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Distinct metal ion binding sites on Ca^{2+}-activated K^+ channels in inside-out patches of human erythrocytes

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Effects of Cd^{2+}, Co^{2+}, Pb^{2+}, Fe^{2+} and Mg^{2+} (1–100 μM) on single-channel properties of the intermediate conductance Ca^{2+}-activated K^+ (CaK) channels were investigated in inside-out patches of human erythrocytes in a physiological K^+ gradient. Cd^{2+}, Co^{2+} and Pb^{2+}, but not Fe^{2+} and Mg^{2+}, were able to induce CaK channel openings. The potency of the metals to open CaK channels in human erythrocytes follows the sequence Pb^{2+}, $Cd^{2+} > Ca^{2+} \geq Co^{2+} \gg Mg^{2+}$, Fe^{2+}. At higher concentrations Pb^{2+}, Cd^{2+} and Co^{2+} block the CaK channel by reducing the opening frequency and the single-channel current amplitude. The potency of the metals to reduce CaK channel opening frequency follows the sequence $Pb^{2+} > Cd^{2+}$, $Co^{2+} \gg Ca^{2+}$, which differs from the potency sequence $Cd^{2+} > Pb^{2+}$, $Co^{2+} \gg Ca^{2+}$ to reduce the unitary single-channel current amplitude. Fe^{2+} reduced the channel opening frequency and enhanced the two open times of CaK channels activated by Ca^{2+}, whereas up to 100 μM Mg^{2+} had no effect on any of the measured single-channel parameters. It is concluded that the activation of CaK channels of human erythrocytes by various metal ions occurs through an interaction with the same regulatory site at which Ca^{2+} activates these channels. The different potency orders for the activating and blocking effects suggest the presence of at least one activation and two blocking sites. A modulatory binding site for Fe^{2+} exists as well. In addition, the CaK channels in human erythrocytes are distinct from other subtypes of Ca^{2+}-activated K^+ channels in their sensitivity to the metal ions.

Introduction

Application of the patch-clamp technique showed that in human erythrocytes the Ca^{2+}-evoked increase in the K^+ permeability is caused by the opening of Ca^{2+}-activated K^+ channels with a single-channel conductance between 10 and 40 pS [1–3]. According to its single-channel conductance and to the pharmacological properties of Ca^{2+}-dependent K^+ efflux [4,5] the Ca^{2+}-activated K^+ channel in human erythrocytes has been identified as an intermediate conductance channel. As described in the previous paper [6], the gating of CaK channels in human erythrocytes is voltage-independent and the Ca^{2+} dependence of the current mediated by these channels is due to an increase of single-channel opening frequency. The open times of these CaK channels remain constant over a wide range of $[Ca^{2+}]_i$. Results of flux experiments in human erythrocytes have suggested that Pb^{2+} and Ca^{2+} interact with the same site to evoke K^+ efflux [7,8]. With the single-channel patch clamp technique it has been demonstrated that CaK channels in human erythrocytes are activated by 10 μM Pb^{2+} [2] and are blocked by 100 μM Pb^{2+} [9]. Millimolar concentrations of Mg^{2+} do not activate erythrocyte CaK channels, but reduce the open probability of Ca^{2+}-activated CaK channels [10].

Since divalent metal ions interfere with membrane functions that are normally regulated by Ca^{2+}, these ions are useful tools to probe the properties of Ca^{2+}-permeating and Ca^{2+}-dependent channels. Some metals permeate through voltage-dependent Ca^{2+} channels, whereas others block Ca^{2+} channels and are used as Ca^{2+} antagonists [11–13]. A Ca^{2+}-dependent K^+ current in *Helix* pacemaker neurons is activated by intracellularly injected Cd^{2+}, Co^{2+}, Mg^{2+} and Pb^{2+} [14]. Conversely, results obtained by similar methods from *Aplysia* pacemaker neurons show that Co^{2+} and Mg^{2+} are ineffective, whereas Cd^{2+} and Fe^{2+} activate a K^+ current [15]. In lipid bilayers big (BK) Ca^{2+}-activated K^+ channels reconstituted from rat skeletal

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muscle are activated by Cd^{2+}, Co^{2+} and Fe^{2+}, but not by Pb^{2+} and Mg^{2+} [16]. In contrast with the result on CaK channels of human erythrocytes millimolar concentrations of Mg^{2+} enhance the open probability of the reconstituted BK channels activated by Ca^{2+} [16,17]. In inside-out patches of N1E-115 mouse neuroblastoma cells Mg^{2+} and Fe^{2+} do not affect small (SK) and big (BK) Ca^{2+}-activated channels either in the presence or in the absence of Ca^{2+}. SK as well as BK channels are activated by Pb^{2+} and Co^{2+}, whereas Cd^{2+} selectively activates SK channels in N1E-115 membrane patches [18]. In general, it appears that subtypes of Ca^{2+}-activated K^+ channels have distinct sensitivities to metal ions. In addition, metal ions may exert activating or blocking effects depending on the subtype of Ca^{2+}-activated K^+ channel and the nature of the effect may change with metal ion concentration.

Using the patch-clamp technique concentration-dependent effects of various metal ions on CaK channels in human erythrocytes are investigated. The ability of these metals to induce Ca^{2+}-activated K^+ channel opening as well as other observed effects of the metal ions on these channels are compared with those of Ca^{2+}.

Materials and Methods

Single-channel currents of Ca^{2+}-activated K^+ channels were recorded from inside-out membrane patches of human erythrocytes. All methods of cell acquisition and electrophysiological recording were as previously described [6]. Patch pipettes had a resistance of 10–40 MΩ and the seal resistance ranged between 16 and 53 GΩ. Patches were voltage clamped at 0 mV. Experiments were carried out at a temperature of 21–26°C.

Data analysis and statistics. Data were obtained by superfusion of inside-out patches with internal solution containing different metal ion concentrations in a semi-random order. The patches were repeatedly superfused with internal solution containing a saturating $[Ca^{2+}]$. Patches with trends in the channel open probability or with a maximum channel open probability < 0.10 were excluded from further analysis. Opening and closing transitions were detected using a half amplitude threshold criterion and a minimum event width of 2.5 ms. The number of channels (N_C) in a patch was taken equal to the maximum number of channels simultaneously open in the presence of saturating $[Ca^{2+}]_i$. The open probability (P_O) was determined by summation of all open times relative to the total observation time. Note that for independently operating ion channels P_O should be proportional to N_C and that $0 \leq P_O \leq N_C$. The frequency of channel opening was determined as the ratio between the number of opening transitions and the total observation time.

Events were divided into classes of approximately equal frequency and presented as frequency density open time histograms [19]. Exponential time constants were estimated by minimizing χ^2 using a nonlinear least-squares algorithm [20]. Dual exponential fitting was performed only when a single exponential distribution was rejected by the χ^2 goodness-of-fit test ($\alpha = 0.05$). Open time frequency density histograms were constructed from single open events only. Results of multiple-channel patches did not deviate from those of apparent single-channel patches.

Results are presented as means ± S.D. Further testing was performed using statistical software (CSS, Statsoft, Tulsa, USA). As post hoc comparison of the analysis of variance (ANOVA) the LSD test was used.

Solutions and chemicals. Solutions were prepared from milli-Q/UF purified water (Millipore, Bedford, USA). The external solution contained (in mM): NaCl, 145; KCl, 5; Hepes, 20; $CaCl_2$, 1.8; and $MgCl_2$, 0.8. The pH was adjusted to 7.4 with approx. 8.4 mM NaOH and the osmolarity was adjusted to 330 mosmol/l with 30 mM glucose. The internal solution contained (in mM): KNO_3, 120; Hepes, 10; citric acid, 10; and various concentrations of metal salts. The pH was adjusted to 7.2 with approximately 30 mM KOH and the osmolarity was adjusted to 300 mosmol/l with 55 mM sucrose. Experiments were performed without intracellular Na^+ to prevent channel block [21,22]. Free metal concentrations were calculated using the procedure of Van Heeswijk, Geertsen and Van Os [23] and using published stability constants [24,25]. Experiments were performed at buffered free metal ion concentrations between 1 and 100 μM, except for Pb^{2+} that was tested up to 90 μM to avoid the precipitation observed at 100 μM. The final maximum amount of contaminants, calculated from the data supplied with the chemicals, was (in μM): Ba, 1.1; Ca, 1.8; Cd, 0.11; Co, 0.20; Mg, 0.57; Na, 7.6; and Pb, 0.075. In internal solution containing 100 μM free Fe^{2+} the Pb contamination was 0.124 μM. In the presence of 100 μM free Co^{2+} the contaminations of Ca, Mg and Pb were 4.3, 2.2 and 0.171 μM, respectively.

KNO_3, $Ca(NO_3)_2$, $Cd(NO_3)_2$, $Mg(NO_3)_2$, $Pb(NO_3)_2$, and KOH (Ultrapure), $FeCl_2$, NaCl and KCl (pro analysi) were obtained from Merck, Darmstadt, Germany; Hepes (MicroSelect) from Fluka, Buchs, Switzerland; $CaCl_2$, $CoCl_2$ and citric acid (AnalaR), glucose and sucrose (Aristar) from BDH, Poole, UK; $MgCl_2$ (Baker Analyzed reagent) from Baker, Deventer, Netherlands.

Results

CaK channels in inside-out membrane patches of human erythrocytes attain maximum open probability during superfusion with internal solution containing 115 μM Ca^{2+} [6]. Consecutive single-channel traces of

CaK channel activity in a single inside-out membrane patch superfused with Ca^{2+}, Co^{2+}, Cd^{2+} and Pb^{2+} and their corresponding open probabilities (P_O) are shown in Fig. 1. When metal ions were removed by superfusion with internal solution containing either 10 mM citric acid or 10 mM EGTA, no CaK channel openings were observed. Except for the 100 μM Co^{2+} containing internal solution the free $[Ca^{2+}]_i$ contaminating the internal solutions containing the various metal ions was always ≤ 0.2 μM, which is not sufficient to activate CaK channels [6]. Mg^{2+} (11 patches) and Fe^{2+} (12 patches) failed to activate the channel at either 10 or 100 μM. In four additional patches, in which 10 mM EDTA was used instead of citric acid, Mg^{2+} up to 100 μM did neither evoke CaK channel openings.

Single-channel records of CaK channels in the presence of various metal ions (Fig. 2) show activating as well as blocking effects in the concentration range of 1–100 μM, in which Ca^{2+} activates CaK channels. All effects were readily reversed after washing with control internal solution and subsequent superfusion with internal solution containing an activating metal ion. The P_O values and opening frequencies of the CaK channel relative to those obtained with 115 μM Ca^{2+} for Ca^{2+}, Cd^{2+}, Co^{2+} and Pb^{2+} between 1 and 100 μM are presented in Table I. A comparison of the two single-channel parameters shows similar variations in P_O and opening frequency with metal ion species and concentration. This is consistent with our previous conclusion that the opening frequency determines the concentration dependence of CaK channels activated by Ca^{2+} [6]. Statistical analysis indicates that the P_O as well as the opening frequency of CaK channels activated by 1 μM Pb^{2+} are higher than those obtained with the other metal ions at the same concentration (LSD: $P < 0.001$). Although it cannot be excluded that some CaK channel block already occurs at 1 μM Pb^{2+} (see below), this result demonstrates that Pb^{2+} is a more potent activator of CaK channels than Ca^{2+} and Co^{2+}. The comparison between Pb^{2+} and Cd^{2+} is complicated by the fact that the activating effects of 1 and 10 μM Cd^{2+} yield similar maximum values of P_O (LSD: $P = 0.48$) and opening frequency (LSD: $P = 0.94$). Since the maximum values of CaK channel activation by Ca^{2+} are obtained at concentrations between 10 and 115 μM, Cd^{2+} appears also a more potent activator of CaK channels than Ca^{2+}. Hardly any CaK channel activation is observed with 1 μM Co^{2+} and a clear increase in P_O and opening frequency occur at 10 μM Co^{2+} (LSD: $P < 0.001$). This result indicates that Co^{2+} is a less potent activator than Pb^{2+} and Cd^{2+}. The blocking effect observed at 100 μM Co^{2+} precludes to decide whether Co^{2+} is less potent than or equipotent to Ca^{2+}. The potency order of metal ions for the activation of CaK channels in human erythrocytes follows the sequence Pb^{2+}, $Cd^{2+} > Ca^{2+} \geq Co^{2+} \gg Fe^{2+}$, Mg^{2+}.

The open time frequency density distributions obtained at 115 μM Ca^{2+} were fitted by the sum of two exponential functions. The mean values of the two time constants of 28 patches were 5.7 ± 2.5 ms and 21 ± 8 ms. In the presence of either 1 or 10 μM Ca^{2+}, 10 μM Cd^{2+}, 10 or 100 μM Co^{2+}, or 1, 10 or 90 μM Pb^{2+} no

TABLE I

Open probability (P_O), opening frequency (F_O) and single-channel current (i) of the CaK channel of human erythrocytes in the presence of different concentrations of metals

The parameters in the presence of the metals were normalized to those obtained with 115 μM Ca^{2+} in the same patch. In addition, time constants of the dual exponential function $A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$ fitted to the open time frequency density histograms of CaK channel in the presence of the various metal ion concentrations are presented. *N* represents the number of patches. The values given for P_O, F_O and *i* are relative to those for 115 μM Ca^{2+}.

Metal	(μM)	N	P_O	F_O	i	τ_1 (ms)	τ_2 (ms)
Ca^{2+}	1	5	0.23 ± 0.07	0.29 ± 0.06	0.97 ± 0.07	3.5 ± 1.2	13 ± 2
	10	3	0.71 ± 0.12	0.69 ± 0.07	0.99 ± 0.04	3.0 ± 1.0	16 ± 7
	115	28	1	1	1	5.7 ± 2.5	21 ± 8
Cd^{2+}	1	3	0.24 ± 0.11	0.30 ± 0.20	0.99 ± 0.01	–	–
	10	5	0.29 ± 0.13	0.30 ± 0.12	0.89 ± 0.02	5.4 ± 1.9	29 ± 9
	100	4	0.09 ± 0.08	0.07 ± 0.06	0.59 ± 0.06	–	–
Co^{2+}	1	3	0.02 ± 0.01	0.02 ± 0.01	1.02 ± 0.02	–	–
	10	7	0.34 ± 0.11	0.32 ± 0.13	0.96 ± 0.05	3.4 ± 1.0	19 ± 2
	100	7	0.08 ± 0.04	0.08 ± 0.05	0.88 ± 0.08	3.3 ± 0.5	18 ± 7
Pb^{2+}	1	4	0.62 ± 0.13	0.69 ± 0.14	1.06 ± 0.06	5.2 ± 1.5	23 ± 4
	10	3	0.33 ± 0.11	0.36 ± 0.16	0.99 ± 0.06	3.2 ± 1.2	20 ± 8
	90	5	0.22 ± 0.20	0.25 ± 0.24	0.77 ± 0.08	5.0 ± 1.7	19 ± 9

difference in the two open time constants was observed (Table I; ANOVA: $P = 0.11$ and 0.29, respectively). With the various metal ions the relative amplitudes of the fast and slow component of the open time frequency density histograms varied between patches and also between different sets of data obtained from the same patch, consistent with previous results on CaK channels in human erythrocytes activated by Ca^{2+} [6]. Since the experimental protocol required the comparison of channel parameters in the presence of Ca^{2+} with those in the presence of metal ions in the same

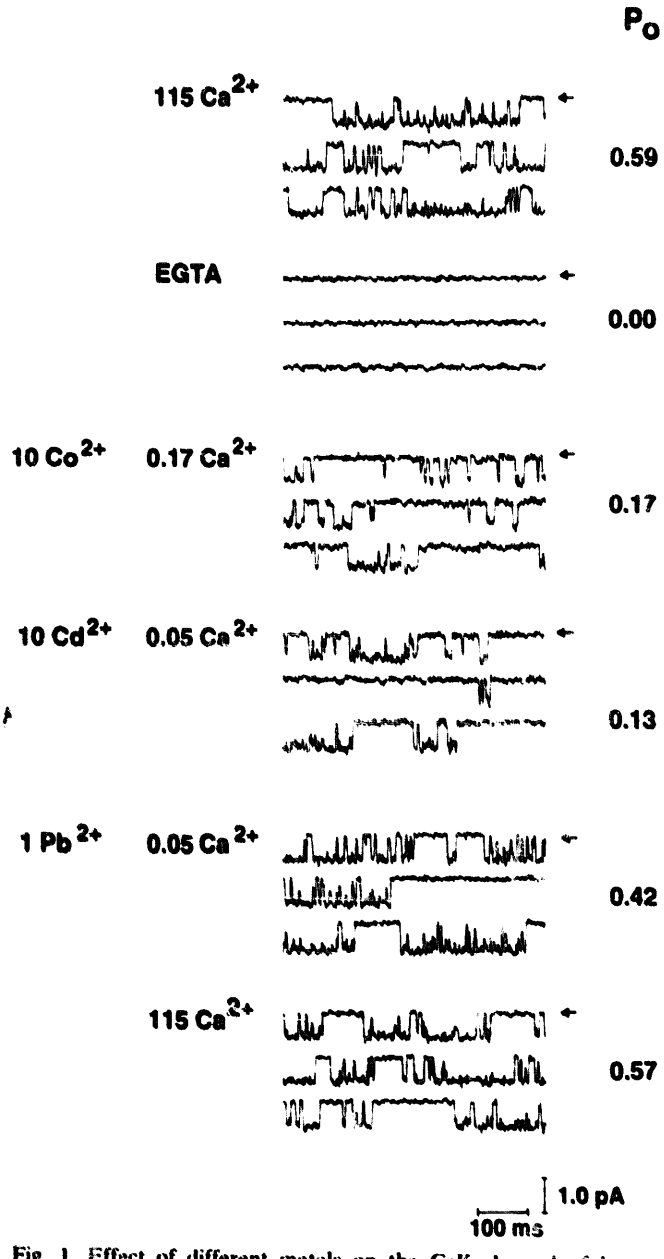

Fig. 1. Effect of different metals on the CaK channel of human erythrocytes at 0 mV membrane potential in an inside-out patch. The final free metal concentrations (in μM) as well as the corresponding P_O are indicated. The concentration of EGTA was 10 mM. Each block represents three consecutive traces and all records have been obtained from the same patch. The closed level is marked by an arrow.

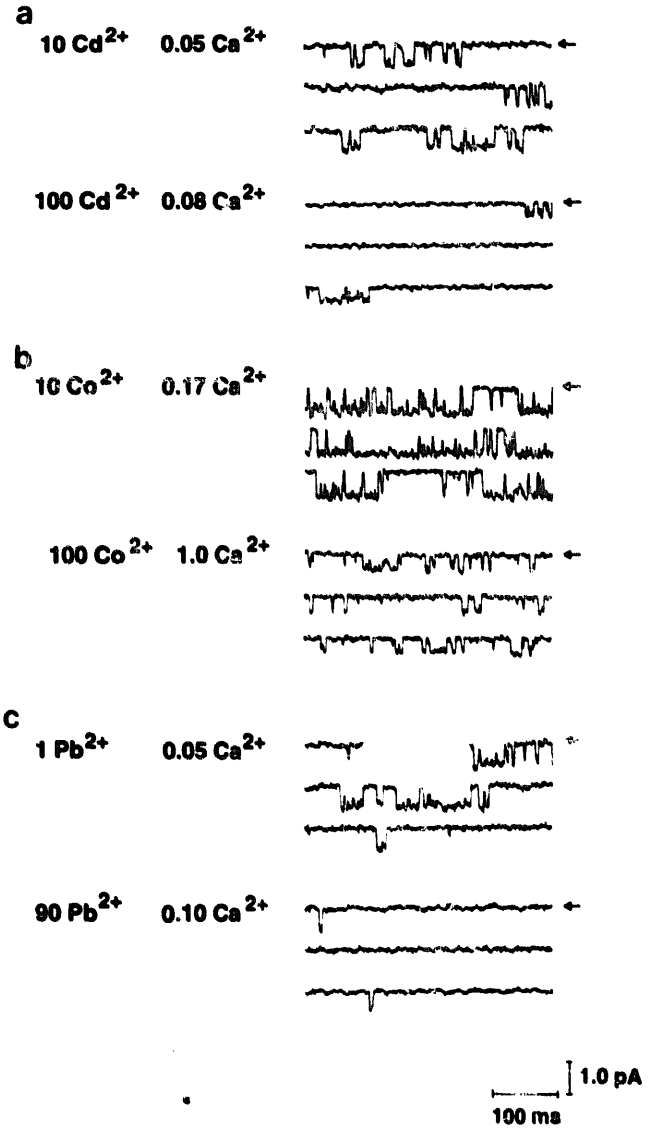

Fig. 2. Effect of high and low concentrations of (a) Co^{2+}, (b) Cd^{2+} and (c) Pb^{2+} on the CaK channel of human erythrocytes at 0 mV membrane potential in inside-out patches. The final free metal concentrations (in μM) are indicated. Each block represents three consecutive traces and all records have been obtained from the same patch. The closed level is marked by an arrow.

patch, the number of events collected was not sufficiently large to obtain reliable open time histograms for 1 μM Co^{2+} and for 1 and 100 μM Cd^{2+}.

Single-channel open probability and opening frequency decreased and in some cases CaK channel activity was diminished to almost zero at the higher concentrations of Cd^{2+}, Co^{2+} and Pb^{2+}. At 100 μM Cd^{2+} or Co^{2+} P_O and opening frequency values were significantly reduced as compared to those at 10 μM (LSD: $P < 0.01$ and $P < 0.05$, respectively). With Pb^{2+} a general decrease in P_O and opening frequency for concentrations beyond 1 μM was observed (LSD: $P < 0.05$ and $P < 0.05$, respectively). The latter result indicates that the maximum activating effect of Pb^{2+} occurs at or below 1 μM and that the P_O and opening

frequency at 1 μM might be below the maximum value due to channel block. The reduction of P_O in the presence of 100 μM Co^{2+} is noteworthy, since the Ca^{2+} contaminating the 100 μM Co^{2+} solution yields the calculated equivalent of 1 μM free Ca^{2+}, which on itself is sufficient to produce a relative P_O of 0.23 (see Table I). This indicates that 100 μM Co^{2+} blocks both Co^{2+}- and Ca^{2+}-activated CaK channels. The potency of the various metals to reduce P_O and opening frequency is less than that to activate CaK channels and follows the sequence $Pb^{2+} > Cd^{2+}$, $Co^{2+} > Ca^{2+}$.

Analysis of the open probabilities and the opening frequency of patches having multiple open levels revealed that the CaK channels did not behave independently. The deviation from independent behaviour was observed not only in the presence of Ca^{2+} as reported in the previous paper [6], but also in the presence of the other metals that activate CaK channels.

Metal ions may also reduce the single CaK channel current amplitude (Table I). Activated by Ca^{2+} these channels carry an average outward current of 0.93 ± 0.10 pA (45 patches) at the holding potential of 0 mV. The mean amplitudes of unitary current obtained at low metal ion concentrations (1 and 10 μM Co^{2+} and Pb^{2+} and at 1 μM Cd^{2+}) could not be distinguished from that in the presence of Ca^{2+} (LSD: $P = 0.30$–0.99). However, the mean unitary current amplitude was significantly reduced by the higher concentrations of Cd^{2+}, Co^{2+} and Pb^{2+} (LSD: $P < 0.05$). The highest block occurred at 100 μM Cd^{2+} (LSD: $P < 0.01$). The results indicate that Cd^{2+} is the more potent blocker of the single CaK channel current amplitude and that Ca^{2+} is ineffective in this respect up to 115 μM.

Effects of Mg^{2+} and Fe^{2+}, which were unable to induce CaK channel opening by themselves, were also investigated in the presence of 115 μM Ca^{2+} (Table II). The P_O and the single-channel current amplitudes did not differ from those obtained with Ca^{2+} only (ANOVA: $P = 0.53$ and $P = 0.99$, respectively). In contrast, the opening frequency of CaK channels activated by Ca^{2+} was reduced in the presence of either 10 or

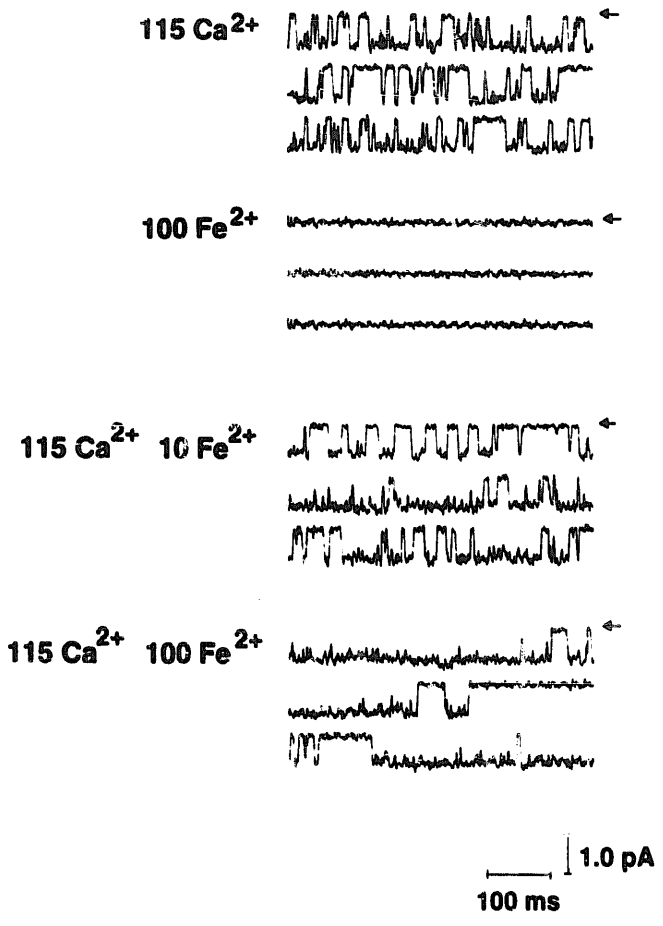

Fig. 3. Effect of Fe^{2+} on the CaK channel of human erythrocytes at 0 mV membrane potential in an inside-out patch. Traces were recorded in the presence of Ca^{2+} and Fe^{2+} alone as well as in the presence of a combination of Ca^{2+} and two concentrations of Fe^{2+}. The final free metal concentrations (in μM) are indicated. Each block represents three consecutive traces and all records have been obtained from the same patch. The closed level is marked by an arrow.

100 μM Fe^{2+} (LSD: $P < 0.05$), but a significant reduction could not be demonstrated in the presence of 100 μM Mg^{2+} (LSD: $P = 0.05$). Simultaneous with the reduction of opening frequency Fe^{2+} causes a prolon-

TABLE II

Open probability (P_O), opening frequency (F_O) and single-channel current (i) of the CaK channel of human erythrocytes in the presence of Mg^{2+} or Fe^{2+} together with 115.2 μM Ca^{2+}

The parameters were normalized to those obtained in the presence of saturating Ca^{2+} only in the same patch. *N* represents the number of patches. The values given are relative to those for 115 μM Ca^{2+}.

Metal	(μM)	*N*	P_O	F_O	*i*
Fe^{2+}	10	4	0.95 ± 0.09	0.82 ± 0.14	1.00 ± 0.09
	100	4	1.07 ± 0.15	0.48 ± 0.27	0.99 ± 0.01
Mg^{2+}	100	3	0.83 ± 0.16	0.82 ± 0.14	1.00 ± 0.01

TABLE III

Parameters of the dual exponential function $A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$ fitted to the open time frequency density histograms of CaK channels of human erythrocytes in the presence of 115 μM Ca^{2+} and either Mg^{2+} or Fe^{2+}

N represents the number of patches.

Metal	(μM)	*N*	$\tau_{O,1}$ (ms)	$A_{O,1}$ (%)	$\tau_{O,2}$ (ms)	$A_{O,2}$ (%)
Ca^{2+}	115	7	5.0 ± 2.5	41 ± 21	21 ± 8	59 ± 21
Fe^{2+}	10	4	9.4 ± 2.5	58 ± 13	49 ± 14	42 ± 13
	100	3	10.2 ± 3.9	49 ± 11	54 ± 22	51 ± 11
Mg^{2+}	100	3	4.0 ± 0.2	39 ± 14	15 ± 1	61 ± 14

gation of the open time of CaK channels activated by 115 μM Ca^{2+} (Fig. 3). Open time frequency density histograms of CaK channels activated by 115 μM Ca^{2+} in the presence of Mg^{2+} and Fe^{2+} were fitted by the sum of two exponential functions (Table III). The short and the long open time constant in the presence of 10 and 100 μM Fe^{2+} are increased with respect to the values obtained with 115 μM Ca^{2+} only (LSD: $P < 0.05$). The two open time constants obtained in the presence of 100 μM Mg^{2+} did not differ from those obtained with 115 μM Ca^{2+} only (LSD: $P = 0.31$ and $P = 0.39$, respectively).

Discussion

Single-channel properties of the intermediate conductance CaK channel in human erythrocytes have been investigated by replacing Ca^{2+} by other metal ions. Of the various metals tested Cd^{2+}, Co^{2+} and Pb^{2+} induce CaK channel openings, whereas no channel activity was observed in the presence of Fe^{2+} and Mg^{2+}. In addition, Cd^{2+}, Co^{2+} and Pb^{2+} are able to block the CaK channels by decreasing the opening frequency and the single-channel current amplitude.

Both the open probability (P_O) and the opening frequency vary with metal ion species and concentration in a very similar way. The Ca^{2+} dependence of CaK channels in human erythrocytes has previously been shown to be determined by an effect of internal Ca^{2+} on the opening frequency and not on open time [6]. By analogy, the various metal ions appear to regulate CaK channel opening frequency, whereas the two open time constants of the channel observed in the presence of Ca^{2+} cannot be distinguished from the two open time constants in the presence of Cd^{2+}, Co^{2+} and Pb^{2+}, irrespective of metal ion concentration. Therefore, it is concluded that the activating effects of metal ions occur through an interaction with the binding site(s) at which Ca^{2+} normally activates the erythrocyte CaK channel.

The metals differ in their potency to induce CaK channel opening. The potency of the metal ions in the activation of CaK channels in human erythrocytes follows the sequence Pb^{2+}, $Cd^{2+} > Ca^{2+} \geq Co^{2+} \gg Fe^{2+}$, Mg^{2+}. From the absence of CaK channel openings in internal solution containing 100 μM Fe^{2+} (see Fig. 3) it can be concluded that the contaminating metal ions, present at relatively high levels in this internal solution, are sufficiently buffered to prevent interfering effects. The Ca^{2+}-dependent K^+ efflux in human erythrocytes was already shown to be more sensitive to Pb^{2+} than to Ca^{2+} [8]. Pb^{2+} is also more potent than Ca^{2+} in stimulating calmodulin and protein kinase C [26,27], while Pb^{2+} as well as Cd^{2+} are equipotent to Ca^{2+} in displacing $^{45}Ca^{2+}$ binding of troponin C [28]. Contradicting results have been reported as well. In studies on molluscan neurons investigating the ability of metals to activate Ca^{2+}-dependent K^+ currents using injection methods, Ca^{2+} was more effective than Pb^{2+} and Cd^{2+} [14,15]. However, these results have to be viewed with caution, since it is difficult to relate the amount injected to the intracellular metal ion concentration. In addition, the capacity of the cytoplasm to sequester the various metal ions is unknown and the injected cations may cause release of intracellular Ca^{2+} to different extents. The BK channel from rat skeletal muscle reconstituted in lipid bilayers is activated by high concentrations of metal ions in order of effectiveness $Ca^{2+} > Cd^{2+} > Fe^{2+} > Co^{2+} \gg Pb^{2+}$, Mg^{2+} [16]. The deviations between this sequence and that found in the present study may have multiple causes, including a range of experimental conditions and differences in the sensitivity of subtypes of Ca^{2+}-activated K^+ channels to metal ions. Differences in metal ion binding sites between subtypes of Ca^{2+}-activated K^+ channels are supported by a comparison of the present results with those previously obtained on SK and BK channels in N1E-115 neuroblastoma cells under identical experimental conditions [18]. The intermediate conductance CaK channels in human erythrocytes are distinct from BK channels in their sensitivity to Cd^{2+} and from both BK and SK channels in the reduction of opening frequency and single-channel current amplitude at the higher metal ion concentrations. Therefore, Ca^{2+}-activated K^+ channels can not only be classified by single-channel conductance and using selective channel blockers, but also according to their sensitivity to various metal ions.

High concentrations of Cd^{2+}, Co^{2+} and Pb^{2+} block the erythrocyte CaK channel. The metal ions reduce the open probability by decreasing the opening frequency. The potency sequence of this effect follows the order of $Pb^{2+} > Cd^{2+}$, $Co^{2+} > Ca^{2+}$. In addition, the single-channel current amplitude decreases gradually with increasing metal ion concentration, Cd^{2+} being the more potent and Ca^{2+} an ineffective blocking metal ion. A decrease in the unitary current is generally interpreted as being caused by partially resolved closures due to blocking and unblocking events too rapid to be detected as complete channel closures. It could be argued that the reduction of CaK channel opening frequency is due to an increase in the numbers of missed events caused by rapid open channel block. However, Pb^{2+} is the more potent metal ion in reducing opening frequency, but Cd^{2+} is the more potent in reducing single-channel current amplitude. The different relative potencies of Pb^{2+} and Cd^{2+} also suggest that the two inhibitory effects are unrelated. This could be accounted for by distinct sites involved in the reduction of opening frequency and of single-channel current amplitude of the CaK channel. Firm conclusions at this point would require competition experiments.

Mg^{2+} is unable to open CaK channels in human erythrocytes by itself, as has previously been described for different types of Ca^{2+}-activated K^+ channels [10,15,16]. However, in the presence of Ca^{2+}, millimolar concentrations of Mg^{2+} enhance the activity of BK channels reconstituted from rat skeletal muscle [16,17]. The present results show that up to 100 μM Mg^{2+} is unable to mimic the effect of Ca^{2+} on CaK channels of human erythrocytes and additionally demonstrate that Mg^{2+} does not enhance, but slightly inhibits activation of this CaK channel by Ca^{2+}. This would be consistent with the previous finding that millimolar concentrations of Mg^{2+} inhibit Ca^{2+}-induced CaK channel activity in human erythrocytes [10]. However, the latter effect has been obtained at a holding potential of -100 mV and possible voltage dependence of CaK channel block has not been investigated.

Fe^{2+}, which is unable to open CaK channels in human erythrocytes by itself, has a remarkable effect on the CaK channel activated by Ca^{2+}. Fe^{2+} reduces the opening frequency of CaK channels activated by Ca^{2+} and enhances the two open time constants. This suggests that a modulatory site for Fe^{2+} exists on the human erythrocyte CaK channel. It remains to be seen whether other metals interact with this modulatory site.

Effects of metals on Ca^{2+}-binding proteins and ion channels are presumed to be related to specific chemical and physical characteristics of the ions as ionic radius, ion polarizability, electronic structure, and hard-soft characteristics [29]. The ability of the metals to activate Ca^{2+}-binding proteins is related to the ionic radius which should be in the range of 1 ± 0.2 Å [16,26,28]. However, in human erythrocytes Co^{2+} and Pb^{2+} with ionic radii of 0.72 Å and 1.20 Å, respectively, are able to activate CaK channels, whereas Fe^{2+} (0.74 Å) is ineffective in this respect. The difference in reducing the opening frequency and the single-channel amplitude by Ca^{2+} and Cd^{2+} in erythrocytes, despite their similarity in size (0.99 and 0.97 Å, respectively), also suggests that the ability of divalent ions to substitute for Ca^{2+} in K^+ channel activation does not simply depend on the ionic radius. More general, the interaction of metal ions with CaK channels cannot be accounted for on the basis of single chemical and physical parameters and different combinations of these parameters may be involved in the interaction with distinct metal ion binding sites on subtypes of Ca^{2+}-activated K^+ channels.

The cations Co^{2+}, Fe^{2+} and Mg^{2+} are essential metals and serve physiological functions in human tissue. Cd^{2+} and Pb^{2+} are xenobiotics and represent serious environmental pollutants. They accumulate in various human tissues, causing neurological, muscular, renal, hematological and bronchial disorders [30]. The finding that Cd^{2+}, Co^{2+} and Pb^{2+} effectively substitute for Ca^{2+} in Ca^{2+}-activated K^+ channel activation is yet another way for these metals to interfere with the Ca^{2+} homeostasis.

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