Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5212–5216, July 1989
Physiological Sciences

Reconstitution of an inwardly rectifying potassium channel from the basolateral membranes of *Necturus* enterocytes into planar lipid bilayers

(small intestine)

JAMES COSTANTIN, SOCORRO ALCALEN, ANGELA DE SOUZA OTERO*, WILLIAM P. DUBINSKY, AND STANLEY G. SCHULTZ†

Department of Physiology and Cell Biology, University of Texas Medical School, Houston, TX 77225

Communicated by Gerhard Giebisch, March 21, 1989

ABSTRACT **Basolateral membrane vesicles from *Necturus* enterocytes, highly (>20-fold) enriched in Na^+,K^+-ATPase, were reconstituted into planar lipid bilayers. The principal channel activity observed is selective for K^+ over Na^+ and Cl^-. This K^+ channel is blocked by Ba^{2+} and *Leiurus quinquestriatus* venom but is *not* affected by Ca^{2+} over the range of 10^{-3} to $<10^{-7}$ M and is *not* inhibited by charybdotoxin. *L. quinquestriatus* venom also markedly reduces the conductance of the basolateral membrane of intact villus cells of *Necturus* small intestine. The open-time probability (P_o) of the channel displays a voltage-dependence characteristic of an "inward rectifier"; i.e., the channel inactivates when the basolateral membrane is depolarized and P_o increases with increasing hyperpolarization of that barrier. Assuming that similar properties prevail under physiological conditions, this characteristic could provide, in part, an explanation for the parallelism between Na^+-pump and K^+-leak activities of the basolateral membrane observed in this epithelium. Thus, an increase in rheogenic Na^+-pump activity at the basolateral membrane would hyperpolarize that barrier and, in turn, increase the open time of this K^+ channel.**

The results of electrophysiological studies on a variety of Na^+-absorbing epithelial cells have disclosed parallelisms among the rates of Na^+ entry across their apical membranes, the Na^+-pump activities at their basolateral membranes, and the K^+ conductances of their basolateral membranes. Although the physiological utility of these "pump–leak" parallelisms is clear, their underlying mechanisms have not been resolved (1, 2).

Two methods have been developed that permit the investigation of the properties and regulation of single ionic channels at the molecular level—i.e., the patch-clamp technique and techniques for reconstituting ionic channels into planar phospholipid bilayers. The application of the patch-clamp technique to the study of single-channel activities in the basolateral membranes of Na^+-absorbing epithelial cells is, however, restricted by the fact that in many instances those membranes are bounded by subepithelial layers of connective tissue, smooth muscle, etc., which preclude the formation of gigaohm seals.

In this paper we describe a method for isolating a highly enriched preparation of basolateral membranes from *Necturus* enterocytes, the reconstitution of these vesicles into planar phospholipid bilayers, and the general properties of a K^+ channel present in those membranes.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Isolation of Basolateral Membranes. Male *Necturus maculosa* were anesthetized by immersion in a 0.1% tricaine solution. The small intestine was removed and immersed in an ice-cold amphibian Ringer's solution, and the mucosal cells were scraped off with a glass slide and transferred to an isolation medium consisting of 180 mM sucrose and 2 mM Tris adjusted to pH 7.4 with Hepes (Tris/Hepes). All of the subsequent steps in the isolation procedure were carried out at 4°C.

The membrane fractionation procedure employed is a modification of that described by Boumendil-Podevin and Podevin (3). Mucosal scrapings, 0.9 g per intestine, were homogenized in 30 volumes of isolation medium, first in a Dounce glass homogenizer and then in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 1000 × *g*; all centrifugations were carried out with the automatic brake off. The resulting pellet was discarded, and the supernatant was centrifuged for 15 min at 22,000 × *g*. This resulted in a pellet consisting of a loosely packed (fluffy) upper portion and a densely packed lower portion. The loosely packed portion was carefully aspirated and resuspended to a final volume of 12 ml in the isolation medium by using a glass homogenizer with a Teflon pestle; the supernatant and the lower pellet were discarded.

The suspension was mixed with 1.4 ml of Percoll and centrifuged for 35 min at 42,000 × *g*. This resulted in two bands located at the extremes of the self-forming gradient. The top 2.4 ml of the gradient consisted of a band enriched in basolateral membranes (BLMV) (see *Results*). To remove the Percoll, this fraction was diluted with a minimum of five volumes of a solution consisting of 60 mM KCl, 60 mM sucrose, and 2 mM Tris/Hepes (pH 7.4) and centrifuged for 90 min at 60,000 × *g* in a Beckman 60Ti rotor. The membrane fraction was carefully removed from the top of the Percoll pellet and resuspended in the isolation medium. This membrane fraction as well as aliquots of the total homogenate were stored in liquid nitrogen. Prior to being assayed, the samples were rapidly thawed at 37°C and immediately transferred to ice. Control experiments performed on fresh preparations established that the pertinent enzymatic activities (see below) are preserved in the frozen samples.

Assay Procedures. Na^+,K^+-ATPase activity was assayed at 22°C by spectrophotometric determination of NADH oxidation with a coupled enzyme system as described previously

Abbreviations: BLMV, basolateral membrane vesicles; LQV, *Leiurus quinquestriatus* venom; CTX, charybdotoxin.

*Present address: Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.
†To whom reprint requests should be addressed.

(4) using a modification of the assay medium described by Kagawa (5). The reaction was initiated by the addition of 10–50 μg (2–10 μl) of membrane protein to two cuvettes containing 0.6 ml of assay medium. The decrease in absorbance at 340 nm was recorded for 2 min. Lubrol WX [final concentration of 0.1% (wt/vol)] was then added to one cuvette, while the other cuvette received an equal volume of water. After 3 min, ouabain (final concentration of 0.3 mM) was added to both cuvettes, and the rate of NADH oxidation was monitored for an additional 5 min.

Alkaline phosphatase, NADH dehydrogenase, and succinic dehydrogenase activities were assayed as described (6, 7). Acid phosphatase activity was determined at 37°C, according to the procedure of Nelson (8). Protein was quantitated by the method of Lowry *et al.* (9).

Channel Reconstitution, Data Acquisition, and Analyses. Mueller–Rudin planar bilayers containing phosphatidylethanolamine (10 mg/ml) and phosphatidylserine (10 mg/ml) dissolved in decane were painted over a 0.33-mm aperture in a Delrin cup inserted into a cut-away polyvinyl chloride block as described elsewhere (10). The cup contained the cis compartment to which the basolateral membrane preparation was added; the trans compartment was formed by the polyvinyl chloride block. In most experiments the cis compartment initially contained 450 mM KCl and the trans compartment contained 50 mM KCl; in addition, in most experiments, both compartments contained 0.5 or 1 mM $CaCl_2$ and were buffered to pH 7.0 with 10 mM Hepes (K^+ salt). As discussed elsewhere, the presence of Ca^{2+} in the cis compartment and the large osmotic difference across the bilayer were employed to promote fusion (11). After the addition of membranes (0.05–0.1 mg of protein per ml), the cis compartment was stirred with a magnetic stirring bar; stirring was terminated when channel activity was detected. Channel activity was observed within 30 min after introducing the membranes to the cis compartment in ≈20% of the attempts. In some experiments, the cis compartment was diluted to a final concentration of 150 mM KCl while the trans compartment contained 50 mM KCl. In the *Results*, we will employ the notation 450/50 or 150/50 to designate the KCl concentrations in the cis/trans compartments, respectively.

Channel activity was monitored by a List EPC-7 amplifier, visualized with a Nicholet digital oscilloscope, and recorded in digital form on video tape employing an analog–digital converter (model PCM-2; Medical Systems, New York). For analysis, the analog signal derived from the digital record was passed through an 8-pole Bessel filter with the corner frequency (−3 decibels) set at 500 Hz and then digitized (with a Keithley system 570) with a sampling frequency of 3000 Hz. Data were analyzed by using a program written by Hubert Affolter and kindly provided by Roberto Coronado. Open events are detected through the use of two discriminators: the first (the closed discriminator) set at 1 SD above the mean baseline current and the second (the open discriminator) set at 1 SD below the mean single channel current. Open events are defined as transitions that cross both discriminators and remain above the open discriminator for at least two sampled points (i.e., >0.66 ms). As per convention, a positive current reflects the flow of cations from the cis to the trans compartment or the flow of anions in the opposite direction. The "holding voltage," V_m, is defined as the electrical potential of the cis compartment with reference to that of the trans (ground) compartment.

At the conclusion of every experiment, the contents of the cis and trans compartments were removed for analyses of K^+ concentrations by flame photometry and, when relevant, Na^+ and pH. Ca^{2+} activities were determined by using an ion-selective electrode (Orion, model 93-20) corrected for ambient KCl concentrations.

Experiments on Intact Tissues. Microelectrode impalements of strips of *Necturus* small intestine were performed as described by this laboratory (12).

All experiments were carried out at room temperature (22°C).

Materials. *Necturus* were obtained from Nasco (Oshkosh, WI) and maintained at 4°C in tap water. Mg^{2+}-ATP, phospho*enol*pyruvate, NADH, *p*-nitrophenyl phosphate, pyruvate kinase, lactic dehydrogenase, Tris, Hepes, EGTA, and *Leiurus quinquestriatus* venom (LQV) were obtained from Sigma. Percoll was obtained from Pharmacia. Lipids were obtained from Avanti Polar Lipids, and mixtures were freshly prepared daily. Charybdotoxin (CTX) was kindly provided by Christopher Miller.

Statistical variance is expressed as the standard error of the mean.

RESULTS

Biochemical and Ultrastructural Characterization of the Basolateral Membrane Preparation. The recoveries and specific activities of marker enzymes in the total homogenate and the BLMV fraction are given in Table 1. It can be seen that while the BLMV fraction comprised only 1% of the total protein in the homogenate, it contained 28% of the total Na^+,K^+-ATPase activity enriched ≈23-fold over that in the homogenate. At the same time, this fraction was minimally contaminated by marker enzymes for the brush border (alkaline phosphatase), mitochondria (succinic dehydrogenase), lysosomes (acid phosphatase), or endoplasmic reticulum (NADH dehydrogenase).

Electronmicrographs of the BLMV pellet (data not shown) revealed the presence of simple membrane structures, many in the form of vesicles, with no evidence of brush-border structures and minimal contamination with mitochondria, lysosomes, endoplasmic reticulum, or nuclear envelopes.

Table 1. Biochemical characteristics of the BLMV fraction

	Homogenate	BLMV
Na^+,K^+-ATPase		
Activity	1.2 ± 0.7	28 ± 6
Enrichment factor	1.0	23.0
Recovery, %	100	28 ± 8
Mg^{2+}-ATPase		
Activity	1.4 ± 0.7	5 ± 1
Enrichment factor	1.0	3.7
Recovery, %	100	3 ± 1
Alkaline phosphatase		
Activity	3.8 ± 0.9	7 ± 2
Enrichment factor	1.0	1.8
Recovery, %	100	2 ± 1
Succinic dehydrogenase		
Activity	0.1 ± 0.5	0.06 ± 0.03
Enrichment factor	1.0	0.4
Recovery, %	100	0.5 ± 0.3
NADH dehydrogenase		
Activity	16 ± 4	31 ± 17
Enrichment factor	1.0	1.9
Recovery, %	100	2 ± 1
Acid phosphatase		
Activity	2.4 ± 1.0	9 ± 3
Enrichment factor	1.0	3.7
Recovery, %	100	3 ± 2
Protein recovery, %	100	1.1 ± 0.5

Activities are expressed in μmol per hr per mg of protein; enrichment factors are the ratios of the enzyme activity in the fraction to that in the total homogenate; recoveries are presented as the percent of the homogenate. Values are means ± SEM of seven preparations except for acid phosphatase, which was determined in three preparations.

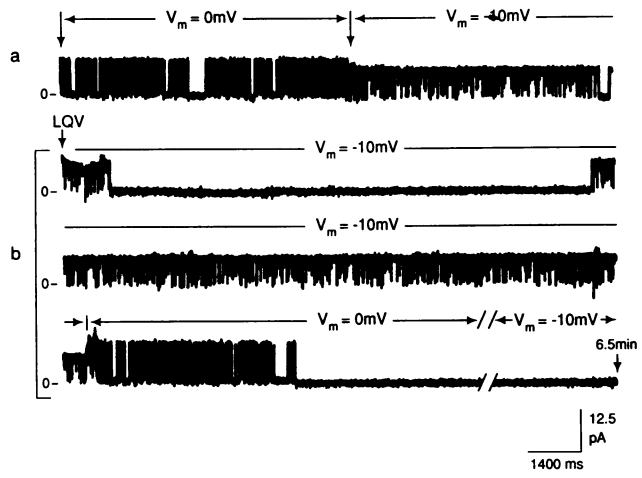

FIG. 1. (*a*) Tracing of a single "flickering" channel when V_m = 0 mV and V_m = −10 mV. (*b*) These three rows are continuous recordings of the channel activity illustrated above following the addition of LQV (arrow) to the trans compartment. This venom brought about a long block of channel activity followed by a brief reappearance of activity that, in turn, was followed by the complete disappearance of activity for 6.5 min.

Kinetics, Current–Voltage Relations, and Ion Selectivity. In virtually every instance, channels reconstituted into the lipid bilayer exhibited cation selectivity as evidenced by upward deflections when V_m = 0 mV. A tracing of channel activity in the presence of 450/50 is shown in Fig. 1*a*.

The current–voltage relation of the channel (I_c vs. V_m) in the presence of 450/50‡ is illustrated in Fig. 2. This relation is linear over the range studied ($r = 0.95$, $P < 0.01$). The slope corresponds to a single-channel conductance, g_c, of 330 pS. The reversal potential, E_r, of −36 mV does not differ significantly from the Nernst equilibrium potential for K^+, which, correcting for activity coefficients, averaged −40 ± 4 mV. The data are consistent with a ratio of P_K/P_{Cl} = 19, where P_i is the permeability coefficient of ion *i*.

I_c vs. V_m relations obtained first in the presence of 150/50‡ and then after the addition of 100 mM or 150 mM NaCl to the trans compartment are shown in Fig. 3*a* and Fig. 3*b*, respectively. In all instances the I_c vs. V_m relations are linear, and g_c averaged 190 pS. In these experiments P_K/P_{Cl} (calculated using the equation for a biionic diffusion potential) averaged 10, and P_K/P_{Na} (calculated using the Goldman–Hodgkin–Katz "constant-field equation"; ref. 13) averaged 12.

Thus, this channel is selective for K^+ over Na^+ and Cl^- and, in view of its high conductance, falls into the category of "maxi" K^+ channels (14).

Other Characteristics. The addition of 5–10 mM Ba^{2+} to the trans compartment resulted in a marked decrease in channel activity. This was observed in the presence of either 450/50 or 150/50. In five such experiments, the effect of this well-established blocker of K^+ channels was limited to a marked decrease in the open-time probability, P_o, with no effect on single-channel conductance.

Channel activity was also completely blocked by LQV (50 μg/ml) added to the trans compartment alone (n = 7) (Fig. 1*b*); this venom was ineffective when introduced into the cis compartment alone even at concentrations as high as 500 μg/ml. However, CTX, a component of LQV, did not affect channel activity when added to the trans compartment to final concentrations as high as 100 nM (n = 9). In one experiment, the addition of 50 nM CTX to the trans compartment had no effect on channel activity, but the subsequent addition of LQV (50 μg/ml) was followed by prompt cessation of that activity.

‡These are the initial concentrations. They differ slightly from the final concentrations due to the small amount of mixing that takes place when the bilayer breaks and is repainted. The predicted values of the reversal potentials were calculated using the measured final concentrations of K^+ and, when relevant, Na^+.

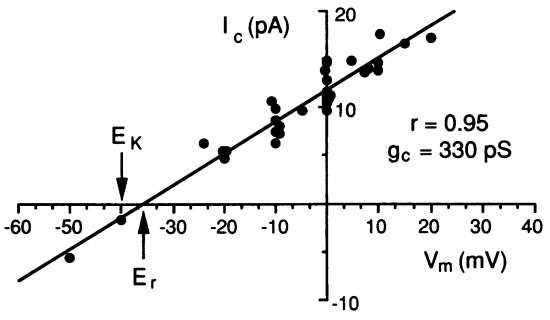

FIG. 2. The I_c–V_m relation in the presence of 450/50. The predicted reversal potential for an ideally K^+-selective channel, E_K, is indicated by the arrow. The 29 data points were obtained from seven experiments.

Finally, channel activity was not affected by the addition of 10 mM EGTA (in the form of the K^+ salt) to the cis and trans compartments, which reduced the Ca^{2+} activities to below 10^{-7} M. Indeed, these channels could be reconstituted into the bilayers in the presence of KCl solutions containing 1 mM EGTA (K^+ salt) to which no Ca^{2+} was added; under these conditions the estimated Ca^{2+} activity is less than 40 nM.

Voltage Gating of the K^+ Channel. An example of the relation between P_o and V_m in the presence of 450/50 is illustrated in Fig. 4. Clearly, P_o approached unity when V_m = −10 mV and sharply declined as V_m was depolarized. In 10 experiments in the presence of (450/50), P_o averaged 0.95 ± 0.01 when V_m averaged −10 ± 1 mV and averaged 0.14 ± 0.02 when V_m averaged 13 ± 2 mV. Similar voltage gating was observed in the presence of 150/50; P_o averaged 0.96 ± 0.04 and 0.28 ± 0.05 (n = 5) when V_m averaged −25 mV and +25 mV, respectively.

Effect of *Leiurus* Venom on Intact Tissues. To strengthen the argument that the reconstituted K^+ channels are derived from the basolateral membranes of villus absorptive cells, a series of studies were performed in which the electrical potential difference across the apical membrane, ψ^{mc}, and the ratios of the slope resistance of the apical membrane (r^m) to that of the basolateral membrane (r^s) (i.e., r^m/r^s) were determined (12) from microelectrode impalements of intact sheets of small intestine before and after the introduction of

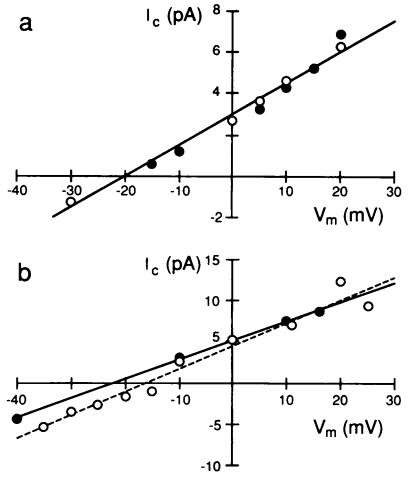

FIG. 3. The relation between I_c and V_m first in the presence of 150/50 (●) and then after the addition of 100 mM NaCl (*a*) or 150 mM NaCl (*b*) to the trans compartment (○).

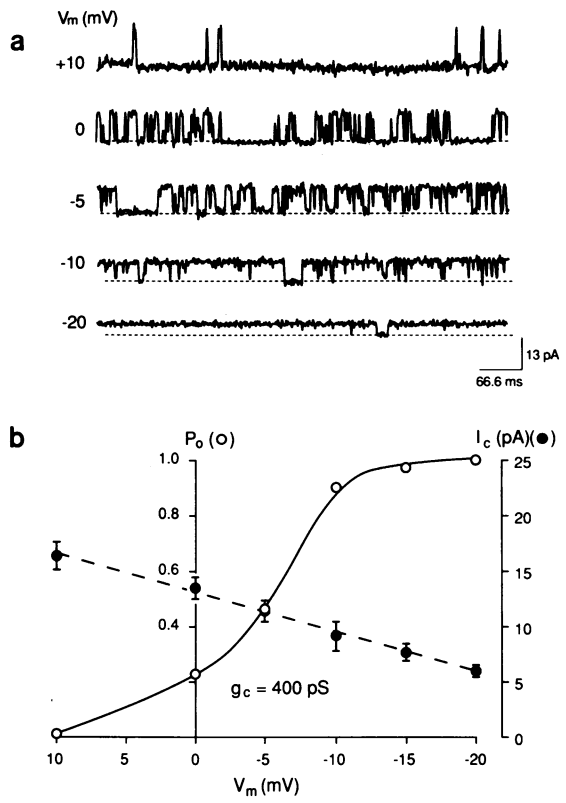

FIG. 4. An example of the effect of V_m on P_o in the presence of 450/50. (*a*) Tracings of channel activities at the indicated values of V_m. (*b*) Relations among P_o, I_c, and V_m.

LQV (0.5 mg/ml) into the solution perfusing the serosal surface of the tissue.

As shown in Fig. 5, after a variable delay of 3–6 min, attributable to the time required for diffusion of the venom through the villus core of connective tissue to reach the basolateral membranes, there is a gradual decrease in (r^m/r^s), which often reached indiscernible values. This decrease is associated with a significant depolarization of ψ^{mc} consistent with the conclusion that LQV blocked K^+-conductive pathways in the basolateral membranes.

These findings together with the finding that LQV blocks the reconstituted channel from the trans side but is ineffective from the cis side establishes the orientation of this channel in the bilayer. Thus, in spite of the fact that the vesicles have a mixed orientation, they always appear to fuse so that the cis chamber corresponds to the intracellular compartment and the trans chamber corresponds to the extracellular (serosal) compartment. It follows that these channels are inactivated when the basolateral membrane of the enterocyte is *depolarized* and thus parallel the behavior of "inward (or anomalous) rectifiers" observed in a wide variety of cell membranes (15).§

DISCUSSION

The results of these studies conclusively demonstrate the presence of a K^+ channel in membranes of *Necturus* enterocytes that can be reconstituted into phospholipid planar

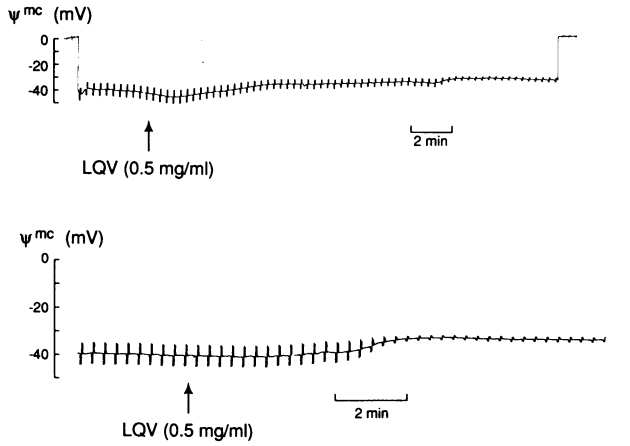

FIG. 5. Effect of LQV on ψ^{mc} and r^m/r^s of two different intact villus enterocytes. As discussed previously (12), the periodic deflections in the tracings of ψ^{mc} are the results of the periodic passage of bipolar constant current pulses across the epithelium. A decrease in the magnitude of these deflections indicates a decrease in r^m/r^s that in these experiments is the result of an increase in r^s.

bilayers. The presumption that these channels originate from the basolateral membranes of these enterocytes is based on the findings that (*i*) the membrane preparation is highly enriched in the Na^+,K^+-ATPase and, by biochemical and ultrastructural criteria, is minimally contaminated by apical or intracellular membranes, and (*ii*) Ba^{2+} (16) and LQV block both the reconstituted K^+ channel and the basolateral membrane conductance of Na^+-absorbing villus cells. Further, in view of the consistent properties exhibited by this K^+ channel, it seems unlikely that it originates, incidentally, from minor contaminants. Nonetheless, patch-clamp studies on the basolateral membranes of isolated villus enterocytes from *Necturus* small intestine will be necessary to unequivocally establish the correspondence between the properties of these reconstituted channels and those of the intact cell. Sheppard *et al.* (17) have recently reported the presence of a Ca^{2+}-activated, maxi K^+ channel in excised patches of membranes from isolated *Necturus* enterocytes that was *inactive* in the cell-attached configuration. Such a channel was not evident in our studies. Further, it could not be responsible for the effect of LQV on the intact villus enterocyte illustrated in Fig. 5. Further studies are necessary to resolve this matter.

The conductance of this channel in the presence of 450/50 and 150/50 is high and clearly places it into the category of maxi channels (see table 1 of ref. 14). At the same time, its activity is not affected by changes in the cis (intracellular) Ca^{2+} activity over the range 1 mM to ≈40 nM. Further, its voltage dependence is diametrically opposite to that described for Ca^{2+}-activated maxi K^+ channels; the latter are activated by depolarizing voltages (18), whereas the present channels are activated by hyperpolarizing voltages. Finally, the channel is not blocked by CTX at a concentration as high as 100 nM. This toxin, which comprises only 0.1% of the crude LQV, is a blocker of Ca^{2+}-activated K^+ channels found in a variety of cells with half-maximum inhibition observed at concentrations less than 10 nM (18–22). On the bases of these findings, we conclude that the reconstituted channel is a high-conductance K^+ channel that is not Ca^{2+}-activated and is inhibited by some component of LQV other than CTX.¶

§The term inward (or anomalous) rectification is traditionally employed to describe macroscopic K^+ conductances that decrease when the membrane is depolarized and increase when it is hyperpolarized with reference to the resting potential; i.e., the conductance for an outward K^+ current is much less than that for an inward current (15). The K^+ channel described in this paper has a voltage-independent single-channel conductance, but P_o decreases when the basolateral membrane is depolarized. Thus, the macroscopic K^+ conductance of that barrier attributable to an ensemble of these K^+ channels would conform with this classic definition.

¶LQV contains approximately 50 proteins (18) including a low molecular weight polypeptide that is distinct from CTX and whose sequence resembles that of noxiustoxin (NTX) isolated from the Mexican scorpion, *Centruroides noxius*. NTX has a number of homologies with CTX but appears to have a broader range of K^+-channel-blocking ability (20–22).

Perhaps the most interesting characteristic of this channel is its inward-rectifying properties. Such channels have been identified in a number of cell types and, in some, have been shown to be regulated by adenine nucleotides, guanine nucleotide-binding proteins, cAMP-dependent phosphorylation, and intracellular Mg^{2+} (21). Thus, at this stage of our investigation, it is impossible to relate the properties of the reconstituted channel reported in this paper to those in the intact cell. Nonetheless, it is intriguing to speculate about the physiological significance of this channel assuming that its voltage-gating properties reflect those of the K^+-conductive pathways in the basolateral membrane of the intact enterocyte. If so, under physiological conditions the channel would be in the activated state and contribute to the conductance of the basolateral membrane and, thus, to the transapical and transbasolateral electrical potential differences; this conclusion is consistent with the findings illustrated in Fig. 5.

In addition, we have previously demonstrated that the addition of galactose or alanine to the solution bathing the mucosal surface of *Necturus* small intestine brings about a rapid depolarization of ψ^{mc}, which often reverses polarity (i.e., the cell interior becomes electrically positive with respect to the mucosal and serosal solutions) and a simultaneous decrease in the value of r^m/r^s. This initial response is followed by a slower repolarization of ψ^{mc} that is paralleled by increases in r^m/r^s and the rate of transcellular Na^+ transport (as reflected by the short-circuit current) (12). This slower response is, at least in part, due to an increase in the K^+ conductance of the basolateral membrane, which can be blocked by metabolic inhibitors (12, 23) and by the presence of Ba^{2+} (16) in the serosal bathing solution.

An explanation for the initial rapid response, supported by past and present findings, is that the rapid depolarization of ψ^{mc} is triggered by the activation of rheogenic and conductive Na^+-coupled sugar and/or amino acid entry mechanisms in the apical membrane (12, 24). This depolarization would reduce the activity of the inward-rectifying K^+ channels in the basolateral membrane which, in turn, would potentiate the depolarization of both limiting membranes. Thus, the accompanying decrease in r^m/r^s would be the result of *both* a decrease in r^m (due to the conductive properties of these Na^+-coupled entry processes) and an increase in r^s due to the voltage dependence of the K^+ channels, which comprise the principal conductive pathways across the basolateral membrane (16, 23).

Lang *et al.* (25) have reported that the activation of Na^+-coupled transport mechanisms in the apical membranes of frog renal proximal tubule cells results in a depolarization of the apical and peritubular membranes and a transient decrease in the K^+ conductance of the latter barrier, which amplifies the depolarization; these investigators suggested the presence of inward-rectifying K^+ channels in the peritubular membrane to explain these findings.

Inward-rectifying properties have also been reported for K^+-conductive pathways in the peritubular membranes of *Necturus* (26–28) proximal tubule, *Amphiuma* collecting tubule (29), and the basolateral membranes of frog skin (30). As suggested by Hille (15) for excitable cells, one physiological function served by these inward-rectifying pathways in the basolateral membranes would be to prevent excessive losses of cell K^+ when that barrier is depolarized.

Finally, with respect to the pump–leak parallelism, it is intriguing to speculate that, while an increase in the rate of Na^+ entry across the apical membranes depolarizes the cell interior and reduces the basolateral membrane K^+ conductance, the subsequent increase in the activity of the rheogenic Na^+,K^+ pumps at the basolateral membrane would tend to hyperpolarize that barrier and reactivate these K^+-leak pathways. This, in turn, would amplify the repolarization of the cell interior and restore the electrical driving force for rheogenic Na^+-coupled entry processes across the apical membrane (1, 2). This scenario is consistent with our finding that metabolic inhibitors that prevent an increase in pump activity also block the repolarization of ψ^{mc} and increase in r^m/r^s (12).

In short, as suggested by Sackin and Palmer (29), Kawahara *et al.* (27), and Horisberger and Giebisch (29) for renal tubule epithelial cells, the combination of rheogenic Na^+,K^+ pumps and inward-rectifying K^+ leaks in the basolateral membranes could provide, at least in part, an explanation for the "pump–leak" parallelism observed in Na^+-absorbing epithelia.

The authors gratefully acknowledge the assistance provided by Dr. Roberto Coronado (Baylor College of Medicine, Houston) in setting up the reconstitution technique in our laboratories. We are also grateful to Dr. Christopher Miller (Brandeis University, Waltham, MA) for providing highly purified CTX. These studies were supported by research grants to S.G.S. (DK-37620) and W.P.D. (DK-38518).

1. Schultz, S. G. (1981) *Am. J. Physiol.* **241,** F579–F590.
2. Schultz, S. G. (1989) *Curr. Top. Membr. Transp.* **34,** 21–44.
3. Boumendil-Podevin, E. F. & Podevin, R. A. (1983) *Biochim. Biophys. Acta* **735,** 86–94.
4. Dubinsky, W. P. & Monti, L. B. (1986) *Am. J. Physiol.* **251,** C721–C726.
5. Kagawa, Y. (1984) *Methods Enzymol.* **10,** 505–510.
6. Langridge-Smith, J. E., Field, M. & Dubinsky, W. P. (1983) *Biochim. Biophys. Acta* **731,** 318–328.
7. Hochstadt, J., Quinlan, D. C., Rader, R. L., Chen-Chung, L. & Dowd, D. (1975) in *Methods in Membrane Biology*, ed. Korn, E. D. (Plenum, New York), Vol. 5, pp. 117–162.
8. Nelson, B. D. (1966) *Proc. Soc. Exp. Biol. Med.* **121,** 998–1001.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
10. Alvarez, O. (1986) in *Ion Channel Reconstitution*, ed. Miller, C. (Plenum, New York), pp. 115–130.
11. Cohen, F. S. (1986) in *Ion Channel Reconstitution*, ed. Miller, C. (Plenum, New York), pp. 131–139.
12. Gunter-Smith, P., Grasset, E. & Schultz, S. G. (1982) *J. Membr. Biol.* **66,** 25–39.
13. Schultz, S. G. (1980) *Basic Principles of Membrane Transport* (Cambridge Univ. Press, Cambridge, U.K.).
14. Latorre, R. & Miller, C. (1983) *J. Membr. Biol.* **71,** 11–30.
15. Hille, B. (1984) in *Ionic Channels in Excitable Membranes* (Sinauer, Sunderland, MA), pp. 109–112.
16. Lau, K. R., Hudson, R. L. & Schultz, S. G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3591–3594.
17. Sheppard, D. N., Giraldez, F. & Sepulveda, F. V. (1988) *J. Membr. Biol.* **105,** 65–75.
18. Latorre, R. (1986) in *Ion Channel Reconstitution*, ed. Miller, C. (Plenum, New York), pp. 431–467.
19. Anderson, C. S., MacKinnon, R., Smith, C. & Miller, C. (1988) *J. Gen. Physiol.* **91,** 317–333.
20. Valdivia, H., Smith, J. S., Martin, B. M., Coronado, R. & Possani, L. D. (1988) *FEBS Lett.* **226,** 280–284.
21. Moczydlowski, E., Lucchesi, K. & Ravindran, A. (1988) *J. Membr. Biol.* **105,** 95–111.
22. Gimenez-Gallego, G., Navia, M. A., Reuben, J. P., Katz, G. M., Kaczorowski, G. J. & Garcia, M. L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3329–3333.
23. Grasset, E., Gunter-Smith, P. & Schultz, S. G. (1983) *J. Membr. Biol.* **71,** 89–94.
24. Lapointe, Y.-Y., Hudson, R. L. & Schultz, S. G. (1986) *J. Membr. Biol.* **93,** 205–219.
25. Lang, F., Messner, G. & Rehwald, W. (1986) *Am. J. Physiol.* **250,** F953–F962.
26. Hunter, M., Kawahara, K. & Giebisch, G. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45,** 2723–2726.
27. Kawahara, K., Hunter, M. & Giebisch, G. (1987) *Am. J. Physiol.* **253,** F488–F494.
28. Sackin, H. & Palmer, L. G. (1987) *Am. J. Physiol.* **253,** F476–F487.
29. Horisberger, J.-D. & Giebisch, G. (1988) *J. Membr. Biol.* **105,** 257–263.
30. Nagel, W. (1985) *Pflügers Arch.* **405** Suppl. 1, S39–S43.