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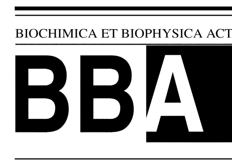

Swelling-activated potassium currents of Ehrlich ascites tumour cells

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Abstract

The K^+ and Cl^- currents activated by Ca^{2+}-ionophore treatment or by hypotonic cell swelling have been studied in Ehrlich ascites tumour cells by the patch-clamp technique. A charybdotoxin-inhibitable K^+ current was activated by increasing intracellular Ca^{2+} concentration. In contrast, the K^+ current activated by cell swelling was insensitive to charybdotoxin as well as to apamin, suggesting that channels different from those sensitive to Ca^{2+} are responsible for regulatory volume adjustments in these cells. The magnitude of the K^+ and Cl^- currents activated by hypotonic challenge was markedly temperature-dependent, possibly reflecting the temperature-dependence of enzymes involved in the intracellular signalling of cell volume regulation. © 1998 Elsevier Science B.V.

Keywords: Regulatory volume decrease; Patch-clamp; Calcium ion dependence; Charybdotoxin

Volume regulation is a property common to all animal cell types investigated and has been extensively reviewed [1,2]. The mechanism for cell volume regulation always involves the modification of the intracellular concentration of organic osmolytes and ions by activation of permeability pathways, leading to osmotically-driven water movements. In response to swelling, the cells lose KCl and organic osmolytes to regain their normal volume in a process known as regulatory volume decrease (RVD).

Ehrlich cells have been studied extensively for their ability to volume regulate. It has been proposed that separate K^+ and Cl^- conductance pathways activate upon cell swelling [1], and hypotonicity has been shown to activate a small conductance Cl^- channel in Ehrlich cells [3]. Furthermore, a small conductance inwardly rectifying Ca^{2+}-dependent K^+ channel has been described and proposed to be responsible for the increased K^+ permeability induced by cell-swelling [3]. Recently, the question of the identity of the conductance pathway mediating K^+ efflux during RVD in Ehrlich cells has been addressed [4]. It was shown that swelling-induced activation of the permeability pathway for K^+ occurred in the absence of any detectable increase in intra-

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cellular free Ca^{2+} concentration and that it was not sensitive to inhibitors of Ca^{2+}-dependent K^+ channels such as charybdotoxin (ChTX). This study investigates by patch-clamp whole-cell recording the presence of a volume-activated K^+ current different from that mediated by Ca^{2+}-dependent K^+ channels.

In order to determine K^+ (I_K) and Cl^- (I_{Cl}) currents developing during cell swelling, a two-pulse protocol was used from a holding potential (V_h) of −30 mV. The membrane potential was clamped alternatively at E_K and E_{Cl}. Because exposure to hypotonicity will lead to cell swelling, and its conse-

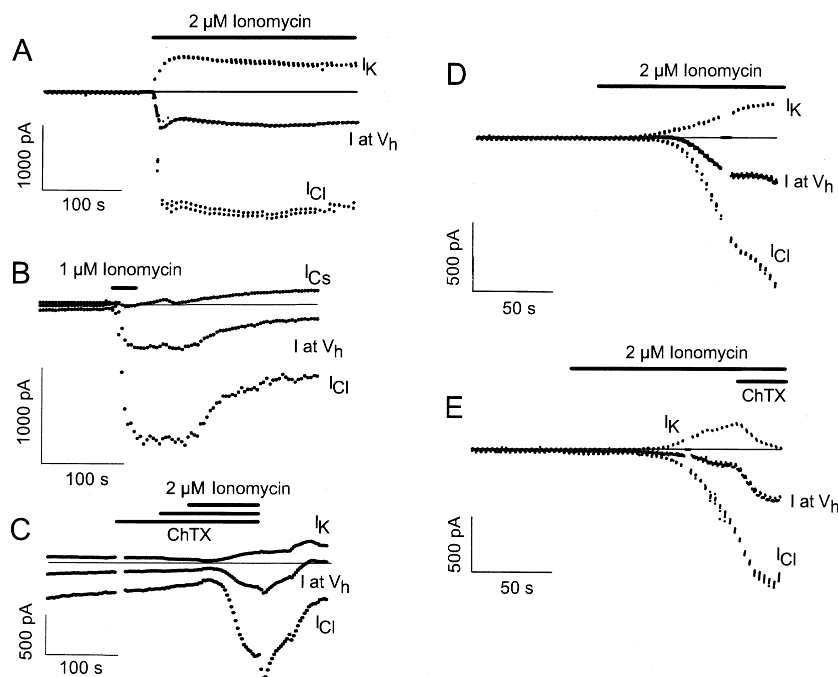

Fig. 1. Effect of ionomycin on I_K and I_{Cl} in Ehrlich cells. Currents were measured in the whole-cell recording mode of the patch-clamp technique, using a holding potential (V_h) of −30 mV and pulsing throughout the experiment to −73 (E_K) or −2 mV (E_{Cl}) with square pulses of 500 ms duration. Intracellular solution composition was, in mM, 93 KCl, 1.2 $MgCl_2$, 100 D-mannitol, 0.1 EGTA, 10 HEPES pH 7.4 adjusted with Tris in panels A, D and E. In the experiments in B and C intracellular solution had in addition 13 mM NaCl and the pulses were to 1 mV and −73 mV. The extracellular solution contained 93 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 100 D-mannitol, 3.3 MOPS, 3.3 TES, 5 HEPES, pH 7.4 adjusted with Tris. ChTX was used at 100 nM and the solution contained 0.01% dialysed bovine serum albumin. In B, intracellular KCl was replaced by an equimolar amount of CsCl. Experiments in A–C were done at room temperature. Those in D and E were done in a chamber thermostated at 37°C. The horizontal line indicates the zero current level. The bars indicate periods of addition of ionomycin or ChTX. In C, the double bar under 2 μM Ionomycin indicates a double addition first of 2 μM and then augmentation to 4 μM ionophore. *Experimental procedure*: the line of Ehrlich ascites tumor cells (hyperdiploid strain) used here was maintained by weekly intraperitoneal inoculation in NMRI (Denmark) or CF-1 (Chile) female mice. Harvesting of the cells was done as described [8]. Cells were transferred to cell culture Petri dishes, which could be mounted directly on the stage of an inverted microscope. The bath was grounded via an agar bridge. Standard whole-cell patch-clamp recordings were performed as described elsewhere [9] using an EPC-7 (List Medical, Darmstadt, Germany) or a RK-300 (Biologic, Claix, France) amplifier. Cells were continuously superfused by gravity with the appropriate extracellular solution and a peristaltic pump was used to remove fluid so that the level in the chamber was kept constant. Local changes in composition of the medium were achieved as described in Ref. [9]. For experiments at 37°C a thermostated chamber (Brook, IL, USA) was used; in this case solution changes were effected by complete replacement of the chamber volume (400 μl). Intracellular solutions contained 0.1 mM EGTA and no added Ca^{2+}. At this concentration EGTA is unlikely to buffer any possible Ca^{2+} transients. Patch-clamp pipettes were made from thin borosilicate glass capillary tubing with an outside diameter of 1.5 mm (Clark Electromedical, Reading, UK). The pipettes had a resistance of 3–5 MΩ. Voltage and current signals from the amplifier were recorded on a digital tape recorder (DTR-1204, Biologic, France) and digitised using a 486-based computer equipped with a Labmaster (Scientific Solutions, USA) or a CED (Cambridge, UK) AD/DA interface. Low-pass filtering was done with an 8-pole Bessel filter (Frequency Devices, USA). The data acquisition and analysis programs used were those written by J. Dempster (University of Strathclyde, Glasgow, Scotland).

quent dilution of intracellular ions, we investigated what correction of equilibrium potentials should be applied. The dependence of V_m upon extracellular K^+ concentration was explored in Ehrlich cells using the current-clamp mode of the whole-cell configuration. All solutions contained 10 μM gramicidin and because the main cations were K^+ and *N*-methyl-D-glucamine (NMDG), V_m should be set by E_K. Data were corrected for calculated liquid junction potential changes [5]. Values at high extracellular K^+ only were considered because it is known that gramicidin shows some degree of permeability to large cations such as NMDG and Tris [6]. V_m dependence upon $\log[K^+]_o$ between 25 and 105 mM can be described by a straight line with a slope of 59 mV/decade and intercept at 91 mM $[K^+]_i$. Upon switching to hypotonicity there was a shift in the V_m vs. $\log[K^+]_o$ relation giving a slope of 58 mV/decade and an intercept at 76 mM $[K^+]_i$. This would translate into a dilution to 83% of that in isotonic medium. We used this figure in subsequent experiments to correct our estimated E_K and E_{Cl} values when doing swelling experiments. It should be noted that swelling in a hypotonic solution containing gramicidin might lead to a different $[K^+]_i$ as compared to control condition; also, if some volume regulation takes place during the actual experiments this is not accounted for, but is considered unlikely as in a similar experimental configuration no recovery of cell volume has been observed [7].

We have used macroscopic current recordings to study K^+ and Cl^- channels in Ehrlich cells. Fig. 1 shows that prior to any treatment of the cells there was only a low level of currents with the voltage protocol used. We presume that the intracellular Ca^{2+} concentration (no added Ca^{2+} and 0.1 mM EGTA) was too low to activate Ca^{2+}-dependent K^+ and Cl^- channels. Fig. 1A shows that K^+ and Cl^- currents are elicited after addition of 2 μM ionomycin in Ehrlich cells. The effect illustrated was sustained, but we have also observed transient increases, reminiscent of previously reported transient ionophore-induced increases in intracellular Ca^{2+} concentration [10]. Upon addition of ionomycin, I_K increased from 16 ± 6 to 405 ± 147 pA and I_{Cl} from -139 ± 48 to -941 ± 160 pA (mean $\pm$ SEM, $n = 5$). Cs^+ is known to be an impermeant cation in Ca^{2+}-dependent inwardly rectifying K^+ channels [11]. As shown in Fig. 1B, replacement of intracellular K^+ by Cs^+ abolished the ionomycin-dependent increase in cur-

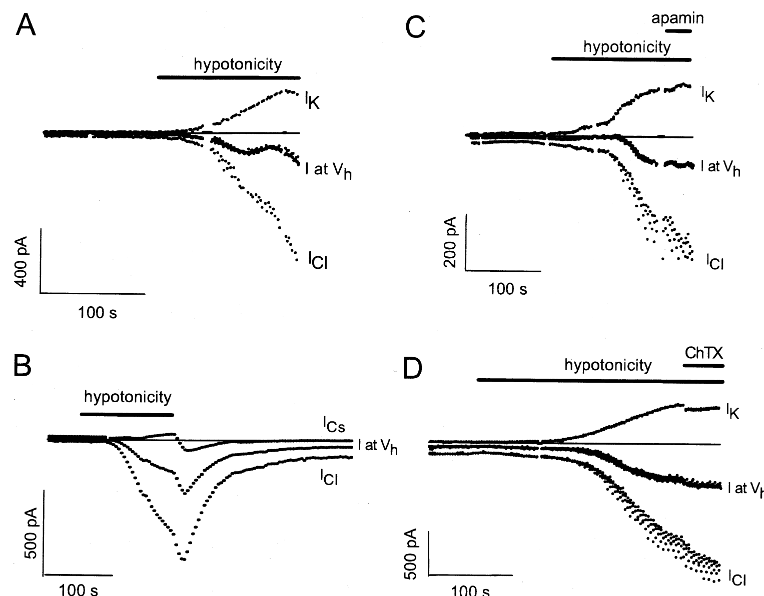

Fig. 2. Effect of hypotonicity on I_K and I_{Cl} in Ehrlich cells. Currents were measured as described in the legend to Fig. 1 but with a voltage protocol corrected as described in the legend to Fig. 1 (holding potential −30 mV, pulses to −6 and −68 mV). Extracellular tonicity was reduced 30% by removal of D-mannitol from the solution. Otherwise the composition of solutions and other details were as in Fig. 1. Apamin was used at 1 μM (C) and ChTX at 100 nM (D). Intracellular KCl was replaced with CsCl in B. Experiments were done at room temperature and those in A, C and D are also included in Fig. 3, middle panels.

rent at E_{Cl} (from 6 ± 9 to 73 ± 58 pA, $n = 3$), but Cl^- current still developed (from -129 ± 50 to -1276 ± 611 pA, $n = 3$).

ChTX is a well known inhibitor of various types of K^+ channels, including Ca^{2+}-dependent inwardly rectifying K^+ channels [11]. Addition of ChTX 1 min after evoking I_K with ionomycin led to a fast decrease in this current with no effect on I_{Cl} (data not shown). In Fig. 1C currents were measured in standard isotonic solution. At the time indicated by the bar 100 nM ChTX was added; then ionomycin was added at 2 μM and then its concentration augmented to 4 μM in the continued presence of ChTX. Under these conditions no development in I_K took place, whilst there was an increase in I_{Cl}. A similar result was obtained in two separate experiments. Fig. 1 panels D and E show experiments performed at 37°C that also demonstrate the development of K^+ and Cl^- currents upon ionomycin addition and blockade of I_K by ChTX. Notice that the perfusion system used at 37°C was slower compared with that used at room temperature. It is concluded that ChTX-sensitive, Ca^{2+}-dependent K^+ currents are present in Ehrlich cells. It is proposed that this corresponds to the activity of low conductance, inwardly-rectifying K^+ channels previously recorded in Ehrlich [3] and other cells [11,12] and probably belonging to the so-called SK channel family [13,14].

In order to look for K^+ and Cl^- conductances activated by cell swelling, a 30% dilution of the bathing medium was effected without altering the ionic composition (removal of mannitol). Fig. 2A shows a typical experiment where, after a delay of about 2 min, currents developed both at E_K and E_{Cl}. A summary of a series of similar experiments is found in the middle panel of Fig. 3. The lack of

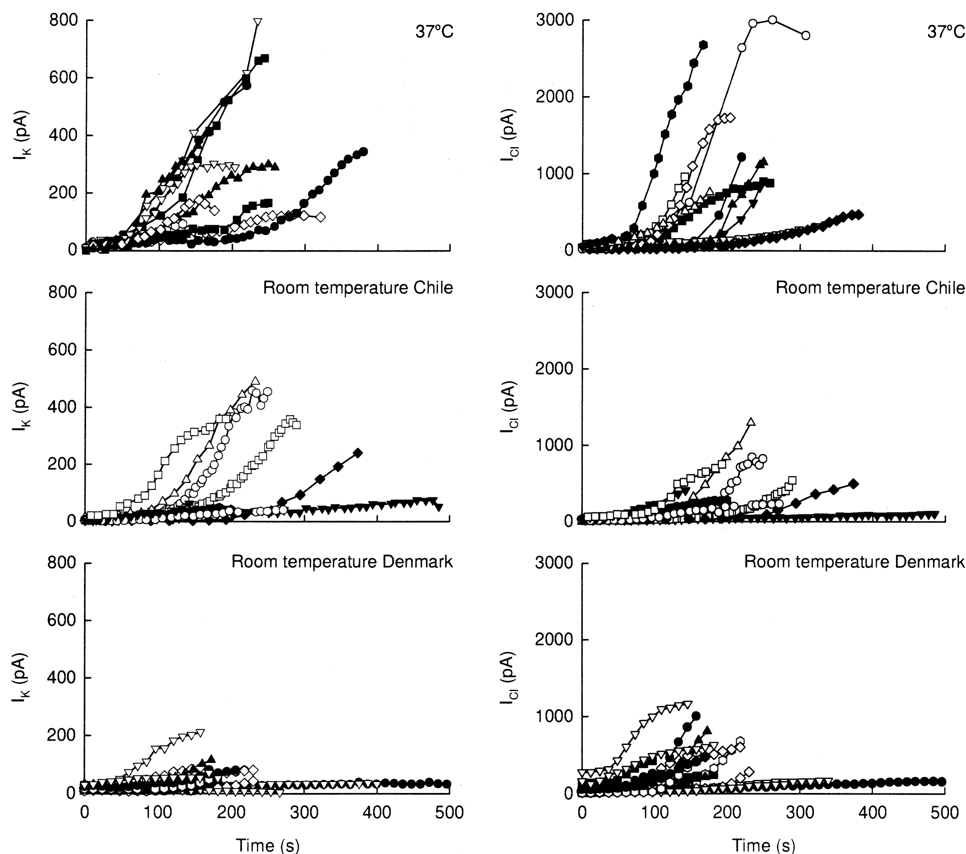

Fig. 3. Effect of temperature on the time course of the development of I_K and I_{Cl} in Ehrlich cells upon exposure to hypotonicity. Different panels show plots of the magnitude of the currents measured at E_{Cl} and E_K from individual cells (different symbols). Two sets of room temperature results are illustrated (see text). Not included in the bottom panel graphs are eight experiments where no increase in I_K was observed. In all of these there was a sizeable hypotonicity-induced I_{Cl}.

correlation between the time courses for the development of K^+ and Cl^- currents points to the presence of independent conductance pathways. The effect of hypotonicity was reversible within 100 s after returning to isotonic solution (data not shown). In order to demonstrate that the outward current observed upon hypotonic challenge was carried by K^+, the effect of cancelling the driving force for this ion was investigated. The outward K^+ current that developed in hypotonic medium was abolished by replacing extracellular Na^+ with K^+ (experiments not shown). Under these conditions there was also a marked increase in inward current at high negative potentials, suggesting that the volume-activated K^+ conductance readily allows permeation of K^+ in the inward direction consistent with an inwardly rectifying channel.

Replacing intracellular K^+ with Cs^+ (Fig. 2B) markedly reduced the development of outward current, with no effect upon I_{Cl}. Similar results were obtained in five separate experiments. The slight increase in outward current followed by the transient inward current increase upon returning to isotonicity observed in Fig. 2B, might be the consequence of the changes in the Cl^- gradient brought about by cell swelling followed by shrinkage. The possibility that the increase in inward current upon returning to isotonicity might be due to transient activation of a Na^+ conductance cannot be discarded, however, as an increase in the permeability to this cation by shrinkage has been reported in Ehrlich cells [15]. To test whether the increase in I_K evoked by cell swelling occurred through the Ca^{2+}-dependent, ChTX-sensitive conductance described above, its sensitivity to the toxin was examined. ChTX caused only a minor decrease in I_K (Fig. 2D) and in eight separate experiments there was no significant effect ($2 \pm 9\%$ increase in I_K upon ChTX addition, mean $\pm$ SEM). Apamin, another inhibitor of SK channels [13], was also without effect as shown in Fig. 2C ($n = 3$). In no case did the toxins affect swelling-activated I_{Cl}. Ba^{2+} at 5 mM reduced I_K by $39 \pm 1\%$ ($n = 4$), which is comparable to its partial blockade of RVD in Ehrlich cells [4].

There was a marked variability from cell-to-cell in the magnitude of currents that developed after cell swelling. This might reflect in part variations in the temperature at which the experiments were performed. In Fig. 3, the lower panels show the development of swelling-activated I_K and I_{Cl} measured in Chile and Denmark, respectively. Room temperature in Chile was 5–7°C higher, and this appears to be reflected in the faster onset and larger currents developed at the higher temperature. That this interpretation might be correct is suggested by the results in the upper panels of Fig. 3 obtained at 37°C. The temperature-dependence of the RVD response has been reported for astrocytes [16]. These results are likely to reflect a high temperature-dependence of the enzymes involved in intracellular signalling of cell volume regulation [1]. In order to verify that additional swelling-induced increase in I_K observed at 37°C is not due to activation of the Ca^{2+}-dependent channels, the effect of ChTX was tested also at this temperature. Fig. 4 summarises results on the effect of the toxin at 37°C on cell swelling-activated I_K and I_{Cl}. There was no significant effect of ChTX on either current.

The results presented here demonstrate, by direct measurement of membrane currents, that in Ehrlich cells increases in K^+ and Cl^- conductance occur as a consequence of cell swelling. We show that the volume-activated K^+ conductance is mediated by channels different from previously described inwardly rectifying, Ca^{2+}-dependent K^+ channels. The development of K^+ conductance upon an increase in cell volume was highly temperature-dependent. This could be the consequence of a high temperature-de-

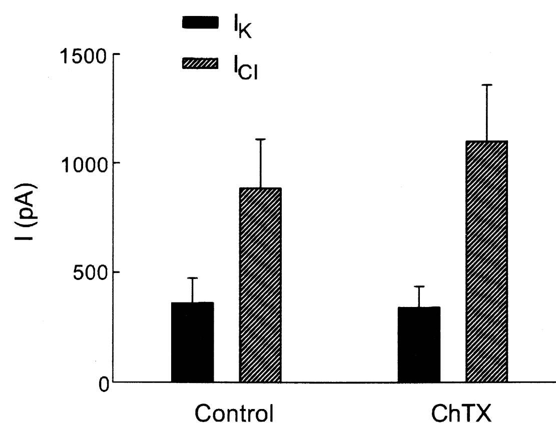

Fig. 4. Effect of ChTX on cell swelling-activated I_K and I_{Cl} in Ehrlich cells at 37°C. Experiments were conducted as described in the legends to Figs. 1 and 2 at 37°C. ChTX (100 nM) was added after a stable level of K^+ current had developed. Results are means $\pm$ SEM of seven experiments.

pendence of enzymes involved in the intracellular signalling pathway for regulatory volume decrease.

Acknowledgements

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