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Leiurus quinquestriatus venom inhibits different kinds of Ca^{2+}-dependent K^+ channels

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A minor protein component of *Leiurus quinquestriatus* venom has been reported to inhibit selectively the apamin-insensitive Ca^{2+}-dependent K^+ channels of mammalian skeletal muscle (Miller, C., Moczydlowski, E., Latorre, R. and Phillips, M. (1985) Nature 313, 316–318). We report the effect of the venom on both the apamin-insensitive channels of the human erythrocyte, the Ehrlich cell and the rat thymocyte and the apamin-sensitive channel of the guinea pig hepatocyte. The venom inhibited Ca^{2+}-dependent K^+ transport in all the cases with a K_i value within the range of 1 to 10 μg/ml, similar to that reported previously in muscle. Valinomycin-induced K^+ transport was also antagonized by the venom but its sensitivity was about 1/10 as much as that of the Ca^{2+}-dependent K^+ channel.

Many animal cells possess membrane channels selective for K^+ and Rb^+ which are activated by the increase of the cytoplasmic Ca^{2+} levels [1–5]. Different kinds of Ca^{2+}-dependent K^+ channels have been defined on the basis of their unitary conductance [3–5] or their sensitivity to apamin, a protein toxin of honeybee venom [6]. It has been reported recently that a minor protein component of the venom of the Israeli scorpion *Leiurus quinquestriatus*, charibdotoxin, inhibits the apamin-insensitive Ca^{2+}-activated K^+ channels of mammalian skeletal muscle [7]. In order to test whether charibdotoxin could help to discriminate among different categories of Ca^{2+}-dependent K^+ channels, the effects of *L. quinquestriatus* venom on both the apamin-insensitive channels of the human erythrocyte [8], the Ehrlich ascites-tumor cell and the rat thymocyte and the apamin-sensitive channel of the guinea-pig hepatocyte [8] were studied.

The Ca^{2+}-dependent K^+ channels were activated in all the cases by increasing the intracellular Ca^{2+} level by incubation of the cells with Ca^{2+} and the divalent cation ionophore A23187, whose concentrations were adjusted to give maximal activation. In the human erythrocyte, the activity of the channels was estimated from the uptake of $^{86}Rb^+$, measured under equilibrium-exchange conditions, or from the net loss of K^+ from cells incubated in low-K^+ medium. Fig. 1 shows the effects of different concentrations of *L. quinquestriatus* venom (LQV, obtained from Sigma) on Ca^{2+}-dependent $^{86}Rb^+$ uptake by human red cells. A logarithmic plot has been made, so that the first-order rate constant for the uptake of $^{86}Rb^+$ can be estimated from the slope of the lines. The venom inhibited the Ca^{2+}-dependent $^{86}Rb^+$ uptake in a concentration-dependent fashion, the concentration of the venom producing half-maximal inhibition being about 3 μg/ml. The inhibitory effect of the venom was the same either at 50 or at 200 μM external Ca^{2+} and with fresh or

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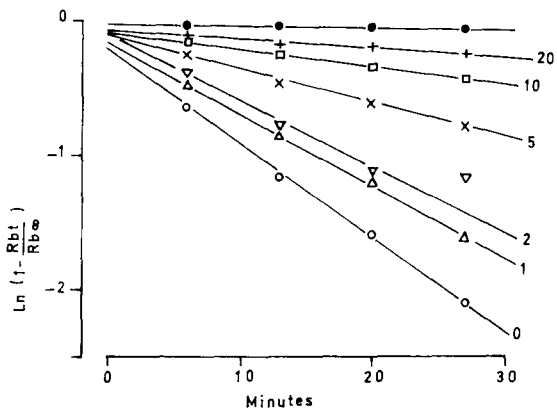

Fig. 1. Effects of *L. quinquestriatus* venom (LQV) on Ca^{2+}-induced $^{86}Rb^+$ uptake by human red cells. Red cells from fresh blood were washed three times and resuspended at about 10% haematocrit in a medium containing (mM): NaCl, 75; KCl, 75; $MgCl_2$, 0.2; $CaCl_2$, 0.1; K-Hepes (pH 7.5), 10; inosine, 10. $^{86}Rb^+$ was added in tracer amounts (about 10^9 cpm/ml). The suspension was divided in several aliquots to which different amounts of LQV (Sigma, stock 5 mg/ml in 0.15 M NaCl) were added. At $t = 0$ the ionophore A23187 (Boehringer Mannheim, stock 2 mM in ethanol) was added under stirring to give a final concentration of 10 μM in the suspension. At the times shown 0.1 ml samples of the cell suspension were mixed with 1 ml of ice-cold incubation medium containing 1 mM quinine and centrifuged immediately over dibutylphthalate oil (BDH) as described before [9]. The radioactivity present in trichloroacetic acid extracts of the cell pellets and aliquots of the cell suspension was measured by Cerenkov counting. A logarithmic plot of the data is shown. The concentration of the venom (in μg/ml cell suspension) is indicated by the figures in the right-hand side of the lines. Closed circles correspond to controls to which the ionophore A23187 was not added. The experiment was performed at room temperature.

ATP-depleted cells (data not shown). The venom, tested at 50 μg/ml, did not modify the kinetics of the uptake of $^{45}Ca^{2+}$ induced by A23187 in ATP-depleted erythrocytes nor the equilibrium level attained (data not shown).

Fig. 2 shows the effects of the venom on the Ca^{2+}-induced net K^+ loss from human erythrocytes. The net salt loss was estimated from changes of cell volume evidenced by light scattering measurements at 650 nm. The permeant anion SCN^- was added to the medium to avoid the limitation of the K^+ loss by the slower Cl^- movements observed under these conditions [10]. Panel A shows the crude records of transmittance at 650 nm and panel B a logarithmic plot of the same data. The value of K_i for the venom estimated from these measurements was about 5 μg/ml. The inhibitory effect of the venom on muscle channels was reversed by washing or by treatment with dithiothreitol [7]. In the human erythrocyte the inhibition was largely reversed by washing, but not relieved by dithiothreitol (Table I). Even the treatment of the venom with 2 mM dithiothreitol for 30 min before contact with the cells was unable to prevent the inhibitory effect either (Results not shown). Modifications of the external K^+ concentration (between 1 and 10 mM) had little effect (less than 15%) on the inhibitory effect of the venom (results not shown). Finally, the venon also antagonized the net loss of K^+ induced by valinomycin, although in this case the K_i was about 50 μg/ml, a value much higher than that obtained for the inhibition of Ca^{2+}-dependent K^+ transport in all the cells tested (Fig. 4, see below).

The activity of the Ca^{2+}-dependent K^+ channels in Ehrlich ascites-tumor cells, rat thymocytes and guinea pig hepatocytes was estimated from the net loss of K^+ from cells incubated in low-K^+ medium, measured using a K^+-selective electrode as described before [8,11,12]. Fig. 3 shows the results of typical experiments, comparing the effects of apamin and *L. quinquestriatus* venom.

TABLE I

EFFECTS OF WASHING AND TREATMENT WITH DITHIOTHREITOL ON THE INHIBITION OF Ca^{2+}-DEPENDENT K^+ TRANSPORT BY *L. QUINQUESTRIATUS* VENOM (LQV)

The activity of Ca^{2+}-dependent K^+ transport was measured in red blood cells by the light scattering procedure, as described in the legend to Fig. 2. The concentration of LQV was in all the cases 28 μg/ml and that of dithiothreitol (DTT) 2 mM.

Expt. No.	Condition	K (min^{-1})	% Inhibition
1	Control	1.46	-
	+ LQV	0.21	86
	LQV washed [a]	0.98	33
2	Control	1.27	-
	+ LQV	0.10	92
	DTT after LQV	0.14	89
	DTT before LQV	0.16	87

[a] After the addition of the venom the cells were washed twice and resuspended in standard medium.

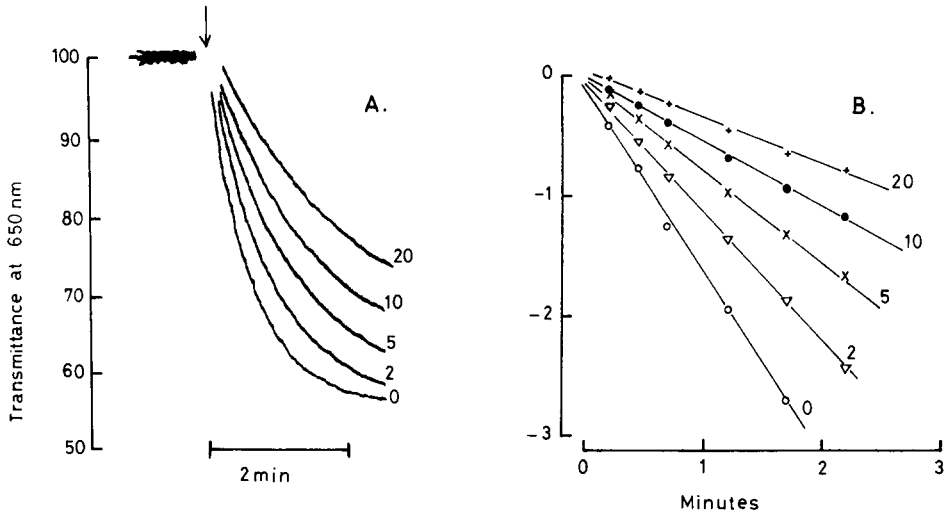

Fig. 2. Effects of *L. quinquestriatus* venom on the Ca^{2+}-induced shrinkage of human red cells. Washed red cells were suspended at 3.3% haematocrit in a solution containing (mM): NaCl, 150; KCl, 3; $CaCl_2$, 1. Immediately before the experiment 1 ml of this cell suspension was mixed in an spectrophotometer cuvette with 2 ml of a solution containing (mM): NaSCN, 75; NaCl, 75; $MgCl_2$, 0.15; K-Hepes (pH 7.5), 10, and different amounts of venom. The experiment was started by the addition of 5 μl of an stock solution of the ionophore A23187 (2 mM in ethanol; marked by an arrow in panel A). The changes of cell volume were followed by recording the changes of the transmittance of the cell suspension at 650 nm. SCN^- was included in the incubation medium in order to accelerate the rate of loss of K^+, which is otherwise limited by the electrogenic permeability to Cl^- [10]. The experiments were performed at room temperature. Panel B shows the same data linearized by plotting in the ordinate $\ln((T_t - T_\infty)/(T_0 - T_\infty))$, where T stands for transmittance at 650 nm and subindexes refer to the time after the addition of ionophore A23187. Figures on right-hand side of the lines indicate the venom concentration in each condition (μg/ml in the final cell suspension). Full inhibitory effect of the venom did not require measurable preincubation periods with the cells.

Apamin blocked the loss of K^+ in the hepatocyte, which is in accordance with previous reports [8] but had no effect in the other two cell types tested confirming previous results obtained by M. Valdeolmillos in our laboratory (unpublished). *L. quinquestriatus* venom at 50 μg/ml almost completely blocked the Ca^{2+}-dependent K^+ loss in all the three cell types (Fig. 3). The effects of several concentrations of the venom on the initial rate of the Ca^{2+}-dependent K^+ loss, studied using the same procedure, are summarized in Fig. 4. The estimated values for K_i were (in μg/ml), 7.2, 8.9 and 1.3 for the Ehrlich cells, thymocytes and hepatocytes, respectively. The inhibition of Ca^{2+}-dependent and valinomycin-induced K^+ (Rb^+) transport in red blood cells is also shown in Fig. 4 for comparison.

Our results point out that the inhibitory effect of the *L. quinquestriatus* venom toxin is not limited to the apamin-insensitive Ca^{2+}-dependent K^+ channels. Indeed the only apamin-sensitive channel tested here seemed to be as least as much and perhaps more sensitive than the others. Reported differences in unitary conductance among the channels studied here [3–5] do not seem to make difference in the sensitivity to *L. quinquestriatus* venom either. Even valinomycin-mediated transport seems to be affected by *L. quinquestriatus* venom, suggesting some structural similarity between the ionophore and the membrane channel. An alternative possibility is that the different categories of Ca^{2+}-dependent K^+ channels are affected by different toxins, all present in *L. quinquestriatus* venom. If that were the case the contents of each toxin within the venom should be such as to give a very similar value of K_i when referred to the crude venom in all the cases. Further work using purified fractions of the venom will help to clarify this question. The inhibitory power of the venom was in the same order of magnitude as that reported previously for muscle channels [7], however, other characteristics such as

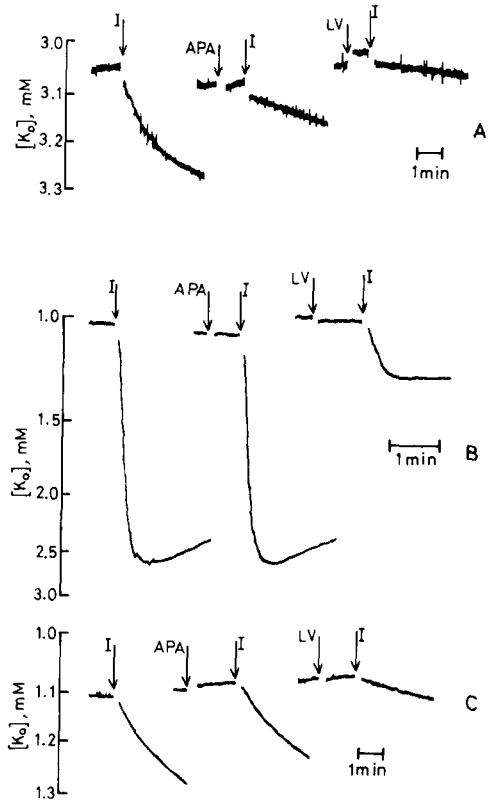

Fig. 3. Comparison of the effects of apamin and *L. quinquestriatus* venom on Ca^{2+}-dependent cell K^+ loss in the guinea pig hepatocyte (A), the Ehrlich ascites-tumor cell (B) and the rat thymocyte (C). Preparation and handling of the cells was as described previously [8,12–14]. The loss of cell K^+ was followed by measuring the K^+ activity of the incubation medium with a Radiometer F2312K K^+-selective electrode [8,11,12]. Experiments with hepatocytes and Ehrlich cells were performed at cytocrits of 5% and 7.1%, respectively. Thymocytes were used at $7.5 \cdot 10^7$ cells/ml. The nominal Ca^{2+} and K^+ concentrations in the incubation media were, respectively, 1.8 and 3 mM for hepatocytes and 1 and 1 mM for the other two cells. All experiments were performed at room temperature. Other details were as described previously [8,12–14]. The additions, marked with arrows, were as follows: I, ionophore A23187, final concentrations, 12.5 μM (A and B) and 8 μM (C); APA, apamin (Sigma), 25 nM; LV, *L. quinquestriatus* venom (Sigma), 25 μg/ml in A and 50 μg/ml in B and C.

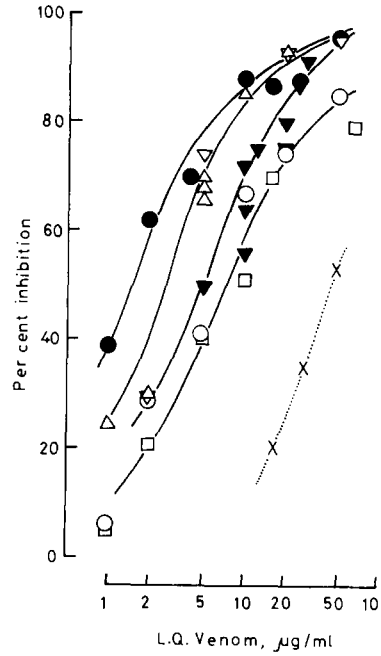

Fig. 4. Inhibition of Ca^{2+}-dependent ^{86}Rb uptake or cell K^+ loss by *L. quinquestriatus* venom in different cells. Cell K^+ loss was measured using a K^+-selective electrode in hepatocytes (●), Ehrlich ascites tumor cells (○) or thymocytes (□) as described in the legend to Fig. 3, or estimated from light scattering in human erythrocytes (▼) as described in the legend to Fig. 2. $^{86}Rb^+$ uptake was measured as described in the legend to Fig. 1 in fed erythrocytes (△) or in erythrocytes depleted of ATP by a previous 3-h incubation with 10 mM inosine and 6 mM iodoacetamide (▽). The effects of the venom on the cell shrinkage induced by valinomycin (3.3 μM), measured in human erythrocytes by light scattering, are also shown (×).

the reversibility by treatment with dithiothreitol [7] could not be reproduced. Concerning to the action mechanism of the venom, the present results suggest that the inhibitory effect is not accomplished by decreasing the affinity for external K^+ or internal Ca^{2+}, as its seems to be the case for other better known inhibitors of Ca^{2+}-dependent K^+ channels in human erythrocytes [15].

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References

1 Lew, V.L. and Ferreira, H.G. (1978) Curr. Top. Membranes Transp. 10, 217–271
2 Meech, R.W. (1978) Annu. Rev. Biophys. Bioeng. 7, 1–18
3 Latorre, R. and Miller, C. (1983) J. Membrane Biol. 71, 11–30
4 Marty, A. (1983) Trends Neurosci. 6, 262–265
5 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696

6 Lazdunski, M. (1983) Cell Calcium 4, 421–428
7 Miller, C., Moczydlowski, E., Latorre, R. and Phillips, M. (1985) Nature 313, 316–318
8 Burgess, G.M., Claret, M. and Jenkinson, D.H. (1981) J. Physiol. (London) 317, 67–90
9 Lew, V.L. and Ferreira, H.G. (1976) Nature 263, 336–338
10 Lew, V.L. and García-Sancho, J. (1985) Cell Calcium 6, 15–23
11 Sanchez, A., García-Sancho, J. and Herreros, B. (1980) FEBS Lett. 110, 65–68
12 Valdeolmillos, M., García-Sancho, J. and Herreros, B. (1982) Biochim. Biophys. Acta 685, 273–278
13 Inui, Y. and Christensen, H.N. (1966) J. Gen. Physiol. 50, 203–224
14 Baran, D.T., Peck, W.A., Frengley, P.A. and Lichtman, M.A. (1973) Biochim. Biophys. Acta 307, 627–639
15 Reichstein, E. and Rothstein, A. (1981) J. Membrane Biol. 59, 57–63