Anaesthetic mechanisms

from C. D. Richards

ANAESTHETICS affect every variety of living system, from microorganisms to mammals; they are very nonspecific drugs which depress a whole battery of cell functions including cell motility, cell division, photosynthesis, oxidative metabolism and electrical excitability. Their mode of action poses one of the fundamental problems in biology. Their clinical value rests solely on their ability (at low concentrations) to impair certain higher nervous functions such as consciousness and memory without disrupting the function of the heart and respiratory system.

Four key observations suggest that anaesthesia is the result of some physical interaction of the anaesthetic with certain constituents of the cell rather than a specific chemical reaction. First, the effects of anaesthetics are freely and rapidly reversible; second, the potency of an anaesthetic is directly related to its lipid solubility; third, anaesthetic properties are shown by a wide variety of chemically unrelated substances; and fourth, the effects of anaesthetics can be reversed by high pressures.

The critical volume hypothesis first discussed by Mullins more than 20 years ago (*Chem. Rev.*, **54**, 289; 1954) seems to be the most successful of the various hypotheses proposed to explain the action of anaesthetics. In its simplest form it proposes that anaesthesia (narcosis) occurs whenever a critical fraction of anaesthetic has been achieved in the membranes of the cell; this critical volume is held to be dependent on the species under study but independent of the nature of the anaesthetic.

In its original form the critical volume hypothesis added little to the well established Meyer–Overton concept of a role for lipid solubility in the mechanism of anaesthetic action, but recent investigations of the reversal of anaesthesia by pressure by Miller *et al.* (*Nature*, **231**, 368; 1971; *Molec. Pharmac.*, **9**, 131; 1973) have led to a more satisfying model. They propose that anaesthetic dissolves in some hydrophobic region of the cell—presumably lipid—causing that region to expand, thereby impairing some vital function. From the amount of pressure required to reverse the effects of various doses of anaesthetic they calculated that during general anaesthesia the hydrophobic region should expand by about 0.4% (v/v). This prediction has subsequently been verified by Seeman and Roth (*Biochim. biophys. Acta*, **255**, 171; 1972) for erythrocyte membranes.

This still leaves four important questions. How is the membrane expansion brought about? How does the membrane expansion lead to impaired membrane function? Which of the many functions of the membrane are impaired by anaesthetics? Does anaesthesia result from the impairment of one or several specific functions?

Membrane effects

Metcalfe *et al.* (*Mol. Pharmac.*, **4**, 87; 1968) found that benzyl alcohol which can act as an anaesthetic increased the fluidity of biological membranes. This observation was later extended by Trudell *et al.* (*Biochim. biophys. Acta*, **291**, 328; 1973) to include clinically important anaesthetics, such as halothane. It was subsequently proposed that anaesthetics cause membrane expansion by increasing the disorder of the fatty acid chains of the phospholipids of the membrane bilayer. During general anaesthesia, anaesthetics occupy only 0.2% (v/v) of the membrane whereas membrane expansion is of the order of 0.4% Seeman has shown, however, that artificial phospholipid bilayers only expand by 0.2% and so he proposed that changes in the conformation of the membrane proteins are the cause of the large expansion of natural membranes (*Experientia*, **30**, 759; 1974). Furthermore, in a recent paper Boggs *et al.* (*Molec. Pharmac.*, **12**, 127; 1976) have shown that concentrations of anaesthetic sufficient to cause general anaesthesia or which block the conduction of impulses along a nerve ("local anaesthesia") have little or no effect on the fluidity of artificial membranes. Although such observations cast doubt on the view that anaesthetics impair membrane function through a generalised perturbation of the structure of the lipid membrane they are consistent with the view that the interface between lipid and protein may be the site of anaesthetic action.

There is now evidence to suggest that at least some membrane-bound proteins require the presence of a layer of tightly bound lipid to stabilise them in their active form. This has been shown for cytochrome oxidase (Jost *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 480; 1973) and for the calcium transport protein of the sarcoplasmic reticulum (Warren *et al.*, *Nature*, **255**, 684; 1975). By studying the effects of temperature on the calcium transport protein reconstituted in defined lipid mixtures, Warren *et al.* (*Biochemistry*, **13**, 5501; 1974) showed that the enzymatic activity of the complex decreased as the lipid became more crystalline. This suggests that the activity of this protein is governed by the fluidity of the lipid in which it is embedded. In the light of this data, Lee (page 545, this issue of *Nature*) has proposed that the sodium channel in nerve membranes may be surrounded by a ring of rigid lipid molecules that serves to keep it open. He suggests that anaesthetics block nerve impulse conduction by fluidising this boundary lipid so causing the sodium channel to collapse inwards. One consequence of the model is that a nerve blocked by an anaesthetic should have its conduction restored when the temperature is lowered, and this has indeed been shown for the action of ethanol on squid axon (Spyropoulos, *J. gen. Physiol.*, **40**, 849; 1957).

Specific interactions

It is not difficult to envisage other membrane proteins such as receptors being similarly surrounded by boundary lipid which serves to keep the protein poised in an optimal conformation. The fact that some anaesthetics affect one function rather than another could then be explained by relatively specific interactions either with the boundary lipid or with the individual protein. Certain volatile anaesthetics have been shown to inhibit the bacterial enzyme luciferase apparently by competing for the substrate binding site (White, in *Molecular Mechanisms in General Anaesthesia*, Ch. 13, Churchill–Livingstone, London, 1974; Middleton and Smith, *Proc. R. Soc. Lond.*, **B193**, 173; 1976).

Moreover, specific interactions of this sort could account for the antagonism of the depressant effects of the steroid anaesthetic alphaxalone by the non-anaesthetic steroid Δ16 alphaxalone observed in isolated preparations of mammalian brain tissue (Richards and Hesketh, *Nature*, **256**, 179; 1975). Nonetheless, such ideas must remain speculative until it becomes possible to isolate receptors and reconstitute them in artificial membranes of defined composition. With the recent developments in understanding the role of lipid-protein interactions in membrane biochemistry, the next period of anaesthetic research should be an exciting one. □