J. Membrane Biol. 138, 181–195 (1994)

The Journal of
Membrane
Biology

Basolateral Potassium Membrane Permeability of A6 Cells and Cell Volume Regulation

J. Ehrenfeld, C. Raschi, E. Brochiero

Department of Cellular and Molecular Biology, Commissariat à l'Energie Atomique, Laboratoire Jean Maetz BP 68, Villefranche/Mer 06230 France

Received: 7 June 1993/Revised: 21 October 1993

Abstract. The K^+ permeabilities ($^{86}Rb(K)$ transport) of the basolateral membranes (J_bK) of a renal cell line (A6) were compared under isosmotic and hypo-osmotic conditions (serosal side) to identify the various components involved in cell volume regulation.

Changing the serosal solution to a hypo-osmotic one (165 mOsm) induced a fast transient increase in Ca_i (max <1 min) and cell swelling (max at 3–5 min) followed by a regulatory volume decrease (5–30 min) and rise in the SCC (stabilization at 30 min). In isosmotic conditions (247 mOsm), the $^{86}Rb(K)$ transport and the SCC were partially blocked by Ba^{2+}, quinidine, TEA and glibenclamide, the latter being the least effective. Changing the osmolarity from isosmotic to hypo-osmotic resulted in an immediate (within the first 3–6 min) stimulation of the $^{86}Rb(K)$ transport followed by a progressive decline to a stable value higher than that found in isosmotic conditions. A serosal Ca^{2+}-free media or quinidine addition did not affect the initial osmotic stimulation of J_bK but prevented its "secondary regulation," whereas TEA, glibenclamide and DIDS completely blocked the initial J_bK increase. Under hypo-osmotic conditions, the initial J_bK increase was enhanced by the presence of 1 mM of barium and delayed with higher concentrations (5 mM). In addition, cell volume regulation was fully blocked by quinidine, DIDS, NPPB and glibenclamide, while partly inhibited by TEA and calcium-free media.

We propose that a TEA- and glibenclamide-sensitive but quinidine-insensitive increase in K^+ permeability is involved in the very first phase of volume regulation of A6 cells submitted to hypo-osmotic media. In achieving cell volume regulation, it would play a complementary role to the quinidine-sensitive K^+ permeability mediated by the observed calcium rise.

Correspondence to: J. Ehrenfeld

Key words: Cell volume regulation — Na^+ transport — Intracellular calcium — TEA — Glibenclamide — K^+ channels

Introduction

A6 cells, a continuous cell line derived from the kidney of *Xenopus laevis* [39], form a Na^+-transporting monolayer epithelium of high resistance when grown on a permeant support [37]. The two barrier model developed by Ussing [50] can be applied to A6 cells, since these cells exhibit a transepithelial sodium transport mediated by amiloride-sensitive Na^+ channels (located on the apical membranes) and by Na/K-ATPase and K^+ channels located on the basolateral membranes [4, 20, 37, 47]. Sodium transport through the cell monolayer has also been found to be stimulated by a variety of hormones including aldosterone, insulin and vasopressin [15, 24].

Recently, it was found that small changes in the osmolarity of the serosal solution bathing A6 cell monolayers, affected the short-circuit current (transepithelial Na^+ transport) by changing the conductance of amiloride-sensitive Na^+ channels [55] and by changing the basolateral membrane (blm) conductance [19]. In addition, the opening of a new quinidine-sensitive K^+ conductive pathway, located on the blm, was demonstrated under hypo-osmotic conditions in amphotericin-treated cells [4].

Under hypo-osmotic conditions, A6 cells are expected to swell by rapid water flow through the basolateral membranes, and as reported above, the transepithelial Na^+ transport increases. The cells are therefore faced with problems of both cell volume regulation and coordination between ion entries and ion exits through the apical and basolateral membranes, respectively ("cross-talk" phenomena). In both cases, the K^+ per-

meability of the basolateral membranes would be modified.

The basolateral membranes of A6 cells grown on permeant support are not directly accessible for electrophysiological techniques, but apical impalements were feasible [19, 21]. Permeabilization of the apical membranes, with ionophores such as amphotericin or nystatin to give indirect access to the basolateral membranes, has also been used on tight epithelia [14, 18, 30, 31] and on A6 cells [4]. However, this technique involves a necessary modification of the ion composition of the apical medium solution (substitution of chloride by impermeant anions, Ca^{2+} free solutions . . .), to avoid changes of cytoplasm composition and cell swelling, since these ionophores allow the passage of cations, anions and water [17, 30]. Furthermore, due to the delay in ionophore insertion into the membranes, rapid phenomena cannot be followed. We therefore decided to adopt a kinetic approach using an isotope (^{86}Rb) to follow the potassium permeability of the basolateral membranes of A6 cells grown on filters, and thus discriminate and characterize the different potassium pathways which develop under resting conditions and after application of a hypotonic serosal solution in relation to cell volume regulation. For this purpose, the effects of various K^+ and Cl^- inhibitors were tested on ^{86}Rb (K) permeability, cell volume, Ca_i and SCC in both osmotic conditions.

Materials and Methods

Cell Culture

A6, a cell line of renal cells of *X. laevis,* was a gift of Dr. Rossier (Lausanne, Switzerland). They had originally been obtained from the American Tissue Type Collection and subsequently cloned (clone A6-2F3) by limiting dilution [52]. Cells were grown between passages 88–98 at 28°C in a humidified atmosphere of 5% CO_2 in air. The amphibian cell medium (AM) [25] was supplemented three times weekly with 10% fetal calf serum (IBF, France) and antibiotics for cell nourishment. The osmolarity of the AM was measured using a vapor pressure osmometer (Model 5500, Wescor, Logan UT) and found to be 247 mOsm.

For transport experiments, the cells were seeded onto transparent collagen-treated membranes (Transwell, 0.45 μm pore, Costar, MA) at a seeding density of 2×10^6 cells per well (5 cm^2). Cell monolayers were then fed for 5–10 days with the amphibian medium (serum-free) supplemented with 2% ultroser-G (GIBCO-IBF, USA-France) to increase their Na^+ transport capacity. The osmolarity of this solution was 247 mOsm.

Transport Experiments

In most transport experiments, we used the cell culture medium (serum free) to avoid intracellular ion and volume changes due to different experimental and culture media.

Electrical measurements (transepithelial potential, short-circuit current and resistance of monolayers) were performed in a homemade modified Ussing chamber designed to fit the Transwell. The volumes of the apical and basolateral bathing solutions were 2 and 2.5 ml, respectively, and the solutions could be changed without interruption of the electrical measurements. The spontaneous transepithelial potential (PD) was measured through Agar-KCl salt bridges and clamped at zero volts, through platinum electrodes, using an automatic voltage clamp (Model VC 600, Physiological Instrument, Houston, TX). The short-circuit current (SCC) and additional pulses (10 mV, 1 sec duration every 60 sec) to measure the monolayer resistance (R) were continuously recorded on a chart paper recorder (SEFRAM, France).

^{86}Rb was used as an index of K^+ permeability through the basolateral membranes of the cell monolayer grown on Transwell. Even though differences in selectivity between Rb^+ and K^+ may exist, the use of ^{86}Rb is widely accepted; it has been, for instance, successfully used in β-cells [27] and MDCK cells [44]. In addition, its ease of use (long half-time, easy availability in Europe) makes the isotope a useful tool in K^+ permeability studies. Cell monolayers were loaded all night from the basolateral side with an ^{86}Rb-containing AM (1 μCi/ml). The cells were then washed twice on their apical and basal sides and the ^{86}Rb efflux measurements during successive 3 min periods were made by simple transfer of the filter supporting the monolayer from one well to another of the 6-well cluster (Costar), each of which contained two ml of AM. At the end of the experiment, samples of the loading solution (20 μl), experimental basal samples (2 ml each), the apical sample at the end of the washout, and the remaining ^{86}Rb activity from the cell monolayer (after 2 hr extraction in NaOH, 1 mM), were placed in counting vials supplemented with 10 ml of ACS (Amersham) for counting in a liquid scintillation counter (Packard Instruments). ^{86}Rb effluxes were usually followed over a 60 min period. The ^{86}Rb activity of the 3 min collecting periods decreased slightly, as would be expected from a single exponential, with a half time of 129 ± 7 min (n = 20). With such a relatively long half-time, the 3 min efflux periods were almost constant during a 1 hr period. ^{86}Rb efflux, nevertheless, was expressed as a fraction of that present intracellularly during the equivalent time period (cpm of the 3 min collecting period divided by the cpm of the cell monolayer at the beginning of the corresponding period).

Intracellular Calcium Measurements

Experiments were performed with isolated A6 cells (obtained by trypsinization and Ca^{2+} chelation) or with intact monolayers. In both cases, acetoxy-methylester of Fura-2 (Fura-2/AM; Molecular Probes, Eugene, OR) was used as a probe of cell calcium.

Isolated cells were loaded with 10 μM Fura-2 at 28°C for 90 min in an amphibian cell culture medium, in a CO_2 incubator. Cells ($3–5 \cdot 10^6$ cells per experimental datum) were then washed and resuspended in the experimental media (*composition below*). The calcium dye loading procedure of A6 cells monolayers was identical to that used with isolated cells and after several fast washings, the filter supporting the monolayer was cut off the Transwell and mounted in an adapted "Ussing chamber" placed in the sample compartment instead of the usual cuvette. The fluorescence was measured with a spectrophotometer system (PTI Deltascan, NJ). The excitation light was set to flicker between 340 and 380 nm (4 nm band pass) at a rate of 100 Hz and the emission was monitored at 505 nm (6 nm band pass). The fluorescence intensity ratio (I_{340}/I_{380}) was measured every second and plotted graphically. Cellular autofluorescence was measured in identical aliquots of cells not exposed to Fura-2 and did not exceed 5% of the fluorescence from dye-loaded cells. This value was subtracted before Ca_i determination. To calculate the calcium concentration, the Tsien equation was used [23]:

$$(Ca^{2+}) = K_d \times B(R - R_{min})/(R_{max} - R),$$

where B is the ratio of fluorescence at 380 nm in the absence and presence of saturating calcium concentrations and K_d is the dissociation constant. Since K_d has been reported to decrease with the ionic strength [48], we measured it in the following 247 mOsm medium (in mM: KCl 105; NaCl 10; HEPES 10; pH 7.20 adjusted with NaOH) and in a medium whose composition was one-third diluted (165 mOsm). Both media contained different amounts of free calcium calculated using computing data according to Chang, Hsiech and Dawson [8], taking into account a correction for $K'Ca^{2+}$ as a function of the ionic strength [26]. Therefore, using $K'Ca^{2+}$ of $6.019 \cdot 10^{-6}$ M^{-1} (ionic strength 0.12 M) and of $6.472 \cdot 10^{-6}$ M^{-1} (ionic strength 0.8 M), we measured a K_d value of 211 and 128 nM for the isosmotic and diluted media, respectively (pH 7.2 at a temperature of 20°C). It should be noted that the 211 nM value is very close to that (224 nM) reported by Grynkiewicz et al. [23] for isosmotic media. R_{max} was determined by the addition of digitonin (20 μM) to the Ringer solution containing calcium (2 mM) and R_{min} was determined by subsequent addition of ethylene glycol-bis (β-amino-ethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) at a final concentration of 20 mM. A small and regular Ca_i rise was observed with isolated cells maintained in an isosmotic medium, and this was interpreted as a Fura-2 leak (permeability) through the cell membranes into the extracellular space. An estimation of the Ca_i error produced by this dye-leakage was a maximum of 10 to 13% at 10 min. A similar value can be deduced from the measurement of the extracellular fluorescence of the medium after centrifugation of the cells. Although the absolute values of Ca_i are subject to various sources of error (autofluorescence, estimation of K_d, leak of Fura-2), Fura-2 is a useful probe to continuously measure calcium variations in different experimental conditions.

Cell Volume Measurement

A Coulter counter was used to measure the volume of isolated cells in suspension. This technique has been used with success in a number of epithelial and nonepithelial dissociated cells and in particular in another kidney cell line, MDCK cells [41, 42]. The cell size distribution and the average volume of a finite number of cells were measured with a Coulter counter multisizer II (256 channels) connected to a PC-type computer. A program developed by Coulter was used to evaluate the average cell volume or the total volume of the cell population counted. Volume changes were presented as a percentage of the initial volume measured in an isosmotic medium.

Statistics

Data variability is expressed as the standard error of the mean (SEM). The Student's test was used for estimating the significance of differences between paired mean data.

Drugs and Ringer Composition

Ouabain, tetraethylammonium (TEA), quinidine, furosemide, gadolinium, bumetanide, glibenclamide and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) were purchased from Sigma Chemical (St. Louis, MO); niflumic acid was a gift from UPSA laboratories; N-phenylanthranilic acid (DPC) was from Fluka (Switzerland). NPPB (5-nitro-2(3-phenylpropylamino) benzoic acid) was a gift of Dr. Greger (Freiburg, Germany).

Ringer solution (in mM) was: NaCl 85; $NaHCO_3$ 24; KCl 2.5; $CaCl_2$ 2; $MgSO_4$ 2; $NaHPO_4$ 2.5; KH_2PO_4 2.5; KH_2PO_4 1.2; glucose 5; pH 7.4 after bubbling with 5% CO_2. When the K^+ channel blocker, Ba^{2+}, was tested, HCO_3^-, SO_4^{2-} and phosphate were substituted by chloride ions to avoid barium precipitation (medium buffered with 10 mM HEPES).

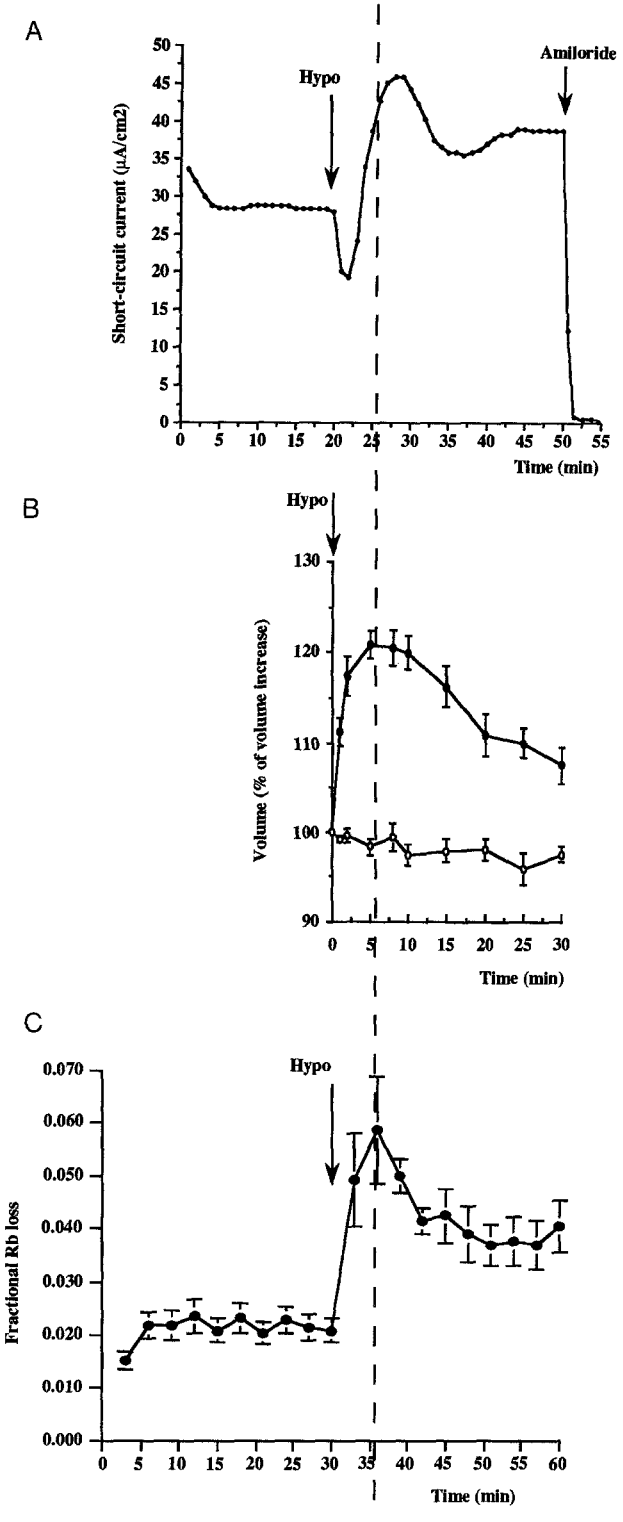
Results

Effects of Hypo-Osmolarity on the Short-Circuit Current, Volume and Calcium Concentration of A6 Cells

Figure 1*A* (typical experiment) and the Table show the time course of the short-circuit current changes observed after replacing the serosal amphibian culture medium (AM, 247 mOsm) by a hypo-osmotic medium (one-third diluted AM, final osmolarity 165 mOsm). After a rapid but transient decline, the short-circuit current (SCC) increased to reach a new steady-state level after 20–25 min, an overshoot usually being observed between 8–10 min. An immediate and transient drop in the transepithelial resistance (R) was observed in hypo-osmotic conditions; the following value in R, however, remained lower than that in isosmotic conditions. The stimulated SCC was completely blocked by amiloride ($5 \cdot 10^{-5}$ M) addition to the apical solution, and the resistance was increased 7–8 times, evidence that in hypo-osmotic conditions, the SCC can be completely accounted for by Na^+ transport.

The cell volume was then followed in isolated A6 cells under isosmotic conditions and after exposure to hypo-osmotic media (Fig. 1*B*). No significant changes in cell volume were observed when the cells remained in isosmotic conditions on a 30 min experimental period. However, when the cells were transferred to a hypo-osmotic solution, rapid cell swelling occurred in the very first minutes (21 ± 2% of maximum increase in cell volume at time 5 min, $P < 0.001$, $n = 17$); it was followed by a recovery phase (usually called regulatory volume decrease, RVD). After 30 min of hypo-osmotic conditions, the cells regulated about two-thirds of their volume.

In epithelial cells, Ca-activated K^+ channels have been suggested to play an important role for K^+ exit during the RVD [7, 12, 29, 46, 49] and a Ca_i rise has been reported in a number of cell systems after a hypotonic challenge [2, 3, 9, 32, 34, 41, 45, 56]. We therefore measured Ca_i in isolated A6 cells or in monolayers, loaded with the fluorescent probe Fura-2, under isosmotic and hypo-osmotic conditions. The mean Ca_i (using a K_d of 224 nM), measured in isolated A6 cells maintained in an isosmotic solution, was 117 ± 10 nM ($n = 29$). When the cells were submitted to a hypo-osmotic solution, Ca_i increased to a peak value of 218 ± 28 nM in less than one minute (peak at 33 ± 3 sec; difference 101 ± 21 nM, $P < 0.001$). A regulatory phase



followed to give a stabilized hypo-osmotic steady value of 176 ± 18 nM (difference between control and stabilization under hypo-osmotic conditions was 59 ± 14 nM, $P < 0.001$ and between peak and stabilization plateau was 42 ± 7 nM, $P < 0.001$). A representative experiment is illustrated in Fig. 2*A*. Experiments performed with intact A6 cell monolayers show a very similar pattern (Fig. 2*B*) but Ca_i concentrations were higher than in isolated cells. The mean Ca_i measured in monolayers maintained in an isosmotic solution was 194 ± 27 nM ($n = 10$). When the cells were submitted to a serosal hypo-osmotic solution, Ca_i fastly increased to a peak value of 370 ± 30 nM (peak at 35 ± 2 sec; difference 176 ± 18 nM, $P < 0.001$) and the stabilized hypo-osmotic steady value was 309 ± 30 nM (difference between control and stabilization under hypo-osmotic conditions was 115 ± 15 nM, $P < 0.001$ and between peak and stabilization plateau was 61 ± 9 nM, $P < 0.001$). It was apparent from these experiments that after a hypo-osmotic shock, the immediate and persistent Ca_i rise may represent an intracellular signal linked to the RVD or to stimulation of the SCC.

Effects of Hypo-Osmolarity on Basolateral Membrane ^{86}Rb (Potassium) Permeability

^{86}Rb effluxes through the apical and basolateral membranes of A6 cells grown on a permeant support were first measured in cell monolayers bathed on both sides with isosmotic media (amphibian culture medium, AM). There was considerable difference between effluxes through the two membranes. Thus, the ^{86}Rb transferred into the serosal solution was 28 ± 3 times greater than that appearing on the apical side of the monolayer ($P < 0.001$; $n = 12$), in agreement with previous findings that the K^+ conductance is localized on the basolateral side of this highly polarized transporting A6 cell monolayer [4, 20, 47].

Since we had previously observed that both cell volume and transepithelial Na^+ transport were considerably affected by bathing the serosal side of the cells with hypo-osmotic solution, we followed the potassium permeability (^{86}Rb efflux) under these conditions.

After a control period (usually 30 min) in isosmotic conditions, exposure of the serosal side of the cell monolayer to a hypo-osmotic solution resulted in an im-

Fig. 1. Effect of serosal hypo-osmotic medium on the SCC (*A*), cell volume (*B*) and Rb (K^+) effluxes (*C*) in A6 cell monolayers. The medium bathing the serosal side of A6 cells was changed from a 247 mOsm solution to a 165 mOsm solution. The dashed line, after 6 min of osmotic shock, permits a comparison of the maximum fractional Rb loss with respect to the cell volume and SCC responses. (*A*) At the end of the experiment, amiloride (10 μM) was added to the apical side. Data from one typical experiment, mean values in the Table. (*B*) Cell volume regulation in isolated A6 cells: the hypo-osmotic medium change was at zero time and A6 cell volume was followed as a function of time (filled circle, $n = 17$). A control experiment is performed in an isosmotic medium (open circle, $n = 17$). (*C*) Effect of serosal hypo-osmotic changes on the ^{86}Rb (K^+) effluxes in A6 cell monolayers: the medium change is indicated by the arrow ($n = 24$).(●) Hypo-osmotic, (○) isosmotic.

Table. Effects of quinidine, TEA or glibenclamide on SCC ($\mu A/cm^2$) and R ($\Omega \times cm^2$) on A6 cell monolayers upon a hypo-osmotic shock

Experimental condition	A		B		C		D	
	SCC	R	SCC	R	SCC	R	SCC	R
Control (iso.)	30.2 ± 2.7	2,378 ± 256	29.2 ± 3.5	2,383 ± 356	22.4 ± 3.8	3,689 ± 605	20.2 ± 3.9	2,904 ± 241
Inhibitor (iso.)	No inhibitor		Quinidine 15.9 ± 2.4[e]	3,946 ± 759[c]	TEA 18.4 ± 3.1[a]	4,493 ± 772	Glibenclamide 7.7 ± 2.2[c]	8,769 ± 2,058[a]
Hypo. 1 min	20.0 ± 2.8[e]	1,008 ± 156[e]	4.1 ± 2.5[f]	1,095 ± 149[b]	9.5 ± 4.1[d]	850 ± 165[d]	5.2 ± 1.5[b]	10,083 ± 2,368
6–8 min	40.6 ± 3.6[e]	1,370 ± 140[e]	15.9 ± 3.2	2,594 ± 350	28.0 ± 5.1[b]	2,200 ± 325[d]	8.0 ± 1.8	8,000 ± 1,733
20–30 min	36.7 ± 3.5[a]	1,370 ± 140[c]	17.1 ± 2.1	2,609 ± 347	23.9 ± 5.0	2,722 ± 424[b]	12.6 ± 1.2[b]	4,839 ± 512
Amiloride	3.1 ± 1.2[e]	12,753 ± 2,811[c]	0.4 ± 0.4[f]	16,666 ± 324[f]	1.5 ± 0.7[f]	9.947 ± 2,520[f]	0.9 ± 0.3[f]	14,666 ± 3,791[b]

(*A*) Effects of a serosal hypo-osmotic shock on the short-circuit current (SCC) and the electrical resistance (R) in all A6 cell monolayers. The medium bathing the serosal side was changed from a 247 mOsm solution to a 165 mOsm solution. At the end of the experiment, amiloride (10 μM) was added to the apical side. After the hypo-osmotic shock, SCC and R are given at times 1 min, at the overshoot (6–8 min) and at the plateau phase (20–30 min; *see* Fig. 1*A*). (*B, C, D*) Effects of serosal hypo-osmolarity on SCC and R in the presence of 200 μM of quinidine (*B*), 10 mM of TEA (*C*) or 0.5 mM of glibenclamide (*D*). Addition of drug is on serosal side, 6 min before the osmotic shock. [a]Significance of the difference between value and related isosmotic value (control-iso). [b]Significance of difference between hypo-osmotic and isosmotic values in the presence of the drug. [a] or [b] $P < 0.025$; [c] or [d] $P < 0.005$; [e] or [f] $P < 0.001$.

mediate and large increase in the ^{86}Rb efflux through the basolateral membrane (Fig. 1*C*). The maximal increase in the ^{86}Rb efflux was usually observed at 3–9 min, followed by a decreasing (regulatory) phase lasting 6–9 min to reach a new steady-state level. ^{86}Rb effluxes at this plateau were significantly higher than those measured in isosmotic conditions (after 30 min of serosal application of a 165 mOsm solution, there was a 81 ± 7% increase of the ^{86}Rb effluxes).

Effects of Various Potassium Transport Inhibitors on the ^{86}Rb Efflux Stimulation Induced by Hypo-Osmotic Shock

To study the nature of the ^{86}Rb (K) pathway(s) involved in the increase in ^{86}Rb efflux observed in hypo-osmotic conditions, we used a pharmacological approach to distinguish between the different possible K^+ transport pathways under isosmotic and hypo-osmotic conditions.

Various drugs and agents affecting the potassium conductances were first tested on the potassium permeability of A6 cell monolayers maintained under isosmotic conditions. Barium is a well-known and potent K^+ channel blocker. Considering its poor solubility in the presence of most anions such as sulphate, phosphate and bicarbonate, a chloride Ringer solution (*see* Materials and Methods for composition) was used instead of the usual amphibian culture medium. At a concentration of 5 mM (serosal addition) this agent blocked the ^{86}Rb efflux (51 ± 4% inhibition at 15 min, $n = 6$, $P < 0.001$) followed by a partial recovery of the ^{86}Rb efflux at $t > 21$ min. Quinidine (200 μM), tetraethylammonium (TEA, 10 mM) and glibenclamide (0.5 mM) also slightly blocked the ^{86}Rb loss through the basolateral membranes of the monolayer. An inhibition of 30 ± 4% ($n = 9$, $P < 0.001$) was found after 15 min of quinidine addition while one of 23 ± 4% ($n = 6$, $P < 0.001$) and 15 ± 4 ($n = 6$, $P < 0.01$) was observed with TEA and glibenclamide, the least effective blockers tested.

These ion channel blockers were therefore tested on the Rb (K^+) effluxes of A6 cells submitted to hypo-osmotic shocks. Barium was added to the serosal side of cell monolayers bathed with a buffered saline solution, at concentrations of 1 and 5 mM. It should be noted that in isosmotic conditions, 1 mM barium blocked the ^{86}Rb efflux by 39 ± 3% ($n = 6$, $P < 0.001$). The use of a buffered saline solution (necessary for barium solubility) instead of the usual culture medium, did not modify the ^{86}Rb efflux response to hypo-osmotic media (*compare* Figs. 1*C* and 3). The response consisted of a transient stimulation, followed by a regulatory phase. However, in the presence of 1 mM barium (Fig. 3*A*), a low concentration relative to those usually investigated (5–10 mM), an unexpectedly greater hypo-osmotically stimulated ^{86}Rb efflux was observed. The time course of the biphasic ^{86}Rb efflux increase after hypo-osmotic shock was similar, but the magnitude of the immediate increase in ^{86}Rb efflux in the presence of barium was twice than in its absence, and the ^{86}Rb efflux re-

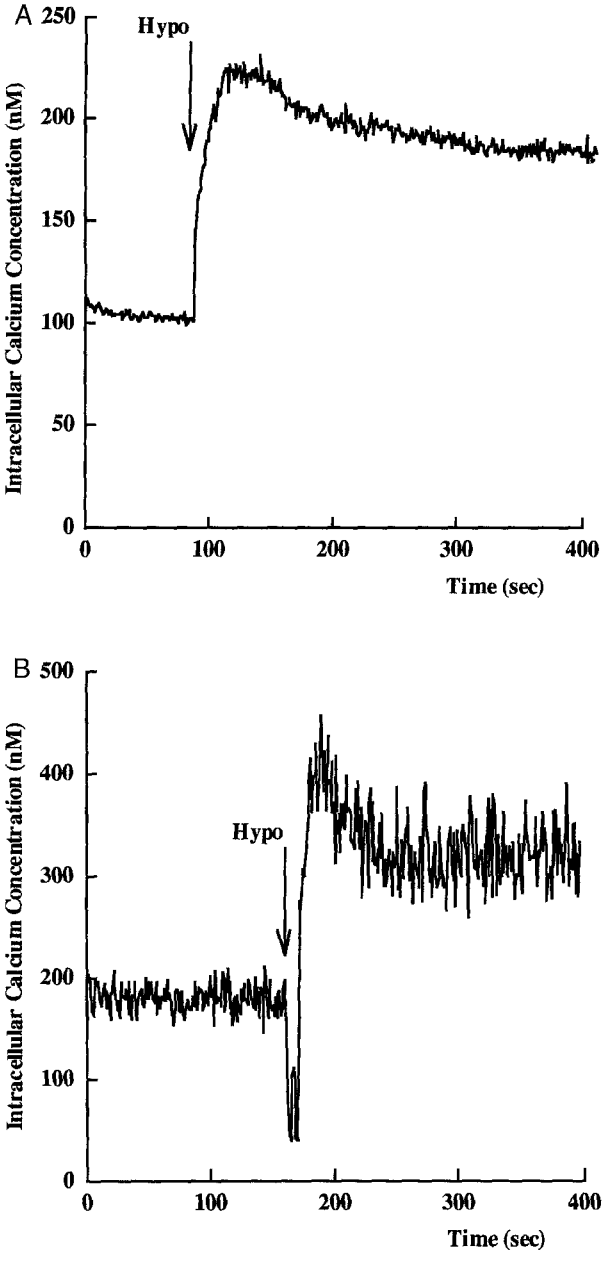


Fig. 2. Effect of hypo-osmotic media on the intracellular calcium (Ca_i) in isolated A6 cells loaded with Fura-2. (*A*) In a typical experiment, the arrow indicates the dilution of the cell suspension to one-third. Mean results given in Results section are corrected from a small artifact due to the dilution of the suspending medium. This figure is not corrected from this artifact (approximately 30 nM). (*B*) Identical experimental procedure repeated with an intact cell monolayer.

mained higher in the presence of the drug than in its absence. The same experiment was repeated with the higher barium concentration of 5 mM (Fig. 3*B*). At this concentration, the initial peak induced by the hypo-osmotic shock was considerably delayed but not prevented since a large increase in the ^{86}Rb efflux was still observed in the presence of 5 mM barium (peaks following the hypo-osmotic shock were at 21 and 6 min with and without 5 mM of barium, respectively).

The effect of quinidine (200 μM) was also tested on the ^{86}Rb efflux stimulation observed under hypo-osmotic conditions. As seen in Fig. 3*C*, this agent did not affect the initial increase of ^{86}Rb efflux induced by serosal application of the 165 mOsm medium. However, quinidine prevented the secondary decline in the ^{86}Rb efflux after the initial transport stimulation, the ^{86}Rb permeability remaining high in the presence of the drug.

We have seen that Ca_i increased under hypo-osmotic conditions. The role of extracellular calcium in the K^+ (^{86}Rb) permeability increase induced by hypo-osmotic media was investigated by comparing the ^{86}Rb effluxes in the presence and absence of calcium in the serosal solution (EGTA addition) (Fig. 3*D*). A serosal remaining free calcium concentration of 17 nM in the presence of 3 mM EGTA was calculated by using computing data according to Chang et al. [8]. Calcium was maintained at its normal concentration in the apical bathing solution to prevent tight junction disruption. The absence of serosal calcium had no effect on the immediate increase in the ^{86}Rb efflux observed under hypo-osmotic conditions, but prevented the subsequent decline in the ^{86}Rb efflux observed in the presence of calcium. The ^{86}Rb efflux stimulation induced by a hypo-osmotic shock in a calcium-free solution is therefore very similar to that reported in the presence of quinidine, since the ^{86}Rb permeability remained elevated throughout the hypo-osmotic period (*compare* Figs. 3*C* and *D*).

Tetraethylammonium (TEA) at concentrations of 2 and 10 mM, was tested on ^{86}Rb (K^+) efflux measured in hypo-osmotic conditions. As seen in Figs. 4*A* and *B*, the initial increase in ^{86}Rb permeability was partly blocked by TEA at the concentration of 2 mM (the maximal ^{86}Rb efflux stimulation after 9 min of serosal hypo-osmotic shock, in the presence of 2 mM TEA, was 64 ± 8% of that measured in its absence, $n = 6$, $P < 0.001$). The ^{86}Rb (K^+) permeability increase was largely blocked by higher doses (10 mM) of TEA (the ^{86}Rb efflux stimulation 9 min after serosal hypo-osmotic shock, in the presence of 10 mM of TEA, was 39 ± 5% of that measured in its absence, $n = 6$, $P < 0.001$). It should be noted that at both TEA concentrations, the late phase of ^{86}Rb efflux stimulation still occurred.

A very similar pattern of inhibition was also found for glibenclamide (Fig. 4*C* and *D*). However, as for SCC experiments (*see below*), relatively large doses were necessary to block the stimulated ^{86}Rb (K^+) efflux measured in hypo-osmotic conditions (after 9 min of osmotic shock, the stimulated ^{86}Rb permeability was inhibited by 50 and 79% with $2 \cdot 10^{-4}$ M and $5 \cdot 10^{-5}$ M of glibenclamide, respectively).

As described above, quinidine and EGTA prevent the regulatory phase following stimulation of the ^{86}Rb

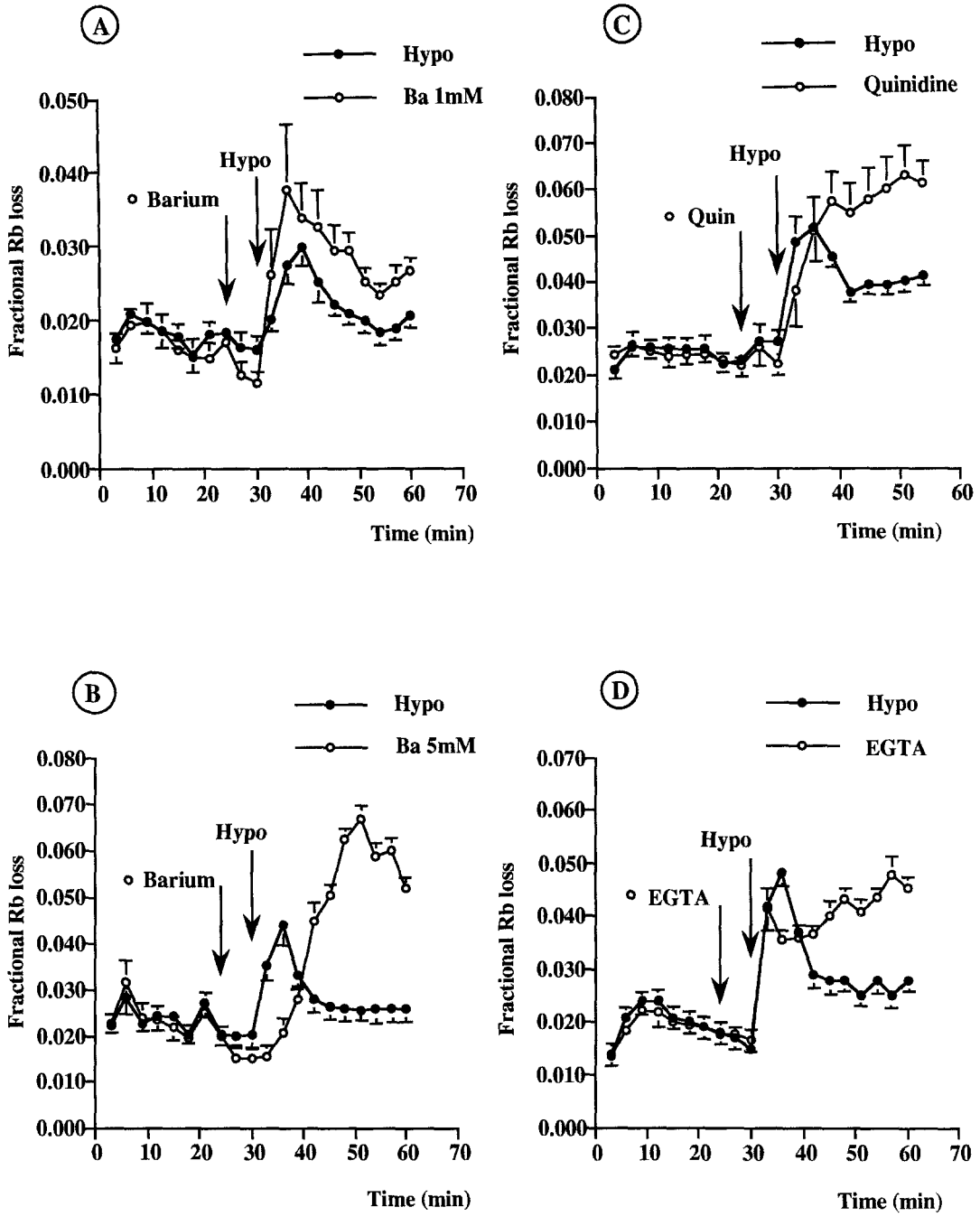


Fig. 3. (*A* and *B*) Effects of barium on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in A6 cell monolayers. Barium was added to the serosal saline solution at the final concentrations of 1 mM (*A*) and 5 mM (*B*), $n = 6$. Control experiments are in full circle (hypo-osmotic stress without drug addition). (*C*) Effects of quinidine (200 μM) on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in A6 cell monolayers ($n = 6$). (*D*) Effects of calcium-free medium (EGTA, 3 mM) on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in A6 cell monolayers. EGTA (3 mM) was added to the serosal side of the monolayer and calcium was not omitted from the apical side ($n = 6$).

(K^+) efflux by hypo-osmotic solutions (*see* Fig. 3*C* and *D*). Final addition of TEA (10 mM) on quinidine- or EGTA-treated monolayers submitted to a hypo-osmotic shock reduced the ^{86}Rb (K^+) efflux in quinidine-treated cells and in EGTA-treated cells to the level of nontreated cells, indicating that the TEA-sensitive component was still present 15 min after the hypo-osmotic shock (Fig. 5). Final addition of glibenclamide (0.5 mM) on quinidine-treated monolayers submitted to a hypo-osmotic shock produced a similar while larger effect than

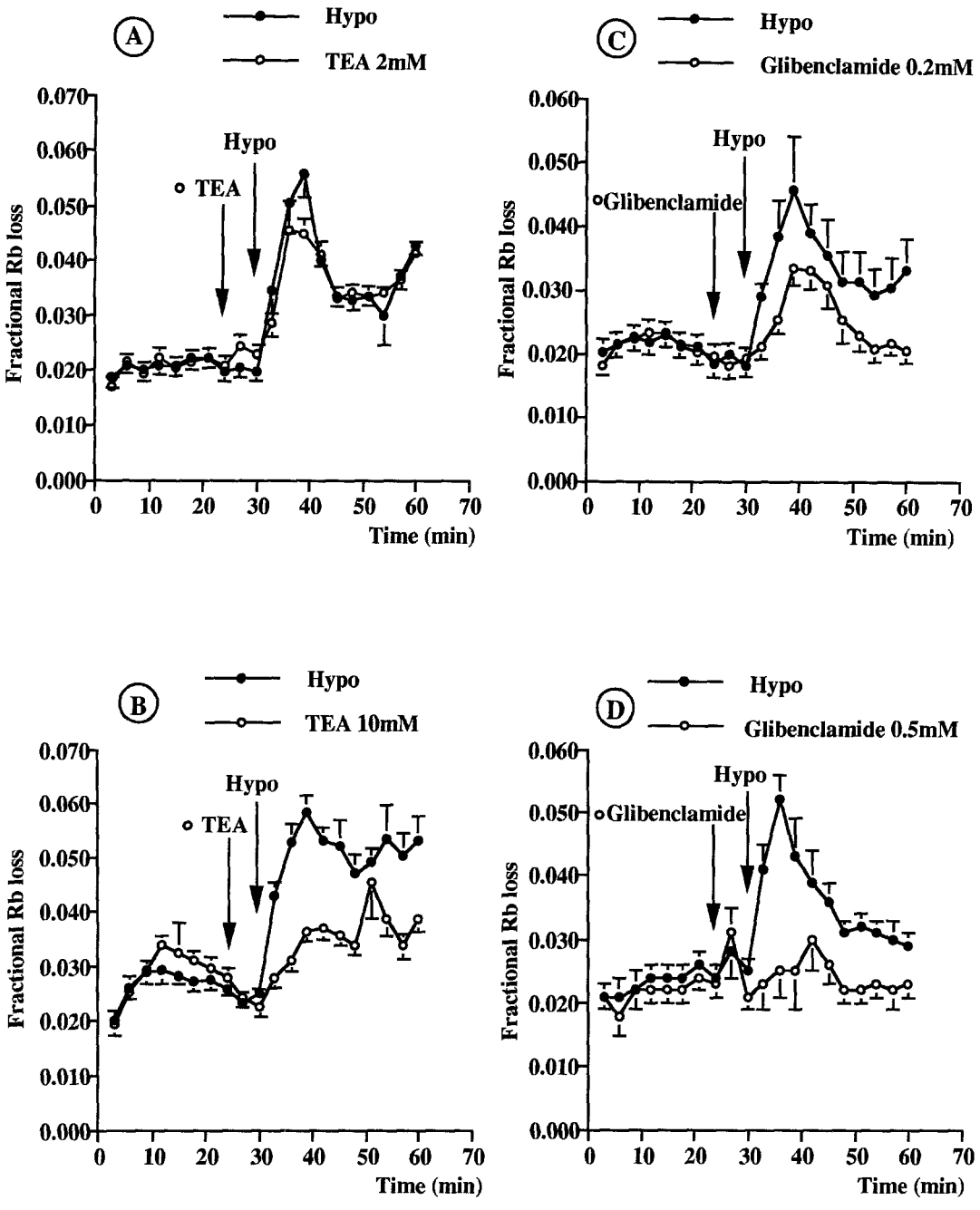


Fig. 4. (*A* and *B*) Effects of TEA on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in A6 cell monolayers. TEA was added to the serosal solution bathing the monolayer at concentrations of 2 mM (*A*) and 10 mM (*B*) ($n = 6$). (*C* and *D*) Effects of glibenclamide on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in A6 cell monolayers. Glibenclamide was added to the serosal solution bathing the monolayer at concentrations of 0.2 mM (*C*) and 0.5 mM (*D*), ($n = 6$).

TEA, blocking the ^{86}Rb (K^+) efflux stimulation to the level of ^{86}Rb (K^+) efflux in isosmotic conditions (Fig. 5*C*).

Gadolinium has recently been described as an effective blocker of stretch-activated K^+ channels and these stretch-activated channels have been shown to be involved in cell volume regulation [43]. Addition of gadolinium to the serosal solution (150 μM) did not modify the ^{86}Rb efflux increase induced by hypo-osmotic conditions. Neither did this drug affect the stimulation of the SCC observed upon hypo-osmotic conditions (*results not shown*).

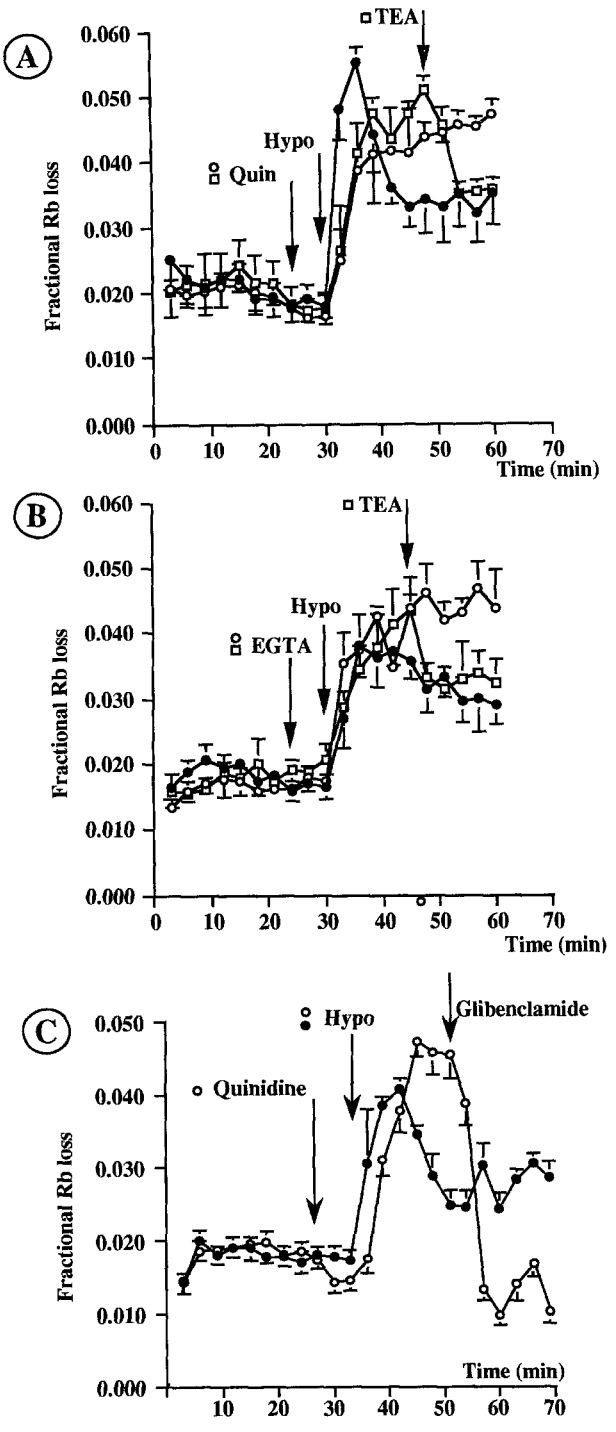


Fig. 5. Effects of TEA on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in quinidine-treated A6 cell monolayers (*A*) and on EGTA-treated cells (*B*). TEA (10 mM) was added 15 min after the hypo-osmotic shock (n = 6). The effect of glibenclamide (0.5 mM) on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in quinidine-treated A6 cell monolayers is represented in Fig. 5*C*. (●) Hypo; (○) quinidine; (□) quinidine + TEA.

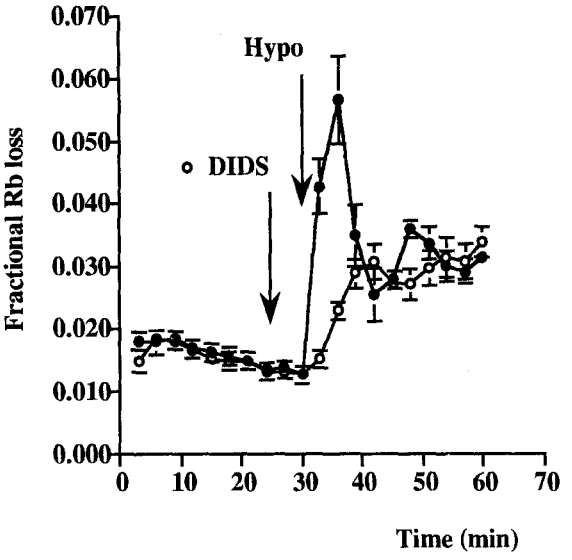


Fig. 6. Effects of DIDS on the ^{86}Rb (K^+) efflux increase induced by hypo-osmotic serosal changes in A6 cell monolayers. DIDS ($5 \cdot 10^{-4}$ M) was added in the serosal solution bathing the monolayer (n = 6). (●) Hypo; (○) DIDS.

With the aim of investigating possible opening of a parallel anion permeability, the effect of DIDS ($5 \cdot 10^{-4}$ M) on the ^{86}Rb efflux was studied (Fig. 6). DIDS added to the serosal side of the monolayer completely blocked the initial increase in the ^{86}Rb permeability, but did not prevent the latter phase of rubidium permeability stimulation. Furosemide (10^{-3} M), bumetanide (50 μM), meclofenamic acid ($5 \cdot 10^{-5}$ M), niflumic acid (10^{-4} M) and DPC ($5 \cdot 10^{-4}$ M) were without effect on the hypo-osmotic-induced increase of ^{86}Rb efflux (*results not shown*).

Effects of Potassium Channel Blockers on the SCC and Cell Volume under Hypo-Osmotic Shock

We have previously seen that the two K^+ channel blockers (TEA and quinidine) exert different inhibitory effects on the Rb (K^+) efflux stimulation induced by hypo-osmotic solutions. We investigated their effects on Na^+ transport and cell volume.

The effects of TEA (10 mM) and quinidine (200 μM) were first assayed on the short-circuit current (Na^+ transport) under isosmotic conditions. In these experimental conditions, quinidine progressively inhibited the SCC (200 μM) of A6 cell monolayers by 51 ± 5% (n = 6), and by 75 ± 3% (n = 6) after 9 and 30 min, respectively, of serosal addition. The inhibitory effect of TEA (10 mM) on the SCC was less pronounced, with a rapid and maximal inhibition (at 6 min) of 18 ± 5% (n = 6). Both drugs were also found to increase the transepithelial resistance (Table), quinidine being the most effective. The SCC changes in serosal hypo-os-

motic conditions were therefore investigated in monolayers previously treated with quinidine or TEA (Table). Quinidine considerably reduced the SCC increase observed after changing the serosal bathing medium of the cell monolayer from a 243 to a 165 mOsm solution (after 20 min of osmotic shock, SCC measurements were of 36.7 ± 3.5 $\mu A/cm^2$ and 17.1 ± 2.1 $\mu A/cm^2$ in nontreated and quinidine-treated cells, respectively). In addition, the transient SCC overshoot (Fig. 1*A*) was not observed in quinidine-treated cells. On the other hand, TEA did not affect the SCC stimulation observed under hypo-osmotic conditions (Table), nor the initial SCC overshoot.

In isosmotic conditions, glibenclamide ($5 \cdot 10^{-4}$ M) inhibited SCC by 59 ± 8% and 72 ± 3%, after 6 and 20 min, respectively, of drug application ($n = 4$, $P < 0.001$) and the resistance of the monolayer was considerably increased (Table). The stimulation of the SCC under hypo-osmotic conditions was also reduced in glibenclamide-treated cells within the first minutes (overshoot) but was still present with longer time of osmotic shock (30 min). It has to be noted too that the immediate decrease in resistance, found in the first minute of submitting the monolayer to a hypo-osmotic shock, was not observed in the presence of glibenclamide (R = 630 ± 146 $\Omega \cdot cm^2$ and 10,083 ± 2,368 $\Omega \cdot cm^2$ in nontreated and treated monolayers respectively, Table); therefore, considering the effect of this potassium channel blocker, it is unlikely that the immediate (and transient) decrease in resistance was due to the opening of a paracellular pathway upon hypo-osmotic shock, and other nonspecific effects on other ion cellular pathways may be involved.

The effects of the different K^+ channel blockers and of the anion channel blockers, DIDS and NPPB were also tested, during an experimental period of 30 min, on the cell volume regulation which is observed when A6 cells are submitted to hypo-osmotic media (Fig. 7). We first investigated the drugs' effects on cell volume in isosmotic conditions: no changes in cell volume were found in quinidine-, TEA-, or DIDS-treated cells nor in Ca^{2+}-deprived or nontreated cells. On the contrary, a 10 ± 1% increase ($n = 5$, $P < 0.001$) in cell volume was found with glibenclamide-treated cells, while a 12 ± 4% increase ($n = 4$, $P < 0.001$) was observed in NPPB-treated cells (after a period of 30 min)—indication that both drugs may affect ion permeabilities involved in cell volume homeostasis.

In hypo-osmotic conditions, incubation in a calcium-free medium or treatment of isolated cells with quinidine (Fig. 7*A* and *B*) resulted in a clear blockage of the regulatory phase which follows the initial increase of the cell volume, indicating the involvement of calcium and of calcium-dependent ion permeability in RVD. However, the two other K^+ channel blockers (TEA and glibenclamide) and the anion channels inhibitors (DIDS and NPPB) also reduced the RVD (Fig. 7*C, D, E, F*).

Discussion

Cellular and Transepithelial Events Following a Serosal Hypo-Osmotic Challenge

Changing the serosal solution from isosmotic to hypo-osmotic causes several cellular and transepithelial events. The Na^+ transport, measured by the amiloride-sensitive short-circuit current, was considerably increased, as had been found in previous studies [55] with the time of maximum activation between 25–30 min, an overshoot usually being observed after approximately 8–10 min. However, the initial and transient decrease in SCC and drop in R (found in the first minute of the osmotic shock) was not reported [55]. The differences in the ionic composition of the solutions used in both studies can probably account for these small discrepancies since, keeping the ionic strength constant (production of a comparable osmotic shock by switching from a 60 mM mannitol-containing solution to a mannitol-free solution) prevents the initial changes in SCC and R without affecting the following increase in SCC (*unpublished results*). Complementary experiments will be useful to determine the origin of this transient initial SCC decrease.

The maximum SCC increase under hypo-osmotic conditions (25–30 min) was preceded by several changes in cell homeostasis: (i) the calcium rose temporarily, to reach an approximate Ca_i peak value (in less than 1 min) and then declined in a regulatory phase to a value which was never the less higher than that measured in isosmotic conditions; (ii) the cellular volume increased rapidly in the very first minutes, followed by a cell volume regulation period (two-thirds of the maximal increase in cell volume being regulated in less than 30 min); (iii) the ^{86}Rb (K) efflux through the basolateral membranes increased considerably, reaching a maximum within the first 3–9 min; this phase was followed by a "regulatory period," the ^{86}Rb (K) efflux, however, remaining significantly higher than that measured in isosmotic conditions.

While K^+ efflux, Ca_i and SCC were measured in intact monolayers, cell volume was determined in isolated cells. It could be objected that cell volume regulation after an osmotic shock may be different in isolated cells or in intact monolayers. This does not seem to be the case considering the very similar time course profile of cell volume regulation (similar maximal swelling time and partial cell volume regulation) recently reported in intact A6 cell monolayers (Van Driessche and colleagues [51]; Dr. N. Wills, *personal communication*).

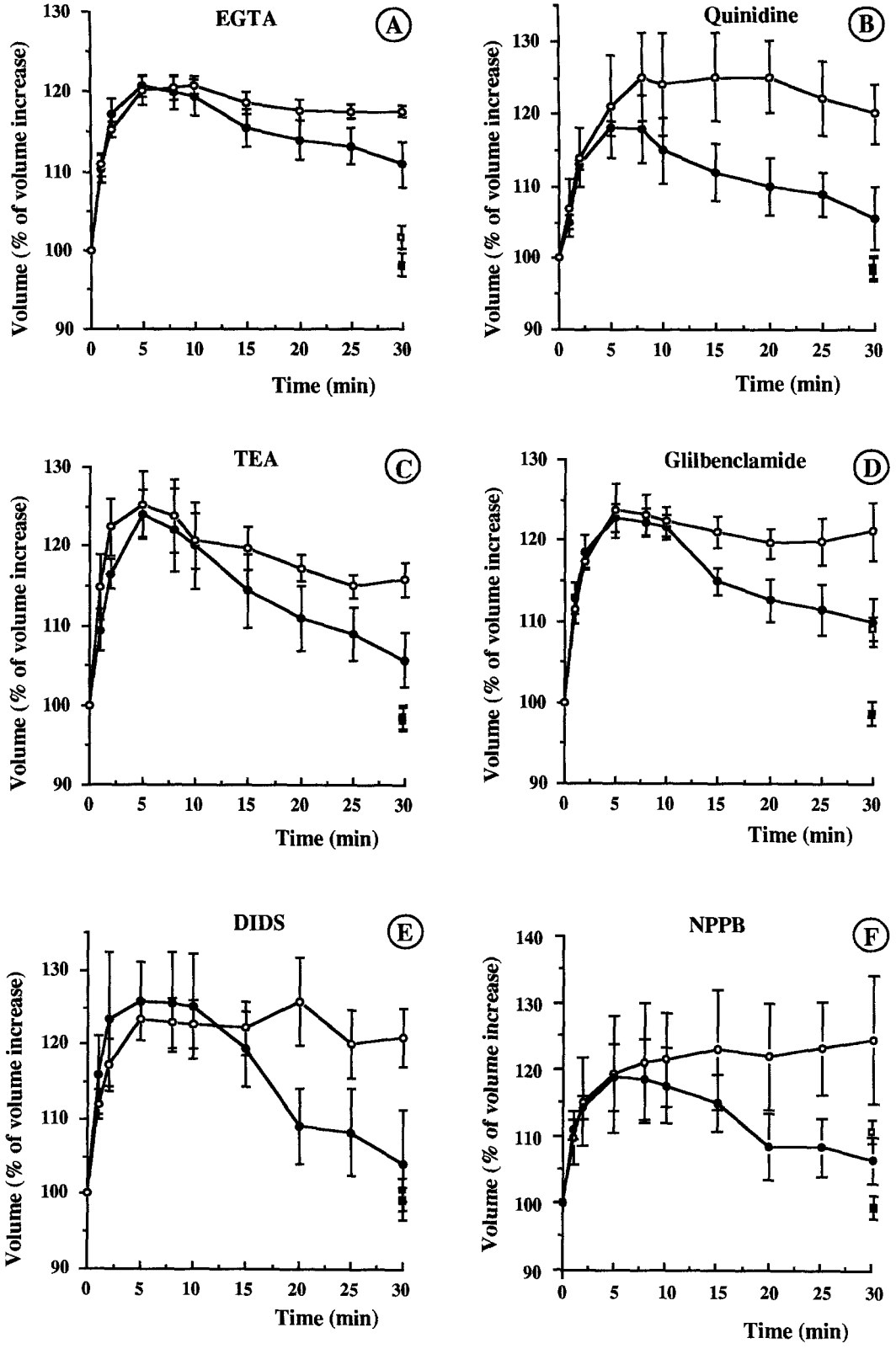


Fig. 7. Effects of calcium-free media and of various potassium and choride channel blockers on the cell volume regulation in isolated A6 cells. The medium was changed from a 247 mOsm solution to a 165 mOsm solution at zero time and A6 cell volume was followed as a function of time (30 min period). The experiment was performed in the presence (filled circles) or in the absence (open circles) of calcium (*A*), (n = 9). Similar experiments were done in the presence (open circles) or in the absence (filled circles) of quinidine (200 μM, n = 4, *B*), TEA (10 mM, n = 5, *C*), glibenclamide (0.5 mM, n = 6, *D*), DIDS (0.5 mM, n = 4, *E*) and NPPB (50 μM, n = 5, *F*). Control experiments were also performed in an isosmotic medium in the presence (open squares) or in the absence (filled squares) of the drug (the effect at time 30 min is reported on the different figures).

Characteristics of the Initial ^{86}Rb (K) Increase Following Hypo-Osmotic Stress

In isosmotic conditions, the ^{86}Rb (K^+) effluxes through the basolateral membranes were partly blocked with barium while quinidine, TEA or glibenclamide addition resulted in a small and progressive inhibitory effect. On the contrary, when the serosal side of the monolayer was submitted to a hypo-osmotic solution, the large and transient initial increase in ^{86}Rb (K^+) efflux which developed was insensitive to quinidine, totally inhibited by TEA and glibenclamide, stimulated by a "low" (1 mM) concentration of barium but delayed by higher doses (5 mM). A very similar type of barium sensitivity was reported in MDCK cells, in which in addition to a barium-sensitive component under isosmotic conditions, a barium-insensitive component of the ^{86}Rb (K^+) efflux was found to develop after subjecting the cells to a hypotonic shock [44].

In our study, the stimulation of the ^{86}Rb (K^+) efflux by low doses of barium may indicate the conductive nature of this pathway following changes in electrochemical driving forces for K^+ exit. Indeed, barium at a concentration of 0.5 mM was reported to depolarize the cell membranes of short-circuited A6 cell monolayers by inhibiting 75% of the basolateral K^+ conductance measured in an isosmotic medium [20]. That the hypo-osmotically induced stimulation of the ^{86}Rb (K^+) efflux observed in the presence of a low dose of barium was greater than in its absence could be interpreted by an increased driving force produced by the cell depolarization and implies too a poor (or no) barium sensitivity of this K^+ efflux.

A change in the basolateral potential (depolarization) of A6 cells by the opening of an outwardly directed anion permeability or an inwardly directed cation permeability could therefore stimulate the initial ^{86}Rb (K^+) efflux observed upon hypo-osmotic perturbation. After a hypo-osmotic shock, a rapid membrane depolarization has been reported in several cell types [28, 41, 49], its origin, however, being controversial. In opossum kidney cells, the pronounced depolarization was found to be the result of the opening of mechanoreceptor-operated ion channels which can be permeable to cations but also to Cl^- ions [49]. In MDCK cells [40, 41] and rabbit proximal convoluted tubule (PCT) cells [29], a transient membrane hyperpolarization followed by a depolarization was observed; this depolarization lasting two minutes was blocked by DIDS [40] and was interpreted as the result of the opening of Cl^- channels [29, 40]. In Ehrlich tumor cells, a nonselective, stretch-activated channel with a single-channel conductance of 23 pS and a 3–7 pS Cl^- channel have been suggested as being responsible for the initial depolarization after cell swelling [34]. In A6 cells, Granitzer et al. [19] observed small variations in membrane potential of short-circuited monolayers with hypo-osmotic perturbations (a small hyperpolarization followed by a depolarization). In preliminary experiments, we observed a membrane depolarization in osmotically shocked cells using bis-oxonol as a membrane potential sensor (*unpublished results*). Furthermore, in view of the large inhibition of the TEA-sensitive ^{86}Rb (K^+) efflux and of the RVD by DIDS and NPPB, it is likely that Cl^- channels are implicated and could be involved in the initial depolarization in hypo-osmotic conditions and the initial cell calcium increase could be implicated.

It is unlikely that the initial increase in the TEA- and glibenclamide-sensitive ^{86}Rb (K^+) permeability is mediated by the stretch-activated cation channel (SAC) as found in the basolateral membranes of *Necturus* proximal tubule [43] and Opossum kidney cells [49]. The lanthanide gadolinium can block a number of SACs at a concentration of 10–20 μM [42]. However, in our A6 cell preparation, gadolinium used at concentrations of 150 μM was ineffective on the ^{86}Rb (K^+) efflux stimulated by hypo-osmotic conditions. It should also be noted that a TEA-sensitive and quinidine-insensitive high conductance voltage-activated K^+ channel has been described in the insulin-secreting cell line RINm5F [16]. Recently, a sulfonylurea-sensitive K^+ conductance was found in the basolateral membranes of A6 cells [5]. In that study, glibenclamide was found to block the ^{86}Rb (K^+) efflux stimulated by hypo-osmotic conditions at relatively high concentrations (larger than 10^{-4} M). Sulfonylurea drugs (glibenclamide or tolbutamide) have been used to specifically block K_{ATP} channels in a variety of nonepithelial [1, 10, 35, 36] and epithelial cells [53, 54]. It has also to be noted that TEA (extracellular) was reported to block ATP-sensitive potassium channels in frog skeletal muscle [11]. It is possible to assume that the initial increase in potassium permeability found in that study is mediated by ATP-sensitive K^+ channels and that both TEA and glibenclamide act on the same pathway. However, differential effects were found in SCC experiments (*see* Table) since TEA did not prevent the drop in R following the hypo-osmotic shock while R continues to increase one minute after the hypo-osmotic shock in glibenclamide-treated cells and TEA did not affect the initial SCC increase (overshoot) while glibenclamide blocked it. In addition, the inhibitory effect on the ^{86}Rb effluxes of quinidine-treated monolayers was larger with glibenclamide than with TEA. Therefore, considering the lack of specificity of most inhibitors (used at relatively high concentrations) in cellular systems, in addition to effects on the basolateral K^+ permeability, indirect effects of the drugs at other cellular or paracellular levels cannot be excluded. The exact nature of this TEA and glibenclamide-sensitive K^+ component remains to be established and patch-clamp studies will be valuable for deciding to which class(es) of channel they belong.

Involvement of a Ca-dependent ^{86}Rb (K^+) Pathway after a Hypo-Osmotic Shock

In cells submitted to hypo-osmotic shock, the period of rapid ^{86}Rb (K^+) efflux increase was followed by a "regulatory phase" in which these effluxes reached a steady value higher than that measured under isosmotic conditions. This could indicate that a second component of K^+ permeability is involved in the hypo-osmotic response. Unlike TEA, quinidine (200 μM) was ineffective on the fast transient increase in ^{86}Rb (K^+) efflux but abolished the "regulatory phase"; a similar response after a hypo-osmotic shock was observed when the cell monolayer was bathed on its serosal side with a calcium-free solution. Cell swelling was previously found to induce a large outward-rectifying K^+ conductance, sensitive to quinidine, through the basolateral membranes of A6 cell monolayers whose apical membranes had been permeabilized by amphotericin B. This increase in potassium permeability was suggested to play a role in cell volume regulation [4]. Similar results have been reported in Na^+-reabsorbing epithelia and in Cl^--secreting epithelia; i.e., a quinidine-sensitive and volume-activated K^+ conductance observed with swollen cells [6, 13]. Our results are not contradictory but complementary. Indeed, we found that quinidine partly blocked the ^{86}Rb (K^+) efflux and the SCC (Na^+ transport) in cells maintained in isosmotic conditions; the SCC stimulation caused by lowering the osmolarity of the serosal solution and the RVD were also inhibited by the drug. In addition, the time course of the plateau phases in the SCC stimulation and the ^{86}Rb (K^+) efflux (after the peak) following an osmotic shock are similar. It is therefore likely that a quinidine-sensitive K^+-conductive pathway is present, not only under isosmotic, but also under hypo-osmotic conditions. In the latter case, the persistence of a high K^+ permeability (absence of "regulatory phase") in quinidine-treated cells and its blocking by subsequent addition of TEA indicates that the initial TEA-sensitive component was still present and not deactivated. Considering the sensitivity of the TEA-blocked K^+ component to voltages, after the likely initial depolarization of the cells by hypo-osmotic conditions (expected to be larger in the presence of quinidine), the absence of membrane repolarization by the quinidine-sensitive K^+ pathway could explain the persistence of a TEA-sensitive ^{86}Rb (K^+) efflux in the quinidine or Ca-free conditions reported above. The large K^+ permeability increase could therefore mask a quinidine-sensitive component.

^{86}Rb (K^+) Pathways and SCC

The involvement of each potassium transport pathway in the SCC increase following a hypo-osmotic shock was more difficult to define considering the relative lack of specificity of K^+ blockers and possible indirect effects of drugs at other levels than the basolateral membranes (apical or paracellular). However, the quinidine-sensitive component was largely represented in the stimulated SCC observed upon a hypo-osmotic stress, while the TEA-sensitive pathway was unrelated to changes in the electrical properties of the monolayer. A membrane depolarization following quinidine application could explain the inhibition of the stimulated SCC by a decrease of the electrochemical driving force for Na^+ entry. Glibenclamide did not completely prevent the long-term (30 min) SCC stimulation but largely affected the electrical parameters in the early osmotic shock, suggesting other site(s) of action than TEA; it has to be noticed too that the glibenclamide inhibitory effect on SCC was predominant in isosmotic conditions (*see* Table).

Role of Ca_i and Rb (K^+) Pathways in Cell Volume Regulation

The transient increase in cell calcium found in this study, either in isolated cells or in intact monolayers (Fig. 3), is consistent with the Ca_i rise usually reported in kidney cells [2, 3, 34, 38, 40, 45] and with the development of a calcium-dependent potassium pathway. In this study, a K_d of 224 nM was taken to calculate Ca_i in both iso-and hypo-osmotic conditions. The absolute values of Ca_i changes, however, would be questionable in a hypo-osmotic medium since the K_d of Fura-2 has been found to be sensitive to the ionic strength of the medium [48, this study], a decrease of K_d (and of Ca_i) being expected with hypo-osmotic media in the very first moment of osmotic shock. The calculation of the "true" K_d *in situ,* in disequilibrium conditions, depends therefore on many cell parameters (cell pH, ionic strength . . .) and could hardly be measured. However, it is unlikely that the relative cell calcium changes found in this study (or in the bulk of publications) under hypo-osmotic conditions are artifactual since the cell calcium rise and its regulation were totally abolished in a Ca-free medium (*unpublished results*). RVD mechanisms in many cells are Ca_i dependent, and it has been suggested that a Ca^{2+} threshold was responsible for the variability of response found in the different cell types [33]. A delay in the RVD was observed with A6 cells submitted to hypo-osmotic calcium-free medium, suggesting the involvement of this signal in A6 cell volume regulation. Quinidine also blocked the RVD, argument in favor of the stimulation of Ca-dependent K^+ channels in cell volume regulation. On the other hand, the RVD was also found to be blocked by TEA and glibenclamide, indicating that several components of K^+ transport through the basolateral membranes, in parallel with the opening of an anion permeability (as demonstrated

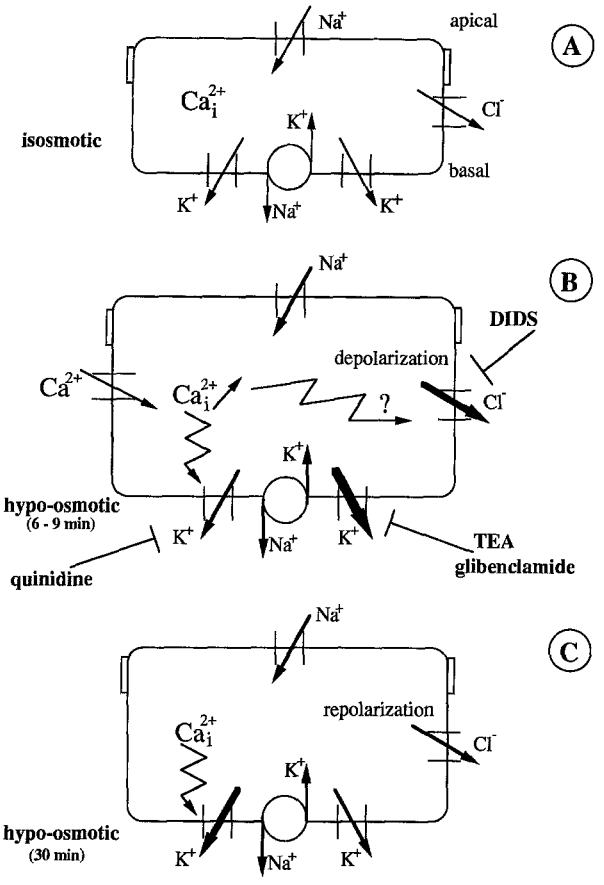


Fig. 8. This functional model represents the working hypothesis for the effects of a serosal hypo-osmotic shock on the basolateral potassium membrane permeability of A6 cells in relation to cell volume regulation. When cells bathed in a serosal isosmotic solution (*A*) are transferred to serosal hypo-osmotic solution (*B, C*), they first swell (*B*) but regulate their cell volume (*C*). We propose that a TEA- and glibenclamide-sensitive but quinidine-insensitive increase in the $Rb(K^+)$ efflux through the basolateral membrane is involved in the very first phase of volume regulation. Membrane depolarization possibly mediated by the opening of DIDS-sensitive Cl^- channels (following initial Ca_i rise?) may cause an increase of the electrochemical driving force for K^+ exit. An increase in the quinidine-sensitive K^+ permeability, mediated by the observed calcium rise could play a complementary role in the late phase. This second component would also contribute to restore the initial membrane potential, thereupon reducing the activity of the TEA-sensitive K^+ channels. The Na^+ transport (SCC) through the monolayer is enhanced in both phases of the osmotic shock.

by DIDS and NPPB blockade of the RVD), could restore A6 cell volume upon osmotic stress.

In conclusion, we suggest that the basolateral membrane potassium permeability is increased following cell swelling because of the activation of two K^+ transport pathways. We propose that a TEA- and glibenclamide-sensitive but quinidine-insensitive increase in the $Rb(K^+)$ efflux through the basolateral membranes is involved in the very first phase of volume regulation of A6 cells submitted to hypo-osmotic media. Transient cell membrane depolarization (calcium-mediated opening of chloride channels?) could be at the origin of this K^+ stimulation. An increase in the quinidine-sensitive K^+ permeability, mediated by the observed calcium rise, would play a complementary role in achieving cell volume regulation, and would restore the membrane potential initially depolarized, thereupon reducing the activity of the TEA-sensitive K^+ channels. A model for the interpretation of our results is given in Fig. 8.

This work was supported by grants from the Commissariat à l'Energie Atomique and the Centre National de Recherche Scientifique URA 638.

References

1. Aschcroft, F.M., Harrison, D.E., Aschcroft, S.J.H. 1984. Glucose induces closure of single potassium channels in isolated pancreatic β-cells. *Nature* **312:**446–448
2. Breton, S., Beck, J.S., Cardinal J., Giebisch, G., Laprade, R. 1992. Involvement and source of calcium in volume regulatory decrease of collapsed proximal convoluted tubule. *Am. J. Physiol.* **263:**F656–F664
3. Breuer, W.V., Mack, E., Rothstein, A. 1988. Activation of K^+ and Cl^- channels by Ca^{2+} and cyclic AMP in dissociated kidney epithelial MDCK cells. *Pfluegers Arch.* **411:**450–455
4. Broillet, M.C., Horisberger, J.M. 1991. Basolateral membrane potassium conductance of A6 cells. *J. Membrane Biol.* **124:**1–12
5. Broillet, M.C., Horisberger, J.M. 1993. Tolbutamide-sensitive potassium conductance in the basolateral membrane of A6 cells. *J. Membrane Biol.* **134:**181–188
6. Butt, A.G., Clapp, W.L., Frizzell, R.A. 1990. Potassium conductances in tracheal epithelium activated by secretion and cell swelling. *Am. J. Physiol.* **258:**C630–C638
7. Chang, D., Dawson, D.C. 1988. Digitonin-permeabilized colonic cell layers. Demonstration of calcium-activated basolateral K^+ and Cl^- conductances. *J. Gen. Physiol.* **92:**281–306
8. Chang, D., Hsiech, P.S., Dawson, D.C. 1988. Calcium: a program in basic for calculating the composition of solutions with specified free concentrations of calcium, magnesium and other divalent cations. *Comp. Biol. Med.* **18(5):**351–366
9. Christensen, O. 1987. Mediation of cell volume regulation by Ca^{2+} influx through stretch-activated channels. *Nature* **330:**66–68
10. Cook, D.L., Hale, C.N. 1984. Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. *Nature* **311:**271–273
11. Davies, N.W., Spruce, A.E., Standen, N.B., Stanfield, P.R. 1989. Multiple blocking mechanisms of ATP-sensitive potassium channels of frog skeletal muscle by tetraethylammonium ions. *J. Physiol.* **413:**31–48
12. Davis, C.W., Finn, A. 1988. Potassium-induced cell swelling in *Necturus* gall bladder epithelium. *Am. J. Physiol.* **254:**C643–C650
13. Dawson, D.C., Richards, N.W. 1990. Basolateral K^+ conductance: Role in regulation of NaCl absorption and secretion. *Am. J. Physiol.* **259:**C181–C195
14. Ehrenfeld, J., Lacoste, I., Harvey, B.J. 1992. Effects of intracellular signals on Na^+/K^+-ATPase pump activity in the frog skin epithelium. *Biochim. Biophys. Acta* **1106:**197–208
15. Fidelman, M.L., Watlington, C.O. 1984. Insulin and aldosterone. *Endocrinology* **115:**1171–1178
16. Findlay, I., Dunne, M.J., Ulrich, S., Wolheim, C.B., Petersen, O.

1985. Quinidine inhibits Ca^{2+}-independent K^+ channels whereas tetraethylammonium inhibits Ca^{2+}-activated K^+ channels in insulin secreting cells. *FEBS Lett.* **185:**4–8
17. Finkelstein, A., Holz, R. 1973. Aqueous pores created in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *In:* Membranes. Vol. 2. Lipid Bilayers and Antibiotics. pp. 377–408. Marcel Dekker, New York
18. Garty, H. 1984. Current-voltage relations of basolateral membrane in tight amphibian epithelium: Use of nystatin to depolarize the apical membrane. *J. Membrane Biol.* **77:**213–222
19. Granitzer, M., Bakos, P., Nagel, W., Crabbe, J. 1992. Osmotic swelling and membrane conductances in A6 cells. *Biochim. Biophys. Acta* **1110:**239–242
20. Granitzer, M., Leal, T., Nagel, W., Crabbe, J. 1991. Apical and basolateral conductance in cultured A6 cells. *Pfluegers Arch.* **17:**463–468
21. Granitzer, M., Nagel, W., Crabbe, J. 1993. K^+ recirculation in A6 cells at increased Na^+ transport rates. *Pfluegers Arch.* **422:**546–551
22. Grinstein, S., Smith, J.D. 1990. Calcium independent cell volume regulation in human lymphocytes. *J. Gen. Physiol.* **95:**97–120
23. Grynkiewicz, G., Poenie, M., Tsien, R.Y. 1985. A new generation of calcium indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260:**3440–3450
24. Handler, J.S., Preston, A.S., Steele, R.E. 1984. Factors affecting the differentiation of epithelium transport and responsiveness to hormones. *Fed. Proc.* **43:**2221–2224
25. Handler, J.S., Steele, R.E., Sahib, M.K., Wade, J.B., Preston, A.S., Lawson, N.S.L., Johnson, J.P. 1979. Toad urinary bladder epithelial cells in culture: Maintenance of epithelial structure, sodium transport, and response to hormones. *Proc. Natl. Acad. Sci. USA* **76:**4151–4155
26. Harrison, S.M., Bers, D.M. 1987. The effect of temperature and ionic strength on the apparent Ca-affinity of EGTA and the analogous Ca-chelators BAPTA and dibromo-BAPTA. *Biochim. Biophys. Acta* **925:**133–143
27. Henquin, J.C., Debuyser, A., Drews, G., Plant, T.D. 1992. Regulation of K^+ permeability and membrane potential in insulin-secreting cells. *In:* Nutrient Regulation of Insulin Secretion. P.R. Flatt, editor. pp. 173–191. Portland Press, London and Chapel Hill
28. Hoffmann, E.K. 1992. Control of volume regulatory ion transport processes in a mammalian cell: Signalling by secondary messengers. *In:* Alfred Benzon Symposium 34. Munksgaard, Copenhagen
29. Kawahara, K., Ogawa, A., Suzuki, M. 1991. Hypoosmotic activation of Ca^{2+}-activated K^+ channels in cultured rabbit kidney proximal tubule cells. *Am. J. Physiol.* **260:**F27–F33
30. Lewis, S.A., Eaton, D.C., Clausen, D., Diamond, J.M. 1977. Nystatin as a probe for investigating the electrical properties of tight epithelium. *J. Gen. Physiol.* **70:**427–440, 1977
31. Lichtenstein, N.S., Leaf, A. 1965. Effect of amphotericin B on the permeability of the toad bladder. *J. Clin. Invest.* **44:**1328–1342
32. McCarty, N.A., O'Neil, R.G. 1991. Calcium-dependent control of volume regulation in renal proximal tubule cells: Roles of dihydropyridine. Sensitive and insensitive Ca^{2+} entry pathways. *J. Membrane Biol.* **123:**161–170
33. McCarty, N.A., O'Neil, R.G. 1992. Calcium signaling in cell volume regulation. *Physiol. Rev.* **72:**1037–1061
34. Montrose-Rafizadeh, C., Guggino, W.B. 1991. Role of intracellular calcium in volume regulation by rabbit medullary thick ascending limb cells. *Am. J. Physiol.* **260:**F402–F409
35. Mourre, C., Widmann, C., Lazdunski, M. 1990. Sulfonylurea binding sites associated with ATP-regulated K^+ channels in the central ontogenesis, and of their localization in mutant mice cerebellum. *Brain Res.* **519:**29–43
36. Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:**147–148
37. Perkins, F.M., Handler, J.S. 1981. Transport properties of toad kidney epithelium in culture. *Am. J. Physiol.* **241:**C154–C159
38. Petersen, O.H., Maruyama, Y. 1984. Calcium-activated potassium channels and their role in secretion. *Nature* **307:**693–696
39. Rafferty, K.A. 1969. Mass culture of amphibian cells: methods and observation concerning stability of cell type. *In:* Biology of Amphibian Tumors. M. Mizell, editor. pp. 52–81. Springer-Verlag, New York
40. Rothstein, A., Mack, E. 1990. Volume-activated K^+ and Cl^- pathways of dissociated epithelial cells (MDCK): Role of Ca^{2+}. *Am. J. Physiol.* **258:**C827–C834
41. Roy, G., Sauvé, R. 1987. Effect of anisotonic media on volume, ion and amino-acid content and membrane potential of kidney cells (MDCK) in culture. *J. Membrane Biol.* **100:**83–96
42. Sachs, F. 1992. Stretch-sensitive ion channels: an update. *In:* Sensory Transduction. D.P. Corcy and S.D. Roper, editors. pp. 242–260. Rockefeller University, New York
43. Sackin, H. 1987. Stretch-activated potassium channels in renal proximal tubule. *Am. J. Physiol.* **253:**F1253–F1262
44. Simmons, N.L. 1991. The effect of hypo-osmolarity upon transepithelial on transport in cultured renal epithelial layers (MDCK). *Pfluegers Arch.* **419:**572–578
45. Susuki, M., Kawahara, H., Ogawa, A., Morita, T., Kawaguchi, Y., Kurihara, S., Sakai, O. 1990. $[Ca^{2+}]i$ rises via G protein during regulatory volume decrease in rabbit proximal tubule cells. *Am. J. Physiol.* **258:**F690–F696
46. Taniguchi, J., Guggino, N.B. 1989. Membrane stretch: a physiological stimulator of Ca-activated K channels in thick ascending limb. *Am. J. Physiol.* **257:**F347–F352
47. Thomas, S.R., Mintz, E. 1987. Time-dependent apical membrane K^+ and Na^+ selectivity in cultured cells. *Am. J. Physiol.* **253:**C1–C6
48. Thomas-Young, R.J., Smith, T.C., Levinson, C. 1993. Regulatory volume decrease in Ehrlich ascites tumor cells is not mediated by a rise in intracellular calcium. *Biochim. Biophys. Acta* **1146:**81–86
49. Ubl, J., Murer, H., Kolb, H.A. 1988. Ion channels activated by osmotic and mechanical stress in membranes of opossum kidney cells. *J. Membrane Biol.* **104:**223–232
50. Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23:**110–127
51. Van Driessche, W., De Smet, P., Raskin, G. 1993. An automatic monitoring system for epithelial cell height. *Pfluegers Arch.* **425:**164–171
52. Verrey, F., Schaerer, E., Zoerkler, P., Paccolat, M.P., Geering, K., Kraehenbuhl, J.P., Rosier, B.C. 1987. Regulation by aldosterone of Na^+, K^+-ATPase mRNAm, protein synthesis, and sodium transport in cultured kidney cells. *J. Cell Biol.* **104:**1231–1237
53. Wang, W., Schwab, A., Giebisch, G. 1990. Regulation of small conductance K^+ channel in apical membrane of rat cortical collecting tubule. *Am. J. Physiol.* **259:**F494–F502
54. Wang, W., Giebisch, G. 1991. Dual effects of ATP on apical small conductance K^+ channel of the rat cortical collecting duct. *J. Gen. Physiol.* **98:**35–61
55. Wills, N.K., Millinoff, L.P., Crowe, W.E. 1991. Na^+ channel activity in cultured renal (A6) epithelium: Regulation by solution osmolarity. *J. Membrane Biol.* **121:**79–90
56. Wong, S.M.E., Debell, M.C., Chase, H.S., Jr. 1990. Cell swelling increases intracellular free (Ca) in cultured toad bladder cells. *Am. J. Physiol.* **258:**F292–F296