MECHANISM OF VARIOUS DRUG EFFECTS ON THE Ca^{2+}-DEPENDENT K^+-EFFLUX FROM HUMAN RED BLOOD CELLS

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1. Introduction

If ATP is reduced to a minimum in red blood cells (RBC) and Ca^{2+} is present in the medium a rapid K^+-efflux sets in unaccompanied by equimolar Na^+-uptake [1]. Propranolol and pronethalol induce this phenomenon even in cells containing normal ATP levels [2]. This work reports a study of the mechanism of these drug actions

By obtaining different results in ATP-containing and ATP-depleted cells propranolol, pronethalol and tetracaine were shown to interfere with ATP–membrane interactions. Various Ca^{2+}–membrane interactions (responsible for the Ca^{2+}-penetration, rapid K^+-transport, Ca-ATPase function and maintenance of the biconcave shape) were also affected. These effects are compared with effects of other membrane active drugs (e.g. histamine, antihistamines, chlorpromazine). Data are presented concerning the relationship between the K^+ channels of RBC and the excitable membranes.

Lew [3] classified the regulating factors of the rapid K^+-transport into four groups: 1) ATP depletion; 2) Ca^{2+} entry; 3) interaction between intracellular Ca^{2+} and the K^+ channel or carrier; 4) movement of K^+ through the K^+ channel or carrier. From our results we introduced slight modifications into this classification and present our results within this frame.

2. Materials and methods

Fresh or depleted human RBC were used. For 2,3-diphosphoglycerate (DPG) depletion, cells were incubated with 2.5 mM iodoacetate (IA) and 15 mM $NaHSO_3$ for 5 hr at 37°C, for ATP depletion with 2.5 mM IA + 10 mM inosine for the next 2 hr at 37°C. Depletion was carried out in the presence of 1 mM EGTA. Haemoglobin (Hb)-free RBC membranes were prepared in a 20 mosmolar NaCl–Tris–EDTA medium. Cation fluxes were determined by flame photometry and by using ^{42}K, ^{22}Na, ^{45}Ca isotopes. Ca^{2+}-binding to the membrane was followed by the supernatant technique [4]. Membrane Ca-ATPase activity was measured according to Bond and Green [5]. The morphology of RBC was observed under a phase-contrast microscope.

3. Results and discussion

3.1. *Intracellular ATP level and compartmentation*

In cells containing a normal ATP level the β-receptor antagonist and local anaesthetic propranolol and pronethalol, as well as the well-known local anaesthetic tetracaine induced the Ca^{2+}-dependent rapid K^+-transport within a concentration range (fig. 1). Since the ATP level is only slightly affected by these drugs it is to be supposed that they alter some ATP–membrane interactions. As a result the protective effect of ATP against the rapid K^+-transport ceases, as well as its protective effect against Ca^{2+}-penetration (see section 3.2). Pendleton et al. [6] found propranolol to cause a 'membrane mediated' decrease of Hb oxygen affinity in the presence of DPG. Oski et al. [7] even demonstrated that propranolol detached DPG from the membrane and caused thereby a higher portion of DPG to get bound to Hb. They suggest a similar shift from the membrane

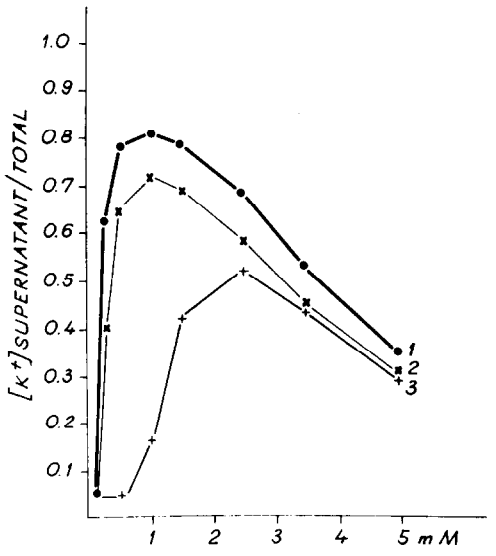

Fig. 1. Effect of propranolol (1), pronethalol (2) and tetracaine (3) on the amount of K^+ loss from human RBC. Temp., 37°C; incubation time, 30 min; Ca^{2+}, 5 mM.

to Hb also for ATP. These findings within the scope of the concept that Hb binds DPG and ATP [8] made it necessary that in addition to the ATP level even its compartmentation should be taken into consideration.

3.2. *Intracellular Ca^{2+}-level and compartmentation*

The above mentioned aspect applies even to the intracellular Ca^{2+}; not only its level is to be considered, but also whether it is bound by a chelator or in a complex (e.g. NaF–phosphate). The intracellular Ca^{2+}-level itself in normal cells is minimal. In our experiments it depends firstly on the rate of Ca^{2+}-penetration which is influenced by many drugs (fig. 2). In the course of the depletion of fresh cells the rate of ^{45}Ca-influx progressively increases (from 0.4 to 4 μmol Ca/ml RBC/hr at 0.3 mM Ca^{2+}). In completely depleted cells it is significantly higher (7–20 μmoles Ca^{2+}/mlRBC/hr). With both cell types histamine at lower concentrations (0.5–1 mM) increases and at higher concentrations (10–20 mM) decreases the penetration rate of Ca^{2+}. Theophylline, on the other hand, increases it in direct proportion to its concentration. Conversely, chlorpromazine (CPZ) decreases Ca^{2+}-penetration (at 0.05–0.25 mM). The effect of propranolol (pronethalol) and tetracaine in the concentration range critical for K^+-transport is different with the two cell types: they increase Ca^{2+}-influx transitorily until cells contain ATP, but in the ATP depleted state they cause a reduction of Ca^{2+}-penetration. Higher concentrations reduce Ca^{2+}-penetration in both cell types. These results will be interpreted together with the findings described in section 3.3.

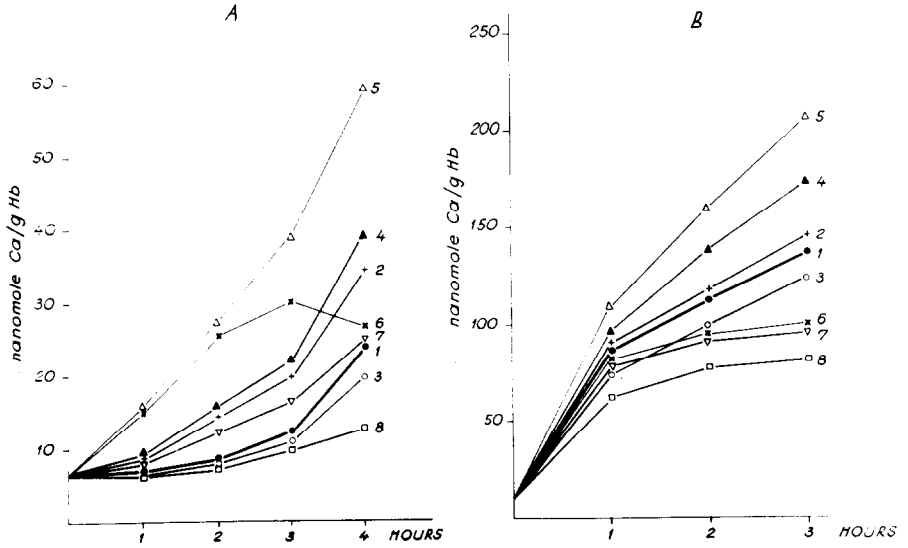

Fig. 2. A: Ca^{2+}-influx in fresh erythrocytes in the presence of 2.5 mM IA and 10 mM inosine; B: Ca^{2+}-influx in phosphate ester-depleted erythrocytes. Temp., 37°C, Ca^{2+}, 0.3 mM. 1) Control; 2) 0.5 mM histamine; 3) 10 mM histamine; 4) 0.5 mM theophylline; 5) 10 mM theophylline; 6) 0.5 mM propranolol; 7) 0.5 mM tetracaine; 8) 0.25 mM CPZ.

3.3. *Interaction between the intracellular Ca^{2+} and the membrane*

The Ca^{2+}-membrane interaction responsible for the rapid K^+-transport is loose, so it cannot be examined by techniques elaborated for tight interactions. We found the 'supernatant-technique' – measuring both the loose and tight interactions – to be the best for investigating our drug effects. Under our experimental conditions the membrane bound 3.9–4.4 nmole Ca^{2+}/mg protein. All the examined drugs reduced the Ca^{2+}–membrane interactions. Histamine at 0.5 mM reduced Ca^{2+}-binding by 10–15%, at 2 mM by 25–30%, at 20–30 mM by 90%. The effect of theophylline differed only in the 20–30 mM range: Ca^{2+}-binding was reduced by only 50% as a maximum. CPZ, tetracaine and propranolol all were very efficient in detaching Ca^{2+}. At 0.1 mM their effects were well detectable, at 0.5 mM they reduced Ca^{2+}-binding by about 50% and at 1 mM they abolished it completely. Various antihistamines as well as another β-receptor antagonist, oxprenolol, behave in the same way.

Comparing these data with functional effects: tight Ca^{2+}–membrane interactions (responsible for the Ca-ATPase activity and the biconcave shape) are affected only by drugs achieving complete Ca^{2+}-release (the CPZ, propranolol, tetracaine group). In the concentration range of incomplete Ca^{2+}-release these drugs exhibit different affinities to various loose membrane – Ca^{2+}-interactions. While CPZ (antihistamines and oxprenolol) disturb the K^+-channel – Ca^{2+}-interaction at very low concentration, propranolol, pronethalol and tetracaine only at higher concentrations. That is the reason why lower concentrations of propranolol, pronethalol and tetracaine may promote interactions between the K^+-channel and Ca^{2+}. Histamine and theophylline do not interfere with the K^+-channel–Ca^{2+}-interactions at all. But high concentrations of histamine appear to disturb the membrane–Ca^{2+} interaction responsible for Ca^{2+}-penetration itself. The maximum curve-like effect of histamine on the K^+-transport as a function of its concentration can be satisfactorily explained by the corresponding changes in the Ca^{2+}-penetration rate.

3.4. *Movement of K^+ through the membrane*

Drugs, ineffective in the previous assays but powerful inhibitors of the rapid K^+-efflux, like oligomycin, dipyridamol and tetraethylammonium salts (TEA) should be considered here. Dipyridamol most probably acts

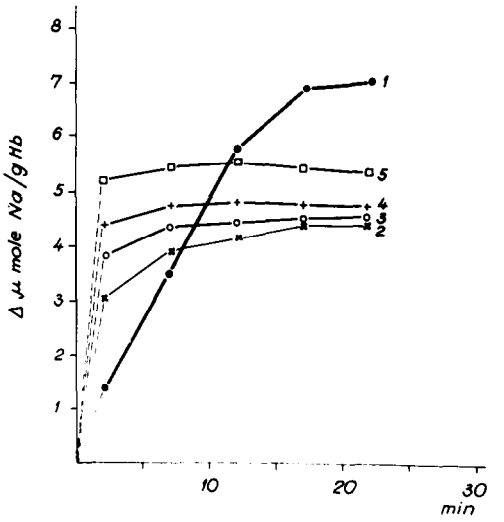

Fig. 3. Effect of carbamylcholine (CCh) on the Na^+-uptake of fresh RBC. Temp., 22°C; Ca^{2+}, 2.5 mM. 1) Control; 2) 10^{-7} M CCh; 3) 10^{-6} M CCh; 4) 10^{-5} M CCh; 5) 10^{-4} M CCh. Cells were separated by centrifuging the samples through an 0.7 M sucrose cushion containing 0.16 M NaCl. Time needed for separation is indicated in the graph.

through the inhibition of the permeability of Cl^-, the ion accompanying K^+. TEA, as it is well known, blocks the K^+-channel of the excitable membranes.

This raises the question, whether the K^+-channel studied in erythrocytes is basically identical with that of excitable membranes. Our preliminary experiments indicate a positive answer. 10^{-7}–10^{-4} M Carbamylcholine caused the transitory decrease of ^{42}K-efflux, and this was more definite in the presence of Ca^{2+}. Simultaneously the transitory activation of the ^{22}Na-uptake which could be demonstrated spontaneously turned into an inhibition (fig. 3). Both the activation and inhibition of Na^+ permeability proved to be tetrodotoxin (TTX)-sensitive. Carbamylcholine effects could be demonstrated even in ATP depleted cells. These findings might suggest that as a result of binding of certain cholinergic agents intramembraneous Ca^{2+}-rearrangement takes place (a detachment or rearrangement of ATP may also play a part here). This opens the TTX-sensitive Na^+ and TTX-sensitive and insensitive Ca^{2+}-channels. When Ca^{2+} reaches the inner surface of the membrane and reacts with the receptors responsible for the formation of the K^+-channel, the membrane conformation, advantageous for K^+-permeability, develops. Further experiments are needed, however, in order to decide whether this assumption is correct.

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