Cadmium as a Tool for Studying Calcium-dependent Cation Permeability of the Human Red Blood Cell Membrane

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Abstract. Three Ca^{2+}-dependent procedures known to increase cation permeability of red blood cell membranes were tested with Cd^{2+} ions which equal Ca^{2+} ions both in their charge and the crystal radius,

1. Increase of non-selective permeability for monovalent cations by incubating the red cells in a Ca^{2+}-free sucrose medium. Addition of Cd^{2+} to the suspension of leaky cells failed to restore the initial impermeability of the red cell membrane while a repairing effect of Ca^{2+} was evident both in the presence and absence of Cd^{2+}. Thus, in low electrolyte medium, Cd^{2+} could neither mimic Ca^{2+}, nor prevent the latter from interacting with membrane structures which control cation permeability.

2. Increase of the K^+-selective permeability by propranolol plus Ca^{2+}. Cd^{2+} added to a Ca^{2+}-free Ringer type medium containing propranolol enhanced K^+ permeability similar to that obtained with Ca^{2+}. No changes of membrane permeability could be detected in the presence of 0.5 mmol/l Cd^{2+} in absence of propranolol. The Cd^{2+}-stimulated K^+ channels were different from those induced by Ca^{2+}. They proved to be insensitive to quinine, exhibited a low K^+/Na^+ selectivity, and showed no tendency to self-inactivation.

3. Stimulation of K^+ permeability by electron donors plus Ca^{2+}. Substitution of Ca^{2+} by Cd^{2+} yielded results similar to those obtained with propranolol.

The ability of Cd^{2+} to overtake the role of Ca^{2+} appears to depend on the system studied. It supplies information allowing to distinguish between the diverse Ca^{2+}-dependent systems in cell membranes.

Key words: Red blood cells — Cation transport — Calcium — Cadmium — Propranolol — Redox reactions — Membrane permeability

Introduction

Being equal to Ca^{2+} in the crystal radius, Cd^{2+} can be used as a probe in Ca^{2+}-dependent processes. Compared to Ca^{2+}, Cd^{2+} as a rule forms more stable complexes with biological ligands, especially with sulfhydryl groups (Aylett 1979; Webb 1979). The acute toxicity of Cd^{2+} can be ascribed to Cd^{2+} displacing Ca^{2+} from Ca^{2+}-binding sites (Järvisalo et al. 1980; Verbost et al. 1988). This process can further be characterized with respect to Cd^{2+}/Ca^{2+} selectivity.

The aim of the present work was to study the ability of Cd^{2+} to substitute Ca^{2+} in modulating cation permeability of the human red blood cells incubated in three different media :(*i*) Ca^{2+}-free sucrose isotonic medium; (Bolingbroke and Maizels 1959; Skulskii and Manninen 1984); (*ii*) Ca^{2+}-free Ringer type medium plus propranolol (Manninen 1970, Manninen and Skulskii 1981); (*iii*) medium (*ii*) without propranolol plus electron donors (Garcia-Sancho et al. 1979; Skulskii and Manninen 1984). Cd^{2+} failed to play the role of Ca^{2+} in preventing the loss of K^+ (^{86}Rb) from red blood cells in low electrolyte medium. On the other hand, Ca^{2+} and Cd^{2+} were both effective, in stimulating ^{86}Rb efflux induced by propranolol or electron donors, though in some respect Cd^{2+}-stimulated efflux was different from that stimulated by Ca^{2+}.

Methods

One ml of human heparinized blood was centrifuged at 3000 × *g* for 10 min at room temperature, and the plasma and the buffy coat were removed. The red blood cells were washed twice with 6% sucrose solution, respuspended in the same medium at about 2% hematocrit, and incubated for 2 h at 37 °C, pH approx. 6,5. After the incubation, the red blood cells were highly permeable to all monovalent cations. The cells were then separated by centrifugation, resuspended in a medium containing 140 mmol/l KCl and 10 mmol/l Tris-Cl, pH 7.5, and incubated for 1 h at 37 °C at 5% hematocrit. At this stage, 80–90 mmol KCl per liter accumulated in the red blood cells. The K^+-loaded cells were concentrated by centrifugation to about 50% hematocrit and, after the addition of ^{86}Rb (5.10^6 cpm/ml), incubated at 4 °C for additional 12–16 h. The labelled cells were washed 3 times with Tris-buffered 140 mmol/l KCl at 0–4 °C and diluted with the same medium to 1% hematocrit at 37 °C to initiate the efflux of ^{86}Rb, which behaves similarly to K^+. Either Ca^{2+} or Cd^{2+} were added to the diluting medium to compare the effect of these cations on the tracer efflux rate. Aliquots of the cell suspension were taken at defined intervals and immediately cooled by mixing with a 10-fold volume of ice-cold isotonic $MgCl_2$ solution. The samples were centrifuged and washed in the counting tubes with the same medium. The loss of tracer during centrifugation and washing was negligible.

Propranolol treatment. These experiments were carried out with fresh human red blood cells loaded with ^{86}Rb or ^{22}Na for 12–16 h at 4 °C, the tracers being added to the whole blood. The double labelled cells were then washed with 0.9% NaCl and suspended at 1% hematocrit in a medium of the following composition (mmol/l): NaCl 135, KCl 5, Tris-HCl 10, pH 7.4, propranolol 1. The tracer efflux rates were measured as described above.

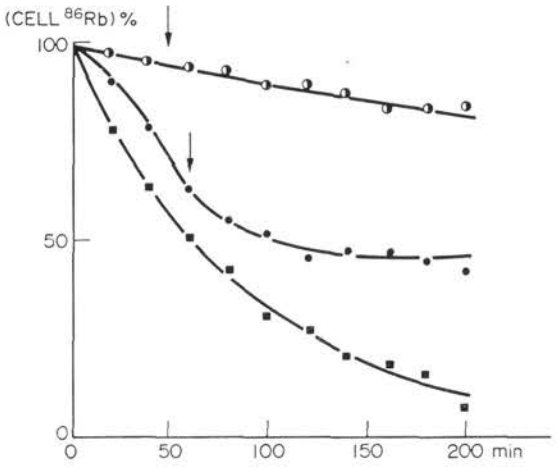

Figure 1. Effects of external Ca^{2+} and Cd^{2+} on ^{86}Rb efflux from sucrose-treated red blood cells into KCl isotonic medium containing (in mmol.l^{-1}): ◑ Ca^{2+} 2.5 (added at zero time), Cd^{2+} 0.5 (indicated by the arrow); ● Cd^{2+} 0.5 (added at zero time), Ca^{2+} 2.5 (indicated by the arrow); ■ no additions.

Electron donor treatment. Ten mmol/l sodium ascorbate plus 0.1 mmol/l phenazine methosulfate were used as the electron donor system. In all other aspects the tracer efflux experiments were similar to those performed with the propranolol treated cells.

^{86}Rb and ^{22}Na were supplied from Amersham (U.K.). Other chemicals were purchased from Sigma (U.S.A.) or from E. Merk (F.R.G.).

Results

Figures 1–8 show the results of the individual experiments. Basically similar results were obtained from 3–4 repetitions. In all cases the effects of Ca^{2+} and Cd^{2+} were compared with the same blood samples.

Sucrose-treated red blood cells. The aim of this series of experiments was to compare Cd^{2+} and Ca^{2+} in their ability to inhibit enhanced cation permeability of red blood cells incubated in a sucrose medium. The efflux of ^{86}Rb into 140 mmol/l KCl solution buffered with 10 mmol/l Tris-HCl, pH 7.4, was measured. High concentrations of external K^+ blocked the development of the K^+ diffusion membrane potential affecting the tracer efflux rate (Skulskii and Manninen 1981). Fig. 1 shows that the efflux of ^{86}Rb from the sucrose treated red cells into KCl isotonic medium, both Ca^{2+} and Cd^{2+}—free is rather rapid. The addition of 0.5 mmol/l Cd^{2+} at the very beginning of the efflux experiment did not substantially affect either the efflux rate of ^{86}Rb or the inhibiting action of Ca^{2+}, added later to the same medium. At

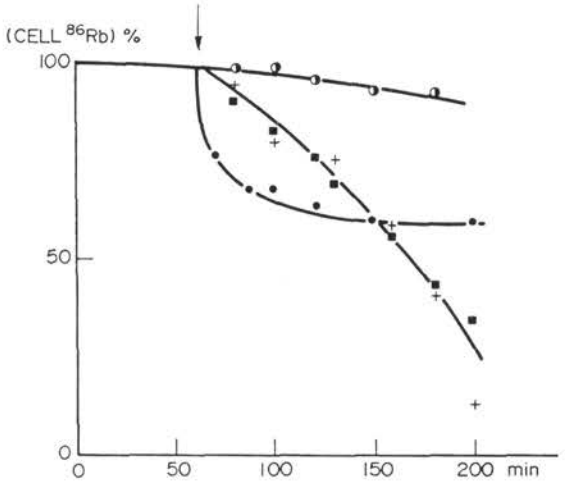

Figure 2. Effects of propranolol plus Ca^{2+}, and propranolol plus Cd^{2+} on ^{86}Rb efflux from red cells incubated in NaCl isotonic medium (see Methods) supplemented with (mmol/l): ◐ propranolol 1 at zero time, Ca^{2+} 2.5 as indicated by the arrow; ● Ca^{2+} 2.5 at zero time, propranolol 1 as indicated by the arrow; ■ propranolol 1 at zero time, Cd^{2+} 0.5 as indicated by the arrow; + Cd^{2+} 0.5 at zero time, propranolol 1 as indicated by the arrow.

higher Cd^{2+} concentrations hemolysis occurred. With Ca^{2+} added to the incubation medium at zero time the efflux rate of ^{86}Rb was nearly as slow as that in intact cells. The addition of 0.5 mmol/l Cd^{2+} to the Ca^{2+}-containing medium failed to raise the ^{86}Rb efflux. In an attempt to yield an effect, the ^{86}Rb-loaded cells were incubated with 0.5 mmol/l Cd^{2+} at 37 °C for 1 h at 50% PCV prior to diluting the suspension for the efflux experiment. In spite of a prolonged exposure to Cd^{2+} the results were similar. No evidence of Cd^{2+}-Ca^{2+} competition was observed.

Propranolol-treated red blood cells. Propranolol induced the formation of K^+-selective channels in human red blood cell membrane (Manninen 1970). The effect has been attributed to a propranolol-stimulated increase in the concentration of intracellular Ca^{2+} (Blum and Hoffman 1972; Szasz et al. 1977; Passow 1980; Simons 1980). This process requires external K^+ (Heinz and Passow 1980) and can be inhibited by quinine (Garcia-Sancho et al. 1979; Reichstein and Rothstein 1981; Alvarez et al. 1984).

The effects of propranolol added to the red cell suspension containing either Ca^{2+} or Cd^{2+} are compared in Fig. 2. Both cations proved to be effective. The Cd^{2+}-dependent cation leakage showed no tendency to self-inhibition typical of the ^{86}Rb efflux induced by propranolol plus Ca^{2+}. The effect of Cd^{2+} plus propranolol

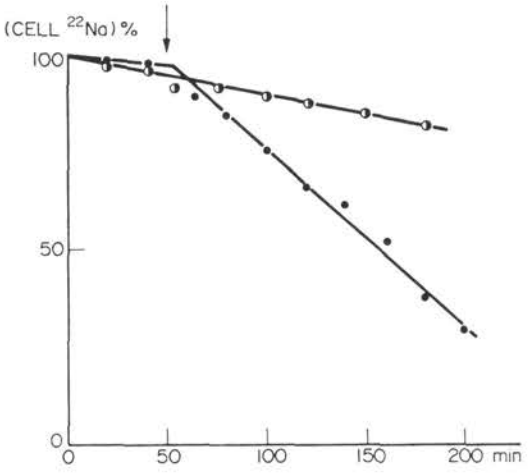

Figure 3. Effects of propranolol plus Ca^{2+}, or propranolol plus Cd^{2+}, on ^{22}Na efflux from red cells incubated in NaCl isotonic medium containing (mmol.l^{-1}): ◑ Ca^{2+} 2.5 or ● Cd^{2+} 0.5, 1 mmol.l^{-1} propranol was added, indicated by the arrow. Ouabain 1.10^{-4} mmol.l^{-1}.

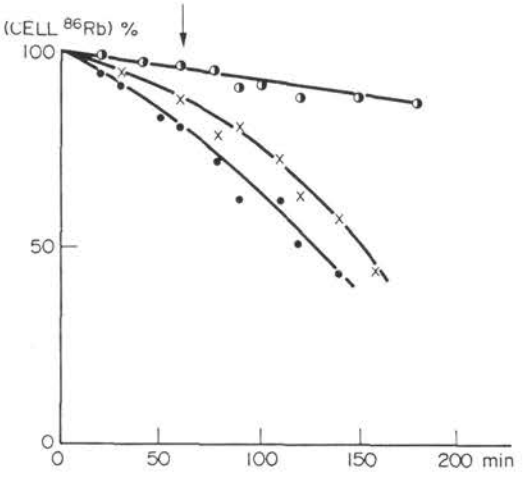

Figure 4. Effect of quinine on the propranolol-induced efflux of ^{86}Rb from red cells incubated in NaCl isotonic medium containing (mmol.l^{-1}): ◑ quinine 1, Ca^{2+} 2.5, K^{+}5. ● quinine 1, Cd^{2+} 0.5, K^{+}5. x quinine 1, Cd^{2+} 0.5, K^{+} 0.1 mmol.l^{-1} propranolol was added, indicated by the arrow.

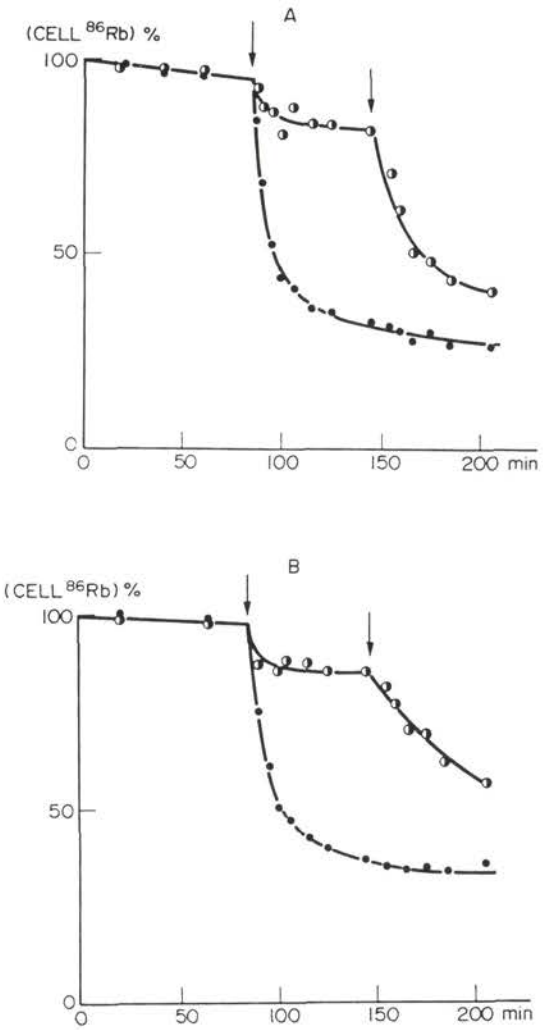

Figure 5. Effects of Ca^{2+} (A) and Cd^{2+} (B) on the ^{86}Rb efflux induced in red cells by electron donors (ascorbate plus phenazine methosulfate) added (first arrow) 85 min after preincubation of the cells in NaCl isotonic medium containing (mmol.l^{-1}): ● Ca^{2+} (A), or Cd^{2+} 0.5 (B) or ◑ both Ca^{2+} and Cd^{2+}-free (A and B). In the latter case 2.5 mmol.l^{-1} Ca^{2+} or 0.5 mmol.l^{-1} Cd^{2+} were added (second arrow) 67 min after addition of the electron donors.

was independent of the sequence of their addition, whereas Ca^{2+}, when added after propranolol, was ineffective (Fig. 2). The Cd^{2+}-dependent channels were highly permeable to both ^{86}Rb and ^{22}Na (Fig. 3). Quinine failed to decrease the

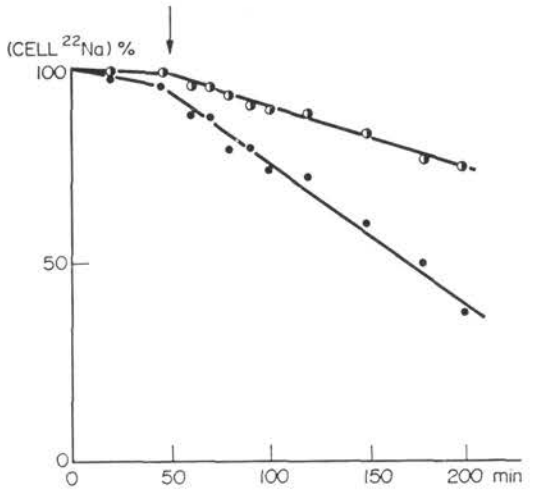

Figure 6. Effects of electron donors (added as indicated by the arrow) on the ^{22}Na efflux from red cells into NaCl isotonic medium containing 1.10^{-4} ouabain and either 2.5 mmol.l^{-1} Ca^{2+} (◑) or 0.5 mmol.l^{-1} Cd^{2+} (●).

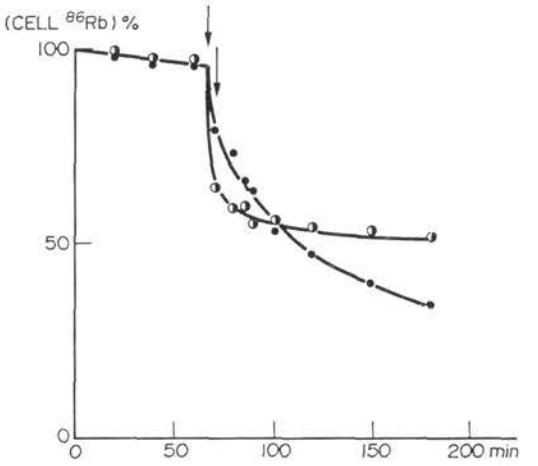

Figure 7. Effects of propranolol on the ^{86}Rb efflux induced by electron donors in red cells preincubated either with 2.5 mmol.l^{-1} Ca^{2+} (◑) or 0.5 mmol.l^{-1} Cd^{2+} (●). Propranolol (second arrow) was added to both media 6 min after addition of the electron donors (first arrow).

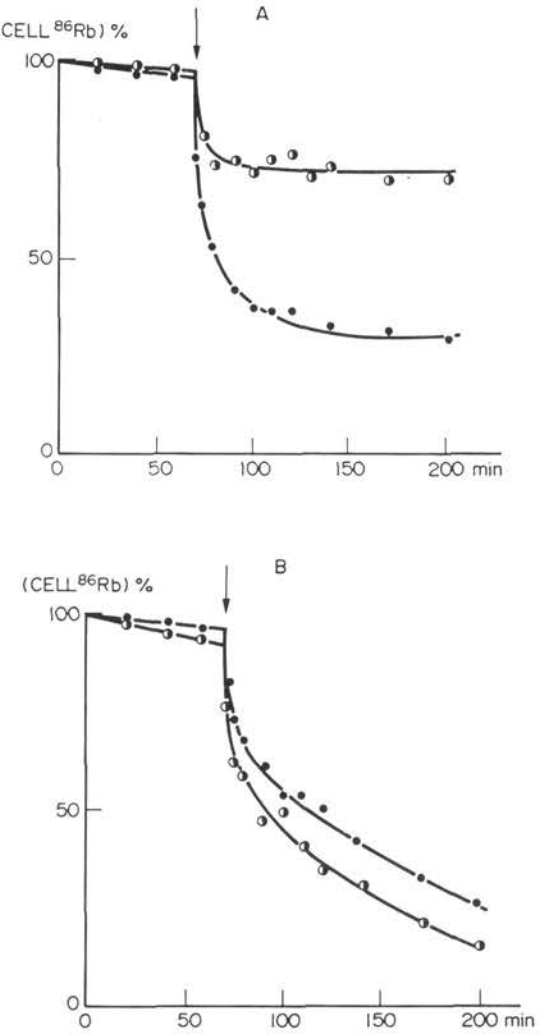

Figure 8. Effect of quinine on the ^{86}Rb efflux induced by electron donors in red cells preincubated in NaCl isotonic medium containing (mmol.l^{-1}): ◑ 1 quinine plus either 2.5 Ca^{2+} (A) or 0.5 Cd^{2+} (B): ^{86}Rb ● no quinine, 2.5 Ca^{2+} (A) or 0.5 Cd^{2+} (B). Electron donors were added as indicated by the arrows.

^{86}Rb efflux induced by propranolol plus Cd^{2+} (Fig. 4). The Cd^{2+}-mediated effect of propranolol did not require external K^+ (Fig. 4).

Red cells treated with electron donors (ascorbate + phenazine methosulfate). The permeability of the red cell membrane for K^+ can be specifically raised by various

reducing agents which also require Ca^{2+} for their action to develop (Garcia-Sancho et al. 1979; Skulskii and Manninen 1984). Fig. 5 compares the effects of Ca^{2+} and Cd^{2+} on the K^+ (^{86}Rb) permeability of red cells in the presence of electron donors. Qualitatively, the effects of Cd^{2+} were similar to those of Ca^{2+}. In both cases the ^{86}Rb efflux was greatly increased, the sequence of addition of the inducing agents being of no influence. The red cells treated with the electron donors maintained their responsiveness to both Ca^{2+} and Cd^{2+} over long periods. By analogy with the channels induced by propranolol plus Cd^{2+} (Fig. 3), the membrane pathways opened with the electron donors plus Cd^{2+} (Fig. 6) were accessible to both K^+ and Na^+, whereas in the presence of Ca^{2+} the electron donors formed K^+-selective channels.

Earlier studies (Skulskii and Manninen 1984) showed that ^{86}Rb efflux induced by electron donors plus Ca^{2+} can be inhibited by propranolol. Fig. 7 shows that this is not the case with the Cd^{2+}-dependent channels, where propranolol has no effect. Preincubation of red cells with quinine substantially decreases the efflux of ^{86}Rb induced by electron donors plus Ca^{2+} (Fig. 8*A*), whereas no inhibition could be detected with Cd^{2+}-dependent cation channels (Fig. 8*B*).

Discussion

Studies using three model systems showed that Cd^{2+} as a probe could distinguish between two kinds of the Ca^{2+}-dependent membrane sites controlling the monovalent cation permeability of the red cell membrane. The behaviors of Cd^{2+} and Ca^{2+} were quite different in the sucrose treated red cells, requiring Ca^{2+} to either maintain or restore the initially low cation permeability of the red cell membrane.

In this case, Cd^{2+} could not inhibit the efflux of ^{86}Rb which was drastically increased in a Ca^{2+}-free sucrose medium, nor could these ions accelerate the efflux by displacing Ca^{2+} from its highly specific membrane sites. Accordingly, Ca^{2+} inhibits the efflux of ^{86}Rb despite the presence of Cd^{2+} at the Ca^{2+}-binding sites suggesting that the closeness of the crystal sizes of Ca^{2+} and Cd^{2+} is not the only basis for these cations being selected by the membrane structures exposed to a low electrolyte medium. Likely, some other factors are also involved (hydration energy, ligand affinity etc.).

A partial similarity between Ca^{2+} and Cd^{2+} could be demonstrated while studying their combined effects with propranolol or electron donors on the cation permeability of red cells (Fig. 2). Both Ca^{2+} and Cd^{2+} proved to be effective in stimulating the efflux of monovalent cations into Ringer type medium.

The effect of Cd^{2+} does not seem to be due to nonspecific interactions of the cation with various membrane components. The incubation of red cells for 1–2 h in a medium containing 0.5 mmol/l Cd^{2+} caused no significant changes in the permeability of the red cell membrane for ^{86}Rb (Fig. 1) (Kunimoto and

Miura 1986). The stimulating effect of Cd^{2+} was observed only in the presence of propranolol or electron donors. Thus, Cd^{2+}, unlike Ca^{2+}, could not act to protect the low cation permeability in low-electrolyte media; however, it was as effective as Ca^{2+} promoting cation leakage from red cells into Ringer medium.

Apart from this apparent similarity, the Cd^{2+}-dependent propranolol-induced cation channels differ in many respects from those formed in the presence of Ca^{2+} plus propranolol.

In the latter case, leakage of ^{86}Rb induced by Cd^{2+} exhibited no tendency to self-inactivation typical of the Ca^{2+}-mediated effect of propranolol (Fig. 2) (Szasz et al. 1977; Skulskii and Manninen 1981; 1984). This inactivation could be explained by the accumulation of propranolol in the red cells (Manninen 1970), followed by the replacement of Ca^{2+} at the internal membrane sites which control K^+ permeability (Szasz et al. 1977). It seems reasonable to suggest that the affinity of Cd^{2+} for these sites is much higher than that of Ca^{2+}, since the former can interact with a variety of biological ligands of the membrane, preferentially SH-groups (Grinstein and Rothstein 1978; Sze and Solomon 1979). Propranolol appears to be unable to displace tightly bound Cd^{2+}, which can penetrate through the red cell membrane (Nguen and Chien 1989) keeping the cation channels open for prolonger periods of time. The next important difference between the Ca^{2+}- and Cd^{2+}-induced channels is that the latters are not K^+ specific, being permeable to both K^+ and Na^+ (Fig. 3). The characteristics of the induced cation channels may depend on minor changes in membrane structure (Sze and Solomon 1979) and as well as on the mode of interaction of Ca^{2+} or Cd^{2+} with functionally important groups which control cation permeability. (Romero and Whittam 1971; Lew and Ferreira 1976).

The Ca^{2+}- and Cd^{2+}-induced propranolol-dependent channels are further distinguished from each other by their responsiveness to activating cations. When added after propranolol, Ca^{2+} is ineffective, whereas the effect of Cd^{2+} is independent of the sequence of its addition (Fig. 2). It has also been established that cation permeability induced by Cd^{2+} plus propranolol could not be inhibited by quinine, presumably because the inhibitory effects of biogenic amines is a result of the replacement of K^+ at the external membrane sites (Heinz and Passow 1980; Reichstein and Rothstein 1981); this appears to be important for the action of Ca^{2+}, but not for Cd^{2+} (Fig. 4). Reducing agents have been suggested to raise the affinity of the internal receptor to Ca^{2+}, thus decreasing its threshold stimulating concentration (Garcia-Sancho et al. 1979). It should be noted that the interaction of Ca^{2+} with a proposed reduced component of the red cell membrane resembles the behavior of Cd^{2+} in that the effect of Ca^{2+} does not depend on the sequence of the addition of Ca^{2+} and reducing agents (Fig. 5), and in that the K^+ efflux stimulated by Ca^{2+} and electron donors is dependent to a lesser degree on external K^+ than it is the case in the channels stimulated by propranolol plus Ca^{2+}. Otherwise, the results obtained with Cd^{2+} plus electron donors were similar to those

obtained with Cd^{2+} plus propranolol. For instance, the cation channels induced by Cd^{2+} in the presence of ascorbate plus phenazine methosulfate were fairly permeable for Na^+ (Fig. 6) and resistant to quinine (Fig. 8). Propranolol inhibited the Ca^{2+}-dependent cation efflux stimulated by electron donors but failed to block the efflux activated by Cd^{2+} (Fig. 7) (Szasz et al. 1979).

A protein possessing functionally important sulfhydryl groups has been suggested to be an essential component of the induced cation channels (Grinstein and Rothstein 1978; Sze and Solomon 1979). Sterically, in the unhydrated form both Ca^{2+} and Cd^{2+} seem to be capable of occupying common sites somewhere in hydrophobic domains of the proposed channel protein, but the hydrophobic domains in the action of Ca^{2+} and Cd^{2+} would depend on the nature of the ligands involved in the cation-protein interaction.

Acknowledgements. This study was supported by the Finnish Society of Sciences and Letters, and by the Wihuri Foundation in Helsinki (grant awarded to Dr. I. A. Skulskii).

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Final version accepted September 20, 1991