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Ion channels in the human red blood cell membrane: their further investigation and physiological relevance

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Abstract

Using the patch-clamp technique, two different ion channels have been characterized further in the human red blood cell (RBC) membrane. We demonstrate that the non-selective cation channel (NSC) is permeable to Ca^{2+} and can be activated by prostaglandin E_2 (PGE_2). Therefore, the physiological role of this channel could be, together with the Ca^{2+}-activated K^+ channel, the participation in the process of blood clot formation. We give also evidence that another channel in the RBC membrane, so far assumed to be a small conductance anion channel, is more likely to be a proton or a hydroxyl ion channel. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ion channel; Erythrocyte; Patch-clamp; Prostaglandin E_2; Clot formation

1. Introduction

In the human red blood cell (RBC) membrane, three different types of ion channels have been described so far. Besides the well-known Ca^{2+}-activated K^+ channel (Gardos channel) [1], conflicting reports [2,3] about a non-selective cation channel (NSC) have been reconciled recently [4]. In addition, there are two reports about a small conductance anion channel in the RBC membrane [5,6]. The classification of this channel as an anion channel was however only based on different measured conductances in chloride and nitrate media.

The aim of the present paper was therefore to further characterize the NSC and the small conductance anion channel. Using the patch-clamp technique, we demonstrate that NSC is permeable to Ca^{2+} and can be activated by prostaglandin E_2 (PGE_2). Based on these findings, we present an idea on the possible physiological role of both the NSC and the Gardos channel in the process of blood clot formation. We take into account a report demonstrating in flux experiments that the Gardos channel is activated by PGE_2 [7]. However, no idea of the mechanism of activation has been presented. In addition, we give evidence that the putative anion channel is more likely to be a proton or a hydroxyl ion channel.

2. Experimental

For experiments, freshly drawn blood from healthy human donors was used. The RBCs were washed three times by centrifugation ($1500 \times g$, 8 min) in physiological NaCl solution containing (in mM): 145 NaCl, 10 glucose, 10 morpholinoethane sulfonic acid/tris(hydroxymethyl)aminomethane (MES/Tris), pH 7.4. Plasma and buffy coat were removed by aspiration. The solutions used in the patch-clamp experiments contained (in mM): 150 or 75 KCl (or 75 $CaCl_2$), 10 MES/Tris, 2.5 $BaCl_2$ and 10^{-7} or 10^{-4} PGE_2 (as indicated). The pH of all solutions was 7.4. Inorganic salts were of analytical grade. EGTA and PGE_2 were obtained from Sigma (St. Louis, USA). MES/Tris was purchased from Fluka Chemie (Buchs, Switzerland). Pipettes were pulled from borosilicate glass (GC150F-10, Harvard Apparatus, UK) and had a resistance of about 10 MΩ. The measurements were carried out at room temperature (23 °C). Inside–out patches were performed by the following procedure. After a gigaseal (5–20 GΩ) was reached, the inside–out configuration was formed spontaneously or by moving the pipette tip through the air–water interface. The measured currents were low-pass filtered at 1 kHz (Bessel filter) and digitized at 3–5 kHz.

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3. Results and discussion

Patch-clamp experiments on human RBCs were performed in the presence of PGE_2 in the extracellular solution (pipette solution). First, experiments were done with a PGE_2 concentration of 10^{-10} M. The physiological PGE_2 concentration is about one order of magnitude lower, the concentration that occurs when the activated platelet release of PGE_2 is about one order of magnitude higher [8]. Although an effect on channel openings could be seen at a PGE_2 concentration of 10^{-10} M, we were not able to evaluate the obtained current traces quantitatively. Therefore, the PGE_2 concentration was increased to 10^{-7} M. In contrast to almost all other inside–out measurements of the NSC in the human RBC membrane [2–4] in the presence of PGE_2, channel openings could also be detected at negative membrane potentials (for current traces at different membrane potentials, see insert of Fig. 1). The data were analysed using all point amplitude histograms. The current–voltage (I–V) diagram is shown in Fig. 1. The channel is slightly outward rectifying, i.e. while at the positive membrane potentials, the conductance is almost constant (35 pS), it is decreasing and reaches a value of about 24 pS as the membrane potential becomes more negative. Although channel openings are detected at negative membrane potentials, it can be assumed that the recorded channel is identical with the previously described NSC in the human RBC membrane. This assumption is based on a comparison of the conductances and characteristics of current traces.

Besides NSC, a Ca^{2+}-activated K^+ channel exists in the human RBC membrane. Its conductance is in the same range as that for NSC [1]. However, since 2.5 mM Ba^{2+} is present in the bath solution (Ba^{2+} inhibits the Ca^{2+}-activated K^+ channel), an activation of the Ca^{2+}-activated K^+ channel can be excluded. An activation of the anion channel described in the RBC membrane (see below) can be also excluded since its conductance in physiological chloride solutions is less than 10 pS [5].

Interestingly, an activation of the Ca^{2+}-activated K^+ channel in the human RBC membrane by PGE_2 was demonstrated in flux experiments [7]. However, this report is lacking for an idea of the mechanism of activation. Furthermore, it has been shown that NSC is permeable for divalent cations [4]. The I–V diagram for Ca^{2+} is shown in Fig. 1. In symmetrical 75 mM $CaCl_2$ solutions, NSC shows a conductance between 15 and 18 pS. The argument that the conductance is indeed caused by NSC is similar as that for the conductance in the presence of PGE_2 (see also Ref. [4]). Based on the data shown above, it seems reasonable to assume a possible mechanism of ion channel activation that could be of importance in the process of blood clot formation. PGE_2 activates the non-selective cation channel. The reversal potential of NSC for intact RBCs in their own plasma is approximately 0 mV. Since the resting potential of human RBCs is about -10 mV, an inward current is expected and cations present in the blood plasma including Ca^{2+} can enter the cells. Although this report shows that the open probability of NSC at a PGE_2 concentration of 10^{-10} M is very low, it was shown in Ref. [4] that even a small open probability of NSC leads to remarkable Ca^{2+} influxes, and hence, to the increase of the intracellular Ca^{2+} concentration resulting in the activation of the Ca^{2+}-activated K^+ channel.

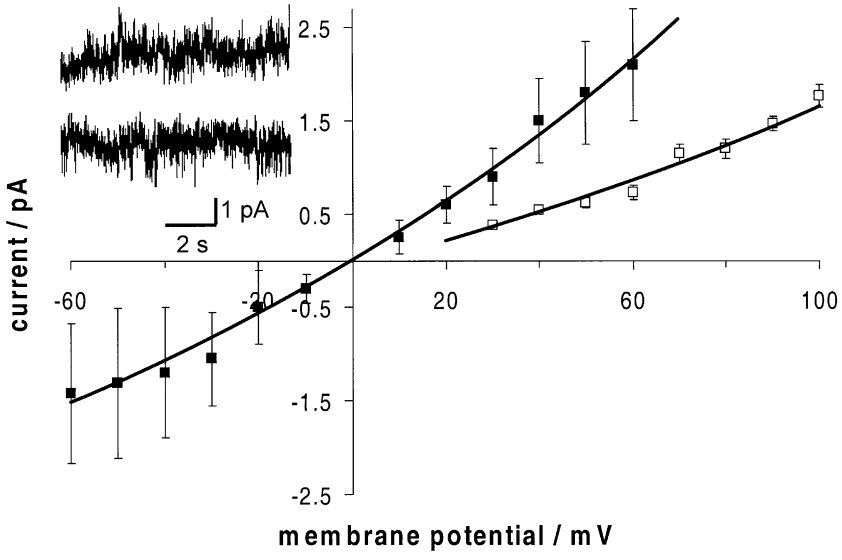

Fig. 1. Current–voltage diagrams of NSC channels from inside–out patches of red blood cells. ■, Symmetrical solution containing (in mM): 150 KCl and 10 MES/Tris. In addition, the pipette and bath solution contained 10^{-7} M PGE_2 and 2.5 mM Ba^{2+}, respectively. The data are mean ± S.D. values from three patches. □, Symmetrical solution containing (in mM): 75 $CaCl_2$ and 10 MES/Tris. In addition, the pipette solution contained 2 μM carbachol to stimulate the channel openings (the channel is coupled to an acetylcholine receptor of nicotinic type [9]). The data are mean ± S.D. values from six patches. The insert shows representative examples of current traces of symmetrical KCl solutions in the presence of PGE_2 in the pipette at membrane potentials of +30 mV (upper trace) and −30 mV (lower trace).

Since for the Ca^{2+}-activated K^+ channel, the transport ratio of K^+ compared to Na^+ is 16:1 [1], the reversal potential for this channel under physiological conditions in the blood is about +70 mV (calculated from the Nernst equation). Therefore, at a resting potential of the human RBC of about −10 mV, an outward current of K^+ is provoked. The maximal resulting efflux can be calculated to be 270 mmol $(l_{cells}\ h)^{-1}$. Although this enormous value is an upper limit of the possible K^+ efflux, the K^+ loss of the cells is so high that it leads to significant shrinkage of the RBCs in a very short time (minutes). This effect seems to play an important role in the thrombus formation. Although it is believed that RBCs themselves are primarily not involved in the clot formation, it is evident that they make up an enormous part of it. However, such proposed mechanism of interaction could explain the physiological importance of both NSC as well as the Ca^{2+}-activated K^+ channel. Their physiological functions were so far unknown.

Besides the two above-mentioned channels, a small anion channel [5,6] was reported to be present in the RBC membrane. This channel showed a conductance of 6 and 15 pS in chloride and nitrate media, respectively. The channel could be inhibited by persantin [6], and the number of channels per cell was estimated to be 100 [5]. Representative examples of current traces of the small conductance channel at different membrane potentials in the chloride media are shown in the insert of Fig. 2. This figure also shows the *I–V* diagrams of the measurements of the channel in symmetrical 150 mM KCl solutions and after the replacement of the bath solution (same patches) by a 75 mM KCl solution. These *I–V* curves allow the conclusion that the channel cannot be either a conventional $K^+(Na^+)$ channel or a chloride channel since a division of the KCl concentration in the bath solution by two does not lead to a significant shift in the reversal potential. Based on the Nernst equation, the reversal potential should change from 0 to approximately +17 and −17 mV for a cation and anion channel, respectively. Even more surprising, the reduction of the KCl concentration in the bath leads to an enhancement of the channel conductance from 8.4 to 17 pS. Thus, the *I–V* curves can only be interpreted assuming that a proton or a hydroxyl ion channel is involved. The classification of this channel as an anion channel [5,6] is only based on the different above-mentioned conductances in chloride and nitrate media. However, this finding already excludes a conventional $K^+(Na^+)$ channel. This statement is supported by the fact that under conditions where the pipette and the bath solution contained 20 and 70 mM Na–tartrate, respectively (in addition, both solutions contained 2.5 mM $BaCl_2$, 10 mM MES/Tris (pH 7.4); sucrose was added to adjust the osmolarity of the solutions to 300 mOsM), a linear *I–V* curve was obtained giving a reversal potential close to 0 mV and a conductance of 9.7 pS (not shown).

Summarising the results, it seems more likely that the low conductance channel described in the present paper represents a proton or a hydroxyl ion channel in the human RBC membrane. In order to support this finding, it would be helpful to measure the channel conductance at different pHs in the bath and pipette solution. To have a pH gradient present at least for several minutes, one has to inhibit the anion exchanger (band 3) in the RBC membrane. In the first approaches, we used DIDS or dipyridamol in the pipette solution (for inside–out patches), but under these conditions, we were not able reach a gigaseal (it seems likely that these substances prevent a seal formation).

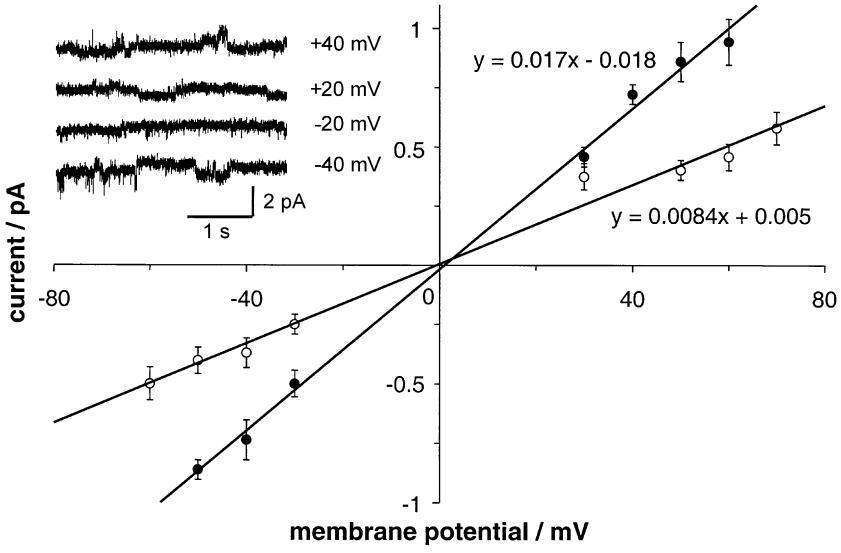

Fig. 2. Current–voltage diagrams of small conductance channels from inside–out patches of red blood cells. ○, 150 mM KCl bath and pipette solution. ●, 75 mM KCl bath solution and 150 mM KCl pipette solution. In addition, all solutions in the figure contained (in mM): 10 MES/Tris and 2.5 Ba^{2+}. The data are mean ± S.D. values from eight patches. The insert shows representative examples of current traces of the small conductance channel at the indicated membrane potentials.

A possible physiological relevance of the channel could be its participation in the oxygen and carbon dioxide transport. CO_2 produced in the tissue diffuses into the erythrocytes. Using the enzyme carbonic anhydrase, CO_2 will be hydrated and H^+ as well as HCO_3^- is produced. Normally, the protons are buffered by hemoglobin [10] and HCO_3^- leaves the cells in exchange for Cl^- via band 3. However, since the process is connected with an acidification of the red blood cells, a physiological relevance could be that protons leave the cell via the proton channel.

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