusion, the effects of TAP^+ on baseline membrane potential, ratio of membrane resistances, cable properties of the epithelium, and K-dependence of the luminal membrane potential, are all consistent, and indicate that TAP^+ reduces apical membrane gK. Our approach does not allow us to demonstrate or exclude effects on other partial ionic conductances at this site.

Moreno (1974) observed that during exposure to TAP^+ the rate of fluid transport by the frog gallbladder was unchanged, whereas the transepithelial potential increased in proportion to the increase in transepithelial resistance. He suggested that the mechanism of transmural Na transport may be electrogenic, and that the TAP^+-induced V_{ms} increase results from reduced paracellular shunting, due to the effect of TAP^+ at the

junctions. Our results suggest, rather , that the principal cause of the change of V_{ms} may be the effect of TAP^+ at the luminal membrane. A selective or dominant reduction of E_a, with E_b constant or reduced by a smaller amount, will result in a mucosa-negative V_{ms} change regardless of the rate of transmural salt transport.

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Appendix

Calculation of Cell Membrane Resistances

$$R_t = \Delta V_{ms}/i_t \tag{1}$$

where ΔV_{ms} is the transepithelial voltage deflection produced by a transepithelial current pulse (i_t) in the linear range of the IV plot.

$$R_a/R_b = \Delta V_{mc}/\Delta V_{cs} = a \tag{2}$$

where ΔV_{mc} and ΔV_{cs} are the voltage delections produced by i_t across the luminal and basolateral membrane, respectively.

$$R_z = 2\pi A \lambda^2/i_c \tag{3}$$

where A and λ are determined from the fit of V_x to the Bessel function K_o, i_c is the intracellular current, and $R_z^{-1} = R_a^{-1} + R_b^{-1}$.

$$R_i = 2\pi A/i_c \tag{4}$$

where R_i is the resistance to horizontal current flow within the epithelial sheet

$$\lambda = (R_z/R_i)^{\frac{1}{2}} \tag{5}$$

$$R_t^{-1} = (R_a + R_b)^{-1} + R_s^{-1} \tag{6}$$

$$a = R_a/R_b \tag{7}$$

$$R_z^{-1} = R_a^{-1} + R_b^{-1}. \tag{8}$$

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