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Voltage Dependence of the Basolateral Membrane Conductance in the *Amphiuma* Collecting Tubule

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Summary. The basolateral potassium conductance of cells of most epithelial cells plays an important role in the transcellular sodium transport inasmuch as the large negative equilibrium potential of potassium across this membrane contributes to the electrical driving force for Na^+ across the apical membrane. In the present study, we have attempted to establish the *I-V* curve of the basolateral membrane of the *Amphiuma* collecting tubule, a membrane shown to be K^+ selective. Transepithelial *I-V* curves were obtained in short, isolated perfused collecting tubule segments. The "shunt" conductance was determined using amiloride to block the apical membrane Na^+ conductance. In symmetrical solutions, the "shunt" *I-V* curve was linear (conductance: 2.2 ± 0.3 mS · cm^{-2}). Transcellular current was calculated by subtracting the "shunt" current from the transepithelial current in the absence of amiloride. Using intracellular microelectrodes, it was then possible to measure the basolateral membrane potential simultaneously with the transcellular current. The basolateral conductance was found to be voltage dependent, being activated by hyperpolarization: conductance values at -30 and -80 mV were 3.6 ± 1.0 and 6.6 ± 1.0 mS · cm^{-2}, respectively. Basolateral *I-V* curves were thus clearly different from that predicted by the "constant field" model. These results indicate that the K^+-selective basolateral conductance of an amphibian collecting tubule shows inward ("anomalous") rectification. Considering the electrogenic nature basolateral Na-K-pump, this may account for coupling between pump-generated potential and basolateral K^+ conductance.

Key Words *Amphiuma* · collecting tubule · basolateral membrane · potassium conductance · *I-V* curve

Introduction

The basolateral potassium conductance of most epithelial cells plays an important role in the transcellular sodium transport inasmuch as the large negative equilibrium potential of potassium across this membrane contributes to the electrical driving force for Na^+ across the apical membrane. Experimental evidence suggests that this basolateral conductance is regulated by the activity of the basolateral Na-K-pump [3, 8, 10]. In general, an increased active pump rate is associated with a higher potassium permeability. Several mechanisms have been proposed for this regulation [2, 32], but no clear consensus has yet been reached concerning the control of the basolateral membrane potassium conductance.

One interesting aspect of the behavior of the basolateral membrane conductance is its relation to the potential difference across this membrane. Current-voltage relations of the basolateral membrane have been obtained in several tight epithelia [26, 31, 33, 40]. However, depending on the technique and the experimental preparation used, the results have been variously described as either conforming or not conforming to the Goldman "constant field" model.

In previous experiments on the collecting tubule of *Amphiuma*, we had observed that the effects of basolateral membrane potential changes induced by lowering the bath K^+ concentration or by blocking the Na-K pump by ouabain were clearly different from those predicted by the "constant field" model; the basolateral K^+-selective conductance was increased by the hyperpolarization immediately following the reduction of the potassium concentration of the bath solution. The K^+ conductance was decreased by the fast depolarization after inhibition of the pump current by ouabain [14]. Similar findings have been reported by Messner, Oberleithner and Lang [25], concerning the basolateral K^+ conductance of the frog proximal tubule.

Thus we were interested in determining the current-voltage relationship of the basolateral membrane of this epithelium, by direct measurement of the transcellular current under voltage-clamp conditions. As stated in previous papers from this laboratory [14, 15], exposing *Amphiuma* to a high potas-

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sium environment induces in the collecting tubule: (i) an increase of the apical membrane conductance, largely a sodium-selective conductance, and (ii) a decrease of the paracellular conductance. Since these conditions allow for optimal measurements of the transcellular current, all the experiments reported here have been performed on potassium-adapted *Amphiuma*.

Materials and Methods

Amphiuma of both sexes were obtained from C. Sullivan (Nashville, TN) and kept for at least 4 days in aquaria containing a 10 mM NaCl + 50 mM KCl solution.

The identification, preparation and perfusion of single-collecting tubules, and the use of intracellular microelectrodes were carried out as described in other papers from this laboratory [15, 16]. Short segments (about 200 μm in length) were dissected and cannulated at both ends for perfusion with control of the intratubular pressure [14]. In short tubules with high transepithelial resistance, current injected at the proximal end of the tubule induced a nearly constant voltage deflection along the whole length of the tubule. Experiments were performed only when the voltage deflection measured at the distal end of the tubule was greater than 80% of the voltage deflection measured at the proximal end. The effective transepithelial voltage (V_{te}) was taken as the mean of the values measured at both ends of the tubule. Intracellular microelectrodes allowed for measurements of the basolateral membrane potential (V_{bl}). The mean distance of the intracellular electrode from the proximal end of the tubule was 37 ± 7% of the whole tubular length.

To obtain basolateral membrane current-voltage curves, we used an approach analogous to that of Thompson, Suzuki and Schultz [37]. By means of a data acquisition system consisting of a microcomputer (IBM-XT), an analog-digital converter (Analog Device, RTI-815) and a software package (UnkelScope, Unkel Software, Lexington, MA), we measured simultaneously with a sampling frequency of 20 Hz: (i) the transepithelial potential (V_{te}) recorded at both ends of the tubule, (ii) the basolateral membrane potential (V_{bl}), and (iii) the transepithelial current (I_{te}). This system also allowed us to control the current injected through the lumen with respect to either the transepithelial potential or the basolateral membrane potential. We thus could voltage clamp either V_{bl} or V_{te}.

V_{bl} was clamped through luminal current injections for 1.5 sec, with 1.5-sec intervals, at alternately positive and negative potentials 10, 20, 30 and 40 mV above or below the level of the basolateral membrane potential measured under open-circuit conditions. Alternatively, V_{te} was clamped for 1.5-sec periods at alternatively positive and negative potentials 40, 80, 120 and 160 mV above or below the transepithelial potential measured under open-circuit conditions. This maneuver was performed under control conditions and in the presence of 10^{-5} M amiloride in the luminal solution. The 10 last-sampled values of each voltage step (i.e., the values sampled between 1.0 and 1.5 sec after the beginning of the voltage step) were averaged to yield the values of the potentials (V_{bl}, V_{te}) and of the transepithelial current (I_{te}) used to establish the *I-V* curves. The effects of serial resistances, measured in the absence of tubule, were subtracted. We have pooled the data obtained by the basolateral membrane voltage clamp (5 experiments) and the transepithelial voltage clamp (11 experiments) as the results of these two techniques were similar.

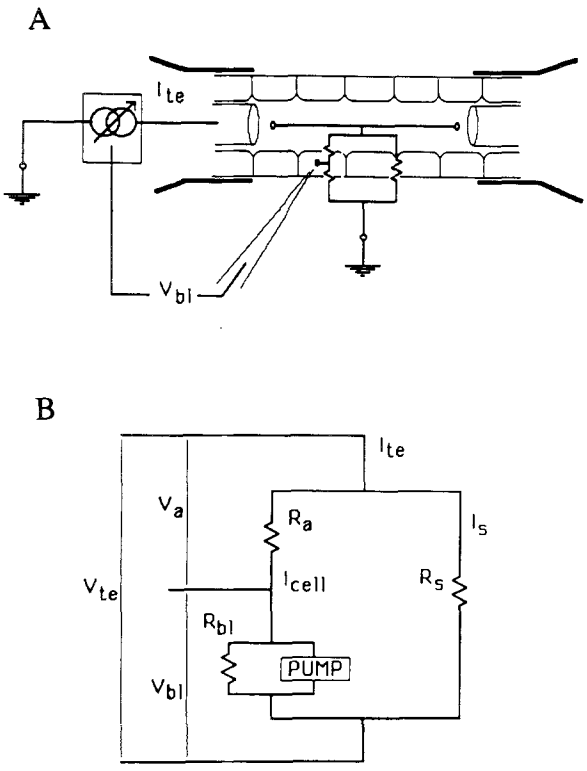

Fig. 1. (*A*) Scheme of the isolated perfused tubule and the equivalent electrical circuit used to calculate the transcellular current. To voltage clamp the basolateral membrane potential (V_{bl}), the current-injecting device was driven by the basolateral membrane potential; the transepithelial current (I_{te}) was adjusted so that V_{bl} was maintained at a determined value (basolateral membrane voltage clamp). (*B*) Equivalent electrical circuit. The transcellular current was calculated as the difference between the transepithelial current (I_{te}) and the "shunt" current (I_s). I_s was estimated from the "shunt" conductance, measured in the presence of luminal amiloride, and the transepithelial potential (V_{te}).

The "shunt" conductance (G_s) was estimated from the transepithelial conductance (G_{te}) measured in the presence of luminal amiloride. We had previously demonstrated that this is a valid method of measuring the "shunt" conductance in the collecting tubule of *Amphiuma* [15, 16]. Accordingly, the transcellular current (I_{cell}) at each V_{te} value was calculated as the difference between the measured transepithelial current (I_{te}) and the "shunt" current (I_s) (*see* equivalent electrical circuit in Fig. 1)

$$I_s = V_{te} \cdot G_s \tag{1}$$

$$I_{cell} = I_{te} - I_s = I_{bl} = -I_o. \tag{2}$$

In six tubules (out of 16), we observed that amiloride did not completely block the apical membrane conductance, in opposition to what we had observed in three previous series of experiments [14–16]. This residual apical membrane conductance was abolished by 2 mM barium added to the luminal fluid. Thus, in these tubules, the shunt conductance was measured as the transepithelial conductance in the presence of amiloride and barium in the luminal fluid.

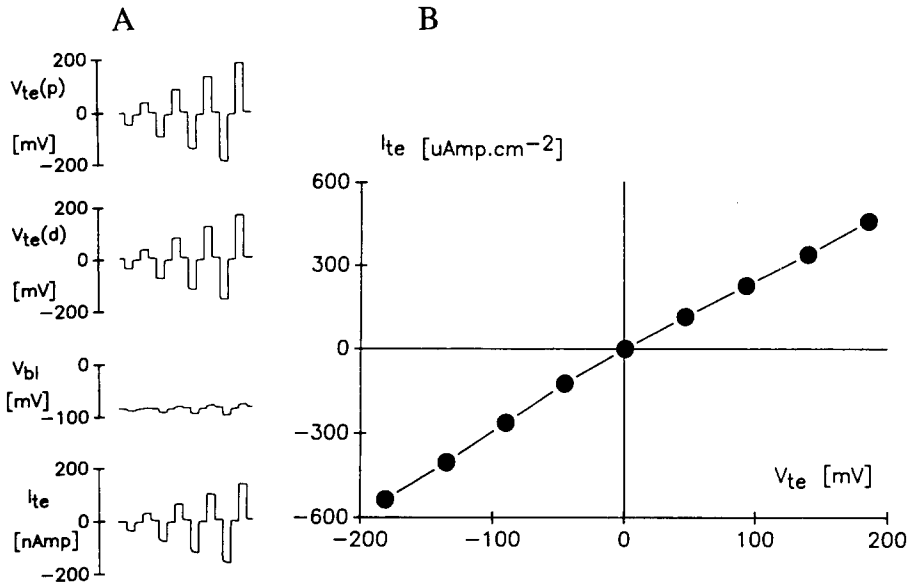

Fig. 2. (*A*). Original tracings of the transepithelial potential recorded at the proximal ($V_{te}p$) and distal ($V_{te}d$) end of the isolated perfused tubule, basolateral membrane potential (V_{bl}) and transepithelial current (I_t) during transepithelial voltage steps under transepithelial voltage-clamp conditions, in the presence of luminal amiloride (10^{-5} M). (*B*). The corresponding transepithelial *I-V* curve (I_{te} *vs.* V_{te}). This curve is linear in the positive potential range and slightly curved in the negative potential range because of the incomplete block of the apical membrane when this membrane is strongly depolarized. The partial relief of the block of the apical membrane conductance in the lumen negative potential range is detectable by the larger deflections of the V_{bl} when negative current rather than positive current is injected into the lumen

Solutions and Drugs

The ionic composition of the bath and luminal perfusate solutions was (in mM) Na^+ 97.0, K^+ 3.0, Ca^{2+} 1.8, Mg^{2+} 1.0, Cl^- 89.6, HEPES/$HEPES^-$ 14.4, and $H_2PO_4^-/HPO_4$ = 0.8. The pH was adjusted to 7.6. In addition, the bath solution contained 2.0 mM glucose and 0.25 mM glycine. All solutions were bubbled with O_2. Amiloride (gift of Merk, Sharp & Dome, West Point, PA) was used at a concentration of 10^{-5} M and barium at 2 mM (as $BaCl_2$ 2 mM replacing 3 mM NaCl).

Statistics

Results are expressed as mean ± SEM (n = number of observations). The statistical significance of the difference between mean values was determined by the Student's t test. When appropriate, the Student's t test for paired data was used, as indicated in the text. $P < 0.05$ was chosen as the level of significance.

Results

Characteristics of the Tubules

The mean length of 16 tubules was 228 ± 10 μm (range 158 to 281 μm). Under control conditions, the transepithelial (V_{te}) and the basolateral membrane (V_{bl}) potentials were −50 ± 5 mV and −59 ± 2 mV (n = 16), respectively. These values are similar to those observed previously in potassium-adapted *Amphiuma* [14, 15].

The "Shunt" Conductance

A representative example of I_{te}, V_{te} (recorded at both ends of the tubule) and V_{bl} and of the corresponding transepithelial *I-V* curves (I_{te} versus V_{te}) in the presence of luminal amiloride, is shown in Fig. 2. Amiloride abolished the transepithelial potential difference and thus blocks the Na^+-selective apical membrane conductance; the remaining conductance is defined as the "shunt" conductance. Careful inspection of the *I-V* curve indicates that it is linear in the positive transepithelial potential range, but slightly curved at negative V_{te} potentials, where the conductance is increased. Inspection of the V_{bl} tracing shows larger deflections with negative than with positive luminal current injection most likely due to a partial relief of the apical membrane block by amiloride (decrease of the resistance of the apical membrane); voltage dependence of the amiloride block of the sodium channel has been demonstrated in tight epithelia [27, 38]. For this reason, the "shunt" conductance was estimated as the slope of the transepithelial *I-V* curve in the positive

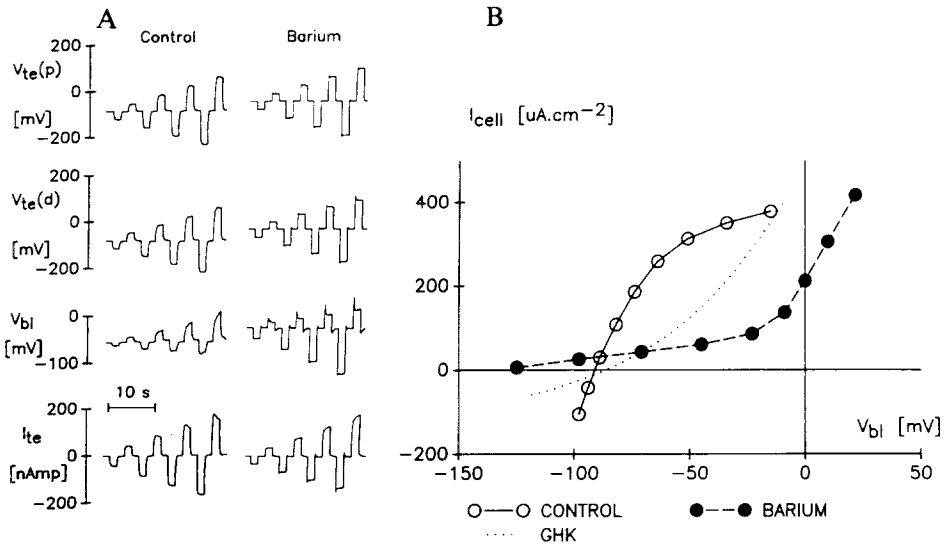

Fig. 3. *I-V* curve of the basolateral membrane under control conditions (○) and in the presence of 2 mM barium in the bath solution (●). (*A*). The original tracings of the transepithelial potential recorded at the proximal ($V_{te}p$) and distal ($V_{te}d$) end of the isolated perfused tubule, of the basolateral membrane potential (V_{bl}) and of the transepithelial current (I_t) during steps of transepithelial voltage clamp. (*B*). The corresponding *I-V* curves and, with a dotted line, an *I-V* curve predicted by the Goldman "constant field" model for "physiological" intracellular (56 mM) and extracellular (2.3 mM) potassium activities

potential range, in the presence of luminal amiloride. The mean value of the "shunt" conductance was 2.2 ± 0.3 mS · cm^{-2} (n = 16).

Basolateral Membrane *I-V* Curves

Figure 3 presents an example of basolateral membrane *I-V* curves obtained under transepithelial voltage-clamp conditions. The reversal potential of this membrane: −75 ± 3 mV, (n = 16) was slightly lower than the estimated equilibrium potential for potassium (E_{K^+}) (intracellular potassium activities measured under similar conditions averaged 56 mM, which yields a E_{K^+} value of −81 mV [14]). This value of V_{bl} very near E_{K^+} is in agreement with the hypothesis of a predominantly potassium-selective conductance of the basolateral membrane. It is important to note that the slope conductance of the basolateral membrane was larger at high negative potential than at low membrane potential, in other words, the basolateral membrane *I-V* curve was inward rectifying. Figure 3 also shows (dotted line) the outward-rectifying *I-V* curve predicted by the Goldman "constant field" model for a K^+-selective membrane with "physiological" bath and cell K^+ activities of 2.3 mM and 56 mM, respectively [14]. This predicted *I-V* curve was clearly different from the observed basolateral *I-V* curve. In 15 experiments, the shape of the *I-V* curve was more or less inward rectifying, sometimes quasi linear. It never presented the outward rectification predicted by the "constant field" model.

To compare the slope conductance at determined membrane potential levels, the 9-point basolateral membrane *I-V* curves were fitted to a 4th degree polynomial using a least-squares fit method. The slope conductance was determined as the slope of the best fitting curve. The mean slope conductance was 3.6 ± 1.0 mS · cm^{-2} at −30 mV and 6.6 ± 1.0 mS · cm^{-2} at −80 mV (n = 15), $P < 0.001$ (paired t test).

The Effects of Barium

As observed in earlier studies [15, 16], barium in the peritubular bath solution had no effect on the paracellular conductance: G_s control 2.2 ± 0.4 (14) *vs.* barium 2.3 ± 0.4 (14) (paired t test: NS). As shown in the example of Fig. 3, barium induced a significant decrease of the slope conductance in the −100 to −30 mV range of V_{bl}. In 14 paired experiments, barium decreased the slope conductance at −60 mV from 5.2 ± 1.0 to 2.7 ± 1.0 mS · cm^{-2}, $P < 0.01$ (paired t test). This reduction of the basolateral conductance by about 50% is somewhat smaller than what could have been predicted from transference number measurements, which had indicated that potassium was responsible for 70 to 80% of the basolateral membrane conductance [15, 16]. Furthermore, at potentials less negative than −30 mV and

at positive membrane potentials, there was a large increase of the basolateral membrane conductance in the presence of barium (*see* Fig. 3). Part of this increase may be explained by the voltage dependence of the barium block of the K^+ conductance [17, 34]; however, as the conductance becomes sometimes even larger than in the absence of barium, some other mechanism must be involved. Although our data do not allow us to determine the nature of the current across the basolateral membrane in the presence of barium, we can suggest that either barium does not completely block the K^+ conductance, or that another ionic conductance appears in the basolateral membrane as a consequence of the effects of barium. By blocking the efflux of K^+, barium may induce cell swelling, and cell swelling has been shown to increase the basolateral membrane potassium and chloride conductance in the rabbit proximal tubule [39].

Discussion

The data reported here indicate that the basolateral membrane conductance of the *Amphiuma* collecting tubule is inward rectifying, or, in other words, is larger at high negative potentials than at low negative potentials. This modification of the conductance occurs in the range of the physiological variations of the basolateral membrane potential in this epithelium [15]. Our direct measurements confirm previous indirect evidence that the basolateral membrane potassium conductance presents inward-rectifying properties [14], opposite to what is predicted by the Goldman "constant field" model in the presence of an outwardly direct potassium gradient.

In the present study, we have measured the total basolateral current, which may include both a pump-current component [14] and various passive ionic currents. In view of the fact that the pump conductance is thought to represent only a very small part of the total membrane conductance [4] and that the basolateral conductance is predominantly potassium selective, 70 to 80% as estimated by the transference number method [15, 16], the observed changes of the basolateral membrane conductance are, at least for a large part, due to modification of the *potassium* conductance.

Several investigators have indeed observed inward-rectifying properties of the basolateral membrane conductance of sodium-transporting epithelia [1, 20, 25, 26]. Others have reported a linear basolateral membrane conductance in conditions where the potassium gradient and the "constant field" model predict outward rectification [31, 33, 35, 36]. In the turtle colon, comparison of potassium fluxes and current across the basolateral membrane indicated that the movement of potassium could not be explained by simple diffusion [18]. However, results consistent with the "constant field" model have been reported for the rabbit colon [40].

Considering the slow time course of the voltage steps, the observed changes of conductance could be due to time-dependent changes in ionic permeabilities, to modifications of the intracellular ionic activities or to "instantaneous" current-voltage characteristics of the conductance. Calculations of the transcellular ion fluxes indicate that the intracellular potassium concentration is modified by at most 5 to 6 mM (less than 10% change of the intracellular K^+ concentration) during the 1.5-sec voltage-clamp period at the highest voltage used. Thus, the K^+ conductance changes cannot be due directly to modifications of the total intracellular K^+ content.

There are at least three other explanations for an inward rectification of the conductance: (i) an inward-rectifying single-channel conductance, (ii) voltage-dependent gating kinetics of the K^+-channel and, (iii) changes in ionic activities in the unstirred layers along the basolateral membrane.

Indeed, patch-clamp studies have shown *inward-rectifying* properties of single-channel conductance in the basolateral membrane of the rabbit proximal tubule [28]. However, other investigators found that a K^+ channel in the basolateral membrane of the same epithelium was inward-rectifying only when high K^+ concentrations were present at both sides of the membrane, while the single-channel *I-V* curve could be well described by the "constant field" equation under physiological conditions [7].

Channel *gating kinetics* controlled by the membrane potential in the direction of an increase of the open probability with membrane hyperpolarization have recently been demonstrated by patch-clamp studies for K^+-channels of the basolateral membrane of amphibian kidney proximal tubules [17, 30]. However, potassium channels with a voltage dependence in the opposite direction have also been found in the basolateral membrane of the rabbit urinary bladder [21] and of the rabbit proximal tubule [7, 28]. It is presently not known which type of K^+-channel is responsible for the potassium efflux under physiological conditions, as several different types of potassium conductances may be present in the basolateral membrane and be activated under specific conditions [5, 6].

Other possibilities include a "chemical channel gating" due to changes in the intracellular ionic (Na^+?, Ca^{2+}?) composition or large modifications of

the K^+ concentration of the unstirred layers close to the very convoluted basolateral membrane of this epithelium. The fact that the studies using a fast time course of voltage displacement (tens or hundreds of milliseconds) [31, 33, 35, 36, 40] found a linear *I-V* curve, whereas studies using methods with a slower time course [1, 26] (and the present study) observed inward rectification may favor this hypothesis. However, our data did not allow us to distinguish between these three possibilities. The purpose of our study was to examine the behavior of the basolateral membrane conductance when the potential difference of this membrane was modified for a relatively long time (1 to 1.5 sec), a period comparable to that of the voltage changes obtained experimentally by bath ionic substitutions or pharmacological block of pump or conductances [14].

Inward-rectifying potassium conductances have been demonstrated in many nonepithelial cell types, including excitable [12] and nonexcitable cells [9, 23, 34]. In heart muscle cells, the inward rectification has been attributed to a fast block by internal Mg^{2+} [24]. The inward-rectifier K^+-channel is thought to provide the conductive pathway, which determine the resting membrane potential to be near the K^+ equilibrium potential [12, 23]. It is then not surprising that an inward-rectifier K^+ conductance is also present in the basolateral membrane of epithelial cells and it may have a similar role in the maintenance of the basolateral membrane potential. The inward rectifier could constitute a "shutdown" device to prevent excessive loss of intracellular potassium in case of pathological depolarization of the basolateral membrane potential [12].

The relationship between basolateral Na-K-pump activity and the basolateral K^+ conductance is of interest. It is well established that the Na-K-pump present in the basolateral membrane of several types of epithelia is electrogenic [19, 22, 29]. Our previous experiments in the collecting tubule of *Amphiuma* indicate that this is also the case in this type of epithelium [13, 14]. Since the activity of the Na-K-pump produces a significant hyperpolarization of the basolateral membrane potential, the inward-rectifying property of this membrane conductance may explain, at least in part, the large decrease of this conductance observed after inhibition of the Na-K-pump [11, 14].

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