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KCl Cotransport: A Mechanism for Basolateral Chloride Exit in *Necturus* Gallbladder

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Summary. K^+ and Cl^--selective double-barreled microelectrodes were used to study the effect of changes in external K^+ concentration on intracellular Cl^- activity (a^i_{Cl}) in epithelial cells of *Necturus* gallbladder. Decreasing the K^+ concentration simultaneously in both bathing solutions produced a decrease in a^i_{Cl}. Steady-state values of a^i_{Cl} were related to the values of the chemical potential gradient for K^+ ($\Delta\mu_K$) across either the apical or the basolateral cell membrane. A similar dependence between a^i_{Cl} and $\Delta\mu_K$ appeared when the K^+ concentration was changed in the serosal solution only. This indicates that a^i_{Cl} depends on $\Delta\mu_K$ across the basolateral membrane. a^i_{Cl} was virtually independent of the membrane potential. This supports the idea that both the mucosal and the basolateral membranes of *Necturus* gallbladder cells have very low passive permeabilities to Cl^-. These results indicate that the exit of Cl^- from *Necturus* gallbladder cells is driven by $\Delta\mu_K$ across the basolateral membrane, and suggest that a KCl electroneutral coupled mechanism in this membrane plays an important role in transcellular Cl^- transport.

Key Words transcellular chloride transport · intracellular chloride activity · intracellular potassium activity · K^+ chemical potential · Cl^- chemical potential · chloride permeability

Introduction

There is now compelling evidence that an electroneutral NaCl transport process is by far the major route for transapical Cl^- entry into the epithelial cells of isolated gallbladder (Frizzell, Field & Schultz, 1979; Reuss & Grady, 1979; Frizzell & Duffey, 1980; Ericson & Spring, 1982). The evidence is equally strong that this mechanism is responsible for Cl^- accumulation, within the cells, to levels above that which corresponds to electrochemical Cl^- equilibrium across the apical cell membrane and that the energy for Cl^- accumulation is derived from the transapical Na^+ gradient (Duffey, Turnheim, Frizzell & Schultz, 1978; Reuss & Grady, 1979; Garcia-Diaz & Armstrong, 1980).

During steady-state Cl^- absorption, the rate of Cl^- exit across the basolateral cell membrane must equal the rate of transapical Cl^- entry. Since, in the isolated gallbladder, the transepithelial potential (V_T) is normally very low ($\leqq 1$ to 2 mV), intracellular Cl^- activity (a^i_{Cl}) also exceeds the level corresponding to electrochemical equilibrium across the basolateral membrane of the cell. In principle, therefore, basolateral Cl^- exit could occur by simple electrodiffusion down the outwardly directed gradient of Cl^- electrochemical potential. However, a number of studies (van Os & Slegers, 1975; Reuss, 1979) strongly indicate that, like the apical membrane (Cremaschi & Henin, 1975; van Os & Slegers, 1975; Garcia-Diaz, Corcia & Armstrong, 1983), the basolateral membrane has a very low Cl^- conductance, far too small to account for observed rates of transcellular Cl^- transport (Reuss, Bello-Reuss & Grady, 1979; Heintze, Petersen & Wood, 1981). One is forced to conclude therefore that basolateral Cl^- exit, like transapical Cl^- entry, is mediated, in large measure, by electroneutral processes in which Cl^- exit from the cell is coupled to the transmembrane movement of another ion.

Coupled exit of Na^+ and Cl^-, analogous to the coupled transapical entry of these ions, is unlikely from an energetic standpoint. The results of Weinman and Reuss (1982) suggest that, for similar reasons, coupled HCl exit is also unlikely. Recent studies in the authors' laboratory (Baxendale & Armstrong, 1983) indicate that Cl^-/HCO_3^- exchange, a mechanism that seems to be an important pathway for basolateral Cl^- exit in a number of epithelial systems (Frizzell et al. 1979; Armstrong & Youmans, 1980; Guggino, Boulpaep & Giebisch, 1982) plays a minor, if any role in transcellular Cl^- transport by *Necturus* gallbladder. A coupled electroneutral exit of KCl, across the basolateral membrane of gallbladder epithelial cells, has been postulated (Reuss, 1979; Garcia-Diaz &

Armstrong, 1980; Gunter-Smith & Schultz, 1982). So far, no direct experimental evidence for this mechanism has been reported. This paper presents results that demonstrate a direct relation between a^i_{Cl} in *Necturus* gallbladder cells and the chemical potential gradient for K^+ ($\Delta\mu_K$) across the basolateral cell membrane. These results point to a coupled basolateral KCl exit mechanism for which $\Delta\mu_K$ is the driving force.

Materials and Methods

Necturus maculosus obtained from Graska Biological Supplies (Oshkosh, Wis.) were kept in a large aquarium at 4 °C. The animals were killed by a blow on the head and a double transection of the spinal cord was performed. The abdominal cavity was opened by median incision. The gallbladder was removed, separated from adhering liver tissue, emptied, and cut longitudinally. It was then washed free of bile residues with Ringer's solution and mounted (mucosal surface upward) as a flat sheet at room temperature (23 ± 1 °C) in a divided chamber. Both the luminal and the serosal surfaces of the tissue were independently and continuously superfused by gravity. The effective exposed area of gallbladder was 0.38 cm^2. Stopcock silicone grease (Dow Corning) was used to prevent edge damage and leakage. The serosal surface was supported by a stainless steel grid. A negative hydrostatic pressure of approximately 20 cm H_2O was applied to the serosal compartment both to attach the bladder to the supporting grid and to drive the serosal perfusion. Both superfusion solutions could be rapidly changed either by manual stopcocks or by an electrically activated solenoid valve (Model 330, Angar Scientific) located near the chamber inlets.

The Ringer's solutions contained, in mM: 102.5 NaCl + KCl, 1.0 $CaCl_2$ and 5.0 Tris-Cl. The pH was 8.2 and both perfusion solutions were continuously bubbled with 100 percent O_2.

Double-barreled microelectrodes were drawn from borosilicate glass capillary tubing (GCF-(2)-150-4; A-M Systems, Inc.) in a vertical puller (Model 700C, D. Kopf Instruments) calibrated to produce tips of 1 μm. They were then stored overnight in a desiccator at room temperature. This appeared to facilitate adequate and reproducible silanation of the barrel chosen to contain the liquid ion exchanger. This barrel was exposed to the vapor of dimethyl-dichloro-silane (Pierce Chemical Co.) at room temperature for 7.5 min and baked for 60 min at 150 °C. The open-tip barrel was back-filled with 1 M sodium formate and a drop of the appropriate liquid ion exchanger (Corning 477913 for Cl^-, and Corning 477317 for K^+-selective microelectrodes) was introduced by the same technique into the tip of the ion-selective barrel. This was then filled with 0.5 M KCl. Microelectrodes prepared by this procedure were calibrated in solutions containing 10, 20, 50 and 100 mM KCl. The slopes (S) obtained ranged from 52.8 to 61.5 mV/decade change in K^+ activity for K^+-selective barrels (mean value 56.1) and from 50.0 to 61.3 mV/decade in Cl^- activity for Cl^--selective barrels (mean value 56.0).

Intracellular K^+ and Cl^- activities were calculated from the equations:

$$a^i_K = (a^o_K + k_{KNa} a^o_{Na}) 10^{(V_K - V_a)/S}$$

$$a^i_{Cl} = a^o_{Cl} 10^{(V_a - V_{Cl})/S}$$

where V_a is the apical membrane potential measured by the open-tip barrel, V_K and V_{Cl} are the changes in potential recorded by the K^+-selective or the Cl^--selective barrel, respectively, upon impalement, S is the slope of the corresponding ion-selective barrel measured during calibration, a^o_{Na}, a^o_K and a^o_{Cl} are the external activities for these ions calculated assuming an activity coefficient of 0.76 and k_{KNa} is the selectivity coefficient of the K^+-selective barrel for K^+ against Na^+. k_{KNa} was measured by the separate solutions method (Moody & Thomas, 1971) in 0.1 M NaCl and KCl solutions (average value = 0.0230).

The electrical circuitry used in these experiments has been described in detail elsewhere (Garcia-Diaz et al., 1983). In brief, the transepithelial potential V_T was continuously measured by two calomel half-cells connected to the mucosal and the serosal solution, respectively, by either 3 M KCl-agar or Ringer's-agar bridges. The tissue was kept in the open-circuit mode except for brief periods when transepithelial current (I_T) pulses were applied via two AgCl-coated silver rings. Microelectrodes were connected through Ag/AgCl wires and a guarded coaxial cable to a high impedance ($>10^{15}$ Ω) FET-input electrometer (Analog Devices 515L) with capacitance neutralization. An electronic current-clamp device was used to control the I_T pulses and to calculate transepithelial resistance R_T and the fractional apical voltage ratio F_{Va}, that is, the ratio between the deflections in apical membrane potential ΔV_a and transepithelial potential ΔV_T produced by the I_T pulses. Each pulse had a duration of either 1.1 or 4.4 sec and the interval between individual pulses was 2.2 or 14 sec, respectively. Throughout these experiments the intensity of the I_T pulses was 15 μA (current density 39.5 μA/cm^2), in the serosal-to-mucosal direction. The electronic current-clamp device provided compensation of R_T and F_{Va} for the resistance of the external solutions. All potentials were measured with reference to the mucosal solution. The five parameters measured (R_T, V_T, F_{Va}, V_a and V_{Cl} or V_K) were displayed in digital panel meters. In addition, the last four were recorded on a four-channel strip-chart recorder (Gould-Brush, Mark 240). The basolateral membrane potential was calculated as $V_{bl} = V_T + V_a$.

Experimental Procedures

Microelectrodes were advanced perpendicularly to the tissue using a piezoelectric positioning device (Inchworm, Burleigh Instruments) attached to a manual micromanipulator (MM-33, Narishige). Initially, the micromanipulator was used to position the microelectrode close to the tissue. This was done under microscopic observation through a window in the chamber. The cells were then impaled through the mucosal membrane by advancing the piezoelectric device in steps of 4 μm. On some occasions an additional 2 μm advance or withdrawal of the microelectrodes was necessary following impalement in order to obtain a stable intracellular potential. Details of the criteria used to determine the acceptability of impalements have been described elsewhere (Garcia-Diaz et al., 1983).

Two different experimental protocols were used. In bilateral substitution experiments, both sides of the tissue were initially bathed with Ringer's solutions containing either 10 or 5 mM K^+. 30 min after mounting, the tissue was impaled with either a Cl^- or K^+-selective double-barreled microelectrode. Following a minimum of four acceptable impalements with one kind of selective microelectrode both the mucosal and the serosal solutions were changed to others containing either 1 or 0.5 mM K^+. In some cases, a microelectrode was successfully kept inside a cell during the change of solutions and a continuous record was obtained. In any case, another four acceptable impalements were obtained with the new bathing solution. In a few experiments it was possible to measure both Cl^- and K^+ activities in the same tissue in two different bathing solutions. Normally, only one ionic activity at two different external K^+ concentrations was measured in each tissue. In some cases, the sequence

of solutions was inverted. Instead of changing from a high to a low external K^+ concentration, experiments were performed in which the external K^+ concentration was increased from a low to a high value. K^+ concentration was always changed by a factor of 10. No difference was observed between these two sequences and the results from them were pooled.

In unilateral substitution experiments the serosal solution only was changed from Ringer's containing 10 mM K^+ to Ringer's containing 1 mM K^+. In these experiments continuous records only were accepted. Moreover, impalements were accepted only if the microelectrode stayed within the cell for at least 20 min following the change in solutions.

Results

Both the mucosal and the basolateral membranes of *Necturus* gallbladder cells are highly permeable to K^+ (Reuss & Finn, 1975*b*; van Os & Slegers, 1975; Reuss, 1979). Because of this, when the K^+ concentration of the solutions bathing one or both of these membranes is reduced, the interior of the cells becomes more negative, i.e., the membrane potential hyperpolarizes. Table 1 shows the values of V_{bl}, the basolateral membrane potential, at different external K^+ concentrations, calculated from impalements with double-barreled microelectrodes in bilateral substitution experiments. As explained in Materials and Methods, two different K^+ concentrations were usually applied to each tissue (either 10 and 1 mM or 5 and 0.5 mM) and at least four acceptable impalements were obtained with each solution. As Table 1 shows, there is a significant hyperpolarization when both external solutions are changed to others containing a lower K^+ concentration. The values of V_{bl} at external K^+ concentrations of 1 and 0.5 mM do not differ significantly. This may be due, to some extent, to the fact that the data shown in Table 1 for these two concentrations are unpaired results obtained with different animals. More importantly, however, although the K^+ concentrations in the bulk solutions are different, the real changes in K^+ concentration close to the membranes may well be too small to produce any significant change in membrane potential. For this reason, it is uncertain whether the V_{bl} values in the right-hand column of Table 1 accurately reflect the values of this parameter at a true external K^+ concentration of 0.5 mM. This uncertainty applies equally to the calculated K^+ chemical potential gradients under these conditions (Table 2 and Fig. 3). Qualitatively similar results, including the lack of changes in the membrane potential when small alterations in K^+ concentration are made at low external K^+ levels, were obtained by Teulon and Anagnostopoulos (1982) in *Necturus* kidney.

Table 1. Steady-state values of V_{bl} at four different external K^+ concentrations (bilateral substitution experiments)

K_o^+ (mM)	10	5	1	0.5
V_{bl} (mV)	46.0±0.9	55.4±1.0	71.5±2.0	69.3±1.6
N	15	15	15	15
P		<0.001	<0.001	>0.3

Average values ±SEM are shown. N=number of animals. Unpaired Student's t-test.

Table 2. Steady-state values of a_{Cl}^i, $\Delta\mu_{Cl}$ and $\Delta\mu_K$ at four different external K^+ concentrations (bilateral substitution experiments)

K_o^+ (mM)	10	5	1	0.5
a_{Cl}^i (mM)	26.5±1.9	19.9±1.0	15.1±1.1	14.5±1.2
P		<0.01	<0.01	>0.7
$\Delta\mu_{Cl}/F$(mV)	−28.6±1.8	−36.0±1.4	−42.9±1.7	−44.8±2.6
P		<0.01	<0.01	>0.6
N	5	10	5	10
$\Delta\mu_K/F$ (mV)	55.6±1.2	75.0±1.4	108.1±2.0	128.2±3.0
P		<0.001	<0.001	<0.001
N	10	5	10	5

Average values ±SEM are shown. N=number of animals. Unpaired Student's t-test.

Figure 1 shows a recording of an impalement with a Cl^- selective double-barreled microelectrode in which the microelectrode was successfully kept inside the cell during the change of solutions. Some of our criteria for the acceptability of impalements can be seen in this Figure. After impalement, there usually is a small depolarization of V_a accompanied by a small decrease of the fractional voltage ratio F_{Va}. This is probably due to damage caused to the cell membrane (Armstrong & Garcia-Diaz, 1981). When the membrane reseals around the tip of the microelectrode (usually in 1 or 2 min), V_a recovers and F_{Va} increases. In this study, at least five more minutes of a stable recording were required before an impalement was accepted. In Fig. 1, V_a and V_{Cl} were stable for 8 min before both bathing solutions were changed (from 10 to 1 mM K^+, note arrows). The recording was then continued until a new steady state was achieved. A further requirement for acceptability (Fig. 1) is that the potential registered by each of the two barrels before and after the impalement should differ by less than 2 mV.

In Fig. 1, during the first half of the recording at 10 mM external K^+, $V_a = -56.3$ mV, $F_{Va} = 0.89$ and $V_{Cl} = -25.4$ mV. This V_{Cl} value is equivalent to $a_{Cl}^i = 24.4$ mM. In the second half of the recording, following the change to a solution containing

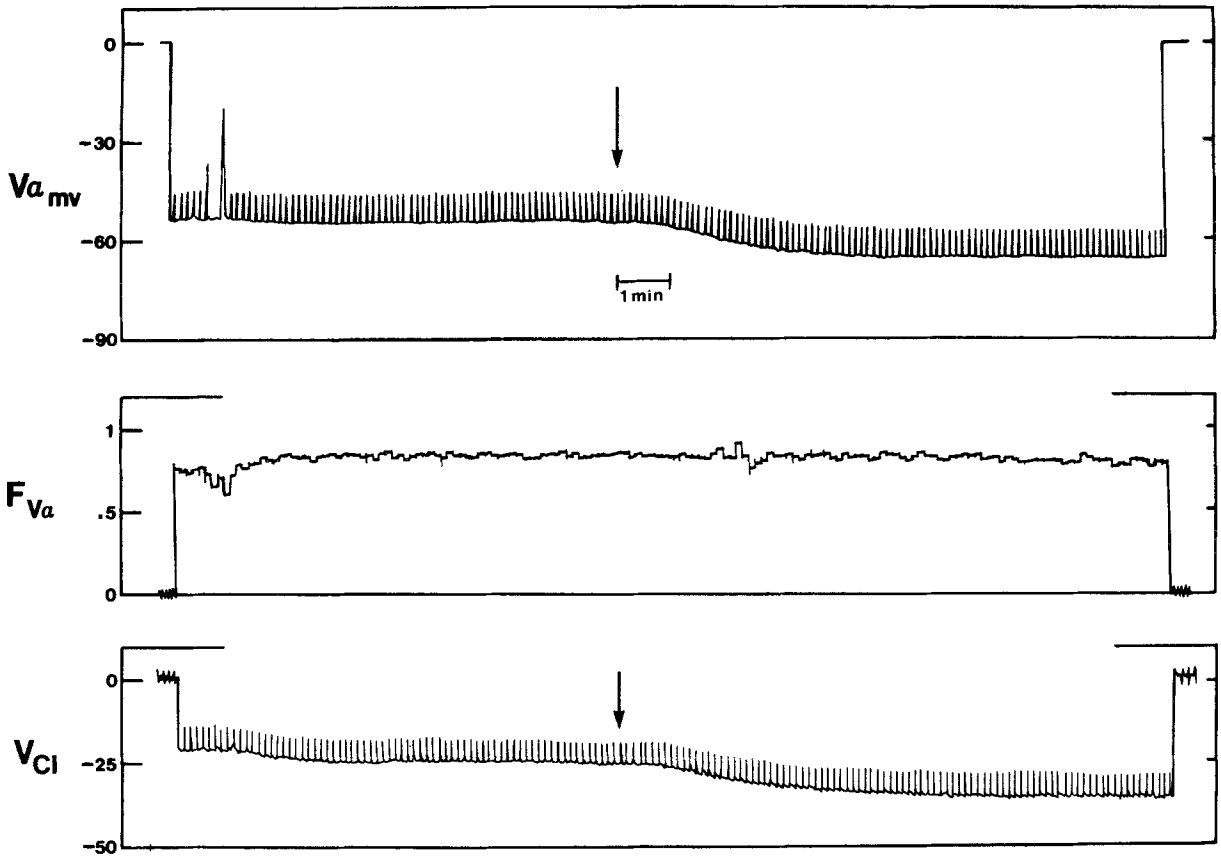

Fig. 1. Effects of bilateral reduction in external K^+ concentration during a single cell impalement. At the arrows, the K^+ content of both bathing solutions was changed simultaneously from 10 to 1 mM. The deflections in the V_a and V_{Cl} traces are due to transepithelial pulses (39.5 μA/cm^2; duration 1.1 sec; interval between pulses 2.2 sec)

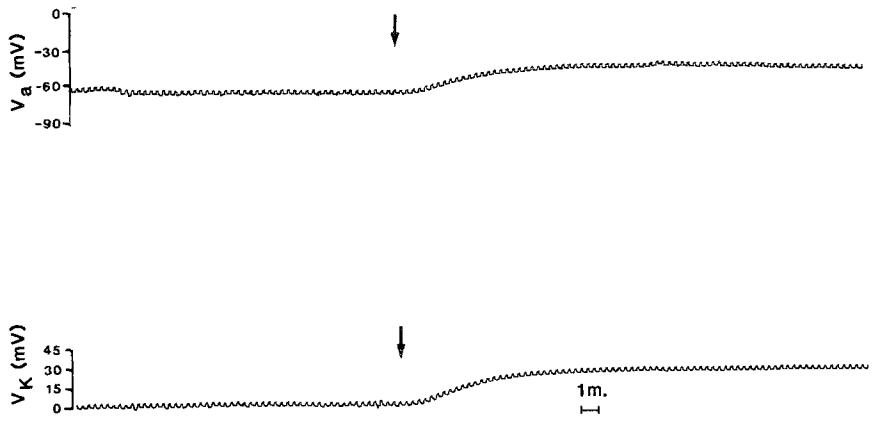

Fig. 2. Effects of a bilateral increase (from 1 to 10 mM) in external K^+ concentration. The arrows indicate the time at which the K^+ concentration of both bathing solutions was changed simultaneously. The transepithelial pulses (note deflections in the traces) had a duration of 4.4 sec and an interval of 14 sec

1 mM K^+, V_a hyperpolarized to -79 mV, F_{Va} decreased slightly to 0.82 and V_{Cl} hyperpolarized to -41 mV (equivalent to $a^i_{Cl}=17.9$ mM). It is clear from this Figure that, when the external K^+ concentration is decreased in both bathing solutions, the membrane potential V_a hyperpolarizes more than V_{Cl}, indicating a decrease in intracellular Cl^- activity. In this respect, Fig. 1 is representative of all the experiments reported herein.

Figure 2 shows a similar continuous record of an impalement with a K^+-selective double-barreled microelectrode during a change from 1 to 10 mM in the K^+ concentration of both external solutions. Note that this is the reverse of the change shown in Fig. 1, and, as already indicated, the direction of this change does not affect the results. In this experiment, the current pulses that produce the deflections in the traces were applied for 4.4 sec

with an interval of 14 sec between pulses, while in the experiment shown in Fig. 1, the shorter and more frequent pulses were applied. In the example shown in Fig. 2, with 1 mM K^+ in the bathing solutions, $V_a = -69.8$ mV and $V_K = +2.3$ mV, equivalent to $a_K^i = 58.3$ mM. At the arrows, both external solutions were changed to others containing 10 mM K^+. In the new steady state, V_a depolarized to -44.2 mV while V_K hyperpolarized to $+31.5$ mV, equivalent to $a_K^i = 67.7$ mM. Although the values of a_K^i obtained in these experiments were somehow lower than those reported previously by others (Reuss & Weinman, 1979; Garcia-Diaz & Armstrong, 1980) and similar to the values reported by Gunter-Smith and Schultz (1982), our criteria of acceptability of impalements rule out any possibility of permanent damage to the cells during impalements. These relatively low intracellular K^+ activities could be due, at least in part, to the pH of the external solutions. It is known that increasing the pH of the external solutions increases the K^+ permeability of both the mucosal and the basolateral cell membranes (Reuss, Cheung & Grady, 1981; Gogelein & Van Driessche, 1981) and that K^+ accumulates inside the cells of *Necturus* gallbladder above the value corresponding to electrochemical equilibrium (Garcia-Diaz & Armstrong, 1980). It is possible then that at pH 8.2, the steady-state intracellular K^+ activity is lower than that reported by others at lower pH values. As in the previous example (Fig. 1) with a Cl^--selective barrel, Fig. 2 shows that when the external K^+ concentration is increased in both bathing solutions by a factor of ten, a_K^i increases. The reverse is also true; i.e. a decrease of K^+ in both bathing solutions produces a decrease of a_K^i.

The measured values of a_K^i and a_K^i enable one to calculate the corresponding chemical potential gradients across the basolateral membrane according to the equation:

$$\frac{\Delta\mu_j}{z_i F} = \frac{RT}{z_i F} \ln \frac{a_j^i}{a_j^o}$$

where a_j^i and a_j^o are the activities of the ion j inside and outside of the cell, respectively, z_i is the valence of j and R, T and F have their usual meanings. Table 2 shows the values of a_{Cl}^i, $\Delta\mu_{Cl}/F$ and $\Delta\mu_K/F$ obtained at four different external K^+ concentrations in the bilateral substitution experiments reported herein. Note that, due to the uncertainty in the value of the membrane potential at $K_o^+ = 0.5$ mM already discussed, the value reported for $\Delta\mu_K/F$ at this K^+ concentration may not be very reliable. It is clear from Table 2 that, as indicated by Fig. 1, there is a relationship between a_{Cl}^i and

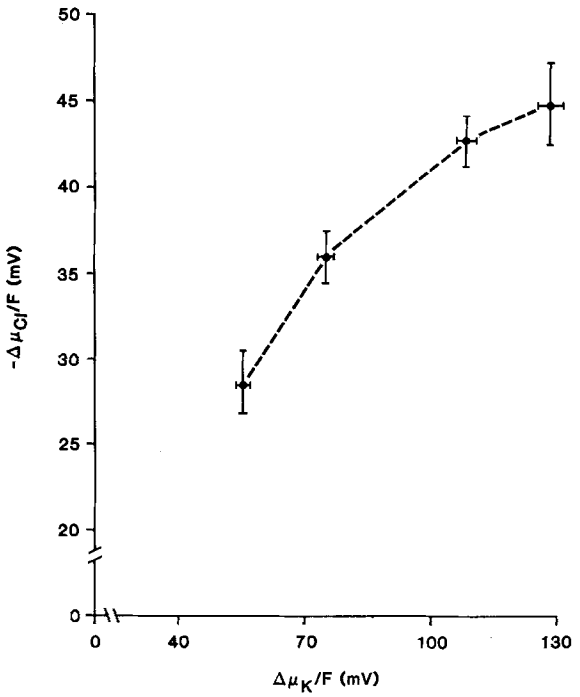

Fig. 3. Relationship between the chemical potential gradient for Cl^- ($\Delta\mu_{Cl}$) and the corresponding gradient for K^+ ($\Delta\mu_K$) across the basolateral membrane of *Necturus* gallbladder cells during bilateral substitution experiments. Average values ± SEM are shown

the external K^+ concentration. This is further illustrated by Fig. 3 which shows $-\Delta\mu_{Cl}/F$ as a function of $\Delta\mu_K/F$. There is an evident dependence, that does not seem to be linear, between $\Delta\mu_{Cl}$ (and, since all the experiments were carried out at constant external Cl^- concentration, $\Delta\mu_{Cl}$ is proportional to a_{Cl}^i) and $\Delta\mu_K$. In other words, the level of Cl^- inside the cells, at constant external Cl^- concentration, seems to depend on the driving force for electroneutral movement of K^+.

From the experimental results so far described it is not clear where the mechanism responsible for the dependence of a_{Cl}^i on $\Delta\mu_K$ is located. Since, in bilateral substitution experiments, $\Delta\mu_K$ is the same across the mucosal and the basolateral cell membranes, this mechanism could be present in either or both of these membranes. Thus, when the K^+ concentration of both bathing solutions is reduced, some at least of the KCl that leaves the cell could be delivered to the mucosal medium. The demonstration of coupled KCl exit to the mucosal solution would not enhance our understanding of transcellular Cl^- movement in the gallbladder under conditions where Na^+ and Cl^- transport is proceeding normally. It is the basolateral exit step in the process that is least understood at present.

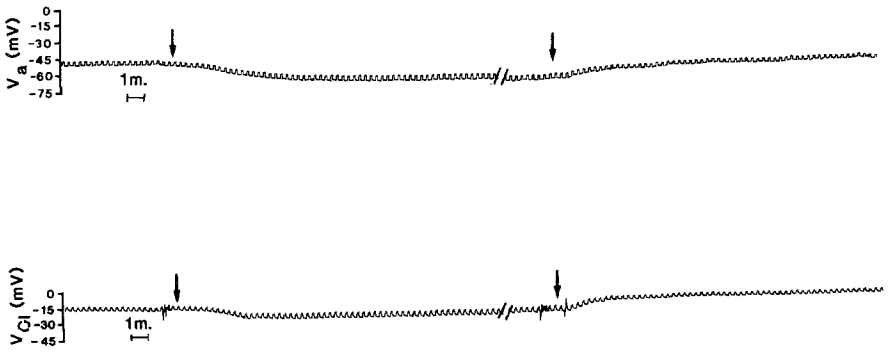

Fig. 4. Effect of unilateral changes in K^+ concentration. At the first arrows, the K^+ concentration of the serosal solution only was changed from 10 to 1 mM. At the second arrows, the K^+ concentration of the serosal solution was changed back to its initial value. The K^+ concentration of the mucosal solution was maintained constant at 10 mM throughout

To investigate the presence of coupled KCl transport in the basolateral cell membrane, unilateral substitution experiments were performed. In these experiments the K^+ concentration of the serosal solution only was changed from 10 to 1 mM or vice versa. The K^+ content of the mucosal medium was kept constant at 10 mM. During the change in the serosal solution, a Cl^--selective double-barreled microelectrode was kept inside a cell. Results were accepted only if continuous recording with the microelectrode was maintained for at least 20 min following the change in solution. To correct for the small diffusion potential arising from the asymmetry of the bathing solutions that resulted from this change, the salt bridge that connected the serosal solution to the calomel electrode was filled with agar plus Ringer's solution containing 10 mM K^+.

Figure 4 shows a record obtained in one such experiment. The record starts with both sides of the tissue bathed in Ringer's solution containing 10 mM K^+ and with a Cl^--selective double-barreled microelectrode in a cell. In this situation, $V_a = -45.7$ mV and $V_{Cl} = -16.3$ mV, equivalent to $a^i_{Cl} = 23.4$ mM. At the first arrows, the serosal solution was changed to Ringer's containing 1 mM K^+ while the mucosal solution remained unchanged. In this experiment, a new steady state was achieved 10 min after the substitution. At this time $V_a = -62.2$ mV and $V_{Cl} = -26.0$ mV, equivalent to $a^i_{Cl} = 17.6$ mM. In this case the steady state remained practically unchanged until 38 min after the change of the serosal solution. At this point (second arrows) the serosal solution was changed back to the original one containing 10 mM K^+. Following this change the values of V_a and V_{Cl} return to similar levels as at the begining of the experiment.

In some experiments, the new steady state after the change of solutions was not achieved as quickly as in the experiment shown in Fig. 4. Moreover, a^i_{Cl} continued to decrease for 10 to 20 min following the change of serosal solution. In one case, a small increase of a^i_{Cl} (1 mM) seemed to occur during the first five minutes after the change of solution. This was followed by the normal decrease and stabilization at around 20 min. Because of these different time courses, the V_{bl} and a^i_{Cl} values presented in Table 3 were obtained at least 20 min after the serosal solution was changed. All of them are steady-state values.

Table 3. Steady-state values of V_{bl} and a^i_{Cl} before and 20 min after the serosal solution was changed from one containing 10 mM K^+ to another containing 1 mM K^+. The mucosal solution remained unchanged with 10 mM K^+.

	Before	After	P
V_{bl} (mV)	48.1 ± 1.4	59.6 ± 2.5	< 0.001
a^i_{Cl} (mM)	23.3 ± 0.7	17.9 ± 1.0	< 0.001

Average ± SEM of 8 experiments. Paired Student's t-test.

Table 3 shows the results obtained in eight unilateral substitution experiments. It is evident that there is a significant hyperpolarization of the membrane potential and a decrease of the intracellular Cl^- activity. Although the results are qualitatively similar to those obtained in bilateral substitution experiments (*see* Tables 1 and 2), it is clear that the hyperpolarization in V_{bl} observed on decreasing the external K^+ concentration is lower in the experiments summarized in Table 3. This is consistent with the fact that both the mucosal and the basolateral membranes of *Necturus* gallbladder cells have high K^+ permeabilities. Therefore, both of them will contribute to the overall hyperpolarization observed when the K^+ concentration of both bathing media is decreased. Because of the highly conducting paracellular shunt pathway in the gallbladder, the different hyperpolarizations observed under these two experimental conditions (Tables 1 and 3) do not accurately reflect the contribution of the mucosal membrane during bilateral substitution.

Table 4. Steady-state values of V_{bl} and a^i_{Cl} before and after the K^+ concentration was changed from 10 to 1 mM, either in both bathing solutions (*A*) or in the serosal solution only (*B*)

	Before			After		
	A	*B*	*P*	*A*	*B*	*P*
V_{bi} (mV)	46.5±1.6	48.1±1.4	>0.6	72.2±3.0	59.6±2.4	<0.005
a^i_{Cl} (mM)	26.5±1.9	23.3±0.7	>0.1	15.1±1.1	17.9±1.0	>0.05
N	5	8		5	8	

Average values ±SEM are shown. *N*=number of experiments. Unpaired Student's *t*-test.

Table 4 compares directly the results obtained in those bilateral (*A*) and unilateral (*B*) experiments in which a^i_{Cl} was measured. The left-hand side of the Table shows the results obtained before substitution. In essence, these are the results obtained in two control groups of experiments under identical conditions (i.e., with 10 mm K^+ on both sides of the tissue). As can be seen, the membrane potential is practically the same in both groups (−46.5 and −48.1 mV; $P>0.6$). The values of a^i_{Cl} are statistically similar, the small difference between them being due to the fact that they represent experiments carried out at different periods of time with two different groups of animals. The right-hand part of the Table shows the values of V_{bl} and a^i_{Cl} after a change to Ringer's containing 1 mM K^+, either on both sides of the tissue (*A*) or on the serosal side only (*B*). It is clear that while the values of V_{bl} are significantly different between the two groups, the values of a^i_{Cl}, although significantly lower than those in the control groups, do not differ significantly from each other.

Discussion

Chloride accumulates within gallbladder epithelial cells to levels above that which corresponds to electrochemical equilibrium across the basolateral membrane of the cells. If the Cl^- permeability of the basolateral membrane were high enough to account for observed rates of transcellular Cl^- transport, Cl^- exit from the cells could be satisfactorily explained in terms of the outwardly directed Cl^- gradient. Most of the evidence so far available runs counter to this possibility. First, Reuss (1979) calculated, on the basis of results obtained in substitution experiments, that the highest possible value for the transport number of Cl^- (t_{Cl}) across the basolateral membrane of *Necturus* gallbladder cells is 0.06. Such a t_{Cl} value would imply a very low Cl^- permeability if the resistance of the basolateral membrane, R_{bl} is higher than 1000 Ω cm^2. Values of R_{bl} higher than this have been consistently obtained during the last ten years by several investigators, e.g. 2880 Ω cm^2 (Fromter, 1972), 2870 (van Os & Slegers, 1975) 2750 (Reuss & Finn, 1975*a*), 2320 (Reuss et al., 1979). Such values of R_{bl}, together with the value of t_{Cl} obtained by Reuss (1979), clearly indicate that the Cl^- permeability of the basolateral membrane of *Necturus* gallbladder cells is much too low to account for the rates of Cl^- transport in this epithelium. Detailed calculations based on these data are given by Reuss (1979). Second, Garcia-Diaz et al. (1983), showed that changes in the membrane potential induced by altering the pH of the solutions bathing *Necturus* gallbladder failed to produce any significant change of a^i_{Cl}. If the Cl^- permeability of one or both cell membranes were sufficiently high, one would expect a^i_{Cl} to depend on the membrane electrical potential.

Recently, a much lower value for R_{bl} has been reported by Suzuki, Kottra, Kampmann and Fromter (1982). These investigators obtained a value of about 200 Ω cm^2 for the resistance of the basolateral membrane of *Necturus* gallbladder cells. It is evident from the calculations outlined by Reuss (1979) that such a low value of R_{bl} would imply the existence of a Cl^- permeability across the basolateral membrane that is high enough to account for the normally observed rates of Cl^- transport. Such a low R_{bl} would enable Cl^- to diffuse passively from the cell into the serosal solution driven by its outwardly directed electrochemical potential gradient. No independent confirmation of these results has been reported and no convincing explanation has been given by Suzuki et al. (1982) for the discrepancy between their results and the results obtained in other investigations including earlier studies from the same laboratory (Fromter, 1972). As already mentioned, the lack of sensitivity of a^i_{Cl} to changes in the membrane potential (Garcia-Diaz et al., 1983), together with some of the results reported in this paper, indicate a low Cl^- permeability across the basolateral membrane and run counter to the idea of a low

R_{bl}. Taking everything into account, it seems questionable that the results obtained by Suzuki et al. are representative of a normal population of *Necturus*. Therefore the conclusion that the Cl^- permeability across the basolateral membrane of *Necturus* gallbladder cells is much too low to account for the observed rates of Cl^- exit across this membrane would appear, at this time, to be justified.

If the exit of Cl^- from the cells does not occur by passive diffusion and is not dependent on the membrane electrical potential, it must be mediated by an electroneutral mechanism. This could be a symport mechanism accompanied by a cation or an antiport mechanism in exchange for another anion. It is worth noting that such an electroneutral mechanism will be driven by the sum of the *chemical* potential gradients for Cl^- and the accompanying ion. From this point of view, Cl^- will be moving uphill, against its chemical potential gradient, since in normal conditions the activity of Cl^- is higher outside the cell than it is inside. The accompanying ion must have a chemical potential gradient that can compensate for the oppositely directed gradient for Cl^-.

Several investigators have raised the possibility that a KCl symport is the major mechanism for Cl^- exit across the basolateral membrane of gallbladder cells (Reuss, 1979; Garcia-Diaz & Armstrong, 1980; Gunter-Smith & Schultz, 1982). The activity of K^+ is much higher inside these cells than it is outside. Consequently, the chemical potential gradient for K^+ is outwardly directed and more than sufficient to compensate for the inwardly directed chemical potential gradient for Cl^-.

The ideal way to demonstrate a link between the chemical potential gradient for K^+ ($\Delta\mu_K$) and the movement of Cl^- out of the cells would be to change, $\Delta\mu_K$, keeping everything else constant, and measure the values of a^i_{Cl} at different $\Delta\mu_K$ values. Unfortunately this is not possible. Both the mucosal and the basolateral membranes of gallbladder cells have high K^+ permeabilities. Any experimental manipulation that would change $\Delta\mu_K$ by altering the K^+ concentration on one side of the membrane, would inevitably affect the membrane electrical potentials. This occurred in the experiments reported herein. There is a clear relationship (Table 1) between the basolateral membrane potential and the K^+ concentration in the bathing solutions. This relationship falls short of the 58 mV per decade change in external K^+ concentration predicted for a perfect K^+ electrode. This indicates that, in *Necturus* gallbladder cells, one or both membranes are permeable to other ions. Since the Cl^- permeabilities of both the apical and basolateral cell membranes and the Na^+ permeability of the basolateral membrane are extremely low (van Os & Slegers, 1975; Reuss, 1979; Garcia-Diaz et al. 1983), the attenuation of the effect of external K^+ on membrane potential probably arises from the Na^+ permeability of the apical membrane. Compared to the K^+ permeability, this has been estimated to lie in the range of 0.10 to 0.37 (Reuss & Finn, 1975; van Os & Slegers, 1975). An apical Na^+/K^+ permeability ratio within this range could, when the effect of the paracellular shunt is taken into consideration, account for the results we observed. However, taking into consideration that a^i_{Cl} in these cells is practically independent of the membrane potential (Garcia-Diaz et al., 1983), the results shown in Table 2 and Fig. 3 show a clear correlation between a^i_{Cl} (or $\Delta\mu_{Cl}$) and $\Delta\mu_K$. Reducing the K^+ concentration in both bathing solutions reduces a^i_K (Fig. 2) but increases $\Delta\mu_K$ (Table 2). This increased $\Delta\mu_K$ drives a^i_{Cl} to a lower value. If one can exclude increases in cell volume under these conditions, these results indicate that increasing $\Delta\mu_K$ increases Cl^- exit of the cells. Although no direct measurements of cell volume were made during the present study, there is evidence that a decrease of K^+ concentration in both bathing solutions produces a reduction in cell volume (M. Larson and K.R. Spring, *personal communication*). If indeed, reduction of external K^+ results in a decrease in cell volume, the increase in Cl^- exit produced by an increase of $\Delta\mu_K$ is even larger than it appears to be from Table 2 and Fig. 3.

It seems unlikely that the results described herein can be attributed to a partial inhibition, with decreasing external K^+ concentration, of the basolateral Na^+-K^+ pump. It could be argued that such inhibition might increase a^i_{Na}, decrease the rate of transapical NaCl entry, and thus lower a^i_{Cl}. However, several aspects of our results do not appear to be consistent with this idea. First, the K_M for the effect of external K^+ on the rate of Na^+ pumping is about 0.5 mM in a number of tissues (Glynn & Karlish, 1975). Assuming that a similar K_M applies to the basolateral Na^+-K^+ pump in gallbladder epithelial cells, it is clear that no significant reduction in pump rate would be expected when the external K^+ concentration is lowered from 10 to 5 mM. Nevertheless (Table 2) this maneuver caused a substantial reduction in a^i_{Cl}. Second, if a lowered pump rate, in addition to KCl symport, were acting to reduce a^i_{Cl} when the external K^+ concentration was decreased to 1.0 or

0.5 mM, one would predict a much greater fall in a^i_{Cl}, under these conditions, than was actually observed (Table 2).

The results obtained in experiments in which the K^+ concentration of both bathing solutions is changed simultaneously, although they indicate a relationship between a^i_{Cl} and $\Delta\mu_K$, leave two questions unanswered. First, where is the mechanism responsible for this relationship located? In bilateral substitution experiments, the same $\Delta\mu_K$ exists across both the mucosal and the basolateral membranes of the cell. It is possible, in principle, that a KCl symport mechanism could be located in either or both of these membranes. It is evident that the existence of such a mechanism in the mucosal membrane, although interesting *per se,* will not explain the mechanism of transepithelial transport of Cl^-. Second, bilateral substitution experiments do not provide a clear-cut proof that a^i_{Cl} does not depend on the membrane potential. As implied earlier in this discussion, the case against such a dependence in bilateral substitution experiments, rests heavily on the Cl^- permeability of the basolateral cell membrane. Given a sufficiently high Cl^- permeability across this membrane, the hyperpolarization of the membrane potential produced by a decrease in external K^+, could be the driving force for increased Cl^- exit and decreased a^i_{Cl}.

The results obtained in the unilateral substitution experiments (Table 3 and Fig. 4) give an answer to these two questions. They show that a^i_{Cl} decreases when the K^+ concentration is reduced in the serosal solution only. In this situation, $\Delta\mu_K$ across the mucosal membrane either does not change or decreases, so that the dependence of a^i_{Cl} on $\Delta\mu_K$ is clearly located in the basolateral membrane of the cells. Moreover, a comparison of the results obtained in the bilateral and unilateral substitution experiments (Table 4) shows that these two manipulations, although producing significantly different hyperpolarizations of the membrane potential, reduce a^i_{Cl} to essentially similar values. This provides a convincing evidence, in our experiments, against an important role for the membrane potential in determining a^i_{Cl}. The small, statistically borderline, difference between the values of a^i_{Cl} observed under the two experimental conditions, may be due to different volume changes in response to unilateral and bilateral changes of K^+ concentration, respectively, or may reflect the small influence that the membrane potential has on a^i_{Cl}.

In any case, the experiments reported in this paper show that the activity of chloride in *Necturus* gallbladder epithelial cells is dependent on the chemical potential gradient of K^+ across the basolateral membrane of the cells. This strongly supports the existence of a neutral symport mechanism in this membrane that transports KCl from the cell into the serosal solution.

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