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Ca^{2+}-Dependent K^+ Transport in Lymphocytes

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The treatment of rat thymocytes with A23187 + Ca^{2+}, ascorbate-phenazine methosulphate or propranolol induced quinine-sensitive fluxes of K^+ (Rb^+) suggesting the presence in the cell membrane of Ca^{2+}-dependent K^+ channels. Concanavalin A induced K^+ channel activation only at very high doses (13 μg/ml). Neither quinine nor the increase of the K^+ concentration in the medium to 30 mM prevented the stimulation of amino acid transport induced by concanavalin A, suggesting that the Ca^{2+}-dependent K^+ channel is not involved in the early phenomena of lymphocyte activation.

Key words: K^+-channel, Ca^{2+}-dependence, Amino acid transport, Lectins, Thymocytes, Lymphocyte activation.

In many cells lines, an increase of the intracellular Ca^{2+} concentration elicits an increase in the membrane permeability to K^+ (Rb^+) by activation of the so-called Ca^{2+}-dependent K^+ channel (12, 14). Recent evidence suggests the presence of such channels in lymphocytes, but it has mostly come as a by-product of experiments concerned with other matters and a systematic study is missing (16). It has been shown that the ionophore A32187 increases the fluxes of ^{86}Rb in a Ca^{2+}-dependent fashion and that this effect is sensitive to quinine and chlorpromazine, two known inhibitors of the Ca^{2+}-dependent K^+ channel in other cells (8, 18). Recent studies using patch-clamp techniques have shown the presence in T-lymphocytes of voltage-dependent K^+ channels which are sensitive to quinine, but their Ca^{2+}-dependence is uncertain (5, 13).

Early phenomena in the activation of lymphocytes by lectins include changes of K^+ permeability and stimulation of amino acid transport (10, 15, 22, 23). Those phenomena are dependent on extracellu-

lar calcium, which is taken up during activation (9, 24), and the cytoplasmic Ca^{2+} concentration increases (19). A concomitant quinine-sensitive membrane hyperpolarization has been reported in thymocytes (14). It has been shown previously that the activation of Ca^{2+}-dependent K^+ transport originates a membrane hyperpolarization in the Ehrlich ascites-tumor cell, and that this hyperpolarization produces and stimulation of Na^+-dependent amino acid transport (21). In this work the existence of Ca^{2+}-dependent K^+ transport in rat thymocytes and human peripheral lymphocytes has been investigated as well as its possible implication on the early phenomena of the activation by lectins.

Materials and Methods

Rat thymocytes were prepared using RPM11640 medium buffered with 20 mM HEPES (2). For net K^+ flux measurements the cells were first incubated at 37°C during 30 min and then suspended at about 5×10^7 cells/ml in a medium of the following composition (mM): NaCl, 145; KCl, 1; $CaCl_2$, 1; $MgSO_4$, 1; Na-HEPES buffer, 10, pH 7.4; glucose 5. Then changes of the K^+ concentration of the medium were continuously monitored using a K^+-selective electrode (17). Net K^+ fluxes were measured at room temperature. Measurements of ^{86}Rb uptake were performed in HEPES-buffered RPM11640 medium at a cell density of about 5×10^7 cells/ml and at 37°C. At the end of the incubation, the extracellular ^{86}Rb was removed by passage through Dowex 50-X-8 resin and cell-associated radioactivity measured by Cerenkov counting (7). For the study of amino acid transport the cells were first incubated in RPM11640 medium as before with or without 1.3 µg/ml of concanavalin A during 4 h at 37°C. Then 2.5 ml-aliquots of these cell suspensions were mixed with 0.5 ml of a 0.6 mM ^{14}C-2-aminoisobutyric acid (AIB) solution in either 0.15 M choline chloride (standard medium) or 0.15 M KCl (high K-medium), and the incubation continued during 20 more min. The incubation was terminated by mixing with two volumes of ice-cold 0.15 M NaCl solution. The cells were sedimented by centrifugation and washed twice with ice-cold 0.15 M NaCl. The cell pellets (about 10^8 cells) were lysed with 1 ml of distilled water, and then 0.1 volumes of 50 % trichloroacetic acid were added. Aliquots of the clear supernatants were used for measurement of the radioactivity by liquid scintillation counting.

Human peripheral lymphocytes were prepared as described (4). The cells ($3-4 \times 10^6$/ml) were loaded with ^{86}Rb by a 3 h incubation at 37°C in a medium of the following composition (mM): NaCl, 119; KCl, 4.8; HK_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; Na-HEPES buffer, 20, pH 7.4. The extracellular ^{86}Rb was removed by repeated washing and centrifugation and the loss of cell ^{86}Rb measured at 20°C by a superfusion procedure on Millipore filters (21) using about 2×10^6 cells/filter.

The chemicals were obtained either from Sigma or Merck, and the radiochemicals from Amersham.

Results

The addition of the divalent cation ionophore A23187 to rat thymocytes incubated in Ca^{2+}-containing medium produced a net loss of cell K^+ which was prevented by quinine (fig. 1 A). Quinine is a known blocker of Ca^{2+}-dependent K^+ transport (1). Other agents that activate Ca^{2+}-dependent K^+ transport in other cells, such as the electron donor system sodium ascorbate + phenazine methosulphate (PMS) or propranolol (6), induced also a quinine-sensitive loss of K^+ (table I). The loss of K^+ was not prevented by 10^{-6} M apamin, a blocker effective in the hepatocytes (3), neuroblastoma cells

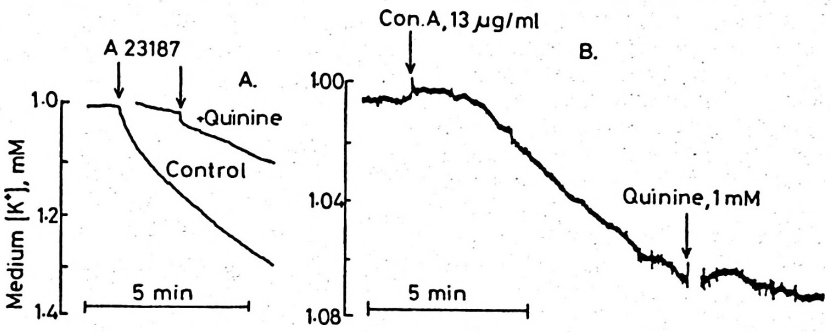

Fig. 1. *Net loss of cell K^+ induced by 1 µM ionophore A23187 (A) or 13 µg/ml concanavalin A (B) from rat thymocytes.*
The K^+ concentration of the medium was continuously monitored with a K^+-sensitive electrode. Note the different ordinate scale in parts A and B.

and rat muscle cells in culture (11), but not in erythrocytes (3) nor in the Ehrlich cells (unpublished observation by M. VALDEOLMILLOS). Both the ionophore A23187 and propranolol stimulated the uptake of ^{86}Rb, the effect being abolished by 0.5 mM quinine (fig. 2). At 75 µM, quinine produced a 30 % inhibition of the A23187-induced uptake of ^{86}Rb under the same conditions of the experiment of fig. 2, and 10 µM trifluoperazine was without effect (not shown).

The effects of A23187 and propranolol on the exit of ^{86}Rb were also tested in superfused human peripheral lymphocytes. Both agents produced an increase of the rate of exodus (fig. 3) which was quinine-sensitive (not shown). The effect of A23187 was dependent on the presence of external Ca^{2+}

The mitogenic agent concanavalin A at 13 µg/ml induced, after a lag period of 1-2 min, a quinine-sensitive net loss of K^+ (fig. 1 B). The effect was comparatively smaller

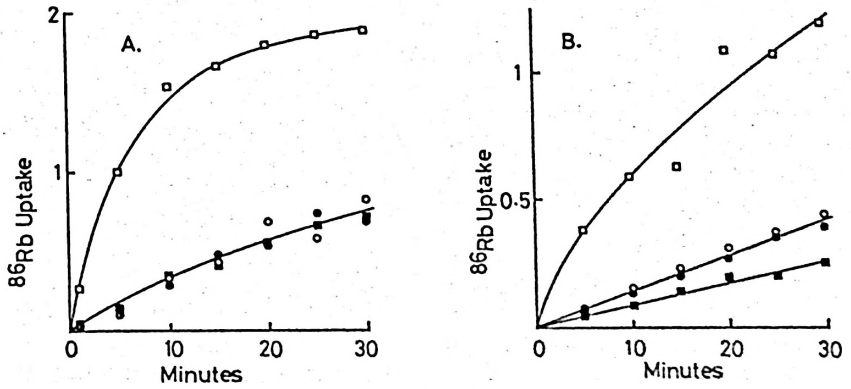

Fig. 2. *Effects of 0.1 µM A23187 (A) and 0.5 mM propranolol (B) on the uptake of ^{86}Rb by rat thymocytes.*
Uptake of ^{86}Rb is expressed as cpm per 10^9 cells/cpm per ml of medium. Symbols: (○), Control; (●), 1 mM quinine; (□), A23187 (A) or propranolol (B); (■), A23187 + 1 mM quinine (A) or Propranolol + 1 mM quinine (B).

Table I. *Effects of several activators of Ca^{2+}-dependent K^+ transport and concanavalin A on the loss of K^+ from rat thymocytes.*
K^+ loss was measured using a K^+ sensitive electrode and the first-order rate constant (k, h^{-1}) estimated as described (17). In the controls with no additions the loss of K^+ was very slow ($k < 0.1$). The results of one out of three similar experiments are shown. The loss of K^+ was inhibited (50-100 %) by 0.5 mM quinine in all the cases.

Additions	Net K^+ loss (k, h^{-1})
A23187 (1 μM)	3.66
Ascorbate (20 mM) + PMS (0.1 mM)	2.41
Propranolol (0.5 mM)	2.33
Concanavalin A (15 mg/l)	0.60

Table II. *Effects of concanavalin A (1.3 mg/l) on the uptake of AIB (0.1 mM) by rat thymocytes.*
Each value is mean ± S.E. of 3 individual data.

Condition	Uptake of AIB (nmole/10^3 cells per min)	
	Control	+ Con. A
Standard medium	0.43 ± 0.04	0.95 ± 0.14
High-K medium*	0.37 ± 0.03	0.69 ± 0.17
Standard medium + 0.5 mM quinine	0.31 ± 0.04	0.66 ± 0.06

* The K concentration was 30 mM replacing an equimolar amount of choline.

than that obtained with the other activators tested (table I) and the K^+ loss could only be evidenced at these high doses.

The effects of concanavalin A on amino acid transport were tested at a lectin concentration of 1.3 μg/ml. After 4 h incubation with the lectin the 20 min uptake of 2-aminoisobutyric acid (AIB) was increased to about twice the basal levels

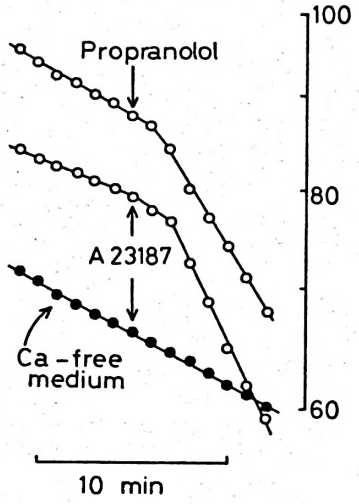

Fig. 3. *Effects of A23187 (1 μM) and propranolol (0.5 mM) on the efflux of ^{86}Rb from human peripheral lymphocytes.*
The experiments were performed using the Millipore superfusion technique. The data are represented in logarithmic scale as per cent of the initial cell ^{86}Rb. Note that the lower two graphs are displaced vertically with regard to the scale at right.

(table II). This stimulation was only slightly decreased by increasing the K^+ concentration of the medium to 30 mM —which prevents the membrane hyperpolarization due to an increase of K^+ permeability (21)— or by the presence of quinine. Both conditions abolished the stimulation of AIB transport observed upon activation of the Ca^{2+}-dependent K^+ channel in the Ehrlich ascites-tumor cells (21).

Discussion

The present results support the existence of a Ca^{2+}-dependent K^+ channel in the membrane of rat thymocytes and human peripheral lymphocytes whose properties are similar to those reported previously in the human erythrocytes and the Ehrlich cells (6, 12, 17, 20). In contrast with a previous report in lymphocytes (8)

the present study shows that 10 μM trifluoperazine does not inhibit the fluxes of K^+ (Rb) induced by A23187 in thymocytes. This inconsistence might be due to the higher cell concentration used here, since it has been found that the effect of trifluoperazine is strogly dependent on the hematocrit in red cells (unpublished results by J. ALVAREZ). The Ca^{2+}-dependent K^+ channels described here fall in the category of the apamin-insensitive ones (11). Their possible relation to the quinine-sensitive K^+ channels found by patch-clamp in T-lymphocytes is uncertain since Ca^{2+}-dependence has not been established in this case (5, 13).

Concanavalin A was able to activate the Ca^{2+}-dependent K^+ transport, but this effect was evidenced only at doses far above those required for lymphocyte activation. On the other hand, the stimulation of amino acid transport could not be blocked by quinine or by increasing the K^+ concentration of the medium to 30 mM to avoid hyperpolarization caused by increased K^+ permeability. These results suggest that the early phenomena of lymphocyte activation are not caused by activation of the Ca^{2+}-dependent K^+ channels. Increased K^+ concentration into the medium has also been reported not to modify the incorporation of ^{3}H-thymidine induced by treatment with the ionophore A23187 (9). On this basis the mechanism by which quinine abolishes concanavalin A-induced ^{3}H-thymidine incorporation (5) deserves further study.

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Resumen

El tratamiento de timocitos de rata con el ionóforo A23187 + Ca^{2+}, el sistema donador de electrones ascorbato + metosulfato de fenacina o con propranolol induce flujos de K^+ (Rb^+) sensibles a la quinina, lo que sugiere la presencia de canales para K^+Ca^{2+}-dependientes en la membrana de estas células. La concanavalina A activa el canal para K^+Ca^{2+}-dependiente solamente a dosis muy altas (13 μg/ml). La estimulación del transporte de aminoácidos inducida por la concanavalina A no es inhibida por la quinina ni por el aumento de la concentración de K^+ en el medio (30 mM). Esto sugiere que el canal para K^+, Ca^{2+}-dependiente no está implicado en los procesos iniciales de la activación de los linfocitos.

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