Activation of red cell Ca^{2+}-activated K^+ channel by Ca^{2+} involves a temperature-dependent step

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We found that vanadate-induced $^{45}Ca^{2+}$ uptake by red cells is maximal at 25°C. At this temperature, the Ca_i-induced increase of the K^+ permeability (the Gárdos effect) shows a lag (up to 8 min) which is not observed at 37°C. This cannot be explained by the lack of availability of Ca^{2+} for the Ca^{2+}-activated K^+ channel, and suggests that its activation by Ca^{2+} is mediated by a temperature-dependent mechanism which remains unknown so far. The lag is not observed when the Gárdos effect was initiated by propranolol. This shows that the putative temperature-dependent step is different from chloride transport.

Red cell; Vanadate; Propronolol; Ca^{2+}-activated K^+ channel

1. INTRODUCTION

The activation of the red cell Ca_i^{2+}-activated K^+ channel can be elicited by several procedures which differ from each other sometimes very substantially [1,2]. The common denominator of their action seems to be their ability to increase the Ca^{2+} concentration in red cell cytoplasm [1,2]. Although the effect of propranolol was controversial in this point it is probable that its effect is also Ca_i-mediated [3,4]. The exceptions to this rule seem to be some divalent cations like Pb^{2+} which may enter the cytoplasm and substitute Ca^{2+} in activating the K^+ channel [5,6].

There are several observations which suggest that opening of the Ca^{2+}-activated K^+ channel is modulated by the internal environment like the ADP/ATP ratio [7], or the redox state of the cell cytoplasm [8,9]. Other authors found that a soluble cytoplasmic protein distinct from calmodulin is necessary for the channel activation ([10], see [2] for review). Although its properties were in part described [10], its precise role remains unknown. It is not known whether it is a part of the protein phosphorylation machinery which was shown to enhance the activity of the Ca^{2+}-activated K^+ channel in snail neurones [11], and heart sarcolemma [12]. Nevertheless, all these observations suggest that the activation of the Ca^{2+}-activated K^+ channel may include the interaction of more than two molecules (i.e. Ca^{2+}, and the channel protein). The observation we present here is within the scope of this notion.

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2. EXPERIMENTAL

Chelatonate-treated blood (5 mM final) was withdrawn from both male and female volunteers, and was used within 3 days after being stored at 0-4°C. The $^{45}Ca^{2+}$ uptake induced by vanadate was measured as described in previous papers [13,14]. The activity of the Ca^{2+}-activated K^+ channel (further referred to as the Gárdos effect) was monitored by the measurement of the net K^+ efflux by flame photometry. In some experiments we measured the Gárdos effect continuously using a K^+-selective electrode, and the double-salt bridge reference electrode.

3. RESULTS

During the study of the transport characteristics of vanadate-induced $^{45}Ca^{2+}$ uptake, we also studied its temperature dependence. It is shown in Fig. 1 that $^{45}Ca^{2+}$ uptake is dependent on the temperature biphasically. At 18°C we always observed a significant uptake which increased with the temperature up to the maximal value at about 25–27°C. On further increasing of the temperature the uptake decreased so that at 37°C the uptake was only slightly but significantly different from the control values which varied between 0.1 and 1.0 $\mu mol/l_{cells}$ at all tested temperatures. It should be noted that the time course of the initial phase of the uptake is linear at 25°C (the uptake starts within the first minute - not shown). At 18°C, however, the lag phase was observed. At both 32°C and 37°C a steady-state was reached within the first sampling interval (6 min). This behaviour of the experimental system is expected when one assumes that the movement of Ca^{2+} is determined by two transport systems: an inward-directed Ca^{2+} carrier, and an outward-directed Ca^{2+}-ATPase which differ in their temperature dependence. The evidence for such a notion was published previously [13,14].

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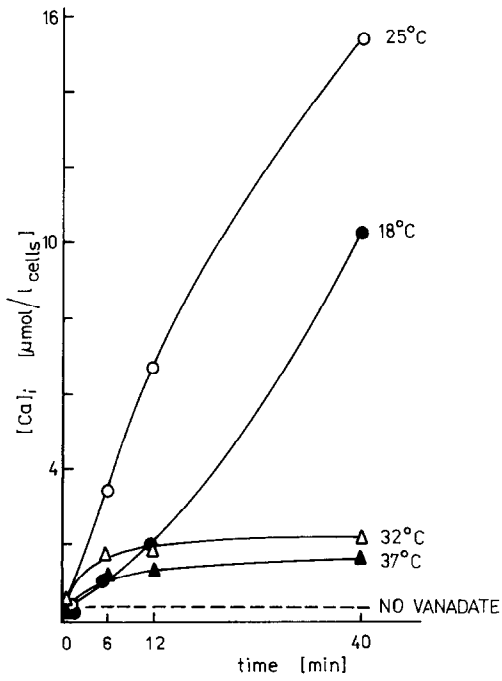

Fig. 1. The kinetics of the vanadate-induced $^{45}Ca^{2+}$ uptake by human red blood cells at various temperatures. Cells were preincubated with vanadate (1 mM) for 15 min at 25°C. Aliquots were equilibrated to 18°C (○), 25°C (●), 32°C (△), 37°C (▲). $^{45}Ca^{2+}$ (2.5 mM final) was added and the transport reaction was stopped at the time indicated by addition of an EDTA-containing medium. After washing out the extracellular radioactivity, the radioactivity content of cell pellets was measured. Experimental points represent an average of two samples treated in parallel. The standard error is less than the diameter of symbols. The dashed line represents the upper limit of the uptake at either temperature without vanadate. Typical of 4 similar experiments.

The effect of the temperature on the kinetics of the vanadate-induced Gárdos effect is shown in Fig. 2. It can be seen that the time course as well as the extent of the K^+ release changes with the temperature. The last one has an optimum at about 25–27°C (see also Fig. 3). The time course was different at various temperatures. From 18°C up to 32°C a distinct lag phase preceded the start of the Gárdos effect which was absent at higher temperatures. (A more pronounced comparison is observed in Fig. 2B where we sampled aliquots more frequently, at 25 and 37°C.) The presence of a lag-phase in the initiation of the Gárdos effect at 25°C contrasts with the linear time course of the $^{45}Ca^{2+}$ uptake, the extent of which is sufficient to activate the Ca^{2+}-activated K^+ channel fully (compare with 37°C) (Fig. 1). These results show that the activation of the Gárdos effect (which represents the activity of the Ca^{2+}-activated K^+ channel) by vanadate requires a temperature-dependent step different from the Ca^{2+}-entry. This step could hardly be identified with the anion transport which was shown to limit the net efflux of KCl from red cells during the Gárdos effect [15]. The first experiment we devised to test this possibility is based on the action of tributyltin, a compound known

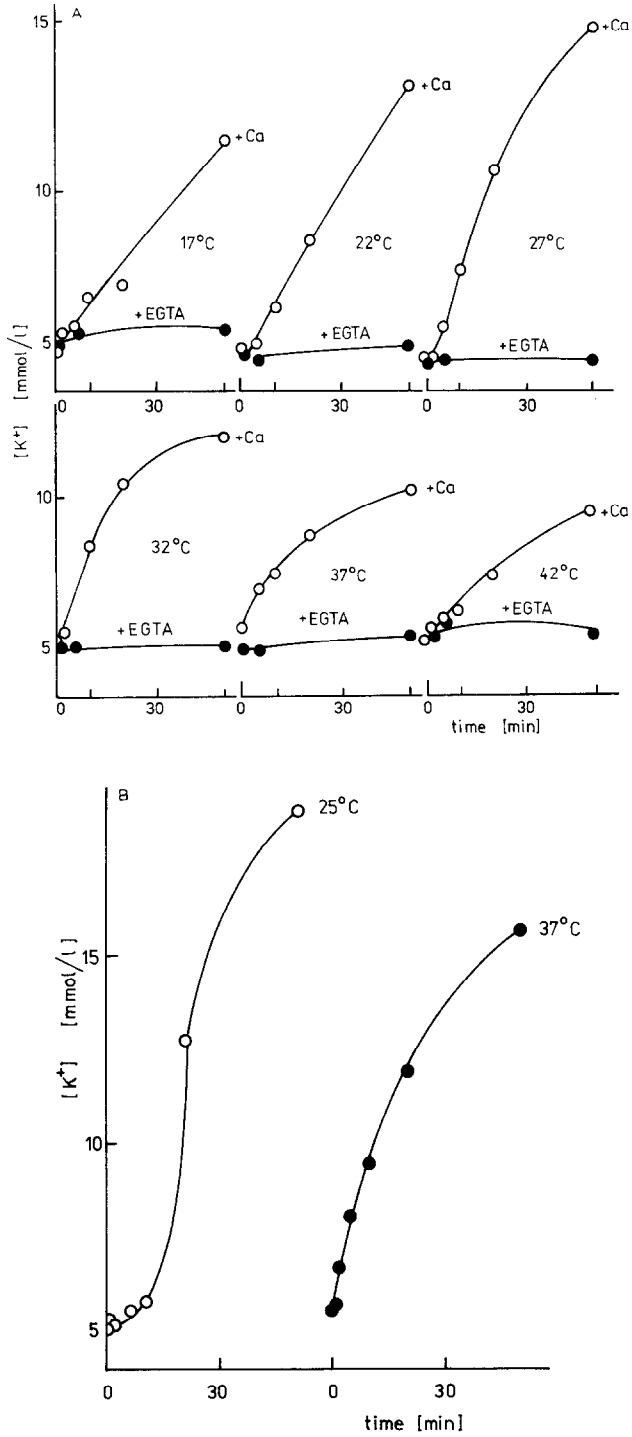

Fig. 2. The kinetics of the vanadate-induced Gárdos effect at various temperatures. (A) Cells were preincubated with vanadate as in Fig. 1. Aliquots were equilibrated at 17°C, 22°C, 27°C, 32°C, 37°C and 42°C. The Gárdos effect was initiated by the addition of $^{40}Ca^{2+}$, and stopped by the centrifugation of the suspension through the layer of silicone oil. Control test tubes with EGTA instead of Ca^{2+} (closed circles) were treated in parallel. The time courses at various temperatures are ordered from the upper left to the lower right side. All experimental points represent an average of two parallel samples, the standard error is less than the diameter of symbols. Typical of three experiments. (B) The comparison of the time course of the Gárdos effect at 25°C (○), and 37°C (●). Experiment independent to this in (A).

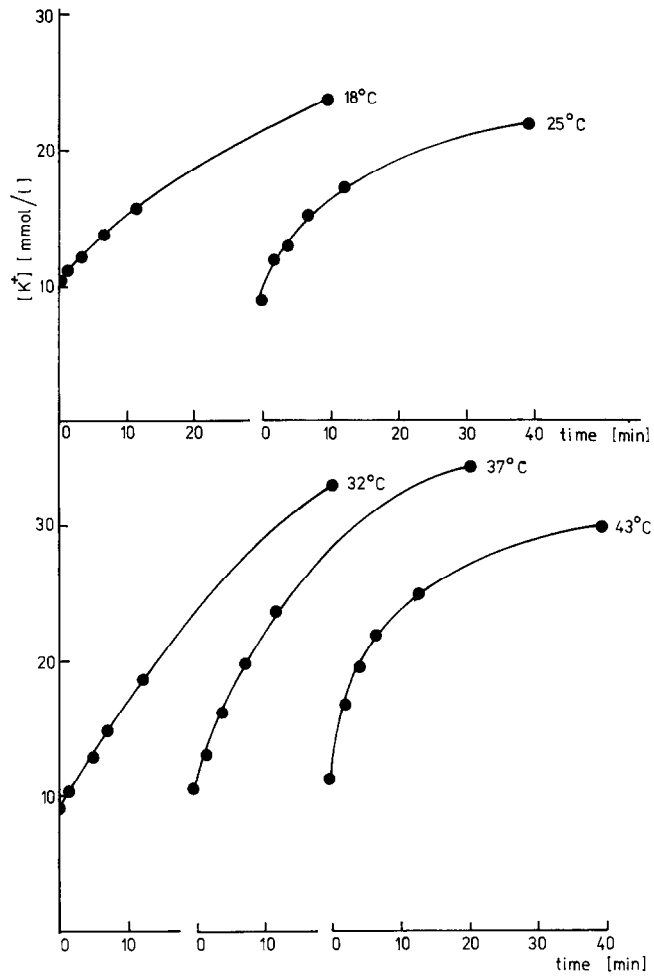

Fig. 3. The kinetics of the Gárdos effect induced by propranolol at various temperatures. The experiment was performed as described in Fig. 2 except that 1 mM propranolol was added instead of vanadate, and the Gárdos effect was measured at temperatures indicated in the figure. One of two experiments.

to have ionophoric properties for chlorides [16]. We have found that this compound did not influence the lag phase up to 100 μmol/l (not shown). The same conclusion can be drawn from the experiment in Fig. 3 which shows the dependence of the Gárdos effect on temperature induced by propranolol (1 mmol/l). It can be seen that the lag phase is completely absent at all tested temperatures. The dependence of the extent of released K^+ on the temperature is similar to that observed in the presence of vanadate (Fig. 2) suggesting that the Gárdos effect once initiated behaves the same regardless of the nature of the inducer. The lag phase was also observed in the ATP-depleted cells. However, ATP-depleted cells differed from both vanadate- and propranolol-treated cells in more aspects than reported here, and these differences deserve a separate treatment. In other experiments we found that sub-threshold concentrations of propranolol, or polymyxin B (an inhibitor of the Ca^{2+}-activated K^+ channel) [18], did not affect the duration and/or slope of the lag phase (not shown). We expected a potentiation of their effects provided that all tested agents act on the same target molecule. This is apparently not the case in our experimental system.

4. DISCUSSION

According to the data summarized in section 1 there are several reasons to expect that the activation of the Ca^{2+}-activated K^+ channel may proceed with a participation of a complementary enzymatic machinery in red cells. Its nature, however, is unknown so far. Our results only add indirect evidence favouring the existence of such a machinery without characterizing it in molecular terms. Our results preclude the idea that the putative temperature-dependent step is the enzyme converting vanadate to vanadayl compound which could activate the Ca^{2+}-activated K^+ channel [17] because the effect of vanadate is strictly Ca-dependent (Fig. 2).

The possibility of the involvement of the protein phosphorylation mechanism in the red cell Ca^{2+}-activated K^+ channel activation is feasible on the basis of comparison to other tissues [11,12]. This, together with the finding that an inhibitor of protein kinase C (polymyxin B) is an inhibitor of the Gárdos effect ([18], and results presented here), may indicate that this is really taking place in red blood cells.

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