EFFECTS OF SEVERAL INHIBITORS ON THE K^+ EFFLUX INDUCED BY ACTIVATION OF THE Ca^{2+}-DEPENDENT CHANNEL AND BY VALINOMYCIN IN THE HUMAN RED CELL

A. SANCHEZ, J. GARCÍA-SANCHO and B. HERREROS
Departamento de Fisiología y Bioquímica, Facultad de Medicina, Valladolid, Spain

Received 26 November 1979

1. Introduction

A number of agents such as Pb^{2+} [1,2], propranolol [3,4] or the divalent cation ionophore A23187 + Ca^{2+} [5,6] produce in the human erythrocyte a loss of K^+ by activation of the so-called Ca^{2+}-dependent K^+ channel. The same effects were obtained treating the cells with the electron donor system ascorbate + phenazine methosulphate (asc-PMS), the K fluxes being prevented in this case by several presumed oxidoreductase inhibitors (chlorpromazine, atebrin, antimycin A). The last results suggest that a redox process is involved in the activation of the Ca^{2+}-dependent K^+ channel [7].

Here we investige whether oxidoreductase inhibitors are also effective when the K^+ channel is activated by procedures other than the treatment with electron donors. To disclose other possible actions not directly related to the K^+ channel, the effects of these inhibitors on the K^+ loss induced by valinomycin were also studied.

2. Materials and methods

Freshly drawn washed human erythrocytes were used immediately for all the experiments except those involving treatment with asc-PMS in which the cells were depleted of ATP by a 6 h incubation at 37°C with 1 mM iodoacetate prior to use [7]. All experiments were carried out at room temperature (18–20°C), 2.5% hematocrit, and an incubation medium of the following composition (mM): NaCl, 135; KCl, 0.2; $MgCl_2$, 1; $CaCl_2$, 1; HEPES/NaOH buffer (pH 7.4) 20.

The net loss of cell K^+ was estimated from the changes of the K^+ concentration in the medium, which was either monitored continuously with a K^+-selective electrode (Radiometer F2312K) or measured by flame photometric analysis of the supernatants obtained by rapid centrifugation of the cell suspensions. Both methods gave identical results. Changes of cell K^+ were expressed as % of their initial contents, which were estimated from the loss of K^+-induced by gramicidin D (4 μg/l). Other details on the experimental procedures have been given in [7] or are included in the legend to the figures and tables.

3. Results and discussion

The time course of the effects of different activators of K^+ channel on the cell contents of K^+ are shown in fig.1. After different lag periods, they produced a decrease of the cell K^+ contents that could be defined by the equation:

$$(K^+)_t/(K^+)_o = e^{-kt}$$

where $(K^+)_o$ and $(K^+)_t$ represent the initial and final cell contents of K^+, and k is an operational rate constant for the loss of K^+. The experimental data fitted this function reasonably well for K^+ losses of $\leqslant$50% of the initial cell contents, which produced an increase of medium K^+ of ~1.8 mEq/l.

The effects of several inhibitors on the rate of K^+ loss induced by different procedures are shown in table 1. Chlorpromazine and atebrin increase the K^+ loss induced by valinomycin. Since this effect was not due to cell lysis, the increase of K^+ loss was mainly balanced by an increase of Cl^- loss (table 2), and the effects of chlorpromazine and atebrin in the absence of valinomycin were negligible (fig.2), it seems that these agents act by increasing the so-called electrogenic permeability to Cl^- [10].

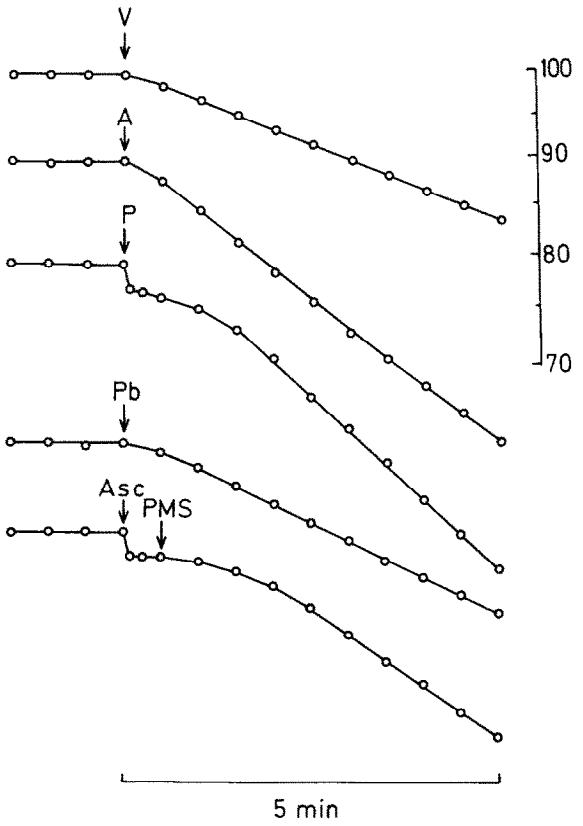

Fig.1. Time course of the changes of cell K^+ contents induced by valinomycin and several activators of the Ca^{2+}-dependent K^+ channel. The cell K^+ contents were estimated from the changes of the concentration of K^+ in the medium, monitored continuously with a K^+-sensitive electrode. The data are represented in logarithmic scale as % of the initial cell K^+. Note that the lower 4 graphs are displaced vertically with regard to the scale on the right. The artefacts produced by the additions of propranolol and ascorbate appeared also in medium containing no cells and did not modify the response of the electrode to K^+. V, valinomycin, 1 μM; A, A23187, 0.5 μM; P, propranolol, 0.5 mM; Pb, $Pb(NO_3)_2$ 0.1 mM (0.1 mM $NaNO_3$ did not have measureable effect) Asc, sodium ascorbate, 20 mM; PMS, phenazine methosulphate, 0.1 mM. Other details are given in the text.

In contrast with its potentiation of the K^+ loss induced by valinomycin, chlorpromazine inhibited consistently the loss of K^+ taking place through the Ca^{2+}-dependent channel (last 4 columns in table 1). Atebrin behaved similarly, except that in this case a modest stimulatory effect was found in most (8 out of 10) of the experiments with A23187. We have also shown that both chlorpromazine and atebrin inhibit the uptake of $^{86}Rb^+$ induced by intracellular Ca^{2+} and electron donors in resealed ghosts [7]. These results indicate that chlorpromazine and atebrin, beside other effects on the cell membrane that could explain the stimulation of the valinomycin-induced fluxes, have a distinct inhibitory effect on the Ca^{2+}-dependent K^+ channel. The stimulation by atebrin in the case of A23187 could arise from the net balance between the inhibition of the Ca^{2+}-dependent channel and the other effects on the membrane permeability.

Antimycin A inhibited the loss of K^+ induced by either valinomycin or the activators of the Ca^{2+}-dependent channel. Dipyridamole, an inhibitor of Cl^- transport [10], behaved similarly (table 1). Dipyridamole does not modify the effect of Ca^{2+} on the K^+ permeability under equilibrium exchange conditions, while it inhibits the net loss of this cation and facilitates the entry of $^{42}K^+$ under non-equilibrium conditions [4]. Thus, dipyridamole acts by decreasing the electrogenic permeability to Cl^- [4]. The same

Table 1
Effects of several inhibitors on the rate of K^+ loss induced by valinomycin and different activators of the Ca^{2+}-dependent K^+ channel

Inhibitor	% Inhibition				
	Valinomycin	A23187	Pb^{2+}	Propranolol	Asc-PMS
Chlorpromazine, 0.15 mM	(−172) ± 22	50 ± 18	73 ± 2	48 ± 8	68 ± 17
Atebrin, 0.5 mM	(−99) ± 37	(−38) ± 20	32 ± 4	43 ± 12	100 ± 0
Antimycin A, 10 mg/l	67 ± 5	30 ± 9	33 ± 10	92 ± 5	48 ± 10
Dipyridamole, 0.05 mM	76 ± 8	77 ± 6	64 ± 2	84 ± 9	52 ± 9
Oligomycin, 10 μM	(−2) ± 2	51 ± 6	41 ± 3	82 ± 5	51 ± 12

Each datum is mean ± SE of 3–8 expt. Other details as in fig.1

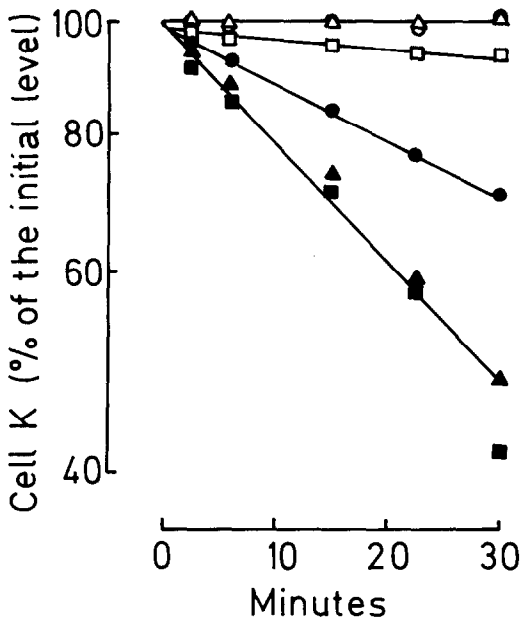

Fig.2. Effects of chlorpromazine and atebrin on the loss of K^+ induced by valinomycin. The cells were incubated in standard medium and the changes of K^+ concentration in the medium measured by flame photometry. Circles, controls; triangles, 0.5 mM atebrin; squares, 0.15 mM chlorpromazine; closed symbols, 1 μM valinomycin; Open symbols, without valinomycin.

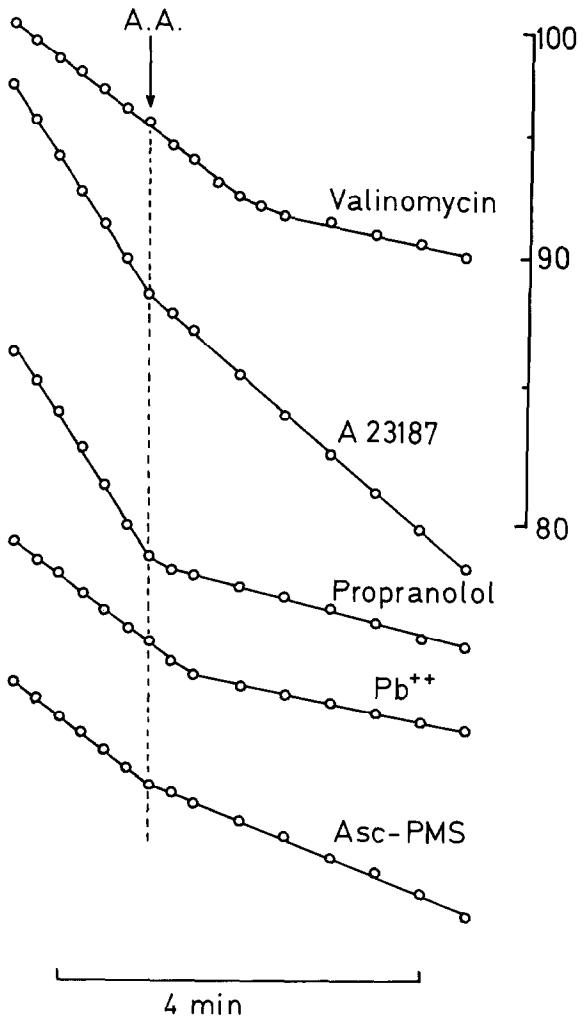

Fig.3. Effects of antimycin A on the loss of K^+ induced by several procedures. Antimycin (10 mg/l) was added at the time marked by the arrow. Other details as in fig.1.

interpretation could be given to the effects of antimycin shown in table 1; however, the observation that antimycin does not stimulate but inhibits the entry of $^{86}Rb^+$ induced by Ca^{2+} and electron donors under non-equilibrium conditions [7] is inconsistent with that interpretation. In addition, the inhibitory effect of antimycin appeared much faster when the loss of K^+ took place through the Ca^{2+}-dependent channel than when it was elicited by valinomycin (fig.3). All these observations suggest that the mechanisms by which antimycin inhibits the efflux of K^+ induced by valinomycin and that taking place through the Ca^{2+}-dependent channel may be different.

Oligomycin, a known inhibitor of the Ca^{2+}-dependent K^+ channel [11], did not modify the loss of K^+ induced by valinomycin (table 1).

Chlorpromazine and atebrin are two known flavin antagonists [12] and antimycin A inhibits electron transport in the mitochondrial chain between cytochromes *b* and c_1 [13]. Atebrin has been also reported to inhibit plasma membrane NADH dehydrogenase [14]. It is shown here that all 3 agents, beside their possible effects on membrane Cl^- conductance, are distinct inhibitors of the Ca^{2+}-dependent K^+ channel, not only when it is activated by electron donors, but also when other procedures are used. These findings are consistent with the hypothesis that a redox process participates in the control of the Ca^{2+}-dependent channel proposed in [7]. Alternative explanations such as interactions with membrane-bound calcium have been given for the inhibition of Ca^{2+}-dependent K^+ efflux by chlorpromazine [15]. Both interpretations, however, do not necessarily exclude each other.

Table 2
Effects of chlorpromazine and atebrin on the net movements of Na^+, K^+ and Cl^- induced by valinomycin

Additions	Net loss (mEq/mg hemoglobin)		
	Na^+	K^+	Cl^-
Valinomycin, 1 μM	−8 ± 5	90 ± 7	81 ± 17
Valinomycin + Chlorpromazine, 0.15 mM	−15 ± 7	130 ± 4	107 ± 7
Valinomycin + Atebrin, 0.5 mM	−2 ± 4	113 ± 7	107 ± 19

Cells were incubated during 30 min (hematocrit 2.5%) in medium containing 5 mM KCl and the additions specified in column 1. At the end of the incubation period the cells were washed twice (1 min centrifugations at 10 000 × *g*) with 30 vol. ice-cold 0.31 M mannitol, and hemolyzed with 5 ml water. Hemoglobin (A_{520}), Na^+ and K^+ (flame photometry) and Cl^- [8] were determined in the hemolysates. Each datum is the mean ± SD of 4 expt. Hemolysis during the incubation period was always <1%

Acknowledgements

Financial support from the Fundación Marcelino Botín and from the Fondo Nacional para el Desarrollo de la Investigación Cientifica is gratefully acknowledged.

References

[1] Riordan, J. R. and Passow, H. (1971) Biochim. Biophys. Acta 249, 601–605.
[2] Riordan, J. R. and Passow, H. (1973) in: Comparative Physiology (Bolis, L. et al. eds) pp. 543–581, North-Holland, Amsterdam.
[3] Manninen, V. (1970) Acta Physiol. Scand. suppl. 355, 1–76.
[4] Gardos, G., Szasz, I. and Sarkadi, B. (1975) in: Biomembranes: Structure and Function (Gardos, G. and Szasz, I. eds) FEBS Proc. vol. 35, pp. 167–180, North-Holland, Amsterdam.
[5] Reed, P. W. (1976) J. Biol. Chem. 251, 3489–3494.
[6] Lew, V. L. and Ferrerira, H. G. (1976) Nature 263, 336–338.
[7] García-Sancho, J., Sanchez, A. and Herreros, B. (1979) Biochim. Biophys. Acta 556, 118–130.
[8] Van Slike, D. D. and Hiller, A. (1947) J. Biol. Chem. 167, 107–124.
[9] Hladky, S. B. and Rink, T. J. (1977) in: Membrane Transport in Red Cells (Ellory, J. C. and Lew, V. L. eds) pp. 115–135, Academic Press, New York.
[10] Fortes, P. A. G. (1977) in: Membrane Transport in Red Cells (Ellory, J. C. and Lew, V. L. eds) pp. 175–195, Academic Press, New York.
[11] Blum, R. M. and Hoffman, J. F. (1972) Biochem. Biophys. Res. Commun. 46, 1146–1152.
[12] Ernster, L. (1967) Methods Enzymol. 10, 309–317.
[13] Slater, E. C. (1967) Methods Enzymol. 10, 48–57.
[14] Crane, F. L. and Löw, H. (1976) FEBS Lett. 68, 153–156.
[15] Szasz, I. and Gardos, G. (1974) FEBS Lett. 44, 213–216.