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Inhibition of Ca^{2+}-dependent K^+ channels by lead in one-step inside-out vesicles from human red cell membranes

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Pb^{2+} modified the apparent threshold sensitivity to Ca^{2+} of individual K^+ channels with a biphasic time-course. At first, the sensitivity to Ca^{2+} was lowered with the result of a decrease of the fraction of activated vesicles at a given Ca^{2+} concentration. Later, Pb^{2+} increased the sensitivity to Ca^{2+} and the fraction of activated vesicles. The increase of Pb^{2+} concentration increased the extent of the initial inhibition but decreased its duration. The inhibitory effect was not observed when the addition of Ca^{2+} preceded the addition of Pb^{2+}. The presence of Mg^{2+} in the incubation medium was also required. In the absence of Mg^{2+}, Pb^{2+} decreased the rate of uptake of ^{86}Rb, but no decrease in the fraction of activated vesicles could be demonstrated.

Lead induces a rapid efflux of K^+ from human red cells by selectively increasing the membrane permeability to this cation [1]. Selective increases in K^+ permeability can also be induced by a variety of treatments which produce an increase of the intracellular Ca^{2+} concentration (for reviews, see Refs. 2–4). On the basis of the similarities between both phenomena, it was proposed that both calcium and lead activate the same membrane channels [5,6]. An alternative view was that lead acted indirectly by increasing the Ca^{2+}-sensitivity or by facilitating the access of Ca^{2+} to the K^+ channels [4]. Recent evidence obtained in intact erythrocytes, ghosts and inside-out membrane patches favors the view that lead acts directly on the Ca^{2+}-dependent K^+ channel [7,8]. In addition, it has been shown recently that larger amounts of lead self-inhibit the K^+ channels and antagonize the activation by Ca^{2+} [7]. We have studied here the effects of lead on ^{86}Rb transport induced by Ca^{2+} in one-step inside-out vesicles [9] derived from human red cell membranes.

The preparation of one-step inside-out vesicles and the measurements of ^{86}Rb uptake were performed as described previously [10,11]. Rb^+ behaves similarly to K^+ for transport through the Ca^{2+}-dependent channels of red cells [12]. The characteristics of ^{86}Rb transport by this preparation of inside-out vesicles have been described previously [9,10]. Individuals K^+ channels respond in an 'all-or-nothing' fashion to Ca^{2+}. Since the mean number of channels per vesicle is low, only a fraction of the total intravesicular space (fraction of activated vesicles) equilibrates with ^{86}Rb at submaximal Ca^{2+} concentrations, whereas the remaining vesicles show low ^{86}Rb permeability, similar to that observed in the Ca^{2+}-free condition. The increase of Ca^{2+} concentration increases the fraction of activated vesicles. The apparent sensitivity to Ca^{2+} can be operationally estimated from the concentration which produces half-maximal activation. Mg^{2+} decreases the apparent sensitivity to Ca^{2+} [10].

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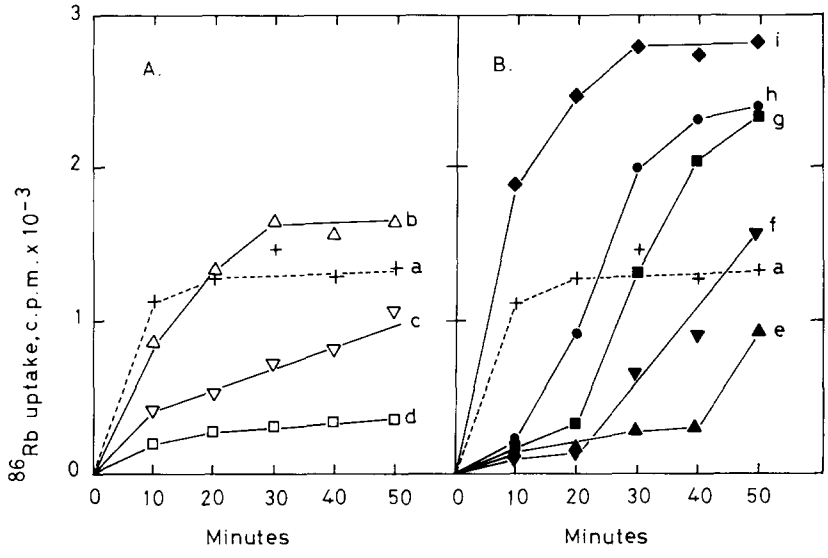

Fig. 1. Effects of lead on the Ca^{2+}-dependent ^{86}Rb uptake by one-step inside-out vesicles in the presence of 3 mM Mg^{2+}. Uptake is expressed as ^{86}Rb that appears inside the vesicles by the end of the time shown in the abscissa (cpm/100 μl sample of vesicles suspension). The uptake without Ca^{2+} (0.4 mM EGTA) has been subtracted. All the data were obtained in the same experiment, but they have been plotted in two panels for convenience. The final incubation medium contained (in mM): KCl, 18; $MgCl_2$, 3; K-Hepes, 16.5 (pH 7.5). Contaminating Ca^{2+}, measured with a selective electrode, was 12 μM. To this medium were added 25 μM EGTA and different amounts of $CaCl_2$ and $Pb(NO_3)_2$, to give the concentrations shown below (in μM). Note that the additions were performed in such a way that the same final Ca^{2+} concentration, 12 μM, was obtained in all cases.

Curve	Added		Estimated	
	$CaCl_2$	$Pb(NO_3)_2$	Ca^{2+}	Pb^{2+}
a	25	0	12	0
b	20	5	12	$1.9 \cdot 10^{-6}$
c	10	15	12	$1.1 \cdot 10^{-5}$
d	3	22	12	$5.5 \cdot 10^{-5}$
e	0	26	12	1
f	0	35	12	10
g	0	65	12	40
h	0	125	12	100
i	0	325	12	300

Fig. 1 shows the effects of different lead concentrations on the Ca^{2+}-dependent ^{86}Rb uptake by inside-out vesicles in a typical experiment. In all the cases the concentration of Ca^{2+} was 12 μM, which activated about 50% of the vesicles under the conditions of this experiment, with 3 mM Mg^{2+} present in the incubation medium. At the lower concentrations ($1.1 \cdot 10^{-11}$ and $5.5 \cdot 10^{-11}$ M), Pb^{2+} decreased the fraction of activated vesicles, this effect increasing with the lead concentration (Fig. 1A). At higher concentrations (1–100 μM) the effect was biphasic. The uptake of ^{86}Rb was strongly inhibited at the shorter incubation periods and then increased, reaching higher values than the control without lead by the end of the incubation period (Fig. 1B). If the incubation period was sufficiently prolonged, the final fraction of activated vesicles was the same in all these cases, similar to that reached at maximal Ca^{2+} concentrations (data not shown). At the highest Pb^{2+} concentration tested (300 μM) the uptake of ^{86}Rb was larger than in the control without lead at all the times tested (Fig. 1B). In all the cases, the uptake of ^{86}Rb was inhibited more than 90% by 1 mM quinine (not shown). It seems, then, that lead causes delayed activation of the K^+ channels. The delay becomes shorter the higher the Pb^{2+} concentration. A possible mechanism for the delay may be a transient increase in the threshold for Ca^{2+} activation. After the delay, ^{86}Rb uptake increases, reaching higher values than the control without lead at the steady-state. Either the delay or the increase of ^{86}Rb uptake at the steady-state may not be apparent at the extreme lead concentrations (under 1 μM or 300 μM) in the experiment of Fig. 1, but this is probably due to the

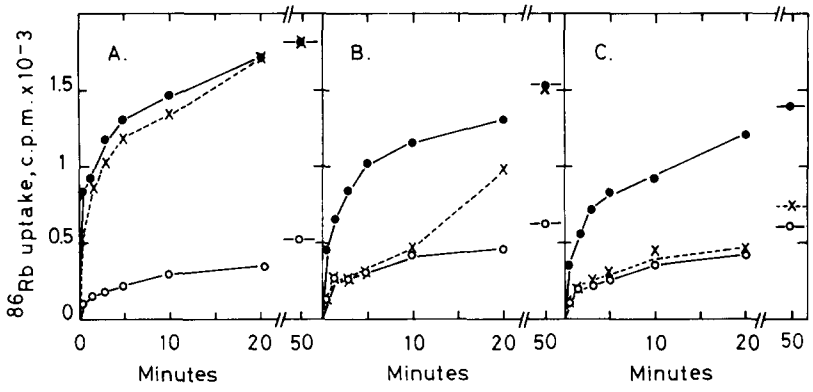

Fig. 2. Effects of Mg^{2+} on the inhibition of Ca^{2+}-dependent ^{86}Rb uptake by lead. The incubation medium contained either no Mg^{2+} (A) or 2 (B) or 4 mM Mg^{2+} (C). The uptake of ^{86}Rb in the absence of Ca^{2+} (0.4 mM EGTA) is shown by open circles. Closed circles and crosses represent the uptake in the presence of 12 μM Ca^{2+} and in the presence of 12 μM Ca^{2+} + 50 μM Pb^{2+}, respectively. Other details as in Fig. 1.

inadequacy of the incubation periods tested, either too short to show the activatory effect or too long to demonstrate the inhibitory effect.

The effect of lead was strongly dependent on the concentration of Mg^{2+} in the incubation medium (Fig. 2). At 4 mM Mg^{2+}, 50 μM Pb^{2+} almost completely prevented the ^{86}Rb uptake induced by 12 μM Ca^{2+} during the whole 50 min incubation period (Fig. 2C). At 2 mM Mg^{2+}, lead strongly inhibited the uptake of ^{86}Rb during the first 10 min of incubation, but the fraction of activated vesicles reached by the end of the incubation period was the same as in the control without lead (Fig. 2B). In the absence of Mg^{2+} lead had no effect on the fraction of activated vesicles, although a decrease in the rate of uptake of ^{86}Rb during the first few minutes was still apparent (Fig. 2A). This result was systematically observed in the absence of Mg^{2+}. In another series of experiments, 10 μM Pb^{2+} increased the half-equilibration time of ^{86}Rb 3.0-times (from 27 to 81 s), and 35 μM Pb^{2+} 4.1-times. In both cases the uptake of ^{86}Rb at the steady state was the same as in the controls without lead. In the absence of Mg^{2+}, EGTA-Pb did not modify either the rate of the Ca^{2+}-induced ^{86}Rb uptake or the fraction of activated vesicles obtained at submaximal Ca^{2+} concentrations (data not shown).

The inhibitory effects of Pb^{2+}, both in the presence and in the absence of Mg^{2+}, were antagonized by increasing the Ca^{2+} concentration (data not shown). On the other hand, if experiments were performed in such a way that the increase in the Ca^{2+} concentration preceded the increase in the Pb^{2+} concentration, the inhibitory effect of Pb^{2+} observed in Mg^{2+}-containing medium was largely prevented (Fig. 3).

Our results seem to point out two different

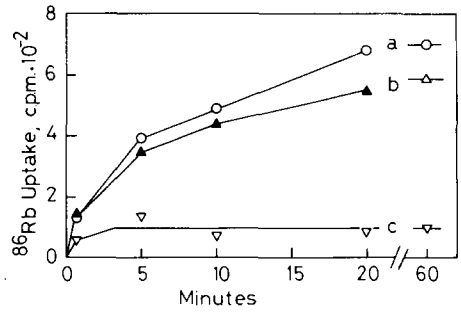

Fig. 3. Effects of the order of the additions of Ca^{2+} and Pb^{2+} on the inhibition of Ca^{2+}-dependent ^{86}Rb uptake by lead. Vesicles were suspended in a medium containing 25 μM EGTA and other constituents as described in the legend to Fig. 1, except for $MgCl_2$ which was 4 mM. Contaminating Ca^{2+} was 12 μM. In curve a 25 μM $CaCl_2$ was added to give a final Ca^{2+} concentration of 12 μM in the suspension. In curve c, concentrations of Ca^{2+} and Pb^{2+} were increased simultaneously to 12 and 50 μM, respectively, by adding 75 μM $Pb(NO_3)_2$. In curve b the addition of Pb $(NO_3)_2$ was done in two steps of 25 (to increase Ca^{2+} to 12 μM) and 50 μM, respectively, separated by a 1 min interval. In all cases, the uptake of ^{86}Rb was started by the addition of the tracer 1 min after the last addition ($t = 0$ in the figure). Uptake is expressed as ^{86}Rb appearing inside the vesicles by the end of the times shown in the abscissa (cpm/100 μl sample of vesicle suspension). The uptake without Ca^{2+} (0.4 mM EGTA) has been subtracted from all the values.

actions of lead on Ca^{2+}-dependent K^+ channels. (1) A decrease in the apparent permeability to ^{86}Rb of the channels with an increase of the half-equilibration time. This effect appears both in the presence and in the absence of Mg^{2+}. It could have the same basis as the decrease of the mean open time of the channels observed in patch-clamp experiments [7]. (2) A biphasic change in the threshold sensitivity to Ca^{2+} of the individual channel units. This interpretation rests on the all-or-nothing interpretation of the channels' response in inside-out vesicles [9,10,13]. The sensitivity to Ca^{2+} first decreases, as evidenced by the decrease of the fraction of activated vesicles, and then increases above the control level at longer incubation periods. Alternatively, the apparent increase of the sensitivity to Ca^{2+} could actually reflect a delayed, direct activatory effect of lead on the K^+ channels. The extent of the inhibitory effect increased with Pb^{2+} concentration, but its duration was inversely proportional to the Pb^{2+} concentration. Mg^{2+} has been shown before to decrease the apparent sensitivity to Ca^{2+} of the K^+ channels in inside-out vesicles [10]. The inhibitory effect of lead reported here was dependent on the presence of Mg^{2+}, and became stronger and/or more lasting by increasing the concentration of Mg^{2+} (Fig. 2).

Both of the inhibitory actions discussed here, the one on the apparent permeability to ^{86}Rb of the K^+ channels and the other on its threshold sensitivity to Ca^{2+}, could contribute to the inhibition of Ca^{2+}-dependent K^+ transport by lead reported in intact cells [7]. This inhibition was reported only for lead concentrations above 15 mmol/l cells, but this low sensitivity could be attributed to the A23187-mediated Mg^{2+} depletion of the cells, which was allowed in the above-mentioned experiments. The proposedly similar inhibitory effect of Co^{2+} reported in the same paper [7] could be explained more simply in terms of competitive blocking of Ca^{2+} influx through the ionophore and fast cell Ca^{2+} extrussion through the Ca^{2+} pump conducive to deactivation of the K^+ channels. This effect of Co^{2+} on Ca^{2+} movements has been documented previously [14].

Our results only circumscribe the effects of lead, but do not allow us to propose a single mechanism of action. The effects of lead reported here could be equally well explained by actions on a modulatory site, probably shared with Mg^{2+}, which modifies the sensitivity of the Ca^{2+}-binding site(s), or by direct interaction of lead with the Ca-binding site(s), or by a mixture of both effects. The antagonizing effect of Ca^{2+} on inhibition by Pb^{2+} seems to favor the view that the Pb-binding site could also accept Ca^{2+}. On the other hand, the transient delay of the activation by Ca^{2+} effected by lead in the presence of Mg^{2+} suggests the existence of slowly reverting conformational changes. Such slow conformational changes could play an important role in determining the all-or-nothing response to Ca^{2+} of the K^+ channels in inside-out vesicles [9,10,13].

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