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THE INFLUENCE OF CHLORPROMAZINE ON THE OSMOTIC FRAGILITY OF ERYTHROCYTES

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Abstract—Protection of erythrocytes by chlorpromazine against osmotic haemolysis has been described before in the literature. This phenomenon has been investigated in some detail, in order to determine its physical chemical background.

Experimental evidence is presented, indicating that the decrease of osmotic fragility is based on a drug induced increase of the critical volume of the erythrocytes.

INTRODUCTION

ATTEMPTING to find a common basis for the diverse biochemical and pharmacological effects of phenothiazine derivatives, Guth and Spirtes suggested that the influence on membrane permeability could serve to explain many of these effects. In a review[1] many examples of proved or probable influences of phenothiazines on membrane permeability are summarized.

The protection of human erythrocytes against haemolysis in hypotonic NaCl solutions has been described by Freeman and Spirtes.[2–4] This phenomenon was ascribed to a drug induced inhibition of passive water transport through the cell membrane. The assumed reduction of transmembrane water transport however, was not measured directly. In connexion with the emphasis laid on the importance of permeability changes induced by phenothiazines, a further investigation of the protection of red cells against osmotic haemolysis seemed worthwhile. The results of this investigation are reported in this paper.

METHODS

Both human and bovine erythrocytes were used in these experiments. Shortly after collection, the heparinized blood was centrifuged, the plasma and buffy coat being discarded. The red blood cells were washed three times in 1% NaCl. Finally the erythrocytes were suspended in 1% NaCl, to give an approximately 50% cell suspension. All NaCl solutions utilized were prepared by dilution of a stock solution, equivalent to 10% NaCl as described by Parpart *et al.*[5] The pH of all the diluted NaCl solutions was 7·33–7·36. The use of buffered solutions in these experiments is desirable, as pH influences osmotic haemolysis.[6]

Osmotic fragility was measured by adding 0·2 ml of the 50% cell suspension to 11 ml NaCl solution of different concentrations. (In the chlorpromazine experiments the drug was added to the NaCl solution in advance.) This mixture was then incubated at 22° in a thermostat water bath for 60 min, unless otherwise stated. This period is sufficient for the haemolytic system to attain equilibrium at this temperature.[7] Further the procedure as described by Mortensen[6] was followed.

Measurement of the mean cellular volume of the erythrocytes under various experimental circumstances was performed as follows: After incubation, as previously described, 2 ml of the cell suspension was centrifuged for 20 min at 3000 rpm in a Hamburger type haematocrit tube. In preliminary experiments it appeared that this time was sufficient to achieve the same haematocrit values as with 5 min centrifugation in a micro-haematocrit ultra centrifuge. The remaining suspension was centrifuged at the same time, and the percentage haemolysis was determined by measuring the percentage haemoglobin, liberated in the supernatant. (Haemoglobin was determined according to Crosby, Munn and Furth.[8] The mean cellular volume of the remaining, intact erythrocytes is calculated as a fractional value of the mean cellular volume in 1% NaCl according to the formula: $V/V_0 = (h/h_0\ (1 - p))$,[9] where V = mean cellular volume; V_0 = mean cellular volume of cells in 1% NaCl; h = measured haematocrit; h_0 = haematocrit value in 1% NaCl; p = fraction of cells which has haemolysed. The use of this formula is justifiable, as it has been shown that osmotic haemolysis is an all-or-none phenomenon.[10]

RESULTS

In preliminary experiments the protection of bovine and human erythrocytes by chlorpromazine against haemolysis in hypotonic NaCl solutions was confirmed (Fig. 1). In following experiments the osmotic haemolysis was studied in the course

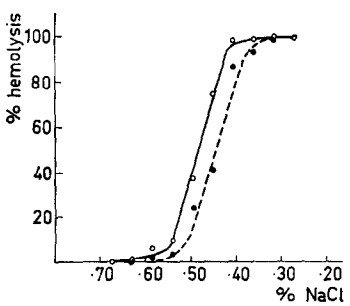

FIG. 1. Osmotic haemolysis of bovine erythrocytes in the absence (○ ○) and in the presence (● ●) of 10^{-4}M chlorpromazine, after incubation for 1 hr at 22°.

of time, with and without chlorpromazine present in the system. A NaCl concentration was chosen, giving, after 1-hr incubation at 22° about 70 per cent haemolysis in the control and about 20 per cent haemolysis in the presence of chlorpromazine. As can be seen from Fig. 2, the percentage haemolysis increases relatively fast during about 20 min, both in the absence and in the presence of chlorpromazine. After that haemolysis increases very slowly. It should be emphasized that the two curves (with and without chlorpromazine) are parallel in the interval from 30 to 210 min (the maximal incubation period).

A number of experiments on osmotic haemolysis was performed in KCl solutions, osmotically equivalent to the usual NaCl solutions. It appeared that utilizing KCl in substitution for NaCl in the haemolytic system did not influence the results. Haemolysis, both in the presence and absence of chlorpromazine is equal in NaCl and in osmotically equivalent KCl solutions (Fig. 3).

The swelling of erythrocytes in hypotonic NaCl solutions was followed by haematocrit measurements. It appeared that in hypotonic NaCl solutions in the presence of chlorpromazine the mean volume of the erythrocytes increases, at least to the same extent as without the drug (Fig. 4). At still lower NaCl concentrations osmotic

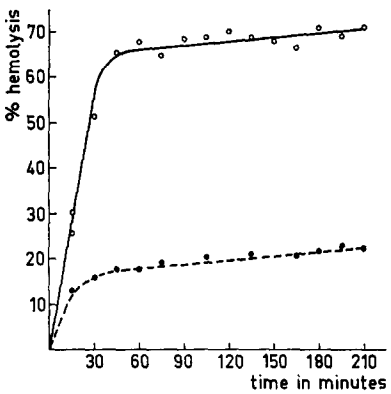

FIG. 2. Osmotic haemolysis in 0·52% NaCl at 22°, in the course of time. ○ ○: control; ● ●: 10^{-3}M chlorpromazine.

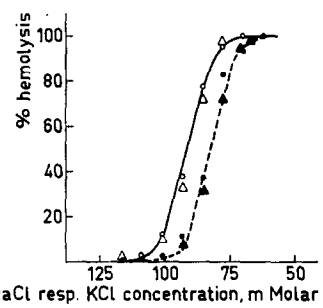

FIG. 3. Comparison of osmotic haemolysis in NaCl and in KCl solutions at 22°. ○ ○: control in NaCl; △ △: control in KCl; ● ●: 10^{-4}M chlorpromazine, in NaCl; ▲ : ▲10^{-4}M chlorpromazine, in KCl.

haemolysis commences, when the erythrocytes attain the critical volume.[11] It appears that in the presence of chlorpromazine the cellular volume increases still further, with haemolysis starting at lower NaCl concentrations, when the cells have swollen beyond the normal critical volume. This is depicted in Fig. 5, where the percentage haemolysis is plotted against the mean cellular volume of the intact cells. The critical volume in the presence of chlorpromazine apparently increases by approximately 10 per cent. An arbitrary value of 100 was attributed to the critical volume of erythrocytes in the absence of chlorpromazine. Statistical treatment of the experimental results revealed a normal critical volume of 100 $\pm$ 3·32 (mean $\pm$ S.D., 32 experiments) increasing to 110·19 $\pm$ 4·43 (mean $\pm$ S.D., 34 experiments) in the presence of chlorpromazine. This increase in critical volume is highly significant and was found both with human and with bovine erythrocytes.

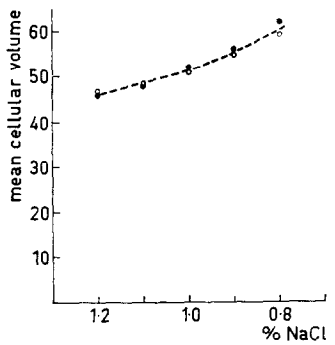

FIG. 4. Swelling of erythrocytes in hypotonic NaCl solutions at 22° in the absence (○ ○) and in the presence (● ●) of 10^{-4}M chlorpromazine.

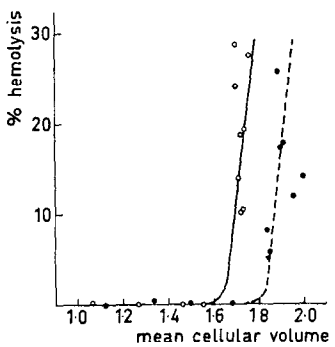

FIG. 5. The relationship between per cent osmotic haemolysis and mean cellular volume at 22° in progressively lower NaCl concentrations. (human erythrocytes). ○ ○: control; ● ●: 10^{-4}M chlorpromazine. Critical volume in the absence of chlorpromazine (± S.D.): (1·66 ± 0·06) V_0; critical volume in the presence of 10^{-4}M chlorpromazine: (1·88 ± 0·04) V_0.

DISCUSSION

The protection of human erythrocytes against osmotic haemolysis by chlorpromazine is a well established phenomenon.[2–4] In the present paper it is shown that the osmotic fragility of bovine erythrocytes is also decreased in the presence of chlorpromazine (Fig. 1). Considering the physical chemical basis of this protection, a number of possibilities must be distinguished.

1. Chlorpromazine may inhibit passive transmembrane water transport, as suggested by Freeman and Spirtes.[4]

2. The drug may increase the membrane permeability to K^+, without concomitant rise of permeability to Na^+. Decreased osmotic fragility on this basis has been described for Pb^{++}-treated erythrocytes.[12, 13]

3. The drug–membrane interaction may induce an increased resistance to membrane stretching, inhibiting further osmotic swelling in hypotonic solutions, once a certain cellular volume has been reached.

4. There may be an increase of the critical volume, caused by the drug–membrane interaction.

The first possibility: inhibition of transmembrane water transport, is rendered unlikely by the experiments on osmotic haemolysis in the course of time (Fig. 2). If inhibition of water transport were the cause of the inhibited haemolysis, one would

have to assume a slight inhibition of water transport during the first 20 min, subsequently changing into a complete blockage. (For any residual water transport would increase the percentage haemolysis, and the haemolysis curve in the presence of chlorpromazine would gradually approach the control, in the course of time). This seems very unlikely. The lack of any inhibition of chlorpromazine on swelling of red blood cells in hypotonic NaCl solutions (Fig. 4) leads to the same conclusion: transmembrane water transport is not inhibited by chlorpromazine.

If increased K^+ permeability, without concomitant rise of permeability to Na^+ would be the background of the protection against osmotic haemolysis, this protection should disappear in a haemolytic system in which NaCl is replaced by KCl. In a KCl system, increased K^+ permeability would not cause a decrease of the osmolarity of the cell contents. Contrariwise: colloid osmotic swelling should be expected to be augmented. The experimental results are in contradiction with these considerations (Fig. 3).

Simultaneous measurements of haematocrit and per cent haemolysis not only exclude the third possibility (inhibition of swelling beyond a certain cellular volume) but moreover confirm the drug-induced increase of the critical volume of the erythrocytes (Fig. 5). An increase of the critical volume of about 10 per cent, as found experimentally, is amply sufficient to account for the observed decrease of osmotic fragility in the presence of chlorpromazine.

Experiments to disclose the physical chemical background of the drug—induced increase of the critical volume are in progress.

Protection against osmotic haemolysis has been described before by Booij *et al* for alcohols.[14, 15] In preliminary experiments a similar protection was found with dodecylpyridinium chloride, cetylpyridinium chloride and sodium dodecylsulfate, in appropriate concentrations. Further experiments are directed at determining whether the protection by these compounds is based on a similar mechanism. The results of these studies will be discussed in a forthcoming paper.

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