Biochemical Pharmacology, Vol. 26, pp. 779–781. Pergamon Press, 1977. Printed in Great Britain.

THE INFLUENCE OF ANESTHETICS ON GLYCEROL AND K^+ TRANSLOCATION ACROSS THE MEMBRANE OF RED BLOOD CELLS

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(*Received* 12 *August* 1976; *accepted* 10 *November* 1976)

Abstract—A wide variety of chemically unrelated anesthetics protects red blood cells against osmotic hemolysis. The protective effects of most anesthetics are mutally additive, suggesting common rather than separate mechanisms of action. Other properties shared by these anesthetics are their inhibition of facilitated diffusion of glycerol into human erythrocytes and their stimulation of passive transport of this solute in bovine red blood cells. With respect to their influence on passive K^+ translocation, anesthetics can be divided into two groups. Neutral and anionic anesthetics cause in isotonic NaCl solutions a large K^+ loss and in hypotonic NaCl solutions a K^+ leakage which decreases rapidly with increasing cell volume. Cationic anesthetics have the opposite effect; no K^+ loss occurs at the optimal protective concentration, whereas at higher concentrations a K^+ leakage occurs which increases with increasing cell volume. The possible significance of these differences in action is discussed.

Anesthetics have several common effects on the membrane of red blood cells. They inhibit hemolysis in hypotonic solutions [1], cause hemolysis at higher concentrations [1], and are responsible for a decrease of cell deformability [2]. Membrane stabilization is coupled with a simultaneous drug-induced membrane expansion [1, 3–5]. In some cases other effects have been described, including anesthetic-induced rearrangement and aggregation of membrane particles as seen in freeze-etch electron microscopy [1, 6].

As Seeman pointed out [1], the effects of anesthetics on transmembrane transport are less clearly understood, due in part to the lack of distinction drawn between passive transport, active transport and facilitated diffusion. A general tendency seems to be drug-induced inhibition of facilitated diffusion and varying effects on passive fluxes [1].

In this context it was decided to study the influence of anesthetics on glycerol and K^+ translocation over the red cell membrane. K^+ translocation, measured as K^+ leakage from the cells, was chosen because previous investigations had revealed that the effect of dimethylsulfoxide on this passive transport depends on cell vol. [7]; the influence of anesthetics on translocation of glycerol in both human and bovine cell species was chosen because glycerol transport should be considered a facilitated diffusion in human red blood cells and passive translocation in bovine erythrocytes [8].

METHODS

Heparinized human and bovine blood were centrifuged shortly after collection. Red blood cell suspensions were prepared as described previously [7]. Osmotic fragility and protection against osmotic lysis by anesthetics were measured as described before [7]. K^+ in the medium was determined with a flame photometer. Two methods were used to measure glycerol permeability: the hemolysis technique as described by Naccache and Sha'afi [9] and the stopped-flow technique according to Owen *et al.* [10]. Similar results were obtained with both methods.

For technical reasons protection against osmotic lysis was measured at a cell concentration of 7 per cent, whereas glycerol permeability was measured with the hemolysis technique at a cell concentration of 0.12 per cent. Although the degree of osmotic hemolysis is influenced by cell concentration, this has no impact on the interpretation of the experimental results: in each set of experiments the results were compared with controls at the same cell concentrations.

RESULTS

For the anesthetics used in the present experiments the protective effect against osmotic hemolysis was measured at a final NaCl concentration of 0.41 per cent, giving 70–80 per cent hemolysis in the absence of anesthetics (Fig. 1). At relatively low drug concentrations the decrease of hemolysis is proportional to the drug concentration. For each drug the concentration at which hemolysis is reduced to about 50 per cent of the initial value was measured ($C_{50\%}$ in Fig. 1). In further experiments the half-$C_{50\%}$ of one anesthetic was supplemented with the half-$C_{50\%}$ of another anesthetic (Fig. 2). Within the experimental error the protective effects appeared to be additive for the anesthetics used (chlorpromazine, dibucaine, methanol, ethanol, *n*-butanol, butane-2,3-diol, octanol, urethane, dimethylsulfoxide and sodium dodecyl sulphate), with one exception. Sodium dodecylsulphate in combination with either chlorpromazine or dibucaine revealed a slight but significant potentiation, which effect is possibly related to the opposite charges of the combined anesthetics.

With respect to the influence on passive K^+ translocation the anesthetics can be divided into two groups. The neutral and negatively charged anesthetics showed the same effects as described previously

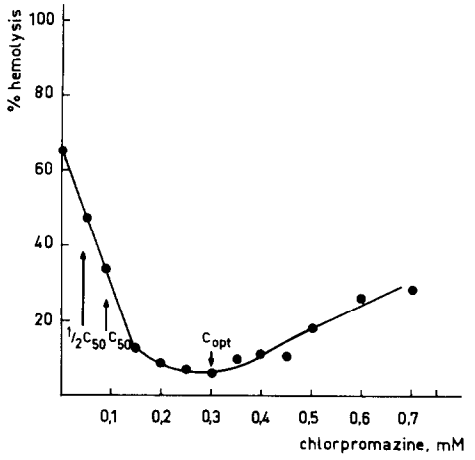

Fig. 1. Protection of a 7% erythrocyte suspension against osmotic hemolysis by varying concentration of chlorpromazine. The final NaCl concentration was 0.41%.

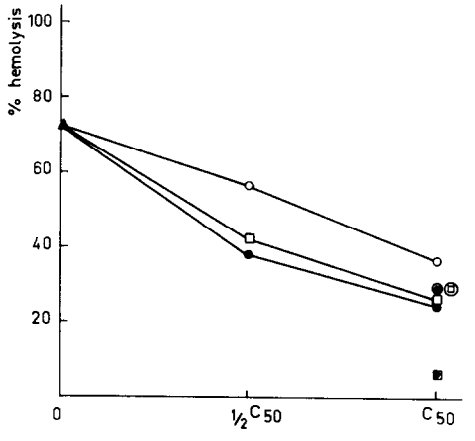

Fig. 2. Protection against osmotic hemolysis by butane-2,3-diol (○——○), sodium dodecyl sulphate (□——□), dibucaine (●——●) and combinations of 1/2 $C_{50\%}$ of butane-2,3-diol and dibucaine (⊙), sodium dodecyl sulphate and dibucaine (⊡) and sodium sulphate and butane-2,3-diol (◎).

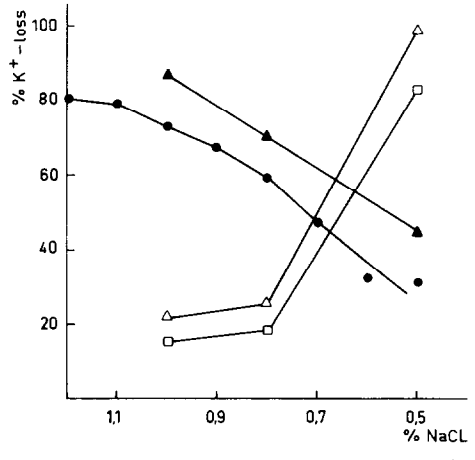

Fig. 3. The relationship between drug-induced K^+ loss from human red blood cells (1.7%) at 25° and the NaCl concentration in the medium. ▲——▲: 1.6×10^{-4} M sodium dodecyl sulphate; ●——●: 2.5×10^{-3} M octanol; △——△: 2×10^{-3} M dibucaine; □——□: 3.3×10^{-4} M chlorpromazine.

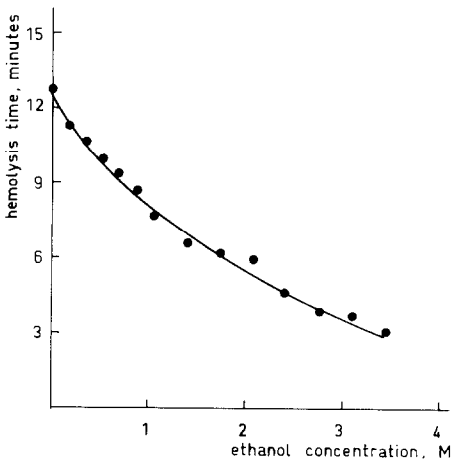

Fig. 4. Hemolysis time of bovine red blood cells (0.12%) suspended in 300 mM glycerol. With the other anesthetics similar results were obtained.

for dimethylsulfoxide [7]. At the optimal protective concentration these drugs provoked a K^+ loss that was very pronounced in isotonic NaCl solution, and decreased rapidly as cell vol. increased in hypotonic NaCl solutions (Fig. 3). The positively charged anesthetics, on the other hand, did not cause K^+ leakage at the optimal protective concentration. At slightly higher concentrations these anesthetics caused K^+ leakage, in this case increasing with increasing cell vol. (Fig. 3).

The effects on glycerol transport were similar for all anesthetics tested. Glycerol transport was stimulated in bovine red blood cells (Fig. 4). In human erythrocytes, however, glycerol transport was strongly impeded at relatively low anesthetic concentrations. At higher concentrations the inhibition was reversed (Fig. 5). In the presence of Cu^{2+} the facilitated diffusion of glycerol in human red blood cells is strongly retarded [8]. The influence of anesthetics on this Cu^{2+}-inhibited transport is also shown in Fig. 5. Under these experimental conditions the anesthetics caused a marked stimulation of glycerol transport.

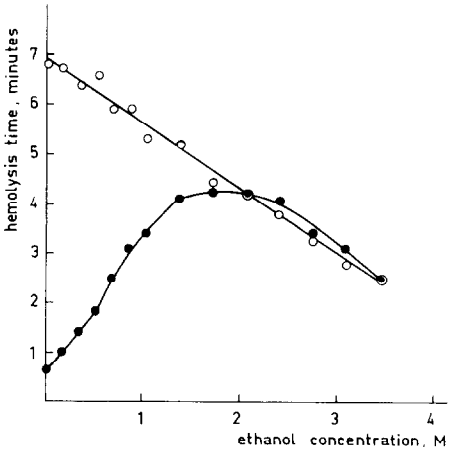

Fig. 5. Hemolysis time of human erythrocytes (0.12%) suspended in 300 mM glycerol in the absence (● ●) and in the presence (○ ○) of 10^{-5} M $CuSO_4$.

DISCUSSION

Although anesthetics comprise a wide variety of chemically unrelated compounds, experiments with combinations of anesthetics indicate that in general there are no separate mechanisms of action of special classes of anesthetics with respect to the effect of anesthesia [1]. This conclusion was reached a.o. from the mutual additive effects of these drugs. However, despite many studies on this subject, the mechanism of action of anesthetics is not yet clear.

It appeared that a similar additive effect existed for the anesthetics used in the present experiments relating to protection against osmotic hemolysis of human erythrocytes (Fig. 2). If an analogy can be drawn with respect to this effect, then the anesthetics share a common mechanism of action.

The effects of glycerol permeability in human red blood cells comply with the general pattern of inhibition of facilitated diffusion by anesthetics. Glycerol transport in bovine red blood cells and in Cu^{2+}-treated human erythrocytes can be considered as passive flux. The passive flux of this highly hydrophilic permeant is strongly accelerated by all anesthetics used in these experiments. The curves in Fig. 5 indicate that the reversal of inhibition of glycerol transport in normal human erythrocytes can be interpreted as accelerated passive diffusion, superimposed on the inhibited facilitated diffusion.

An indication of possible separate mechanisms of action of anesthetics emerges from the effects on K^+ leakage. The cationic anesthetics have a different effect from the neutral and anionic drugs. At the optimal protective concentration no drug-induced K^+-loss was observed, but at higher concentrations a K^+ leakage appeared which increased with increasing cell vol. The effect of the other anesthetics was quite different, with strong K^+ leakage at the protective concentration in isotonic medium and sharply decreasing K^+-loss in hypotonic medium (Fig. 3).

As noted previously for dimethylsulfoxide, the influence of the cellular vol. on the effects of anesthetics is presumably caused by a conformational change in receptor sites of the cell membrane [7]. Not only the dimethylsulfoxide-induced K^+-loss but also the effects of this drug on cell deformability were influenced by the cellular vol. [7]. According to Kregenow, such a volume-dependent conformational change in receptor sites is physiologically involved in the volume-controlling mechanism of red blood cells [11, 12].

The observation that a volume-dependent conformational change enhances K^+-loss, induced by cationic anesthetics, and inhibits K^+-loss caused by neutral and anionic anesthetics, may be of considerable importance. Elucidation of the mechanism of this physiologically important conformational change could lead to a better understanding of the effects of anesthetics on biomembranes.

Acknowledgements—We thank Miss K. Christianse for her skilful technical assistance. These studies were carried out under auspices of the Netherlands Foundation for Biophysics and with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

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