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VOLTAGE GATING OF CA^{2+}-ACTIVATED POTASSIUM CHANNELS IN HUMAN LYMPHOCYTES

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SUMMARY The effect of membrane potential on Ca^{2+} activated K^+ channels was studied on human peripheral lymphocytes. Membrane potential was monitored using bis-oxonol and flow cytometry. 1 mM Ca^{2+} in the presence of 2 μM ionomycin depolarized the control cell population, while 100 μM Ca^{2+} caused hyperpolarization. However 1 mM Ca^{2+} had a hyperpolarizing effect on previously partially depolarized cells. Potassium channel blockers did not influence the depolarization, while they inhibited the hyperpolarization.

Based on the experimental evidence a voltage gating of Ca^{2+} activated K^+ channels is suggested.

The regulation of membrane potential changes during cell stimulation is dependent on the $[Ca^{2+}]_i$ level, the Ca^{2+} activated K^+ channels and different pump (Na^+-K^+; Ca^{2+}) activities (1-4).

Ca^{2+}-activated K^+ channels, originally discovered by Gárdos in erythrocytes, play an important regulatory role in cell metabolism (5). Their existence was also demonstrated in lymphocytes (6-9). Ishida and Chused (10) provided evidence for T cell-specific calcium sensitive potassium channel and claimed that B cells had not possess a Ca^{2+} sensitive potassium channel which produced membrane hyperpolarization at $[Ca^{2+}]_i$ above 200 nM.

It is an intriguing question how the physical events of membrane potential changes (triggered by specific or non-specific stimuli and mediated through changes in channel activities) are coupled to biochemical reactions, finally leading to the activated state of the cell (2).

The present paper reports on the Ca^{2+} and ionophore concentration dependence of the Ca^{2+} activated K^+ channel activity in human peripheral blood mononuclear cells, mostly T lymphocytes. It is suggested that partial depolarization of lymphocytes, caused by an isoto-

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nic increase in the extracellular K^+ concentration, facilitates the responsiveness of the Ca^{2+} activated K^+ channels.

MATERIALS AND METHODS

Cells: Human peripheral lymphocytes were obtained from the blood of healthy donors by the Ficoll-Hipaque isolation method (11). The cells were washed and resuspended at a density of 10^7 cells/ml in phosphate buffered saline, without calcium (PBS, pH 7.4), at room temperature before the experiments. The viability was always above 95 % as tested by the fluorescein diacetate uptake and propidium iodide exclusion (12). The desired Ca^{2+} concentration of the PBS was adjusted by $CaCl_2$ solution as indicated in the texts in the figures.

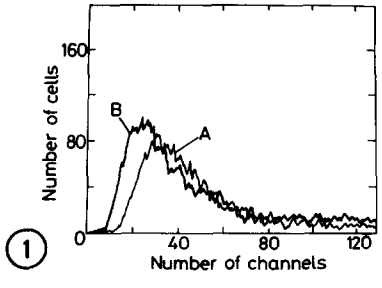
Flow Cytometric Membrane Potential Measurement: The membrane potential was determined by addition of 100-150 nM bis-oxonol ($DiBAC_4(3)$, bis-(1,3-dibutylbarbituric acid)trimethine oxonol), to 10^6 cell/ml as described earlier (2).

The fluorescence intensity histograms were taken in a modified Becton-Dickinson FACS III flow cytometer, using the 488 nm line of an argon ion laser for excitation and a 520 nm long pass filter for the detection of emission after incubating the cells for 3 min at 37°C with bis-oxonol. Each fluorescence intensity histogram contains data from 10000 cells collected at a speed of 300 cells/s.

Chemicals: All chemicals were of analytical or spectroscopic grade (Merck, Darmstadt, FRG) and applied to the samples in quantities as described in the text to the figures.

RESULTS AND DISCUSSION

The existence of Ca^{2+} activated K^+ channels in human peripheral lymphocytes was demonstrated by addition of 100 μM Ca^{2+} to a sample previously equilibrated with 100 mM oxonol and 2 μM ionomycin (Fig. 1). The immediate decrease in the uptake of the voltage sensitive fluorescent dye, bis-oxonol indicates the hyperpolarization of the cells. Fig. 2. shows that increasing the extracellular Ca^{2+} level to the physiologic 1 mM, depolarizes the majority



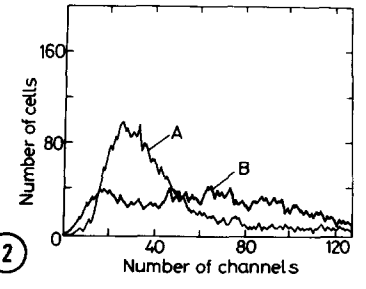


Figure 1. **Hyperpolarizing effect of 100 μM Ca^{2+} on human lymphocytes.** Trace A shows the bis-oxonol fluorescence intensity distribution of control sample. 10^6 cells were incubated with 100 nM bis-oxonol for 3 minutes at 37°C with 2 μM ionomycin Trace B indicates the effect of addition of 100 μM $CaCl_2$.

Figure 2. **Depolarizing effect of 1 mM Ca^{2+} on human lymphocytes.** Trace A shows the fluorescence intensity distribution of the control sample and trace B the effect of 1 mM $CaCl_2$. The experimental conditions were identical to that of in Fig. 1.

of lymphocytes. A small fraction of the cells kept its resting membrane potential. This non-responding population was also observed when the cells were depolarized by high extracellular potassium. The depolarization may be due, at least in part, to a Ca^{2+} induced inactivation of the voltage sensitive K^+ conductance (13), or the steady calcium influx mediated by the ionomycin. In the latter case the increased permeability constant of the Ca^{2+} may explain the depolarization on the basis of the Goldmann equation. On the other hand a significant increase in the positive charges at the immediate vicinity of the inner surface of the plasma membrane alone may also explain the depolarization.

An increase of ionomycin concentration from 2 μM to 5 μM, significantly enhanced the depolarization occurring in the presence of even lower concentration of Ca^{2+} (0.5 mM). Thus it seems that the amount of ionophore, the calcium concentration and other physical parameters as temperature and the responsiveness of the calcium activated potassium channel together will determine the response of the cell, monitored by membrane potential.

It is interesting to note that after activating the Ca^{2+} sensitive potassium channels by modest doses of Ca^{2+}, a further increase in the extracellular Ca^{2+} did not depolarize the cells. The triggering of Ca^{2+} activated K^+ channels was inhibited by quinine, addition of EGTA and proved to be insensitive to 4-aminopyridin or ouabain. The depolarizing effect of Ca^{2+} was not affected by quinine (data not shown).

Fig. 3 demonstrates the effect of ionomycin and extracellular Ca^{2+} on the membrane potential of cells partially depolarized by an isotonic increase in the extracellular K^+ to 35 mM (an equivalent of about 20 mV change in the membrane potential). The same doses of ionophore and extracellular calcium which depolarized the lymphocytes in PBS, caused a hyperpolarization. Flow cytometric membrane potential measurements generally have a 1-2 min lag-time between two samples, therefore the hyperpolarization must occur within this time.

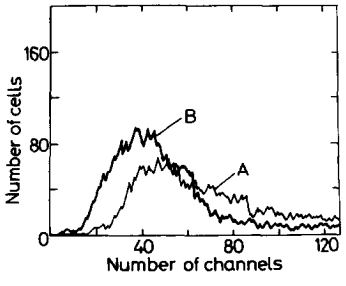


Figure 3. **Hyperpolarizing effect of 1 mM Ca^{2+} on depolarized human lymphocytes.** Trace A shows the fluorescence intensity distribution of human lymphocytes equilibrated with 100 mM oxonol and 2 μM ionomycin in 35 mM K^+ containing PBS. Trace B indicates the effect of 1 mM $CaCl_2$.

A higher degree of depolarization, prior to addition of Ca^{2+} to trigger the Ca^{2+} sensitive K^+ channels, provided that the increase of the extracellular K^+ was between 73-140 mM, resulted in a further depolarization upon addition of even modest Ca^{2+} doses (100-500 μM). These doses generally induce hyperpolarization at 35 mM extracellular [K^+].

It was suggested that depolarization of the plasma membrane potential has an inhibitory effect on divalent cation influx catalyzed by ionomycin, however depolarization does not influence the equilibrium Ca^{2+} concentration just the influx rate (14). Since in our experiments the hyperpolarization of previously depolarized lymphocytes was not followed by depolarization, the lower Ca^{2+} influx rate alone does not explain the phenomenon. The first conclusion to be drawn, regarding the regulation of the Ca^{2+} activated K^+ channels in lymphocytes was that these channels are also voltage regulated.

A similar phenomenon was observed by Sarkadi et al., who very recently have shown that membrane depolarization selectively inhibited receptor (CD-3) operated calcium channels in human T (Jurkat) lymphoblasts. Although they found an effect pointing to the opposite direction i.e. to an inhibition of the Ca^{2+} activated K^+ channels upon depolarization, we believe that both phenomena belong to the same category of regulatory events (15).

The functional behavior of membrane proteins is highly dependent on their conformation (3). The transient and induced dipoles in the membrane generated by the transmembrane potential are reversibly spanning proteins and other molecules across the membrane. This type of electric regulation is reflected e.g. by the voltage gating of channel activities.

Even the accessibility of chemical groups, epitopes or regulatory sites at the cells surface is altered by changes in the membrane potential (12). The facilitated responsiveness of calcium activated K^+ channels by a modest depolarization was clearly shown in our case. The receptor operated Ca^{2+} influx, probably inhibited in the case of Jurkat cells may have a mixed background as the binding of anti-CD-3 antibody to the respective antigen alone, did cause a partial depolarization in an earlier work of us (12).

Herewith the following model is offered which may provide a general explanation for the known experimental facts: Transient and induced dipoles may provide a significant contribution to the regulation of intramembrane molecular interactions. This can be modified through fine modulations of membrane potential by external trigger signals or metabolic processes. The recently discovered similarities between the cells of nervous and immune systems, and the ever accumulating evidence that proximity and dynamic behavior of membrane proteins and their lipid-domain dependent conformational changes can significantly influence, up or down regulate membrane functions, may also be at least in part refer to the above mechanism (16).

Thus we suggest that only a relatively narrow potential window is optimal for those intermolecular interactions which dominate membrane ion-channel and enzyme activities i.e. the initiation and propagation of transmembrane signaling at the plasma membrane level.

While studying the stimulatory signals, generated by antigen or mitogen, using calcium influx dependent pathway, one has to take into account the high, physiological extracellular Ca^{2+} concentration, although these signals will initiate a smaller and more controlled change in the calcium influx, as compared to an ionophore. However, taken together these events with those concerning the changes in membrane potential accompanying stimulations, a combined voltage and calcium regulated potassium channel seems to be more sensitive and adaptable to extracellular conditions than a calcium sensitive channel alone.

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