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# THE EFFECT OF GENERAL ANAESTHETICS ON ACTIVE CATION TRANSPORT IN HUMAN ERYTHROCYTES

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SUMMARY

The effects of the general anaesthetics ether and chloroform on the active cation transport in human erythrocytes are reported. The rate of active $K^+$ and $Na^+$ transport is enhanced by the presence of anaesthetics. The level of activation of the $Na^+$ pump depends on the internal $Na^+$ and the external $K^+$ concentrations and by varying these concentrations it was shown that the anaesthetics act by increasing the degree of activation of the active transport process. Anaesthetics do not affect the maximum possible pump rate.

## INTRODUCTION

Theories of general anaesthesia have frequently been based on the suggestion that anaesthetics perturb the transport of ions through cell membranes. The experimental evidence is often contradictory and some workers have proposed that anaesthetics inhibit transport[1,2] whereas others have suggested that the presence of anaesthetics facilitates ion transport[3]. Anaesthetics presumably inhibit cation fluxes across nerve cell membranes during the transmission of impulses since they block the transmission[4,5] and work on isolated toad bladder[6] and frog skin[7] preparations has indicated that active cation transport may be affected by general anaesthetics. In this paper we report on studies of the transport of $Na^+$ and $K^+$, both active and passive in human red blood cells in the presence of anaesthetic agents. The method is based on the fact that cold-stored erythrocytes lose $K^+$ and gain $Na^+$ (ref. 8). On incubation in the appropriate medium the cells re-establish normal cation levels by active transport. The extent of active transport under steady conditions may be determined by the use of the cardiac glycoside, ouabain which inhibits active transport but not passive ion movements[9]. In an earlier report[10] it was shown that no gross changes in the pattern of ion transport occur except in the presence of anaesthetic concentrations sufficient to cause significant haemolysis, though at lower concentrations some potentiation of active transport was noted. In this work the potentiation of the active transport was investigated in greater detail using two general anaesthetics, ether and chloroform.

## CATION TRANSPORT[11]

Since large concentration differences of $Na^+$ and $K^+$ exist between the inside and outside of the human red cell "passive" transport down the concentration

gradient inevitably occurs. This transport is independent of metabolism and is proportional to the concentration gradient across the membrane. Active transport on the other hand is the movement of ions against the concentration gradients and requires a supply of energy. The activity of the pump depends not only on the energy source but on the internal $Na^+$ and external $K^+$ concentrations. The pump is inhibited by cold and cardiac glycosides. The stoichiometry of the active transport process is such that 3 $Na^+$ are transported for every 2 $K^+$ (ref. 12).

A further type of transport, exchange diffusion, can be observed using radioactive tracers but not in the experiments carried out in this work. In this type of transport similar ions are transported across the membrane in both directions producing no net changes in intracellular ion concentrations. As this process exhibits many of the features associated with active transport it is believed that it operates by the same mechanism.

Two general models of active transport have been invoked. (1) Pore theory: in which access to an aqueous channel is controlled by an enzyme. (2) Carrier mechanism[13]: in which ions are transported combined with a suitable carrier molecule.

In practice it is difficult to discriminate between the two models.

## EXPERIMENTAL PROCEDURE

The experiments were performed with red blood cells obtained from whole blood (treated with acid–citrate–dextrose) that had been stored at 4–6 °C prior to use. The cold stored erythrocytes lose $K^+$ and gain $Na^+$. On incubation in the appropriate medium normal cation levels are restored by active transport.

Erythrocytes for the experiments were prepared by centrifuging the whole blood and removing the plasma and leucocytes. After a number of washings with isotonic phosphate buffer solution (pH 7) 1-ml aliquots of the cell suspension were added to 4 ml of incubation medium. The solutions were incubated at 37 ± 0.5 °C with gentle agitation at hourly intervals. Experiments were performed in the presence of ouabain and various anaesthetic agents. After incubation the samples were quenched in ice, centrifuged and washed carefully with 150 mM choline chloride solution (kept at 0 °C). The cells were haemolysed with concentrated ammonia and the resulting clear solution made up to 25 ml with distilled water. $Na^+$ and $K^+$ concentrations were determined using an EEL flame photometer previously calibrated using solutions of known ion concentrations. Haemoglobin was estimated spectrophotometrically after the addition of Drabkin's reagent to the solution.

The solutions used for incubation and washing were largely the so-called "Active" and "Passive" media recommended by Whittam[14]. In some of the experiments the composition of the incubation solution was modified as suggested by Post *et al.*[12] so that the levels of external $K^+$ concentrations could be varied in the range 0–15 mM. Isotonicity was maintained using choline chloride.

In order to enrich the internal $Na^+$ concentration for some of the experiments, separated and washed erythrocytes were stored in a high $Na^+/K^+$-free medium at 4–6 °C. Storage in such a medium increases the susceptibility of the cells to haemolysis but by frequent replacement of the storage solution a suitable increase in $Na^+$ content to as much as 0.13 μequiv/mg haemoglobin (approx. 42 mM/l of cells) can be achieved after about 8 days of the treatment[15]. The normal level of $Na^+$ within

cold-stored erythrocytes is 0.6–0.8 μequiv/mg haemoglobin (20–27 mM/l of cells).

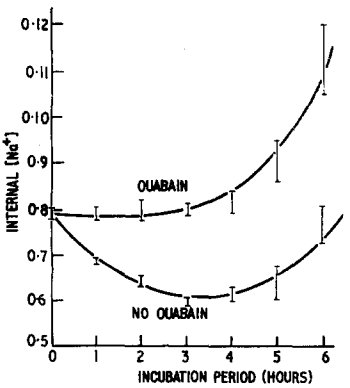
Experiments in the presence of various concentrations of ouabain added showed that a concentration of $5 \cdot 10^{-4}$ M was sufficient to totally inhibit active transport. Ouabain does not affect overall cation transport when the pump is already inhibited by the lack of the three pump requirements, external $K^+$, internal $Na^+$ and internal ATP. Thus it was concluded that ouabain does not alter passive cation transport.

## RESULTS

### *Changes in intracellular cation concentrations during incubation*

The cold-stored red blood cells lose $Na^+$ in the absence of ouabain. After 4–6 h incubation at 37 °C, depending on the age of the blood, haemolysis occurs accompanied by an increase in the sodium flux due to passive leakage[10] (Fig. 1). When studying active transport it is desirable that the difference between the 'ouabain' and 'no ouabain' curves should be as large as possible and an incubation period of 3 h was used in subsequent experiments.

Though it is not possible to determine the intracellular $K^+$ concentration with the same accuracy as the $Na^+$ (ref. 16), Fig. 2 shows that the $K^+$ is actively pumped out during incubation.



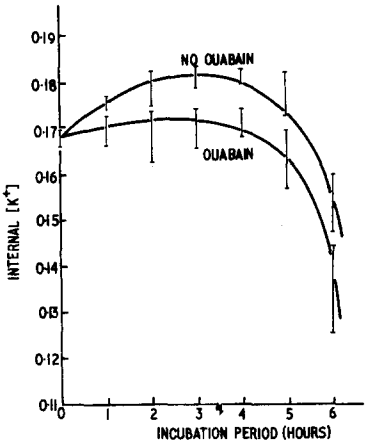




Fig. 1. Changes in intracellular $Na^+$ concentrations (μequiv/mg haemoglobin) during the incubation at 37 °C of cold-stored erythrocytes. Ouabain concn, $5 \cdot 10^{-4}$ M.

Fig. 2. Changes in intracellular $K^+$ concentrations (μequiv/mg haemoglobin) during incubation at 37 °C of cold-stored erythrocytes. Ouabain concn, $5 \cdot 10^{-4}$ M.

### *Dose–response curve for ether*

Fig. 3 shows the effect of varying concentrations of ether on the red cell $Na^+$ after 3 h incubation. The diagram shows four main features:

(i) At relatively low anaesthetic concentrations there is a decrease in membrane permeability (represented by a slight drop in the 'ouabain' curve).

(ii) At higher concentrations of anaesthetic the permeability of the cells shows a marked increase.

(iii) At relatively low anaesthetic concentrations there is a distinct stimulation of the $Na^+$ pump (represented by the maximum in the $Na^+$ pump curve which is the difference between the 'ouabain' and 'no ouabain' curves). Calculation of the $Na^+/K^+$ active flux gave the ratio 1.5 ± 0.3, in agreement with the generally accepted value 1.50 ± 0.06 (ref. 12). Thus anaesthetics do not appear to affect the stoichiometry of the pump.

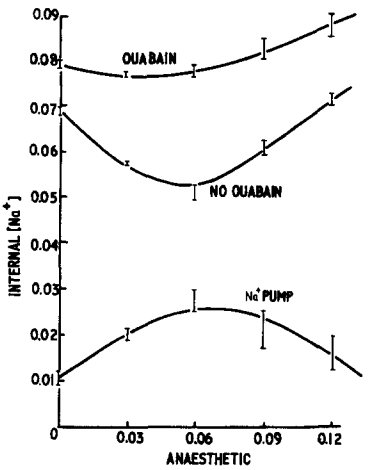



Fig. 3. Changes in intracellular $Na^+$ concentrations ($\mu$equiv/mg haemoglobin) after 3 h incubation at 37 °C in the presence of various concentrations of ether (arbitrary units).

(iv) At higher concentrations the $Na^+$ pump appears to be depressed, however, as at these concentrations there is a large increase in the leakage of ions and significant haemolysis, the determinations of the pump activity became increasingly uncertain.

It should be noted that even the relatively low concentrations of ether referred to above are high compared with the anaesthetic doses that are required to produce loss of consciousness in mammals (*e.g.* maximum stimulation of the $Na^+$ pump occurred at 0.14 atm partial pressure for ether compared with a normal clinical dose which is equivalent to 0.03 atm).

*Effect of external $K^+$*

The $Na^+$ pump is sensitive to the levels of external $K^+$ and internal $Na^+$ concentrations. Fig. 4 shows the effect on the sodium pump of external $K^+$ (isotonicity maintained with choline chloride). In this experiment the internal $Na^+$ concentration was typical of that of stored cells (approx. 23 mM/l of cells) and below the level required for maximum stimulation of the pump. Variation of external $K^+$ did not affect passive transport.

The effects of anaesthetics on the $Na^+$ pump in media containing 3 and 15 mM $K^+$ were investigated. These concentrations represent half-maximum and maximum activation with respect to the external $K^+$ concentration. The internal $Na^+$ concentration was the same for both sets of experiments. Fig. 5a illustrates results for a typical pair of runs. The stimulation of the pump due to the anaesthetic was less when the pump was more highly activated by high external $K^+$ concentrations.

*Experiments with Na+-enriched cells*

Maximal pump activity with respect to both internal $Na^+$ and external $K^+$ can only be obtained with $Na^+$-enriched cells. These were prepared by the method of Post[17], as described in the experimental section above. Half-maximal activity with respect to $Na^+$ occurs at about 0.06 $\mu$equiv/mg haemoglobin (approx. 20 mM/l of cells). Storage for 8 days in $Na^+$ enrichment medium led to a concentration of

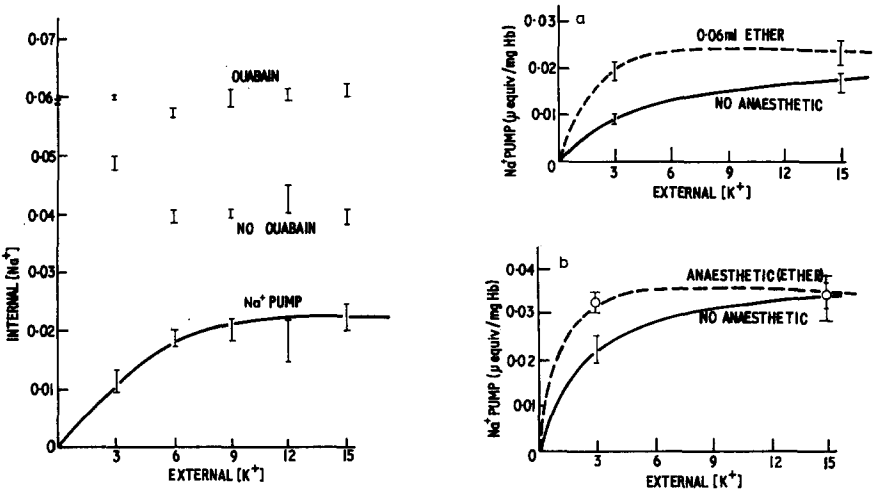



Fig. 4. Effect of external $K^+$ concentrations (mM) on the active transport of $Na^+$ ($\mu$equiv/mg haemoglobin). Incubation: 3 h at 37 °C.

Fig. 5. (a) Effect of anaesthetics on active $Na^+$ transport ($\mu$equiv/mg haemoglobin in 3 h) at various concentrations of external $K^+$ (mM) for cells stored normally (internal $Na^+$ concentration, 23 mM/l of cells). (b) Effect of anaesthetics on active $Na^+$ ($\mu$equiv/mg haemoglobin in 3 h) at various concentrations of external $K^+$ (mM) for $Na^+$-enriched cells (internal $Na^+$ concentration, 40 mM/l of cells). Incubation in both cases: 3 h at 37 °C.

approx. 0.12 $\mu$equiv/mg haemoglobin (approx. 40 mM/l of cells). Experiments to test the effects of anaesthetics on the pump showed that it was stimulated when the external $K^+$ was 3 mM (half maximal); but in the 15 mM $K^+$, when the pump activity was maximal, there was no significant difference in the samples incubated with and without anaesthetic (Fig. 5b).

*Results with chloroform*

Experiments with chloroform showed similar features to those carried out with ether. Chloroform is effective at lower concentrations but causes haemolysis more readily at doses which produce equivalent effects on the $Na^+$ pump. As indicated in Table I, both anaesthetics required considerably higher than clinical doses to produce maximum stimulation of the $Na^+$ pump. The relative potency of the two agents, approx. 3, for the $Na^+$ pump stimulation is comparable to that observed in mammalian experiments (*e.g.* loss of righting reflex in mice).

TABLE I

EFFECTS OF CHLOROFORM AND ETHER

| | *Maximum stimulation of $Na^+$ pump* | | *Clinical dose for righting reflex in mice* |
|---|---|---|---|
| | *Molarity* | *Partial pressure (atm)* | *Partial pressure (atm)* |
| Ether | 0.089 | 0.14 | 0.03 |
| Chloroform | 0.0068 | 0.05 | 0.008 |

DISCUSSION

The results confirm earlier conclusions that the activity of the sodium pump may be enhanced by the presence of general anaesthetics. This enhancement depends on the degree to which the pump is activated in that when the pump is working at its maximum level anaesthetics cause no change in the active ion transport. It is only when the levels of external $K^+$ and internal $Na^+$ are such that the pump activity is sub maximal the presence of anaesthetics can result in increased active transport of the cations. Thus the general anaesthetics investigated would appear to modify the way in which the $Na^+$ pump is regulated rather than the pump 'mechanism' itself. As the mode of operation and the regulation of the pump are still not understood it is not possible to speculate on the mode of action of anaesthetics on the system.

Results on the $Na^+$ transport in other preparations are contradictory. Gottlieb and Saveran[7] using an inverted frog skin preparation found that the $Na^+$ pump was inhibited by pressure above 6.8 atm. of $N_2O$. No stimulation of the pump was reported. Recent work on sodium transport in the isolated toad bladder[6] showed that the anaesthetics cyclopropane, $N_2O$, ether and halothane all exert a biphasic effect on sodium transport similar to those observed in the red blood cells.

The concentrations of anaesthetics required to perturb both the active and passive transport of cations through the red blood cell membrane are considerably greater than the doses required to produce loss of consciousness in mammals. There is therefore no reason to suppose that the effects observed in red blood cells are relevant to the mechanism of general anaesthesia. Nevertheless it is interesting to note that the relative potencies of the anaesthetics investigated are comparable for sodium pump activation and the induction of general anaesthesia in mammals. However, the role of the membrane, which plays an important part in most proposed mechanisms of general anaesthesia, is not clarified by this work. The results give no indication that a general perturbation of membrane processes is an essential feature of the mode of action of general anaesthetics at clinical concentrations. This does not conflict with evidence that general anaesthetics may influence the change of permeability to $Na^+$ in membranes of excitable tissues which accompany the action potential. The results do suggest, however, that the mechanism of general anaesthesia is more specific than has frequently been supposed and is not related necessarily to the ability of these agents to perturb cation fluxes through biological membranes in general.

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