The release of acetylcholinesterase from human erythrocytes by sodium taurocholate

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Glycocholate and taurocholate have been shown (Coleman & Holdsworth, 1976; Billington & Coleman, 1978) to release significant amounts of membrane proteins and phospholipid from erythrocytes before the occurrence of significant cell lysis. Our particular interest was to study in detail the release of acetylcholinesterase from human erythrocytes.

Heparinized human blood was centrifuged at 2500 g for 10 min. After removing the plasma and buffy coat the cells were washed four times with approx. 30 vol. of 154 m~NaCl~/1.5 mm Bicine [\(\text{Na}^+\)-bis-(2-hydroxyethyl)glycinel buffer, pH 7.4. A 1:1 suspension of the washed cells in 105 m~NaCl~/50 mm-Bicine buffer (pH varied) was made. Of this suspension 1 vol. was incubated at 37°C (time varied) with 3 vol. of the above buffer containing different concentrations of sodium taurocholate (Wedde) Pharmaceuticals, Clwyd, N. Wales LL139 PX, U.K.). Supernatants were obtained by centrifuging at 14000 g for 2 min. Acetylcholinesterase activity in the supernatant was assayed spectrophotometrically (Ellman et al., 1961). The extent of erythrocyte lysis was determined by measuring the absorbance of the supernatants at 525 nm. The 100% values (acetylcholinesterase activity and haemoglobin) was determined on uncentrifuged controls. It was assumed that enzyme activity was not affected by the treatment. The optimum concentration of sodium taurocholate causing release of acetylcholinesterase is approx. 0.3% (w/v) (6 mM). Between about 0.3 and 0.8% haemolysis increases, whereas enzyme release remains roughly the same; above 0.8% the two processes parallel each other. The time course of the release process is complete in 20 min.

The extent of both acetylcholinesterase release and haemolysis was found to depend on the age of the erythrocytes in vitro. The older the erythrocytes (stored in their plasma at 4°C) the greater the amount of enzyme release and haemolysis. When the erythrocytes were stored as washed cells re-suspended in 105 m~NaCl~/50 mm-Bicine buffer, pH 7.4, the release of enzyme and haemolysis with age was even greater. Fig. 1 shows a typical result. The spontaneous release of acetylcholinesterase was found to be approx. 0.8% after 2 days, 1.1% after 3 days and 7.2% after 5 days. The corresponding haemolysis values were approx. 0.6%, 0.8 and 1.8%. Thus it seems that as the cells age, changes occur in the membrane that facilitate both the spontaneous and the detergent-induced release of proteins. These changes occur more readily in washed cells, probably because of the removal of plasma factors that stabilize the membrane.

Varying the pH of the incubation medium, using fresh erythrocytes, revealed that more acetylcholinesterase is released by 0.3% taurocholate in 20 min at pH 9.0 (27.15 ± 0.97%) than at pH 7.4 (19.7 ± 1.37%). This may reflect the greater efficiency of bile salts as detergents at alkaline pH values (Tzagoloff & Penefsky, 1971), although a pH effect on the membrane is also possible since more spontaneous release of enzyme occurs in cells stored at pH 9.0 than at 7.4.

On washing the detergent-treated erythrocytes, much more haemolysis occurred than was found in the first supernatant obtained after incubation with taurocholate. At pH 9.0, there was about 2% haemolysis in the first supernatant and about 10, 11 and 6% in subsequent washings. A possible explanation of this could be that the detergent actually has a stabilizing effect on the membrane (Helenius & Simons, 1975) and when it is washed off, the cells become more fragile. Including human albumin in the extraction medium at the concentration found in plasma (45 g/litre), drastically decreases acetylcholinesterase release and haemolysis. Adding albumin to the wash, after extraction, does not measurably decrease cell fragility, indicating that albumin is not an effective membrane stabilizer. Its action in the incubation medium could be by binding the detergent; Helenius & Simons (1975) have shown that albumin possesses high-affinity sites for detergents.

Fig. 1. Effect of erythrocyte age in vitro on acetylcholinesterase release and haemolysis by sodium taurocholate

Fresh human erythrocytes were washed and re-suspended in 105 m~NaCl~/50 mm-Bicine buffer, pH 7.4. They were incubated with sodium taurocholate at pH 7.4 and 37°C for 10 min and results are for 0 days (●, O), 2 days (■, △) and 5 days (▲, Δ) after initial washing. Closed symbols represent acetylcholinesterase release, open symbols represent haemolysis. Background acetylcholinesterase release and haemolysis was subtracted.

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