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Modulation of the Ca^{2+}- or Pb^{2+}-activated K^+-selective channels in human red cells. I. Effects of propranolol *

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To study the effect of propranolol on the Ca^{2+}- or Pb^{2+}-activated K^+ permeability in human erythrocytes, K^+ effluxes were compared with single-channel currents. The results demonstrate that propranolol has a twofold effect: (1) it renders the channel protein more sensitive to Ca^{2+} or Pb^{2+}; and (2) it simultaneously inhibits channel activity and slightly reduces single-channel conductance. The number of active channels is not affected.

Introduction

The adrenergic β-receptor antagonist propranolol specifically increases K^+ permeability in human red cells. As a consequence, red cells respond in a physiological environment with a pronounced net efflux of K^+ [1]. This effect on the K^+-selective membrane permeability is distinct from the interference of propranolol with β-receptors which are not present in human erythrocytes. It was suggested [2–4] that in the human red cells propranolol causes an elevation of the concentration of intracellular free Ca^{2+} by release of membrane-bound Ca^{2+}. This in turn would stimulate the Ca^{2+}-activated K^+ permeability (Gardos phenomenon [5]).

In addition to activation, propranolol can cause inhibition of the K^+ efflux if applied in the presence of a maximally activating Ca^{2+} concentration [3]. Skulskii and Manninen [6] also demonstrated an inhibitory effect after activation of the Ca^{2+}-dependent K^+ permeability by the electron donors ascorbate plus phenazine methosulfate.

It has been demonstrated that the Gardos phenomenon is mediated by Ca^{2+}-gated K^+-selective pores which can be detected in voltage-clamped membrane patches [7–10]. In this contribution, we analysed effects of propranolol on the single Ca^{2+}-activated K^+ channel by the patch-clamp technique and compared the results with flux measurements that were performed in parallel. Part of these results have been reported previously [11].

Methods

Flux measurements

Measurements of net fluxes of Na^+ and K^+ across the erythrocyte membrane were performed on red cells suspended in 150 mM $NaNO_3$ (suprapur Merck, Darmstadt), 1 mM KNO_3 and 10 mM Hepes buffered with Tris to pH 7.6 (hematocrit 0.5%). The NO_3^- medium was used in order to avoid limitations of high K^+ fluxes by the less permeable Cl^- anions [12]. Before the flux measurements, traces of Ca^{2+} were removed by washing the cells twice in 150 mM $NaNO_3$, 1 mM EDTA and 20 mM Hepes, and twice in the suprapur solution (Ca^{2+} at a concentration below 0.2 μM). The K^+ permeability was activated by adding 0.5 μM of the Ca^{2+} ionophore A23187 plus a defined activity of Ca^{2+} or 20 μM Pb^{2+} to the bath medium. At various times after the K^+ permeability was elicited samples of the cell suspension were added to an ice-cold solution of 113 mM $MgCl_2$ [12]. After hemolysing the cells by addition of 0.01% lithium and by ultrasound, the cell contents of sodium and potassium were determined by flame photometry and expressed per kg hemoglobin; the hemoglobin was measured at the isobestic point for oxy- and methemoglobin at 527 nm [13]. All experiments were performed at 37 °C.

* Dedicated to Prof. Dr. K.J. Netter on the occasion of his 60th birthday.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

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Measurements of single-channel currents

The activity of single Ca^{2+}-activated K^+-selective channels was measured in excised inside-out membrane patches (for details see Ref. 14). The single-channel events were recorded at a constant hold potential of −80 mV. The pipette solution contained 70 mM NaCl, 70 mM KCl, 1 mM $MgCl_2$ and was adjusted to pH 7.4 by 5 mM Mops; the bath solution contained instead of the NaCl additional 80 mM KCl and defined activities of Ca^{2+} (buffered with 1 mM EGTA). The single-channel events of the K^+ channels were not effected if NO_3^- were used instead of Cl^- as anions. The concentrations of free Ca^{2+} was calculated according to the Hagiwara and Nakajima [15]. The experiments were performed at about 20 °C.

Results

Flux measurements

In flux experiments, the Gardos phenomenon can be induced by artificial elevation of the intracellular concentration of free Ca^{2+}; this can be achieved by metabolic depletion and, hence, inhibition of the Ca^{2+} pump [5], or by adding a Ca^{2+} ionophore to a bath medium containing micromolar concentrations of free Ca^{2+} [16]. If the Ca^{2+} ionophore A23187 is added to 'suprapur' medium (containing less than 0.2 μM free Ca^{2+}), no loss of K^+ can be induced. If, however, the bath medium contains in addition 1 mM propranolol, application of the ionophore elicits, even in the suprapur medium, a small but significant specific loss of K^+ ($6 \pm 1\%$ ($n = 3$), see Fig. 1A and B). The Na^+ content does not change even in the presence of propranolol. Fig. 1B shows how K^+ loss in the presence of 1 mM propranolol depends on the concentration of free Ca^{2+} in the bath medium. Potassium loss can be detected at Ca^{2+} concentrations below 0.5 μM in the bath medium. A curious finding is that net re-uptake of K^+ occurs about 1 min after the

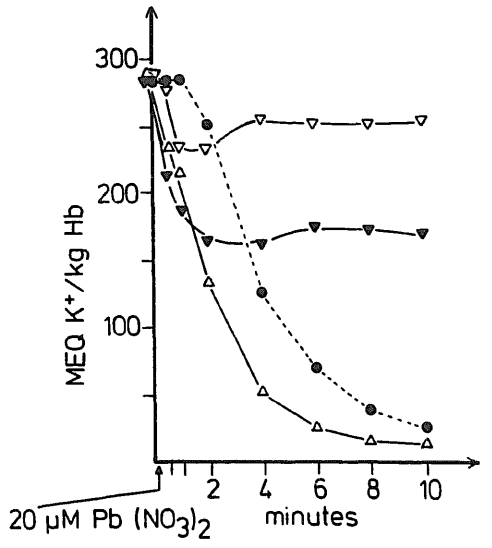

Fig. 2. Effect of different propranolol concentrations (as indicated) on K^+ content as a function of time. Activation of the K^+ permeability is achieved by addition of Pb^{2+}. We omitted values for Na^+ because there was no change in Na^+ content detectable. ●, control; △, 10 μM propranolol; ▼, 0.5 mM propranolol; ▽, 1.0 mM propranolol. MEQ, milliequivalents.

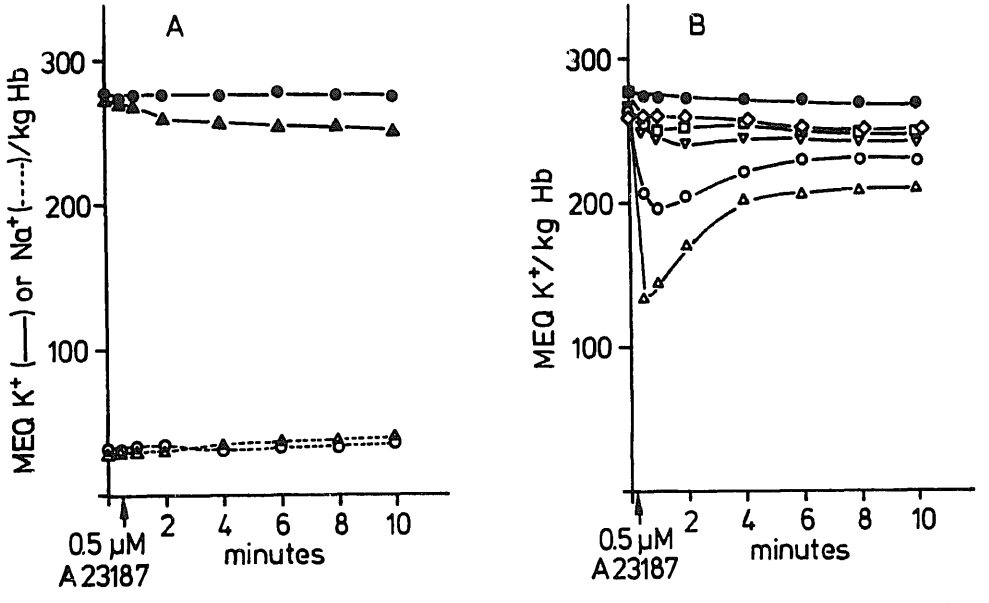

Fig. 1. Effect of 1 mM propranolol on Na^+ and K^+ content of red cells as a function of time. The Gardos effect is elicited by addition of the Ca^{2+} ionophore A23187 (see arrows). (A) Changes of K^+ content (closed symbols) and of Na^+ content (open symbols) in suprapur (Ca^{2+}-free) solution. Circles refer to measurement without and triangles to measurements with propranolol in the bath medium. (B) Changes of K^+ content with 1 mM propranolol in the bath medium for different concentrations of free Ca^{2+} in the medium; diamonds, 0.1 μM; squares, 0.5 μM; downard-pointing triangles, 1.0 μM; open circles, 1.5 μm; upward-pointing triangles, 10 μM. Closed circles refer to measurements without Ca^{2+} and without propranolol. We omitted values for Na^+ in the figure because there was no change in Na^+ content detectable. MEQ, milliequivalents.

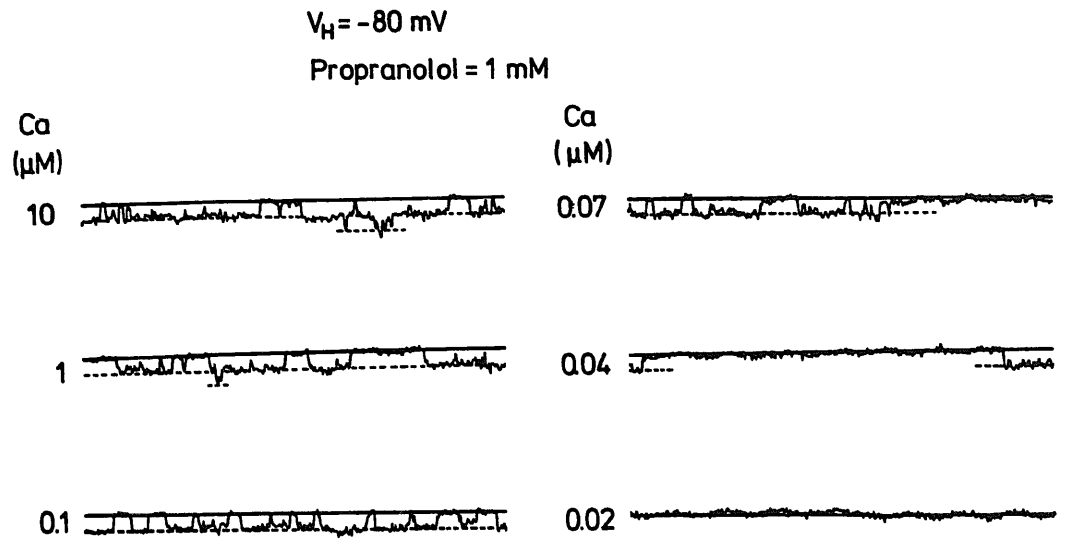

Fig. 3. Single-channel recordings at 1 mM propranolol in the bath solution for different concentrations of free Ca^{2+} at the internal membrane surface.

K^+ permeability has been stimulated. It could be demonstrated that this re-uptake of K^+ in the presence of 1 mM propranolol was accompanied by an additional uptake of $^{35}SO_4^{2-}$ (17% more than in control cells without propranolol, not shown). This suggests a propranolol-induced shift in the Donnan distribution that would be large enough to act as a driving force for the re-uptake of the K^+.

Like Ca^{2+}, micromolar concentrations of Pb^{2+} also activate the K^+-selective channels; concentrations of Pb^{2+} exceeding 20 μM, on the other hand, produce inhibition [17]. Fig. 2 shows the effect of various propranolol concentrations on Pb^{2+}-activated K^+ channels. If the Pb^{2+} concentration is slightly below the maximally activating concentration, addition of low concentrations of propranolol (10 μM) has a stimulating effect on the K^+ loss; higher concentrations of propranolol, on the other hand, result in inhibition. The same observation can be made with Ca^{2+} and the ionophore A23187 as activator of channel openings (not shown). This is in accordance with previous observations by Porzig [3] and Szasz et al. [4].

The stimulating effect of propranolol has been attributed to an elevation of intracellular free Ca^{2+} [3,4]. In the flux measurements on cell suspensions, one cannot distinguish whether the effect of propranolol is indeed due to an uncontrolled modulation of the free Ca^{2+}, to a modulation of the channel number or of the sensitivity of the channel protein to Ca^{2+}. To investigate this question, we performed voltage-clamp experiments on excised membrane patches of single red cells. This technique with inside-out membrane patches allowed us to buffer the Ca^{2+} activity at the internal membrane surface, and modulation of characteristics of the single-channel protein could then be investigated.

Single-channel measurements

Concentrations of free Ca^{2+} exceeding 0.5 μM elicit openings of the K^+-selective channels in cell-free membrane patches of human red cells [9]. Fig. 3 dem-

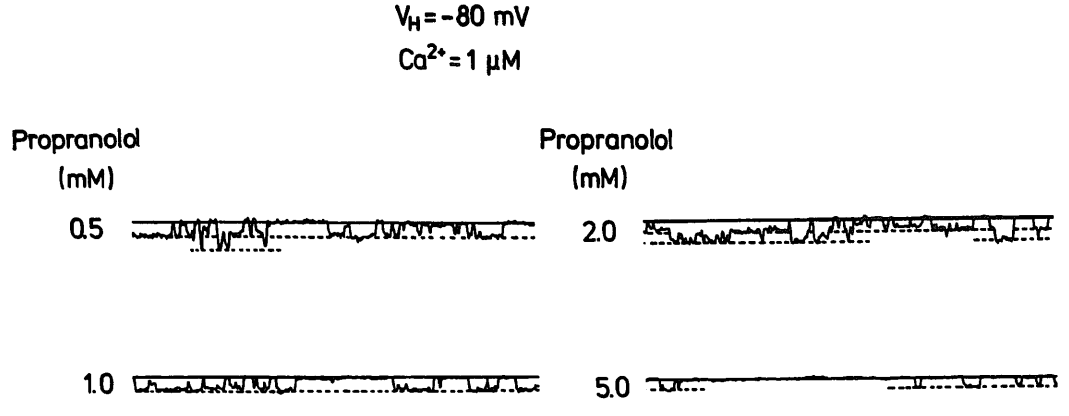

Fig. 4. Single-channel recordings at 1 μM free Ca^{2+} for different concentrations of propranolol in the bath medium.

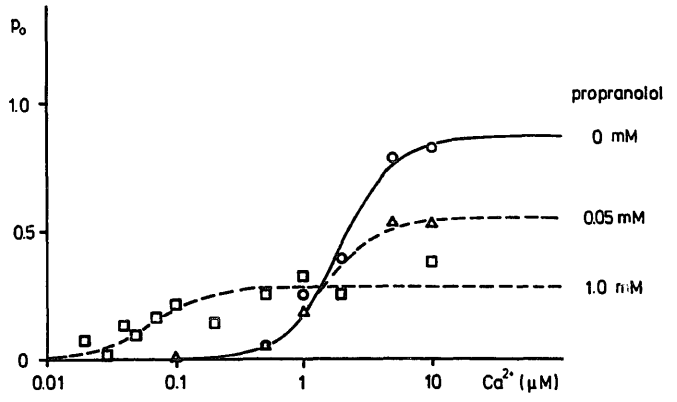

Fig. 5. Dependence of the probability of the open-channel state on the concentration of free Ca^{2+} at the internal membrane surface for different propranolol concentrations. The lines represent least-squares fits to the data of the following equation:

$$p = p_m \frac{[Ca^{2+}]^2}{K_m + [Ca^{2+}]^2}$$

The parameters fitted to the above equation are: For 0 propranolol: $p_m = 0.87 \pm 0.05$; $K_m = 3.83 \pm 0.97$. For 50 μM propranolol: $p_m = 0.54 \pm 0.04$; $K_m = 2.08 \pm 0.12$. Finally, for 1 mM propranolol: $p_m = 0.28 \pm 0.02$; $K_m = 0.04 \pm 0.02$.

onstrates that in the presence of 1 mM propranolol in the bath medium single-channel activity can be detected even at 0.04 μM Ca^{2+}, a concentration one order of magnitude lower than is necessary for activation without propranolol. These concentrations of free Ca^{2+} are in direct contact with the internal membrane surface, and clearly indicate an increased Ca^{2+} sensitivity of the single-channel protein.

As already demonstrated in the flux experiments, application of propranolol has a twofold effect on the Ca^{2+}-activated K^+ permeability: stimulation at low and inhibition at high concentrations. This can also be shown in patch-clamp experiments. In addition to its stimulating effect on the Ca^{2+} sensitivity, higher concentrations of propranolol cause inhibition of channel activity (Fig. 4). At 5 mM propranolol, channel openings become a very rare event. A more detailed analysis of the probability of finding a channel in the conducting state in comparison to control measurements is shown in Fig. 5. This presentation demonstrates that propranolol shifts the sensitivity for Ca^{2+} to lower concentrations, but simultaneously reduces the probability of channel openings. The inhibitory effect is amplified slightly by a reduction in single-channel conductance (compare the traces at the lowest and highest propranolol concentrations in Fig. 4).

Discussion

Our experiments confirm the observations of Porzig [3] and Szasz et al. [4] that concentrations of propranolol below 1 mM increase the Ca^{2+}-activated K^+ permeability, but that higher concentrations have an inhibitory effect. The patch-clamp measurements with cell-free inside-out membrane patches clearly demonstrate that propranolol has a direct effect on the single-channel activity by increasing the sensitivity for Ca^{2+}. This conclusion can be drawn, since in the patch-clamp experiments the concentration of free Ca^{2+} at the internal membrane surface is buffered by EGTA. A release of membrane-bound Ca^{2+}, as suggested by Porzig [3], may supplement stimulation of K^+ loss from red cells in suspension.

Stimulation of Ca^{2+}-dependent K^+ permeability in vascular smooth muscle has also been reported for the anti-hypertensive benzopyran derivative BRL 34915 [18]. This raises the question whether the stimulation of K^+ permeability by these substances can be related to their action as adrenergic β-receptor antagonists. In the red cells, we observed no effect of BRL 34915 (kindly provided to us by Drs. Englert and Lang from Hoechst AG (Pharma Synthese)) on K^+ channel gating (Schwarz, W. and Keim, H., unpublished data). This suggests that the stimulation of the K^+ permeability by propranolol cannot be related to its action as an adrenergic β-receptor antagonist.

The dependence of channel activity on the Ca^{2+} concentration can be described by a Hill coefficient of $n = 2$ independent of the presence or absence of propranolol (see lines in Fig. 5). But 1 mM propranolol reduces the K_m value by about two orders of magnitude (see K_m values in legend to Fig. 5) and simultaneously reduces the single-channel activity. Hence, depending on the intracellular Ca^{2+} activity, stimulation or inhibition of the K^+ permeability is possible. These results can explain the observations made in flux experiments that low concentrations cause stimulation (i.e., making the channel more sensitive to Ca^{2+}), and that high concentrations cause inhibition (i.e. overcompensating for increased sensitivity). Consequently, the inhibitory effect can be detected primarily at maximally activating concentrations of Ca^{2+} or Pb^{2+}, whereas stimulation is detectable only at low concentrations of Ca^{2+} or Pb^{2+}.

Skulskii and Manninen [6] suggested that the inhibitory effect of propranolol may be due to an effect on formation of active channels in the presence of reducing agents. In previous experiments [19], we demonstrated that the gating of the Ca^{2+}-activated K^+ channels can be modulated by drugs that stimulate or inhibit a membrane-bound oxidoreductase. The following paper [20], on the other hand, shows that the action of propranolol on channel activity is not related to effects on this membrane-bound oxidoreductase activity.

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