VOLTAGE-DEPENDENT K CONDUCTANCE AT THE APICAL MEMBRANE OF *NECTURUS* GALLBLADDER

J. F. GARCÍA-DÍAZ, W. NAGEL, AND A. ESSIG
Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118.

ABSTRACT The epithelial and cellular effects of clamping the transepithelial potential (V_t, mucosa reference) have been investigated in the *Necturus* gallbladder. Following initial equilibration at short circuit, tissue conductance g_t was 4.1 ± 1.2 (SD) mS/cm², the apical potential V_a was −76 ± 8 mV, and the apical fractional voltage on brief voltage perturbation ($f_a = \Delta V_a/\Delta V_t$, reflecting the ratio of apical membrane to transcellular resistance) was 0.72 ± 0.11 (21 gallbladders, 34 impalements). On clamping V_t at positive values, V_a depolarized and f_a decreased; at the same time g_t decreased. Clamping V_t at negative values produced converse effects. All of the above changes were related directly to the magnitude of the clamping potential V_t and were reversed on return to the short circuit state. Effects of V_t on f_a are not due to changes in the extracellular pathway resistances (which, however, contribute to g_t). Furthermore, the effects of V_t on f_a were abolished by the mucosal application of TEA or Ba, or acidification of the mucosal solution. Thus, these experiments disclose the presence of a voltage-dependent apical K conductance that increases with apical membrane depolarization. The calculated dose-response curve of TEA inhibition of apical conductance and the values of the apparent dissociation constant were in good agreement with those found for K channels in excitable tissues. Mucosal application of the Ca ionophore A23187 shifted the voltage dependence curve of f_a to more negative values of V_a without altering its shape. This effect of A23187 suggests a possible role for intracellular Ca in the modulation of the apical K channels.

INTRODUCTION

The conductance of both apical and basolateral cell membranes in gallbladder epithelium is mainly due to the electrodiffusive movement of K (Van Os and Slegers, 1975; Reuss and Finn, 1975 *b*; Reuss, 1979). When the gallbladder is bathed by symmetrical Ringer's solutions, the intracellular K activity is well above the value expected for passive distribution (Reuss and Weinmann, 1979; García-Díaz and Armstrong, 1980; Gunter-Smith and Schultz, 1982). Thus, net diffusive transport of K across both membranes is directed from the inside of the cell to the outside. There is evidence that, as in other epithelia, the level of intracellular K is maintained by the operation of a Na-K pump mechanism located at the basolateral membrane (Reuss et al., 1980).

The nature of the apical K conductance in gallbladder epithelium has been investigated with noise analysis techniques (Van Driessche and Gögelein, 1978; Gögelein and Van Driessche, 1981 *a*). The fluctuation of transepithelial current in voltage clamped toad and *Necturus* gallbladders contains a Lorentzian component that arises from conductance fluctuations of apical K channels that open and close randomly. The similarities (permeability sequence, inhibition by tetraethylammonium and acid pH) between these K channels and those found in excitable cells (Gögelein and Van Driessche, 1981 *a*) raise the question of whether they are also voltage dependent. Although Gögelein and Van Driessche (1981 *b*) observed a direct relation between the magnitude of the Lorentzian component and the transepithelial clamping voltage, they ascribed this to changes in the driving force for K efflux, without invoking any voltage dependence of the apical K conductance.

In this study we investigate the voltage dependence of the apical membrane conductance in *Necturus* gallbladder. We show that variation of transepithelial potential between −60 and +80 mV causes changes in apical membrane conductance attributable to a voltage-dependent K conductance. This voltage-dependent K conductance appears to be modulated by intracellular Ca. Some of these results have been presented at the Third Conference on Membrane Biophysics, Oxford, MI (García-Díaz et al., 1983 *b*).

MATERIALS AND METHODS

Gallbladders from *Necturus maculosus* (Graska Biological Supplies Co., Oskosh, WI) were mounted as a flat sheet in a horizontally divided chamber. Details on the chamber and perfusion system are described elsewhere (García-Díaz et al., 1983 *a*; Nagel et al., 1983, Fig. 1). The perfusion solutions contained 110 mM NaCl, 1 mM $CaCl_2$, and 2.5 mM $KHCO_3$, equilibrated with air to a pH of 8.1. In some experiments we used 2.5 mM KOH and 3.1 mM HEPES (pH 8.0) instead of $KHCO_3$. The same results, described below, were obtained when we used a solution

Dr. Nagel's permanent address is Physiologisches Institut der Universität München, 8000 München 2, Federal Republic of Germany

similar to that described by Fisher et al. (1981) containing 10 mM HCO_3 and saturated with 1% CO_2 in air (pH 7.7). Tetraethylammonium chloride (TEA) was substituted on an equimolar basis for NaCl. The Ca ionophore A23187 was dissolved (5 mg/ml) in dimethyl sulfoxide (DMSO) before addition to the Ringer's solution. The final concentration of A23187 in the bathing solution ranged from 0.02 to 1 μM. Addition of DMSO alone (up to 0.01% vol/vol) had no effect on the electrical parameters of *Necturus* gallbladder. The solutions were perfused at rates between 10 and 20 ml/min.

The transepithelial voltage, V_t, was measured with two calomel half cells connected by floating KCl bridges to the bathing solutions ~0.5 mm from the tissue surfaces. Transepithelial current, I_t, was applied via two AgCl-coated silver rings located 5 mm above and below the tissue. The microelectrodes were connected through a Ag/AgCl wire to a high-impedance ($>10^{15}$ Ω), low-bias current ($<3\ 10^{-13}$ A), FET-input electrometer (Analog Devices Inc., Norwood, MA, model 515J) with negative capacitance compensation. Microelectrode resistance, R_{ME}, was continously monitored throughout the experiment. The bladders were voltage clamped to desired values of V_t by means of an automatic clamping device (Frankenberger and Nagel, in preparation; see also Fig. 2 in Nagel et al., 1983). Apical membrane potential, V_a, and V_t are referred to the mucosal solution. Transepithelial conductance, $g_t = \Delta I_t/\Delta V_t$, and apical fractional voltage, $f_a = \Delta V_a/\Delta V_t$, were measured with voltage pulses $\Delta V_t = +10$ mV from the holding V_t value. The values of I_t and V_a were each obtained by means of two sample/hold amplifiers (Intersil, Inc., Cupertino, CA, model IH5110) appropriately triggered to sample values of I_t and V_a for 2 ms immediately before the onset and before the very end of the pulse. The duration of the pulse (150–467 ms) was selected in each experiment so as to obtain a steady-state trace of V_a in the oscilloscope after the capacitive transients had dissipated. The pulse frequency ranged from 0.5 to 1.5 Hz. All of these six parameters, V_t, I_t, g_t, V_a, f_a, R_{ME}, were continously recorded on a six-channel strip-chart recorder (BBC-Metrawatt/Goertz, Edison, NJ, model 460). In addition, V_a was observed on a storage oscilloscope (Tektronix, Inc., Beaverton, OR, model 5115).

Micropipettes were drawn from Kwik-Fil borosilicate glass capillary tubing (1.2 mm OD, W.-P. Instruments, Inc., Westhaven, CT) in a horizontal puller (Industrial Science Assoc., model M1) and back filled with 1.5 M KCl. The microelectrodes had resistances between 20 and 80 MΩ when immersed in Ringer's solution. Cells were impaled through their mucosal surface by advancing the microelectrode perpendicularly to the tissue using a stepping-motor micromanipulator (Frankenberger, Germering-Munich, FRG). On some occasions the cells were punctured by overcompensating for the capacitance of the input stage once the microelectrode was located against the cell membrane. Criteria for the acceptance of microelectrode impalements were as described previously (Armstrong and García-Díaz, 1981; García-Díaz et al., 1983 *a*), except that an increase of up to 20% of R_{ME} upon impalement was considered acceptable, provided that it did not increase further during the experiment. Most of the impalements were stable for periods of up to 3 h. After crossing the epithelial cell layer, f_a was between 0.92 and 0.97. The data in the figures presented here were not corrected for this factor.

RESULTS

Immediately after mounting the bladders the open-circuit V_t was ≤1 mV. The bladders were then short-circuited and ~20 min were allowed for stabilization before impaling the cells. In 21 gallbladders used g_t was 4.4 ± 1.4 (SD) mS/cm^2 initially and decreased during the first 10–20 min to a stable value of 4.1 ± 1.2 mS/cm^2. These values of g_t are in agreement with previous observations (Frömter, 1972; Van Os and Slegers, 1975; Reuss and Finn, 1975 *a*; Reuss et al., 1981; Cereijido et al., 1982; García-Díaz et al., 1983 *a*) but are about three times smaller than the values recently reported by Frömter and associates (Frömter et al., 1981; Suzuki et al., 1982). Fig. 1 shows the histogram of 34 acceptable impalements in 21 gallbladders under short circuit conditions. The values of V_a ranged from −60 to −92 mV and were uniformly distributed around a single maximum at about −75 mV. The mean value, −76 ± 8 (SD) mV, is in agreement with recently reported data in the same preparation under similar conditions (Reuss et al., 1981; Suzuki et al., 1982; García-Díaz, et al., 1983 *a*). The mean corrected f_a under short-circuit was 0.72 ± 0.11 (SD).

The response of V_a, f_a, and g_t to clamping of V_t to positive and negative values in a typical study is shown in Fig. 2 *A*. When V_t was successively clamped to +30, +40, and +60 mV, V_a depolarized from the short-circuit value and f_a decreased very dramatically. Clamping of V_t to −30 mV hyperpolarized V_a and increased f_a. At the same time g_t decreased with positive V_t and increased at negative V_t. All of these changes were reversible upon returning to the short-circuit state ($V_t = 0$). The changes in g_t are in the same direction as those reported for the bullfrog (Bindslev et al., 1974) and *Necturus* (Reuss and Finn, 1977) gallbladder with current clamping, and, for the most part, can be ascribed to changes in the paracellular shunt pathway. In the following we are concerned only with the cellular events evoked by alterations of V_t.

Fig. 2 *B* shows the values of f_a, V_a and the basolateral membrane potential, $V_b = V_a - V_t$, at different V_t clamps for the cell impalement shown in Fig. 2 *A*. It can be seen that f_a decreases very dramatically for $V_t > +20$ mV. In this particular experiment, there is only a small increase in f_a for $V_t < 0$. The shape of the curve relating f_a with V_t was much the same in all the experiments. Thus, when the short circuit value of f_a was low, 0.5 – 0.6, on clamping to

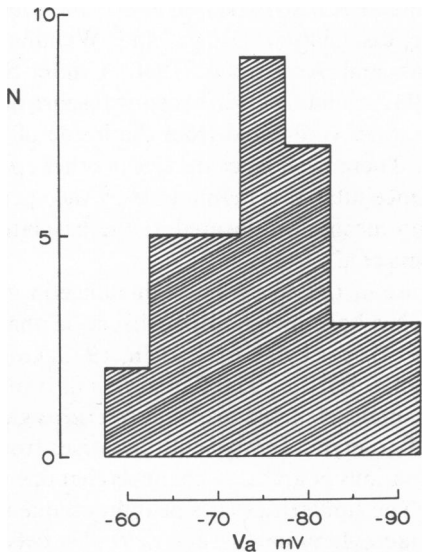

FIGURE 1 Frequency distribution of 34 successful membrane potential measurements in 21 gallbladders. Ordinate: number of observations (N) in 5 mV intervals.

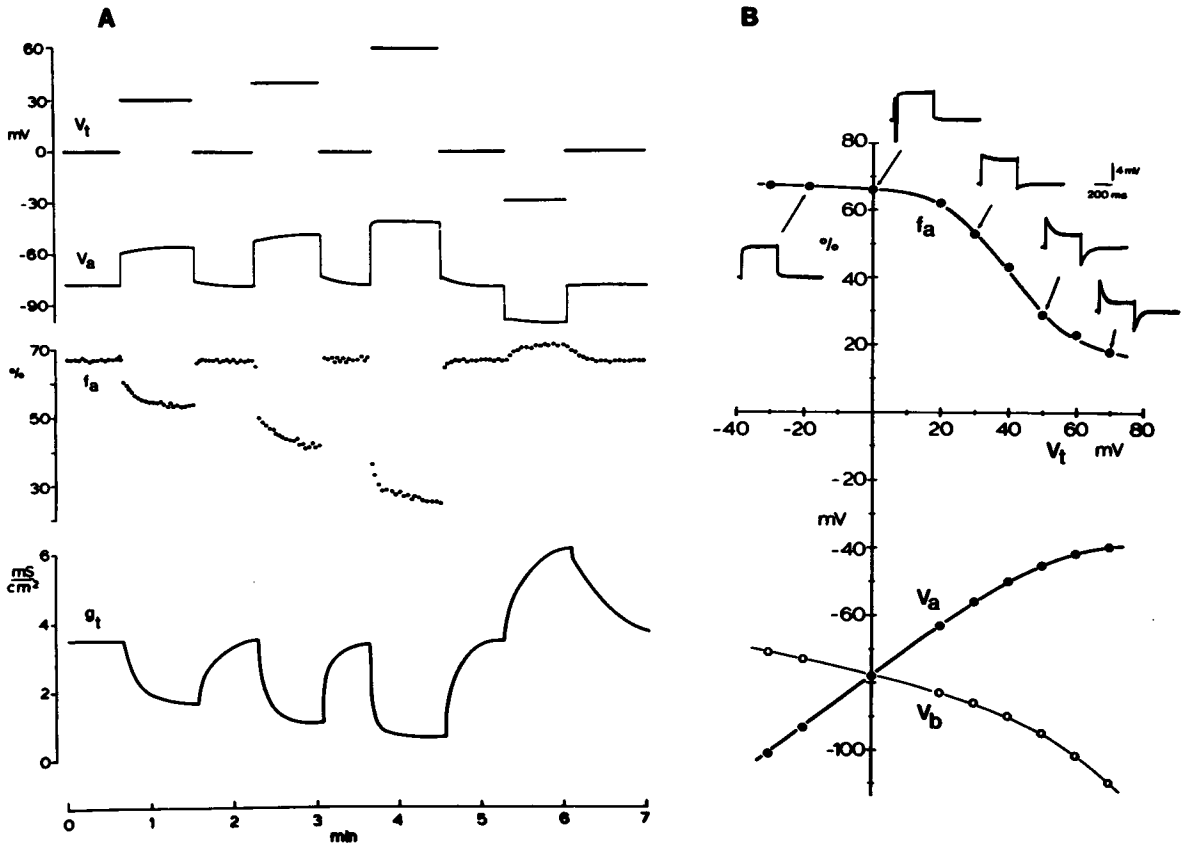

FIGURE 2 (*A*) Response of apical membrane potential, V_a, apical fractional voltage, f_a, and transepithelial conductance, g_t, to clamping of V_t to positive and negative values. In this experiment the on and off time constants of the f_a response are different. This was not always observed. (*B*) Stable values f_a, V_a, and V_b as functions of the clamp voltage V_t. These data were obtained from the same impalement shown in *A*. The insets in the f_a graph show the oscilloscope traces of V_a after the V_t pulse for several holding values of V_t.

negative V_t, f_a increased substantially, reaching a maximum of approximately 0.8 at $V_t = -50$ mV. In association with the drop in f_a, the depolarizations in V_a at $V_t > 0$ become smaller, resulting in a curvilinear relation between V_a and V_t. For $V_t > 80$ mV, further increases of V_t failed to produce additional depolarization of V_a.

The insets on the f_a graph allow a more detailed analysis of the behavior of f_a with variation of V_t. These are the oscilloscope traces of the ΔV_a produced by the +10 mV ΔV_t pulses used to measure f_a and g_t. After the initial upwards deflection upon application of the pulse, V_a either stayed constant or increased slightly at $V_t < 0$. For $V_t > 0$, V_a decreased exponentially to a final steady value, which was smaller with more positive holding V_t value. As explained in the Materials and Methods section, the difference between this final value and the one before the onset of the pulse is the ΔV_a used to calculate f_a. The inset at $V_t = 0$ also shows the up and down deflections immediately before the V_t pulse that were used to measure the microelectrode resistance, R_{ME} (in this case 59 MΩ).

Fig. 3 shows a juxtaposition of the V_a deflections at $V_t = 0$ (*a*) and $V_t = +50$ mV (*b*), for a different bladder than in Fig. 2. The initial upstroke at the onset of the pulse is too fast to be resolved with the time resolution in this figure.

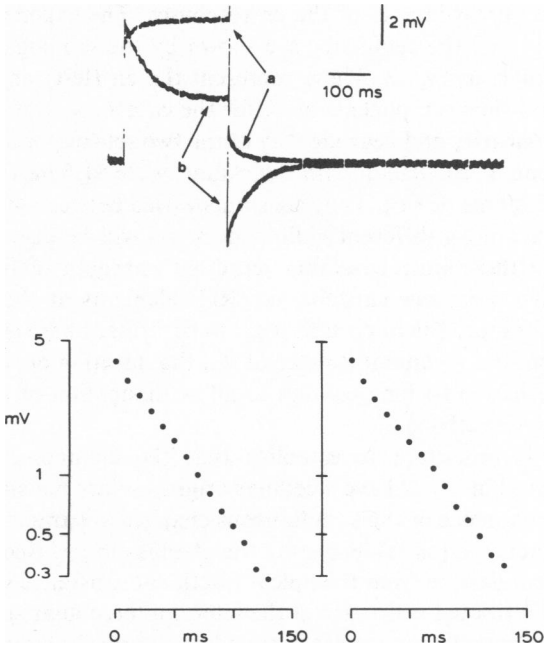

FIGURE 3 Juxtaposition of the oscilloscope V_a traces for V_t clamps of 0 mV (*a*) and +50 mV (*b*). The semilogarithmic plots are for the on (*left*) and off (*right*) values in trace *b* above.

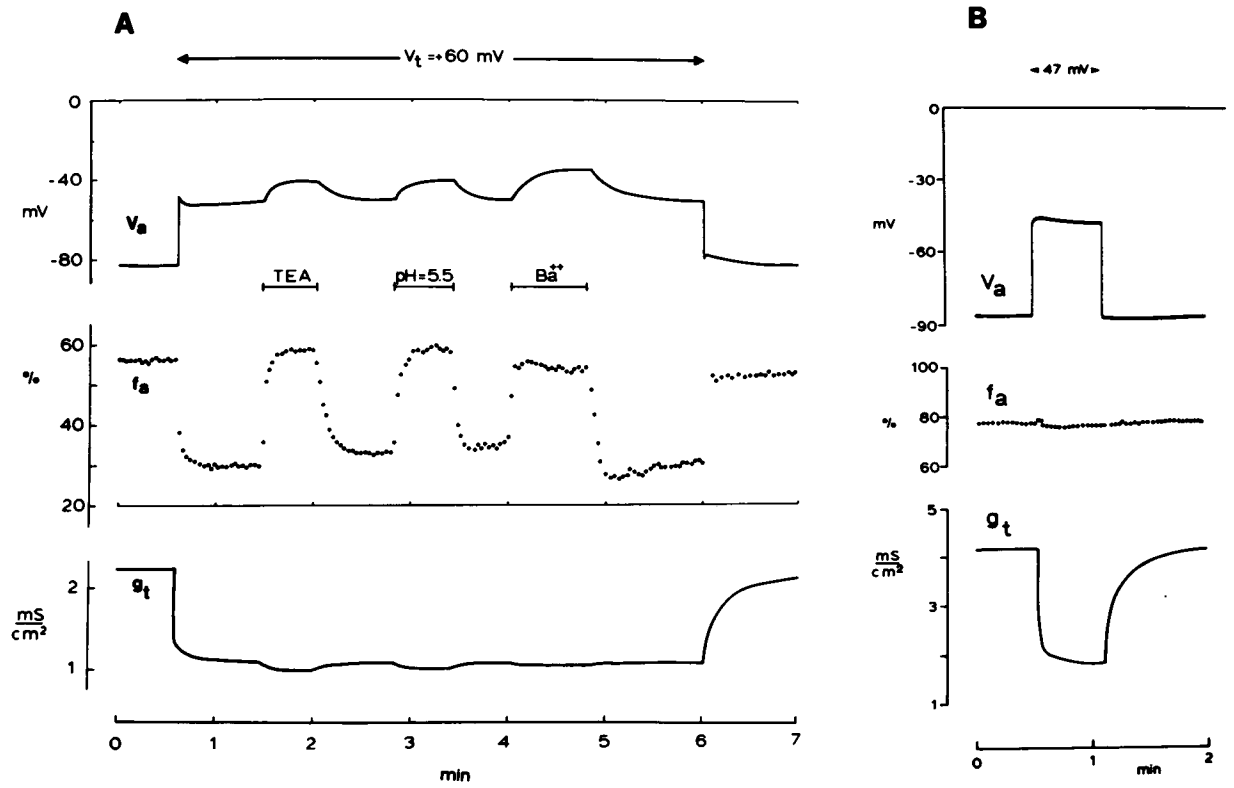

FIGURE 4 (*A*) Reversal of the voltage-induced decrease in f_a by mucosal addition of TEA (5 mM), lowering of pH, or addition of Ba (5 mM). At pH 5.5, the Ringer's solution was buffered with 10 mM MES instead of 3.1 mM HEPES. (*B*) Inhibition of the voltage-induced decrease in f_a by the presence of 20 mM TEA in the mucosal solution.

The second, slow component exponentially increases (V_t = 0) or decreases (V_t = +50 mV) towards a steady-state value. The off responses after the end of the pulses are exact mirror images of the on responses. The exponential decays and the symmetry are shown by the semilogarithmic plots in Fig. 3. These represent the on (left) and off (right) slow components of V_a for the case V_t = +50 mV. The linearity and near identity of the two sets of points are evident. The calculated time constants were 51.5 ms (ON) and 52.9 ms (OFF). Time constants varied between 40 and 95 ms among different gallbladders. As will be discussed below these time constants represent charging of linear passive resistance-capacitance (RC) elements at the cell membranes. The important point to be stressed here is that to obtain a meaningful value of f_a, the duration of the V_t pulse has to be long enough to allow dissipation of these capacitive effects.

It is important to establish that the changes in f_a observed in Fig. 2 have a cellular origin and are not simply a consequence of the simultaneous changes in paracellular conductance (as evidenced by the changes in g_t). Because f_a may deviate from the apical fractional resistance when the distributed resistance of the lateral intercellular spaces (LIS) contributes significantly to the paracellular resistance (Boulpaep and Sackin, 1980), it might seem that the changes in f_a result from alterations in the relative values of junctional and LIS resistances.[1] However, Fig. 4 shows that this is not the case. In Fig. 4 *A*, V_t was clamped to +60 mV from the initial short-circuit state. As previously shown (Fig. 2), f_a and g_t decreased at about the same time. Addition of 5 mM TEA to the mucosal solution rapidly reversed the decreased in f_a, with only a minimal effect on g_t. The same results were obtained with acidification of the mucosal solution or the addition of 5 mM Ba. The oscilloscope traces of V_a produced by the 10 mV V_t pulses on top of the holding V_t changed from the type *b* shown in Fig. 3 (control conditions) to type *a* (in the presence of TEA, Ba or pH 5.5). Fig. 4 *B* shows that, in the presence of 20 mM TEA in the mucosal solution, clamping of V_t to positive voltages does not affect f_a. However, as in the absence of TEA, there was a large decrease in g_t, consistent with an effect of voltage on paracellular conductance.

Additional evidence for the lack of a significant contribution of changes in paracellular conductance to the observed voltage dependence of f_a was obtained in experi-

[1]Apical fractional resistance is defined as $R_a/(R_a + R_b)$, where R_a and R_b are the lumped input resistances of the apical and basolateral membranes, respectively. With junctional and lateral space resistances R_j and R_l, respectively, a lumped circuit analysis shows that for a system with cell resistance ($R_a + R_b$) >> R_l, as in the present case, $R_a/(R_a + R_b) \simeq (1 + R_l/R_j) f_a$. (See Essig, 1982, Eq. 43).

ments where 2 mM $LaCl_3$ was added to the mucosal solution (Fig. 5). Approximately 10 min after the addition of La, clamping of V_t to +50 mV elicited the usual decrease in f_a, but failed to produce any change in g_t. When La was present for at least 15 min, the response of g_t was the opposite to that observed in the absence of La, i.e., g_t increased. However, f_a again showed the usual decrease. This effect of La on the response of g_t to transepithelial current has been observed previously by other investigators (Bindslev et al., 1974; Reuss and Finn, 1977) and related to the reversal of the ionic selectivity of the extracellular pathway (Wright and Diamond, 1968). Thus, the above experiments clearly dissociate the effects of voltage clamping on the cellular conductances, as seen by the behavior of f_a, from those on the paracellular conductance, which is the main contributor to g_t (Frömter, 1972).

Experiments such as those shown in Fig. 4 provide further insight into the nature of the voltage dependence of f_a. First, the changes in f_a upon voltage perturbation are attributable to the apical membrane, since the fall in f_a at positive V_t is abolished by the addition of 10–20 mM TEA to the mucosal solution, while serosal TEA does not affect f_a at any value of V_t. Second, these conductances changes result from changes in the K conductance, since it is well known that TEA and Ba specifically inhibit the K conductance of several cell types (Armstrong and Binstock, 1965; Hagiwara et al., 1978; Hermann and Gorman, 1979 and 1981; Hille, 1967; Stanfield, 1970), including *Necturus* gallbladder (Reuss, 1981). Although acidification of the external solution is not as specific an inhibitor of the K conductance (Hille, 1968; Drouin and The, 1969) its inhibitory effect on luminal membrane K permeability in *Necturus* gallbladder has been recently shown (Reuss et al., 1981). In addition, in this epithelium the relaxation noise of apical K current is abolished by all of the above agents (Gögelein and Van Driessche, 1981 *a*). We also found that the response of f_a to variations of V_t shown in Fig. 2 persists after the removal of Na or Cl from the mucosal solution.

All the above observations indicate the presence of a voltage-dependent K conductance at the apical membrane of *Necturus* gallbladder, similar to K conductances familiar in excitable cells. Assuming constancy of the basolateral conductance, g_b, the voltage dependence of the apical membrane conductance, g_a, and its inhibition by TEA can be evaluated as shown in Fig. 6 *A*. These data were obtained during a single cell impalement from an experiment such as that shown in Fig. 2 *A*. The ratio between apical and basolateral cell conductances, g_a/g_b, was calculated as $(1/f_a) - 1$. This has to be taken as a first

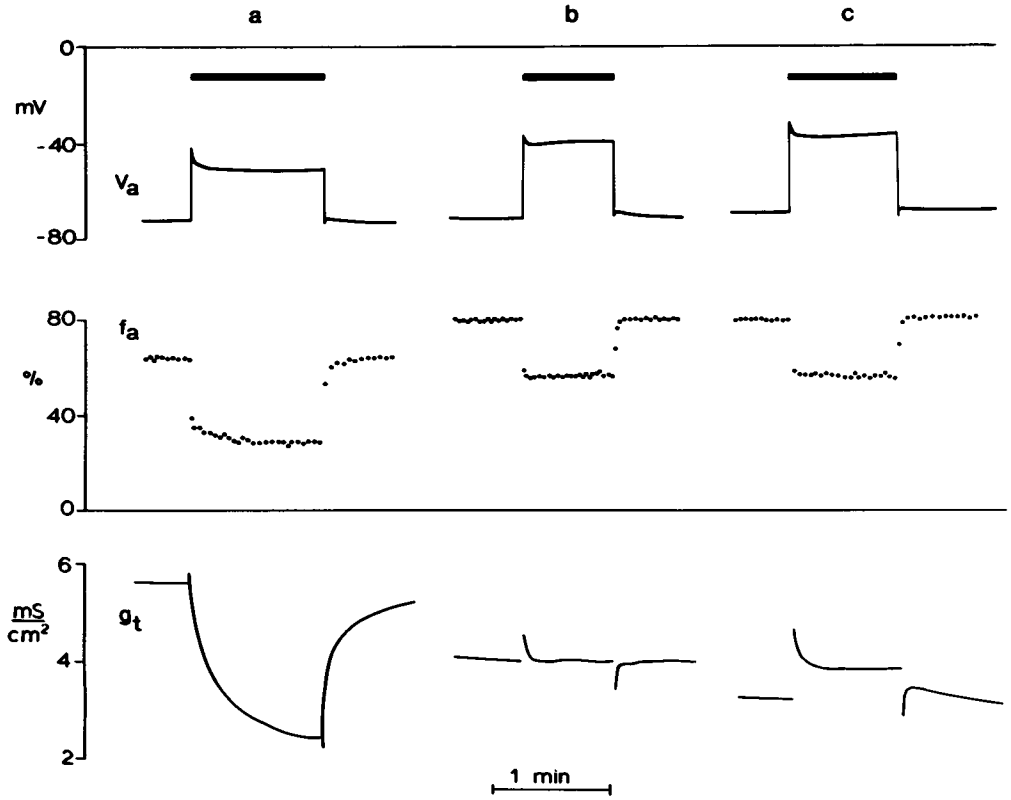

FIGURE 5 Effect of mucosal La (2 mM) on the responses of V_a, f_a, and g_t to +50 mV clamping of V_t (period marked by the bars). (*a*) Control, in the absence of La. (*b*) 10 min after the addition of La. (*c*) 15 min after the addition of La. Note that f_a always decreased with positive V_t clamping in spite of the direction and magnitude of the g_t changes. Addition of La increased f_a, perhaps due to an inhibition of apical K conductance. Data recorded from a single cell impalement.

approximation of the actual ratio, since distribution of resistance in the LIS in association with a highly conductive junction could lead to a value of f_a appreciably lower than the apical fractional resistance (Boulpaep and Sackin, 1980). Nevertheless, the data in Fig. 6 *A* show that the strong dependence of g_a/g_b on apical membrane potential, V_a, was markedly depressed by the presence of 15 mM TEA in the mucosal solution. Assuming only little effect of TEA on paracellular conductance, the solid line in Fig. 6 *A* presumably reflects the maximum possible influence of the junctional conductance on $1/f_a$.

Fig. 6 *B* shows the dose-response of apical membrane conductance, at $V_t = +50$ mV, to mucosal TEA. The ordinate in this graph is the ratio between the apical membrane conductance at a given TEA concentration, g_a^{TEA}, and the maximum apical conductance (in the absence of TEA), g_a, both at a holding V_t of +50 mV. Assuming again constancy of g_b, this ratio was calculated as

$$\frac{g_a^{TEA}}{g_a} = \left(\frac{1}{f_a^{TEA}} - 1\right) \div \left(\frac{1}{f_a} - 1\right). \quad (1)$$

Given the assumptions implicit in the above equation and the fact that V_a changed in response to increasing doses of TEA, the points were in reasonable agreement with a Michaelis-Menten expression indicated by the solid curve in Fig. 6 *B*. This latter was calculated assuming that one TEA molecule binds to a single receptor site R (Hermann and Gorman, 1981), that

$$\text{TEA} + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R\text{-TEA}$$

where k_1 and k_{-1} are the forward and reverse rate constants of the reaction. It can then be shown that

$$\frac{g_a^{TEA}}{g_a} = \frac{K_{TEA}}{K_{TEA} + [TEA]} \quad (2)$$

where $K_{TEA} = k_{-1}/k_1$ is the apparent dissociation constant of the reaction. In two experiments, we found the values of K_{TEA} to be 0.56 and 0.24 mM. The dose-response plot of TEA inhibition and the values of K_{TEA} found in the above experiments are consistent with results obtained for the K current in molluscan neurons (Meech and Standen, 1975; Hermann and Gorman, 1981). In particular, the K_{TEA} values are close to the ones found for the Ca-activated K current in molluscan neurons, 0.4 mM (Hermann and Gorman, 1981), and for a Ca-activated K channel isolated from rabbit skeletal muscle, 0.29 mM (Vergara and Latorre, 1983).

Since the discovery some 14 years ago that an increase in intracellular Ca can activate changes in membrane permeability to K ions in red blood cells (see Lew and Ferreira, 1978 for review), Ca-mediated K permeability changes

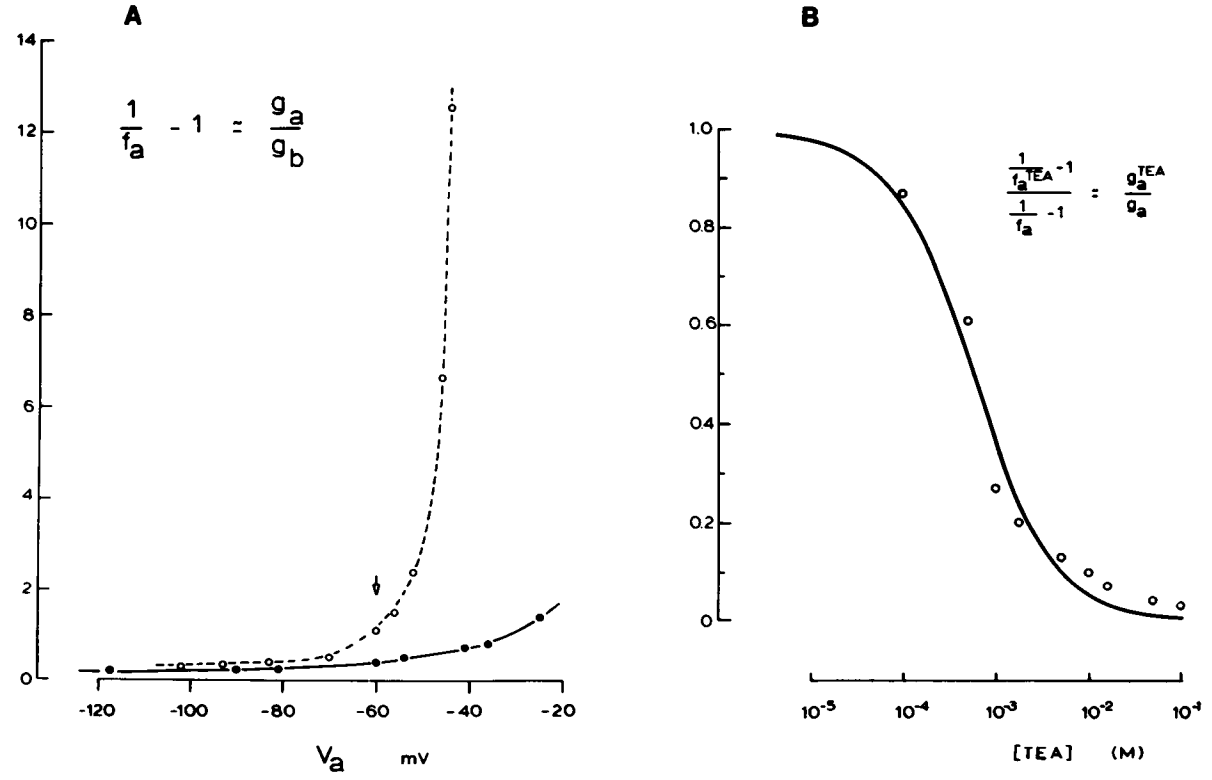

FIGURE 6 (*A*) Voltage dependence of the estimated apical-to-basolateral conductance ratio in the absence (○) and presence of (●) of 15 mM TEA in the mucosal solution. The arrow indicates values at short circuit. (*B*) Dose-response of apical membrane conductance to mucosal TEA at a holding $V_t = +50$ mV. For details see text.

have been found in a variety of nonexcitable and excitable cells (Meech, 1976 and 1978). There is also strong evidence that the apical membrane K conductance of *Necturus* gallbladder is sensitive to changes in intracellular Ca (Bello-Reuss et al., 1981). To investigate the possible Ca sensitivity of the voltage-dependent apical K conductance in gallbladder, we examined the voltage dependence of f_a before and after the mucosal addition of the Ca ionophore A23187. The rationale for this is as follows. It has been shown (Latorre et al., 1982; Wong et al., 1982) that an increase in the free Ca concentration at the cytoplasmic side of a membrane containing Ca-activated K channels shifts the voltage sensitivity of the K conductance towards more negative values of membrane potential. In other words, when intracellular Ca is raised, a given level of K conductance will be attained with a less positive membrane potential than at a lower intracellular Ca level. Thus, if the voltage-dependent K channels at the apical membrane of *Necturus* gallbladder are activated by intracellular Ca one would expect that after the addition of the ionophore the voltage-dependence curve of f_a would be displaced toward more negative values of V_a. Fig. 7 shows that this is indeed the case. In three experiments like the one shown in Fig. 7, the addition of A23187 to the mucosal solution shifted the voltage response curve of f_a toward the left along the voltage axis without changing its shape. These results suggest that intracellular Ca modulates the voltage-dependent K conductance at the apical membrane of *Necturus* gallbladder.

Some additional observations on the effect of the ionophore are worth mentioning. (*a*) Addition of A23187 to the serosal solution, up to 1 μM, had no effect on the electrical parameters of *Necturus* gallbladder. (*b*) The effects of mucosal addition of A23187 in the short-circuit state (hyperpolarization of V_a and decrease of f_a) were concentration dependent in the range we tested (2×10^{-8} to 10^{-6} M). (*c*) With nominal zero Ca and 2 mM EGTA (calculated free Ca = 10^{-10} M) in both bathing media, addition of 0.1 μM A23187 to the mucosal solution also hyperpolarized V_a and decreased f_a. This indicates that A23187 induces release of Ca from intracellular stores, as has been shown for other cell types (Steinhardt et al., 1974; Rose and Loewenstein, 1976; Steinhardt et al., 1977).

DISCUSSION

The values of g_t found in this study (4.1 ± 1.2 mS/cm^2) are in agreement with all previous observations in *Necturus* gallbladder (Frömter, 1972; Van Os and Slegers, 1975; Reuss and Finn, 1975 *a*; Reuss et al., 1981; Cereijido et al., 1982; García-Díaz et al., 1983 *a*) with the exception of the recent data of Frömter and associates (Frömter et al., 1981; Suzuki et al., 1982). These authors found values of g_t three times larger (~13 mS/cm^2). The reason for their anomalously high g_t is not clear. They suggested that it might be due to a faster perfusion rate and better oxygenation of the gallbladder than in previous studies. Although we found that extremely low perfusion rates slightly decreased g_t, this does not seem to be the explanation, since the perfusion rates used in this study were between 6 and 12 times faster than that used by Suzuki et al. (1982).

Suzuki et al. (1982) have analyzed in detail the transients in cell potential after the imposition of a transepithelial current pulse. They showed that these are charging effects of the cell membranes that behave like RC elements in a circuit. The shape of the V_a they normally observed is the same as in the present experiments with V_t zero or negative (see Fig. 2 *B* and trace *a* in Fig. 3). Under these conditions V_a does not show an overshoot with a subsequent decline. In terms of the analysis of Suzuki et al. (1982) this would seem to indicate that the apical membrane time constant ($\tau_a = R_aC_a$) is equal or larger than that of the basolateral membrane ($\tau_b = R_bC_b$). It is not possible to compare directly our results obtained with constant voltage pulses with those of Suzuki et al. where constant current pulses were applied. However, we would point out that the explanation for the overshoot in V_a at low cell potentials suggested by Suzuki et al., i.e., leakage from improper sealing of the microelectrode to the cell membrane, does not apply to our findings. As shown in Figs. 2 *B* and 3, we always obtained an overshoot in V_a after clamping V_t to positive values. On clamping to negative V_t, the overshoot disappeared. On occasion, we found that V_a exhibited an overshoot under short-circuit conditions. However, this overshoot disappeared after clamping V_t to negative values or on addition of mucosal TEA. Such findings are most unlikely to result from impalement artifacts, and we are thus inclined to attribute them to voltage-dependent K conductance at the apical membrane.

The transients in V_a discussed above were also observed by Reuss and Finn (1977) after current clamping the *Necturus* gallbladder. Because the relatively large capacitance of the cell membranes of this epithelium was not then known ($C_a = 8$ μF/cm^2, $C_b = 26$ μF/cm^2, Suzuki et al. 1982), they assumed that the transients were attributable to polarization (i.e., changes in the emf of the cell membranes) rather than charging (capacitive) effects combined with a voltage-dependent apical conductance. In their interpretation, the polarization was induced mainly by accumulation or depletion of K at the subserosal layer, and indeed, when the tissue was bathed with serosal K-Ringer's solution, the transients in V_a during serosa-to-mucosa current clamp disappeared. This, however, could be due to the increase in basolateral membrane conductance and consequent shortening of the basolateral time constant.

The experiments reported here show that the steady state value of V_a, and thus f_a evaluated after the capacitive transients, is voltage dependent. The cellular origin of this dependence is revealed by the experiments where the effects on f_a and g_t of transepithelial voltage clamping are largely dissociated. Thus, TEA, low pH, or Ba abolished the decrease in f_a induced by positive V_t clamping (Fig. 4) with only minimal effects on g_t. The slight decreases in g_t

observed are consistent with a decrease in apical conductance. On the other hand, La inhibited or reversed the changes in g_t while leaving the response of f_a to voltage clamping essentially unaffected (Fig. 5). The inhibition by TEA or Ba of the changes in f_a indicates that they are due to a voltage-dependent K conductance, inasmuch as these agents are specific inhibitors of K channels in several cell types (Armstrong and Binstock, 1965; Hagiwara et al., 1978; Hermann and Gorman, 1979 and 1981; Hille, 1967; Stanfield, 1970).

Our localization of this voltage-dependent K conductance (and thus the changes in f_a) at the apical membrane rests on the rapid inhibition by mucosal TEA, Ba, or low pH of the voltage dependence of f_a (Figs. 4 and 6 *A*). Furthermore, although the basolateral membrane of *Necturus* gallbladder is almost exclusively K permeable (Van Os and Slegers, 1975; Reuss, 1979), when TEA was present in the serosal solution, even for periods as long as 20 min, f_a did not change, and the voltage response of f_a was unaltered. Serosal Ba decreased the control value of f_a, but failed to prevent the voltage response. Thus it seems clear that the changes in f_a are due to a voltage-sensitive K conductance at the apical membrane that increases with depolarization of V_a. There may be some contribution from a voltage-dependent basolateral K conductance, but it was not evident in these experiments. It is possible, of course, that the voltage sensitivity of basolateral K channels resides outside the range in which V_b was varied (-55 to -120 mV). On the other hand, the basolateral K channels may be of an entirely different type from those at the apical membrane, insensitive to variations in membrane potential. In this respect, it is interesting that Gögelein and Van Driessche (1981 *a*) could not observe fluctuating K channels at the basolateral membrane, although fluctuations from the basolateral membrane could be attenuated beyond the detection limit of their technique.

The experiments with the Ca ionophore A23187 shown in Fig. 7 provide evidence for the Ca sensitivity of the apical K channels, on the assumption that addition of A23187 increases intracellular Ca in this preparation. These results agree with the observations of Bello-Reuss et al. (1981) that cyanide (which induces release of Ca from intracellular stores) at either side of the *Necturus* gallbladder epithelium, or A23187 in the mucosal solution hyperpolarizes the cell membrane potential and increases the depolarization produced by substituting K-Ringer's for Na-Ringer's solution. Because A23187 produces the same effects in a virtually Ca-free solution as in standard Ringer's solution, the elevation of intracellular Ca activity is attributable to release from intracellular stores (perhaps mitochondria), as has been observed in other cell types (Steinhard et al., 1974; Rose and Loewenstein, 1976; Steinhardt et al., 1977). Our experiments do not answer the question of whether the voltage-dependent K conductance at the apical membrane of *Necturus* gallbladder responds directly to an elevation of intracellular Ca, as it does in different types of excitable cells (Marty, 1981; Pallota et al., 1981; Wong et al., 1982; Latorre et al., 1982) or is mediated by a secondary event, e.g., increase in intracellular pH, as has been shown in sea urchin eggs (Whitaker and Steinhardt, 1982). A definite answer has to await monitoring of intracellular Ca and H activities. It is also possible that Ca modifies a K conductance other than that activated by voltage at the apical membrane.

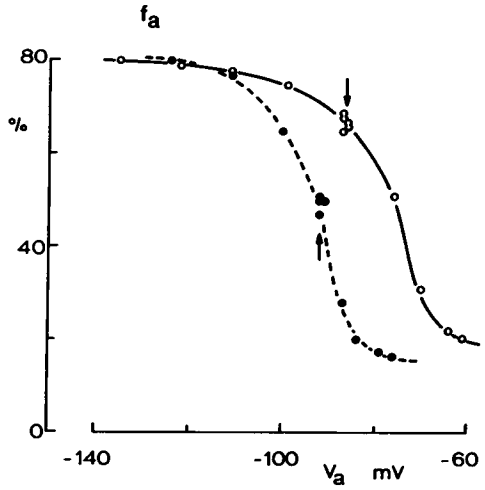

FIGURE 7 Voltage dependence of f_a before (○) and after (●) the addition of the Ca ionophore A23187 (0.1 μM) to the mucosal solution. All observations were made during a single cell impalement. The bathing solution contained 1 mM Ca. The arrows indicate the values at the time the bladders were short-circuited.

The voltage dependence of the apical K conductance does not appear to be mediated by influx of Ca induced by depolarization of V_a, since in two experiments with virtually Ca-free solutions (mucosal and serosal solutions containing 2 mM EGTA) the voltage dependence of f_a was still present. In other tissues containing Ca-dependent K channels it has been found that the voltage dependence is an intrinsic property of the gating mechanism (Marty, 1981; Pallota et al., 1981; Wong et al., 1982; Latorre et al., 1982).

As mentioned in the Introduction, Gögelein and Van Driessche (1981 *b*) found that the relaxation noise of apical K current fluctuations in *Necturus* gallbladder was enhanced by clamping V_t to serosa positive values and abolished by negative V_t (note that their polarity convention is opposite to ours). In the absence of any measurement of apical conductance, they assumed that the V_t dependence of the K current fluctuations was simply due to the effect of V_t on the driving force (and consequently on the efflux) of K across the apical membrane. However, in the present study we show that alterations of V_t from -60 to $+80$ mV cause marked changes in apical membrane K conductance. Thus the increase in K efflux induced by a

positive V_t is due not only to the larger driving force for K (depolarization of V_a), but also to the increased K conductance of this membrane under these conditions.

In conclusion, the present experiments show that the K channels at the apical membrane of *Necturus* gallbladder are voltage dependent, and possibly are modulated by intracellular Ca. Recently, Nagel and Essig (1982) found that the apical Na conductance of another epithelium, frog skin, was voltage dependent. In this case depolarization induced a decrease in conductance. The nature and physiological significance of these voltage-dependent conductances in nonexcitable tissues are yet to be assessed.

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