INHIBITION OF CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE OF HUMAN ERYTHROCYTES BY CALMODULIN INHIBITORY DRUGS

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1. Introduction

A special class of plasma membrane K^+-conductance specifically activated by cytoplasmic $[Ca^{2+}]$ was first described in human red blood cells [1], and later shown to occur in a large variety of vertebrate cells, where it plays an important regulatory role [2]. The mechanism by which Ca^{2+} activates K^+ conductance is, however, unknown.

The ubiquitous calcium-binding protein calmodulin, on the other hand, has been shown to mediate a very large number of intracellular effects of calcium [3]. A number of phenothiazines, such as chlorpromazine, trifluoperazine and fluophenazine; of diphenylbutyl piperidine neuroleptics, such as pimozide, penfluridol and fluspirilene [4–6]; or the drug *N*-(6-aminohexyl)-5-chloro-1-naphtalenesulfonamide (W-7) [7] within a certain concentration range, specifically inhibit the calcium–calmodulin complex.

These experiments were done to find out whether calmodulin participates in the mechanism by which Ca^{2+} activates plasma membrane K^+-conductance. They were prompted by the finding that high chlorpromazine concentrations (0.15 mM) are able to inhibit Ca^{2+}-activated K^+-conductance [8]. For this we have measured, using a K^+-sensitive electrode, net K^+-efflux from human red blood cells under control conditions or when Ca^{2+}-influx was raised by using the ionophore A-23187, and tested the effect of 6 of the above calmodulin-specific inhibitors, on this Ca^{2+}-activated K^+-conductance. To test whether the marked inhibitory effects found with these drugs were specific, we have compared them with those reported, that the same drugs have on human erythrocyte Ca^{2+}-activated ATPase, a well established calmodulin-requiring enzyme [9,10], or on basal membrane Mg^{2+}-ATPase of the same cells, a calmodulin-independent enzyme, that is inhibited non-specifically only by high drug concentrations [5–7].

2. Materials and methods

The rate of net K^+-efflux from human blood bank erythrocytes was measured essentially as in [8]: Red cells were washed twice by centrifugation at room temperature, for 10 min at 1000 × *g*, in 0.15 M NaCl. Each time, before resuspending the pellet, the upper 1/4 of it was aspirated, to eliminate non-red cells. A third wash was done in the medium (mM): NaCl 135; KCl 0.2; $MgCl_2$ 1; $CaCl_2$ 1; Hepes–NaOH (pH 7.4) buffer 20) [8]. Finally the cells were incubated at an hematocrit of 2.5%, at 25°C with stirring, in a final volume of 10 ml, in the above medium [8]. Potassium in the medium [8] was measured continuously by means of a Radiometer F2312K electrode. Ionophore A-23187 was added in 9 μl absolute ethanol, to give a 0.5 μM final conc. At the end of the experiment, the red cells were lysed by adding 2 μl Triton X-100, so that their total $[K^+]$ could be estimated.

Chlorpromazine and W-7 were kind gifts of Professors C. Muñoz and H. Hidaka, respectively; pimozide, penfluridol and fluspirilene were donated by Janssen Pharmaceutica, and fluphenazine by Schering.

3. Results

In the absence of A-23187, the rate constant (k_1) of net K^+-loss from the erythrocytes was found, at 25°C, to be 0.00245 ± 0.00032 min^{-1} (N = 18) (fig.1), and did not change over 45 min incubation,

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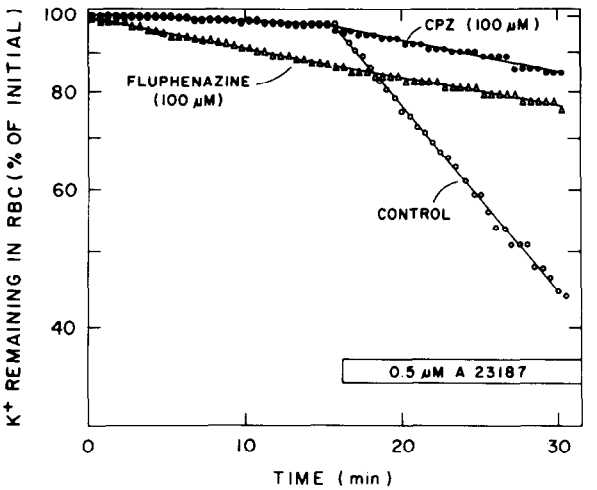

Fig.1. Effect of ionophore A-23187, fluphenazine and chlorpromazine on K^+-loss. Potassium remaining in the erythrocytes (log scale), is plotted against time. In all cases 0.5 μM A-23187 was added at 16 min; k_1 is the rate constant of K^+-loss prior to A-23187; k_2 is that seen following addition of A-23187. k was calculated as $2.303/t \log K_1/K_2$, where K_1 is the $[K^+]$ at the beginning of any interval, K_2 that at the end of the interval, and t the duration of the interval.

or when ethanol up to 1% was added (not shown). When 0.5 μM A-23187 was present (fig.1), the rate constant (k_2) of K^+-efflux increased 20-fold to $0.05032 \pm 0.00118\ min^{-1}$ ($N = 18$). This Ca^{2+}-stimulated K^+-loss remained exponential at least until 80% of the cell K^+ was lost (not shown). Chlorpromazine, even at the highest concentration tested (100 μM) did not change k_1 (fig.1), but all the other drugs tested increased k_1 in variable proportion. This was most marked with high concentrations of fluphenazine (fig.1) or W-7, that markedly increased k_1, but only moderate with diphenylbutyl piperidine neuroleptics, where k_1 increased at most 2-fold, against 10-fold seen with 0.25 μM W-7. Because of these effects on k_1, drug effects are better expressed by measuring their action on the change in rate constant (i.e., $k_2 - k_1$) induced by A-23187.

The diphenylbutyl piperidine neuroleptics pimozide, penfluridol and fluspirilene were all potent inhibitors of Ca^{2+}-activated K^+-conductance (fig.2). This effect had a concentration-dependence that was quite similar to their effects on calmodulin-dependent Ca^{2+}-ATPase, but markedly different from non-specific effects on calmodulin-independent (basal) ATPase of human red blood cells. Chlorpromazine also showed a similar concentration-dependency in the inhibition of Ca^{2+}-activated K^+-conductance relative to Ca^{2+}-ATPase, while fluphenazine deviated from the general pattern (fig.3). Methylene blue, that also has a phenothiazine nucleus, up to 0.5 mM, and tested at 25°C or 37°C,

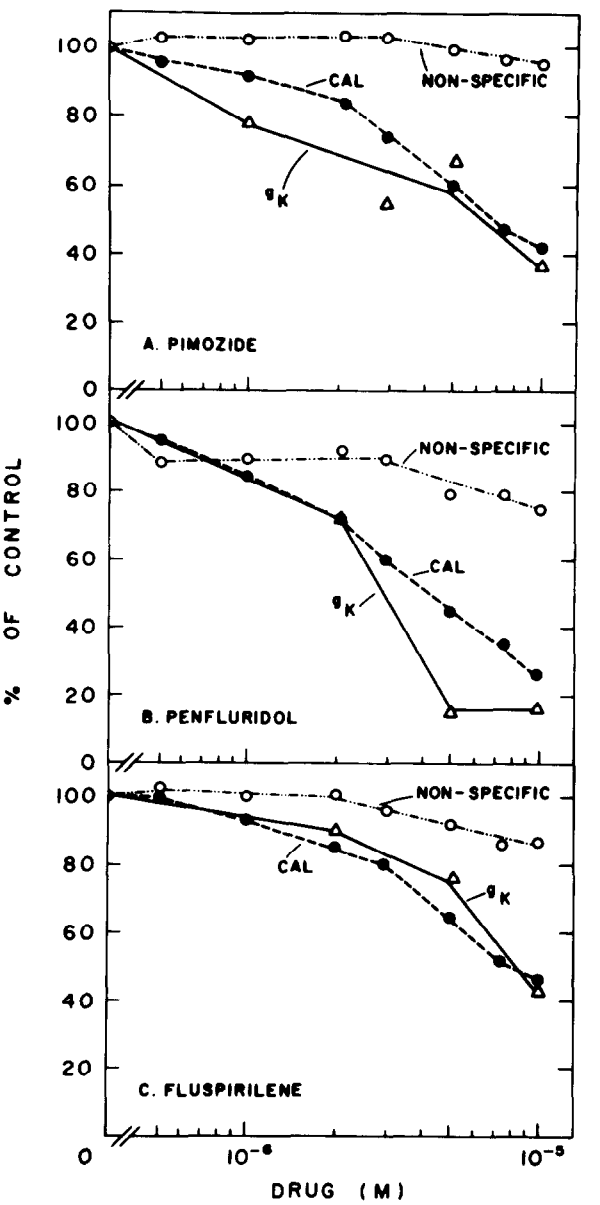

Fig.2. Inhibition of Ca^{2+}-activated K^+-conductance (g_K) by pimozide, penfluridol and fluspirilene; % inhibition is plotted against drug concentration (log scale). Inhibition of g_K is compared with that of calmodulin-activated Ca^{2+}-ATPase (CAL), and with basal, calmodulin-independent Mg^{2+}-ATPase (non-specific), obtained with the same drugs in [5].

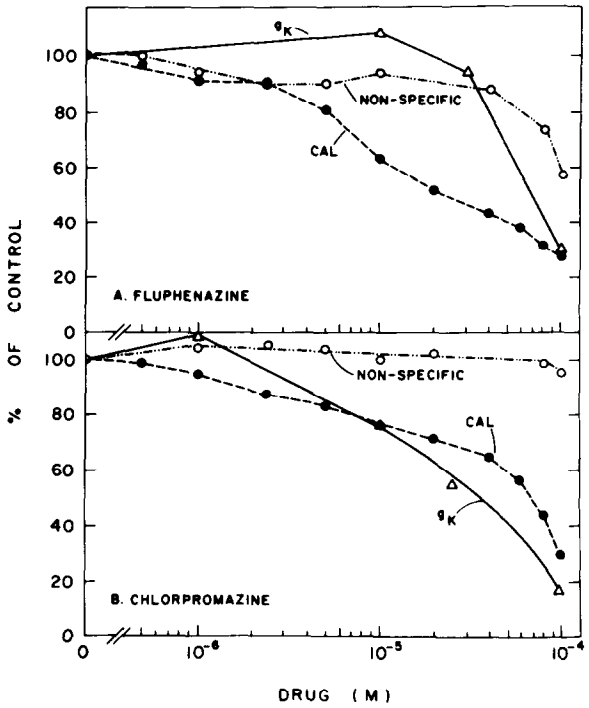

Fig.3. Inhibition of Ca^{2+}-activated K^+-conductance (g_K) by fluphenazine and chlorpromazine. Symbols as in fig.2. Inhibition of Ca^{2+}-ATPase (CAL) and of Mg^{2+}-ATPase (non-specific) was calculated from [5].

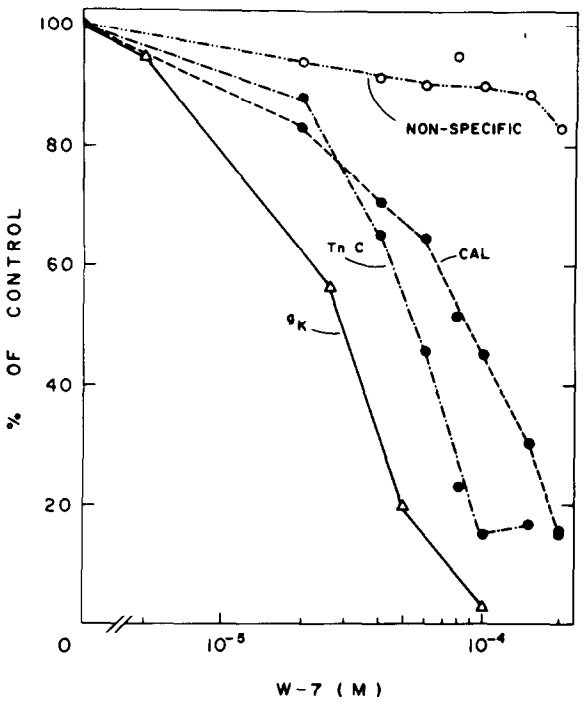

Fig.4. Inhibition of Ca^{2+}-activated g_K by W-7. CAL and TnC represent calmodulin or troponin C-activated Ca-ATPase, respectively, while non-specific indicates basal ATPase. The 3 latter values were calculated from [7].

was entirely inactive on Ca^{2+}-activated K^+-conductance (6 expt, not shown). The calmodulin-inhibitor W-7 was also quite active as an inhibitor of Ca^{2+}-activated K^+-conductance, and its potency was comparable to that shown on calmodulin or troponin C-activated Ca^{2+}-ATPase, but quite different from those on basal Ca^{2+}-ATPase (fig.4).

Inhibition of Ca^{2+}-activated K^+-conductance by the calmodulin inhibitors studied, with the exception of fluphenazine, showed a high degree of correlation ($r = 0.988$) with the effect of these drugs on human red blood cell Ca^{2+}-ATPase, a well-established, calmodulin-dependent enzyme (fig.5).

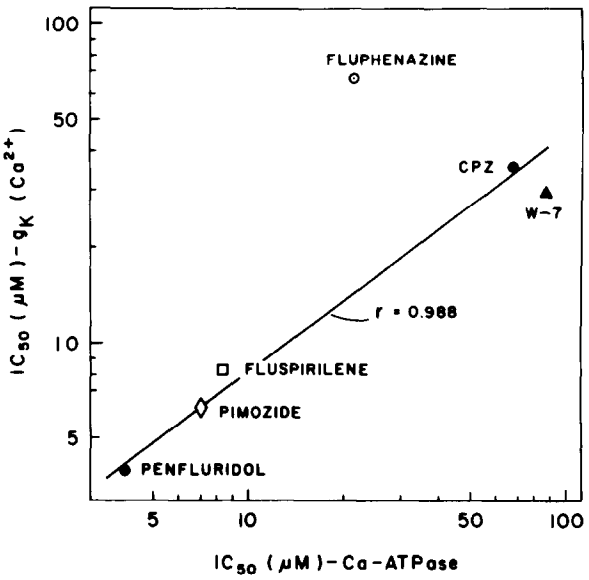

Fig.5. Correlation of inhibition of Ca^{2+}-activated K^+-conductance with that of calmodulin-activated Ca^{2+}-ATPase. Correlation coefficient r is calculated for all drugs except fluphenazine. Slope of the regression line for the same group of drugs was 0.694. For pimozide, penfluridol and fluspirilene r was 0.987 and the slope 1.02.

4. Discussion

The finding that several of the drugs used here are potent inhibitors of Ca^{2+}-activated K^+-conductances is of interest, because of the small number of substances presently known to inhibit that mechanism [8,11–13].

Although it is now established that calmodulin is not the receptor for the neuroleptic effect of phenothiazines or diphenylbutyl piperidines [6,14], several of these drugs do, nevertheless, inhibit numerous calmodulin-mediated calcium effects, at drug concentrations that are ~10-fold lower than those at which nonspecific drug effects are seen [5–7,15]. The high correlation found between inhibition of Ca^{2+}-activated K^+-conductance and $(Ca^{2+} + Mg^{2+})$-ATPase, an established calmodulin-dependent enzyme, as well as the marked differences with the calmodulin-independent Mg^{2+}-ATPase, suggest that calmodulin, or a closely related protein, may mediate Ca^{2+} effects on K^+-conductance. However, this should be confirmed by isolation and purification of the membrane components responsible for this mechanism.

The possible participation of tightly-bound calmodulin in Ca^{2+}-activated K^+-conductance is of interest, because this mechanism is known to exist in 2 states: one with high and the other with low affinity for calcium [16]. The latter form could be due to the presence of an endogenous inhibitor of calmodulin, which, when lost, could switch the system to one with high Ca^{2+} affinity.

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