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EFFECTS OF HALOTHANE ON THE INCORPORATION OF [^{14}C]-SERINE INTO PHOSPHOLIPID IN THE GUINEA-PIG ILEUM

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1 The effects of halothane on the incorporation of L-[3-^{14}C]-serine into phospholipid were studied in the resting, innervated longitudinal muscle preparation of the guinea-pig ileum.

2 The anaesthetic, at clinical concentrations, caused a dose-dependent, partial inhibition of incorporation. The effect was rapid and reversible, and did not show characteristics of competitive inhibition.

3 The incorporation was reduced by a decrease in the Ca^{2+} concentration of the Krebs incubation buffer. Part of the activity persisted in the absence of added Ca^{2+} and this was most susceptible to inhibition by halothane. Sensitivity to external Ca^{2+} was not influenced at the halothane concentrations studied.

4 Evidence in support of the ^{14}C-incorporation being due to L-[3-^{14}C]-serine-phospholipid base-exchange activity included: (a) the rapid appearance of radioactivity in phosphatidylserine; (b) the kinetics of this incorporation in relation to that in phosphatidylethanolamine; (c) its dependence on Ca^{2+}, and (d) its insensitivity to 2,4-dinitrophenol and its sensitivity to temperature.

5 It is concluded that this preparation makes it possible for a membrane-bound lipid-dependent activity (L-serine-phospholipid base-exchange) to be studied in conditions of cellular integrity under which the normal functional effects of lipophilic drugs can be simultaneously tested.

6 A rapid gas-chromatographic assay for halothane from an aqueous medium is also described.

Introduction

There is now good evidence that lipophilic drugs, particularly anaesthetics, act by solution in hydrophobic cellular structures, usually the cell membrane. Much of this evidence is physicochemical or biophysical in character (Miller, Paton, Smith & Smith, 1973; Miller & Miller, 1975; Gage, McBurney & Schneider, 1975; Adams, 1976). Effects of lipophiles on membrane-related biochemical processes have been less extensively studied (Miller, 1975; Lenaz, Curatola, Mazzanti, Parenti-Castelli, Landi & Sechi, 1978; Taussig, 1979) despite the likelihood that the metabolism of lipid constituents might be particularly sensitive to lipophilic interference and that any process which is critically dependent on its lipid environment might be affected.

This paper describes the effects of the lipophilic anaesthetic, halothane, on the incorporation of radioactively labelled L-serine into phospholipid. This incorporation is dependent on membrane-associated processes and in mammalian tissues the immediate formation of the product, phosphatidylserine, is believed to be totally dependent on the membrane-bound activities of phospholipid base-exchange (Thompson, 1973; Arienti, Brunetti, Gaiti, Orlando & Porcellati, 1976; Miura & Kanfer, 1976). Such a process involved in membrane phospholipid metabolism could therefore be a sensitive indicator of lipophilic interference.

It was a particular objective of these experiments to study the base-exchange dependent process of incorporation, not as is usually done, in fragmented tissues, but in an intact tissue, so that the effects observed could be subsequently correlated with physiological responses. For this purpose the guinea-pig ileum strip was used. This tissue, while less complex than the CNS, shares a number of similarities, including cholinergic transmission and sensitivity to opiates. Its functional activity is now well established since Paton & Zar (1968) showed that the source of acetylcholine causing the smooth muscle of the ileum to contract was the nervous tissue normally present in the longitudinal muscle strip. Although the effects of anaesthetics on this preparation do not enable predictions to be made about the effects of anaesthetics in the whole animal (Rang, 1964), Speden (1965) showed that with halothane, for example, there was good agreement in the concentrations required for its depressant effect on functional activity in the ileum *in*

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vitro and those required to produce anaesthesia *in vivo*. Its action on the ileum was shown to be due to simultaneous effects on the post-ganglionic nerve fibres and on the muscle itself. Part of this work has been described to the Biochemical Society (Wing & Paton, 1980).

Methods

L-[3-^{14}C]-*serine incorporation*

Fed guinea-pigs (400 to 600 g) of either sex were supplied by the local University Farm. Strips of innervated longitudinal smooth muscle from the ileum were prepared as described earlier (Paton & Zar, 1968). Twelve strips weighing approximately 25 mg each were usually obtained from one ileum. Each strip was incubated at 37°C in 4.4 ml Krebs buffer solution (ionic composition as in Rang, 1964), gassed with 95% O_2 and 5% CO_2, contained in a screw-capped glass vial. To maintain concentrations of halothane, when present in the incubated buffer, close to those concentrations at the start of the incubation, the residual gas space in the vial was less than 0.4 ml, or less than 10% of the total volume of the aqueous phase. A teflon-coated, silicone disc sealed the hollowed centre of the cap. The tissue and buffer, with or without modifications, were equilibrated in the enclosed vial for 15 min, then L-[3-^{14}C]-serine in saline (0.9% w/v NaCl solution) was injected in μl quantities through the disc into the Krebs solution and was well mixed. Incubations in the presence of L-[3-^{14}C]-serine proceeded for periods up to 2 h. When halothane was required in the Krebs buffer, the anaesthetic liquid was added to a large excess of the pre-gassed buffer in an enclosed glass jar at room temperature. The liquids were equilibrated for 4 h with regular vigorous mixing. Samples of the upper layer of buffer containing dissolved halothane, assayable as described below, were used either undiluted or diluted with buffer not containing halothane.

In early experiments, before the introduction of halothane in this study, strips were incubated in 2 ml Krebs buffer. Data from these were plotted in Figures 1 and 2 only. Later experiments showed that similar results were obtained using the standard volume of 4.4 ml (e.g. compare Figure 2 with the controls in Figure 6).

Tissue extractions and lipid chromatography

At the end of the incubation, the muscle strip was rinsed well in saline (0.9% w/v NaCl solution), blotted and weighed. It was then homogenized in methanol with a hand-operated ground-glass homogenizing tube and pestle. Chloroform was added to give a 2:1 (v:v) mixture and lipids were extracted according to the procedure of Steinberg & Durell (1971), based on the method of Folch, Lees & Sloane-Stanley (1957), the extract being washed with 0.2 vols 0.73% w/v aq. NaCl and repeatedly with 0.2 vols methanol: 0.58% w/v aq. NaCl: choloroform (48:47:3, by volume). This extract was taken to dryness under nitrogen and the lipids were dissolved in 100 μl chloroform. When chromatography of the lipids was applied, 20 μl samples were then spotted onto Whatman SG-81 silica-gel loaded paper and chromatographed in a solvent system containing chloroform: methanol: 5 N ammonia (64:34:4, by volume) using rhodamine 6G (1.2×10^{-3}% w/v) as dye with visualization in ultra-violet light (Wuthier, 1976). This system, used with standards for reference, gave separation of most of the main phospholipids, but some overlap of the phosphatidylserine and sphingomyelin spots occurred. Since ^{14}C-radioactivity can be specifically incorporated from L-[3-^{14}C]-serine into sphingomyelin through the synthesis of sphingosine (Thompson, 1973), chromatograms from control incubations were run using the solvent mixture chloroform: methanol: acetone: acetic acid: water (50:16:20:15:5, by volume) to give separation of phosphatidylserine from sphingomyelin (Wuthier, 1976). Less than 1.5% of the total radioactivity incorporated into lipid was present in sphingomyelin.

Assay of radioactivity

Either 20 μl aliquots of the total lipid extract applied to the SG-81 paper or the individual phospholipid spots cut from the chromatogram were counted in scintillation fluid containing 4 g PPO/l toluene (scintillation grade) using a Philips PW4510/01 Automatic Liquid Scintillation Analyser. The counts were corrected to 100% efficiency by the channels ratio method. Results are expressed per 100 mg wet weight of tissue.

Assay of halothane

Because the nominal concentration of halothane to which the tissue was exposed could be lowered by evaporative loss into vial headspace or otherwise, or by tissue uptake, an assay for halothane was developed. Samples of Krebs buffer containing halothane in solution were shaken with half their volume of benzene, to which an internal standard, dichloromethane (concentration 1 μl/ml benzene) had been added. Halothane was quantitatively extracted into the upper benzene layer, samples (up to 1 μl) of which were assayed for the anaesthetic using the Varian 2400 gas chromatograph fitted with flame ionization detectors and 2 m × 2 mm (i.d.) glass columns packed with 80–100 mesh Porapak Q. The injector and detector block temperatures were 195°C and the nitrogen flow rate was 30 ml/min.

Peak-height ratios for halothane: dichloromethane were measured and these gave a straight-line relationship through the origin when plotted against halothane concentration. In the present study, in which quantities of material for assay were not limiting, 1 to 5 μg halothane were normally subjected to gas chromatography. The mean value for the peak-height ratio of between five and ten determinations had a standard error of ± 1.0%.

Materials

L-[3-^{14}C]-serine (specific activity, 56 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks, halothane ('Fluothane') was from ICI Ltd., L-serine and 2,4-dinitrophenol (DNP) were from the Sigma Chemical Co. Ltd., and 2,5-diphenyloxazole (PPO) and phospholipid standards for chromatographic reference were from Koch Light Labs. Ltd. Porapak Q was supplied by Waters Associates, Inc., Maple St., Milford, Mass. 01757, U.S.A. All other chemicals were purchased from BDH Ltd. and were of 'Analar' grade.

Statistics

Mean values are given ± s.e. mean with the number of tests in parentheses. In Table 1 the significance of the differences between two means was tested by Student's *t* test. A value for $P < 0.05$ was taken as significant.

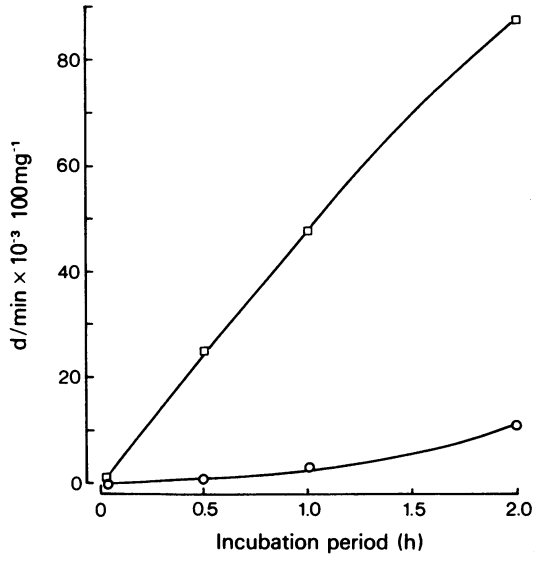

Figure 1 Effect of incubation period on L-[3-^{14}C]-serine incorporation into phosphatidylserine and phosphatidylethanolamine. The incorporation of L-[3-^{14}C]-serine (4.5 μM) into phosphatidylserine (□) and phosphatidylethanolamine (○) was measured as described in Methods. The data represent analyses of four separate strips, each incubated for a different period.

Results

L-[3-^{14}C]-serine incorporation into phospholipid

The innervated longitudinal muscle of the guinea-pig ileum incorporated ^{14}C-radioactivity from L-[3-^{14}C]-serine into lipid; 85.5% (± 1.4, s.e. mean) of the total radioactivity incorporated during routine incubations was present as phosphatidylserine. Figure 1 shows that this incorporation was linear for a period of at least 2 h, with a late, small increase in radioactivity appearing in the second most labelled fraction, phosphatidylethanolamine. Less than 7% of the total radioactivity incorporated into lipid appeared in other fractions, so in most experiments individual phospholipids were not routinely separated.

By raising the concentration of the substrate, L-serine, the rate of its incorporation into lipid rose to a plateau level (Figure 2a). This pattern of increase followed a hyperbolic curve as indicated by the straight-line relationship when a double reciprocal plot was used (Figure 2b).

When the incubation temperature was lowered to 25°C, there was almost a halving of the rate of incorporation of L-[3-^{14}C]-serine (0.75 μM), mean activities at 25°C being 54.4% ± 1.36 of those at 37°C during a 1.5 h incubation period. Linearity of incorporation at this L-serine concentration, which was used in subsequent experiments, was maintained over the time course at both temperatures.

There was no significant inhibition of L-serine incorporation in the presence of DNP (Table 1). The concentration of DNP used was sufficient to uncouple oxidative phosphorylation in muscle (Beresford, 1978). In a further experiment no significant effect of ouabain was found (Table 1).

The influence of Ca^{2+} concentration on L-[3-^{14}C]-serine incorporation is shown later in the control experiments of Figure 5. A reduction in incorporation occurred as the Ca^{2+} concentration in the buffer was lowered, and it was lowest when, without added Ca^{2+}, the chelating agent EGTA was present. In experiments without Ca^{2+}, replacement of the divalent cation with Mg^{2+} (2.54 mM) did not bring about any increase in the low rate of L-serine incorporation.

Chromatography of the phospholipids when the rate of incorporation was low, as in the presence of EGTA, showed that radioactivity was still found predominantly in the fraction containing phosphatidylserine.

Table 1 The effects of 2,4-dinitrophenol (DNP) and ouabain on the rate of L-[3-^{14}C]-serine incorporation into lipid

	d/min × 10^{-3} 100 mg^{-1} h^{-1}
Expt. 1	
Control	12.88 ± 1.12 (6)
2,4-Dinitrophenol (0.05 mM)	11.99 ± 0.70 (6)^{NS}
Expt. 2	
Control	14.29 ± 2.80 (5)
Ouabain (1.0 μM)	12.75 ± 2.07 (5)^{NS}

2,4-Dinitrophenol (0.05 mM) and ouabain (1.0 μM) were present, as indicated, in the incubations of the strips. The incorporation of L-[3-^{14}C]-serine (0.75 μM) into lipid in 1 h was measured as described in Methods. Mean values ± s.e.mean are given, with the number of tests in parentheses. ^{NS} = not significant.

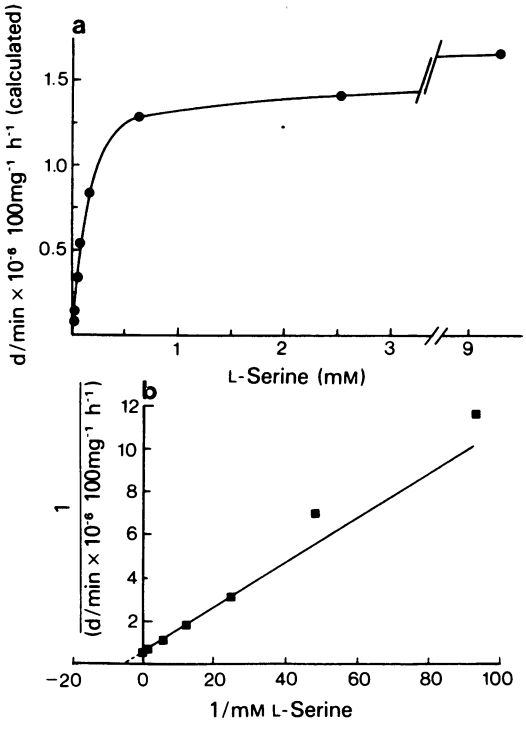

Figure 2 Effect of L-serine concentration on the rate of incorporation of L-[3-^{14}C]-serine into lipid. (a) Each strip was incubated for 2 h in the presence of both L-[3-^{14}C]-serine (0.75 μM) and unlabelled L-serine to give the final concentrations shown. The incorporation of L-[3-^{14}C]-serine into lipid was measured as decribed in Methods. The rate of incorporation of radioactivity was calculated to a constant specific activity for L-[3-^{14}C]-serine of 56 mCi/mmol, consistent with other data presented. The calculation was made from the observed rate of incorporation of radioactivity at each concentration of L-serine tested. Each value is the mean of two tests. (b) Double reciprocal plot of (a). The line was fitted by linear regression analysis by the method of weighted least squares (Roberts, 1977).

Effects of halothane

In control experiments, because of the volatility of halothane, and possible reduction of initial concentrations by tissue uptake, the concentrations of halothane in the buffer were measured during the course of the incubations, both to obtain an accurate value corrected for any losses, and to verify that any particular concentration was maintained for the duration of the radioactive incorporation studies. The results, both in the presence and absence of tissue, are shown in Figure 3. After the initial equilibrium period of approximately 15 min, the halothane concentration was stable in incubations at the lower doses, and only further slight falls were recorded at the higher doses. In the presence of tissue at the higher doses of halothane a proportionately lower concentration of the anaesthetic remained in the buffer than in the absence of tissue, indicating that a considerable uptake of halothane by the tissue is possible (up to 3.5% by weight in the reported experiments). Other tests showed that the halothane concentrations in the buffer were not affected by the absence of Ca^{2+} or the presence of EGTA during the incubations.

The effect of this range of halothane concentrations on L-[3-^{14}C]-serine incorporation is shown in Figure 4. An inhibitory effect was found at all concentrations studied, including the range (between 5 and 25 mg%) necessary in blood for the maintenance of different anaesthetic states (Paton & Payne, 1968). The effect of the anaesthetic is rapid: thus the degree of inhibition of L-[3-^{14}C]-serine incorporation, during a 30 min incubation, was only 10% greater after a 15 min preincubation of tissue in the presence of halothane than when, without preincubation, tissue was added to buffer containing both halothane and L-[3-^{14}C]-serine. The inhibitory effects of halothane (at 4.2 mM) are also reversible. For this test, tissue was incubated in Krebs buffer containing 4.2 mM halothane for the combined duration of a preincubation (15

min) and a test incubation (1 h) period, it was then transferred for 1 h to buffer without halothane but containing L-[3-^{14}C]-serine; the normal inhibition of incorporation seen with 4.2 mM halothane was found to be abolished.

The influence of Ca^{2+} concentration on L-[3-^{14}C]-serine incorporation in the presence of halothane is shown in Figure 5. The pattern of reduced incorporation as the Ca^{2+} concentration in the buffer was lowered was similar to that seen in the absence of the anaesthetic. However, in the presence of halothane the rates of incorporation were consistently lower at all Ca^{2+} concentrations, including the experiments in which Ca^{2+} was not added to the buffer and when EGTA was present. The degree of inhibition was greater with 4.2 mM halothane than with 1.6 mM, consistent with the results already described.

The inhibitory effect of halothane was studied over a range of higher L-serine concentrations. The effects of two doses of the drug are shown in Figure 6a, and are represented by a double reciprocal plot in Figure 6b.

Discussion

L-[3-^{14}C]-*serine incorporation into phospholipid*

The properties of the incorporation indicate that a process of phospholipid base-exchange was being observed. Incorporation into phospholipid was primarily to form phosphatidylserine, with no evidence of L-[3-^{14}C]-serine having significant gluconeogenic activity. The lack of inhibition by DNP and the sensitivity to a specific alteration in the buffer Ca^{2+} concentration are consistent with the properties of phospholipid base-exchange, a process not dependent on mitochondrial adenosine triphosphate (ATP) pro-

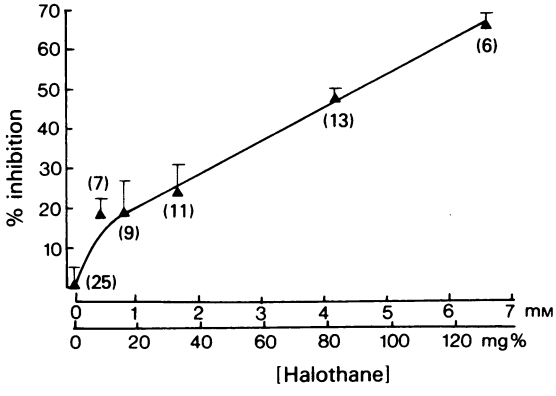

Figure 4 Effect of halothane concentration in the buffer on the rate of L-[3-^{14}C]-serine incorporated into lipid. Strips were incubated with different halothane concentrations in the buffer (the initial concentrations are plotted) and the observed inhibitory effect on the incorporation of L-[3-^{14}C]-serine (0.75 μM) into lipid in 1 h was expressed as a percentage of the control in the absence of halothane. Mean values are given (vertical lines show s.e.mean), with the number of tests in parentheses.

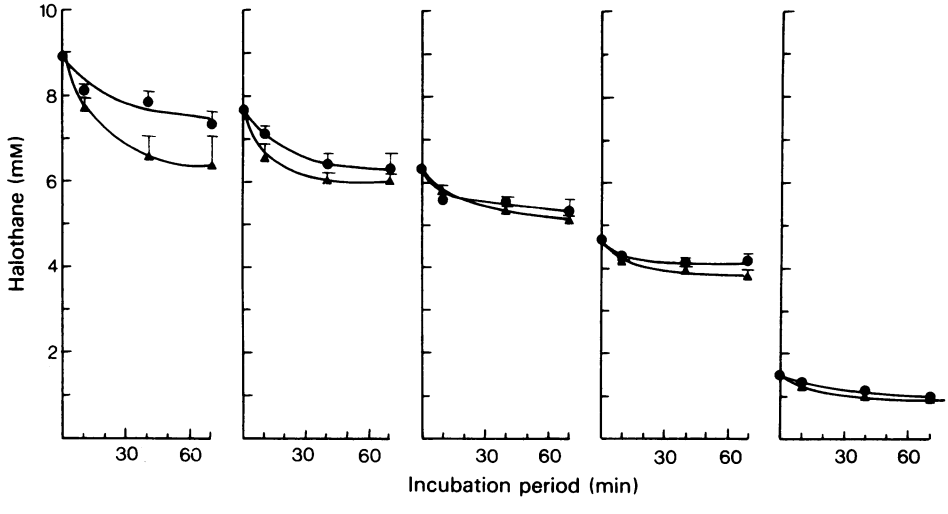

Figure 3 Effects of initial halothane concentrations in the buffer, of the presence of tissue and of the period of incubation on final halothane concentrations. Incubations were paired, with (▲) or without (●) tissue, for the periods and halothane concentrations indicated. At the end of the incubations, halothane was extracted from the total buffer and assayed as described in Methods. Means of four experiments are plotted, with s.e.mean (vertical lines), except at the lowest halothane concentration where the means of two experiments only are given. Tissues, when present, showed no significant differences in weight between the incubation systems. The range of mean tissue weights was from 22.8 mg to 25.4 mg.

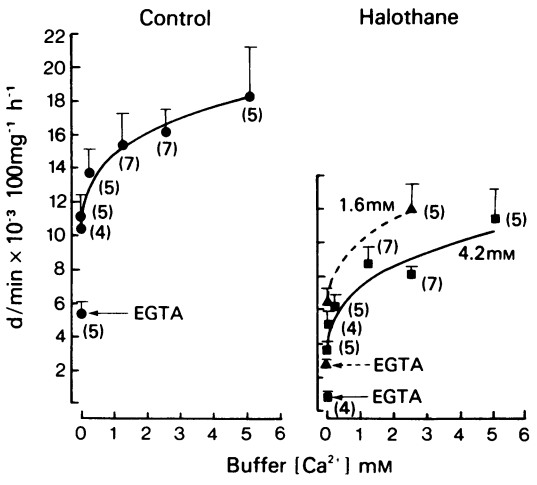

Figure 5 Effect of Ca^{2+} concentration in the buffer on the rate of L-[3-^{14}C]-serine incorporation into lipid. Influence of halothane and EGTA. Ca^{2+} ions were either absent from the buffer or present at the concentrations indicated. Ethyleneglycolbis-(aminoethylether) tetra-acetic acid (EGTA), at a concentration of 0.5 mM, was also added in the absence of Ca^{2+} ions. Strips were incubated in these buffers in the absence (●) or presence of halothane (4.2 mM, ■ ; 1.6 mM, ▲) and the incorporation of L-[3-^{14}C]-serine (0.75 μM) into lipid in 1 h was measured as described in Methods. Mean values are plotted (vertical lines show s.e.mean) with the number of tests in parentheses. The usual Ca^{2+} concentration in Krebs buffer is represented by 2.54 mM Ca^{2+}.

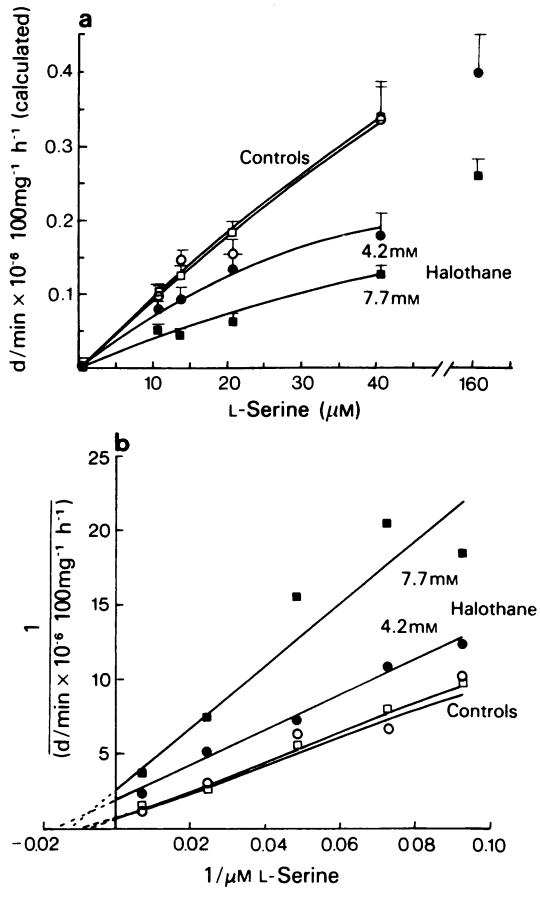

Figure 6 Effect of L-serine concentration on the inhibition by halothane of the rate of L-[3-^{14}C]-serine incorporation into lipid. (a) Each strip was incubated in the presence of both L-[3-^{14}C]-serine (0.75 μM) and unlabelled L-serine to give the final concentrations shown. Halothane was absent or present at initial concentrations of either 4.2 mM or 7.7 mM. The incorporation of L-[3-^{14}C]-serine into lipid in 1 h was measured as described in Methods. The rate of incorporation of radioactivity was calculated to a constant specific activity for L-[3-^{14}C]-serine of 56 mCi/mmol, consistent with other data presented. The calculation was made from the observed rate of incorporation of radioactivity at each concentration of L-serine tested. Mean values are plotted (vertical lines show s.e.mean), with 3–5 tests for each point. The rates of incorporation for the controls at 160.75 μM L-serine were 0.734×10^6 d/min 100 $mg^{-1}h^{-1}$ $\pm$ 0.019×10^6 (s.e.mean) as control for 7.7 mM halothane, and $0.731 \times 10^6 \pm 0.156 \times 10^6$ as control for 4.2 mM halothane. Halothane 7.7 mM (■); its control (□); halothane 4.2 mM (●); its control (○). (b) Double reciprocal plot of (a). The lines were fitted by linear regression analysis by the method of weighted least squares (Roberts, 1977).

duction but with a specific requirement for Ca^{2+} (Arienti *et al.*, 1976).

Although the location of the incorporation process in the tissue was not studied, one possibility was that it was at sites that were sensitive to variations in amino acid transport into the preparation. At the low concentrations of amino acid used in this study, active uptake dominates in smooth muscle and is inhibited by DNP (Osman & Paton, 1971). However, serine incorporations in these experiments were not affected by DNP, despite the use of a concentration of DNP that inhibits active amino acid uptake (Tsukada *et al.*, 1963) and uncouples oxidative phosphorylation (Beresford, 1978). Further evidence that the incorporation process was not primarily dependent on amino acid transport stems from the lack of inhibition by ouabain which, at 10^{-6} M, causes a marked reduction in amino acid uptake (Osman & Paton, 1971). Ca^{2+} lack, on the other hand, does not cause inhibition of amino acid uptake in smooth muscle (Osman & Paton, 1971) but did diminish the incorporation. In addition, at the highest concentrations of L-serine used in the present work, the incorporation into phospholipid did not show the secondary unsaturable process typical of amino acid uptake

into smooth muscle at concentrations above 1 mM (Osman & Paton, 1971). The slow appearance of label in phosphatidylethanolamine, relative to its incorporation into phosphatidylserine, indicates that a little of the newly-labelled phosphatidylserine eventually appears at the mitochondria where decarboxylase activity, necessary for the conversion to phosphatidylethanolamine, is highest (Dennis & Kennedy, 1972).

It is concluded that effects on the observed incorporation into phospholipid indicate effects on phospholipid base-exchange activity at a location, probably on the plasma membrane, which does not depend on inward transport of the amino acid. Because the incorporation diminishes as external Ca^{2+} concentrations are reduced, it is most likely to be regulated at externally accessible sites on the plasma membrane.

The effects of halothane

In addition to showing a reduction of L-serine incorporation at clinically relevant concentrations of halothane, two pieces of evidence as to the mechanism of action were obtained. Firstly, it appeared not to be mediated by interference with the role of added Ca^{2+} in the base-exchange mechanism. Thus it was the component of serine incorporation that persisted both in the absence of externally added Ca^{2+} and with EGTA that appeared to be primarily sensitive to inhibition by halothane; and when activity was mostly inhibited by the anaesthetic, the increase in incorporation which occurred as the external Ca^{2+} concentration was raised, was similar to the control system (Figure 5). The fact that halothane was totally without influence on the incorporation that was dependent on added Ca^{2+} minimizes any possibility that the ionic effect could be an artefact arising from tissue damage. It is proposed that less exposed sites on the membrane, hydrophobic in nature, mediate the actions of halothane, consistent both with its lipophilic nature and with its rapid and readily reversible inhibitory effects.

Secondly, a double reciprocal plot of the increase in rate of incorporation with increase in L-serine concentration fits a straight-line reasonably well (Figure 2b) (Lineweaver & Burk, 1934). If applicability of Michaelis-Menten kinetics is assumed, then the anaesthetic is not acting as a competitive inhibitor (Figure 6). Such an assumption is satisfactory if the observed phospholipid base-exchange activity is present on the plasma membrane as discussed above. The plasma membrane of excitable tissues, notably the neuronal perikaryal membrane and the synaptosomal membrane, do contain significant phospholipid base-exchange activities (Goracci, Blomstrand, Arienti, Hamberger & Porcellati, 1973; De Medio, Trovarelli & Porcellati, 1977). Previously, inhibitory effects of halothane on phospholipid metabolism have been noted in the acetylcholine-stimulated turnover of phosphatidic acid and phosphatidylinositol in synaptosomes (Miller, 1975).

The sites and mechanism of action of halothane may in fact depend on its concentration since inhibition was not fully linear with dose at all doses tested (Figure 4) and the tissue uptake of halothane relative to the drug's added concentration can be disproportionately high at higher concentrations (Figure 3). This recalls the higher partition co-efficients seen between membranes and their aqueous buffer when the concentration of an anaesthetic was increased (Metcalfe, Seeman & Burgen, 1968). These authors suggest that at high concentrations of anaesthetic there is dissociation of lipid from the protein stroma of membranes.

The significance of base-exchange activity is not net synthesis of phospholipid but the redistribution of the pattern of phospholipid bases (and therefore of membrane charge) that will occur when the exchange is heterologous. Since only about 5% of membrane phospholipid is available for base-exchange activity (Arienti *et al.*, 1976) and phosphatidylserine is a minor membrane phospholipid, the observed effects could indicate a high degree of specificity. Aspects of the biochemical significance of phospholipid headgroup specificity are now becoming apparent (for example, Bennett, Smith, Houslay, Hesketh, Metcalfe & Warren, 1978).

The present experiments show that it is possible to study the phospholipid base-exchange process with intact tissues, and under more physiological conditions than with the isolated membranes usually employed. Extension of this work to other lipophilic drugs will determine whether phospholipid base-exchange activities are equally sensitive to other hydrophobic perturbations of the membrane under conditions of cellular and tissue integrity and whether they can provide a biochemical basis for their mechanisms of action. The longitudinal muscle preparation of the guinea-pig ileum will assist a simultaneous functional assessment of such actions.

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References

ADAMS, P.R. (1976). Drug blockade of open end-plate channels. *J. Physiol.*, **260**, 531–552.

ARIENTI, G., BRUNETTI, M., GAITI, A., ORLANDO, P. & PORCELLATI, G. (1976). The contribution of net synthesis and base-exchange reactions in phospholipid biosynthesis. In *Function and Metabolism of Phospholipids in the Central and Peripheral Nervous Systems*. ed. Porcellati, G., Amaducci, L. & Galli, C. pp. 63–78. New York: Plenum.

BENNETT, J.P., SMITH, G.A., HOUSLAY, M.D., HESKETH, T.R., METCALFE, J.C. & WARREN, G.B. (1978). The phospholipid headgroup specificity of an ATP-dependent calcium pump. *Biochim. biophys. Acta*, **513**, 310–320.

BERESFORD, R.A. (1978). Lack of correlation between strength of contractions and oxygen consumption of drug-treated, directly stimulated rat diaphragm. *Br. J. Pharmac.*, **62**, 450P.

DE MEDIO, G.E., TROVARELLI, G. & PORCELLATI, G. (1977). Relationships between base-exchange reaction and synaptosomal phospholipid pool in the rabbit brain *in vitro*. *Ital. J. Biochem.*, **26**, 202–214.

DENNIS, E.A. & KENNEDY, E.P. (1972). Intracellular sites of lipid synthesis and the biogenesis of mitochondria. *J. Lipid Res.*, **13**, 263–267.

FOLCH, J., LEES, M. & SLOANE-STANLEY, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.*, **226**, 497–509.

GAGE, P.W., McBURNEY, R.N. & SCHNEIDER, R.G.T. (1975). Effects of some aliphatic alcohols on the conductance change caused by a quantum of acetylcholine at the toad end-plate. *J. Physiol.*, **244**, 409–429.

GORACCI, G., BLOMSTRAND, C., ARIENTI, G., HAMBERGER, A. & PORCELLATI, G. (1973). Base-exchange enzymic system for the synthesis of phospholipids in neuronal and glial cells and their subfractions: a possible marker for neuronal membranes. *J. Neurochem*, **20**, 1167–1180.

LENAZ. G., CURATOLA, G., MAZZANTI, L., PARENTI-CASTELLI, G., LANDI, L. & SECHI, A.M. (1978). A conformational model of the action of general anaesthetics at the membrane level. III Anaesthetics and the properties of membrane-bound enzymes: mitochondrial ATPase. *Ital. J. Biochem.*, **27**, 431–449.

LINEWEAVER, H. & BURK, D. (1934). The determination of enzyme dissociation constants. *J. Am. Chem. Soc.*, **56**, 658–666.

METCALFE, J.C., SEEMAN, P. & BURGEN, A.S.V. (1968). The proton relaxation of benzyl alcohol in erythrocyte membranes. *Mol. Pharmac.*, **4**, 87–95.

MILLER, J.C. (1975). Anaesthetics and phospholipid metabolism. In *Progress in Anaesthesiology, vol I Molecular Mechanisms of Anaesthesia* ed. Fink, B.R. pp. 439–447. New York: Raven.

MILLER, J.C. & MILLER, K.W. (1975). Approaches to the mechanisms of action of general anaesthetics. In *MTP International Review of Science, Biochemistry Series One*. ed. Blaschko, H.K.F. Vol. 12, pp. 33–76. London: Butterworth.

MILLER, K.W., PATON, W.D.M., SMITH, R.A. & SMITH, E.B. (1973). The pressure reversal of general anaesthesia and the critical volume hypothesis. *Mol. Pharmac.*, **9**, 131–143.

MIURA, T. & KANFER, J. (1976). Studies on base-exchange reactions of phospholipids in rat brain. Heterogeneity of base-exchange enzymes. *Archs Biochem. Biophys.*, **175**, 654–660.

OSMAN, F.H. & PATON, D.M. (1971). Transport of α-aminoisobutyric acid in rabbit detrusor muscle. I General characteristics of the uptake *in vitro*. *Biochim. biophys, Acta*, **233**, 666–675.

PATON, W.D.M. & ZAR, M. ABOO (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194**, 13–33.

PATON, W.D.M. & PAYNE, J.P. (1968). Control of consciousness: 1. In *Pharmacological Principles and Practice*. pp. 20–63. London: Churchill.

RANG, H.P. (1964). Stimulant actions of volatile anaesthetics. *Br. J. Pharmac. Chemother.*, **22**, 356–365.

ROBERTS, D.V. (1977). The computation of enzyme parameters by curve fitting procedures (Appendix II). In *Enzyme Kinetics*, pp. 285–298. Cambridge: Cambridge Univ. Press.

SPEDEN, R.N. (1965). The effect of some volatile anaesthetics on the transmurally stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **25**, 104–118.

STEINBERG, H.R. & DURELL, J. (1971). Effects of denervation on the incorporation of ^{32}P into phosphatidylinositol and other phospholipids of rat diaphragm. *J. Neurochem.*, **18**, 277–286.

TAUSSIG, P.E. (1979). Biochemical mechanisms in anaesthesia. *Int. J. Biochem.*, **10**, 183–191.

THOMPSON, G.A. (1973). Phospholipid metabolism in animal tissues. In *Form and Function of Phospholipids*, ed. Ansell, G.B., Dawson, R.M.C. & Hawthorne, J.N. pp. 67–96. Amsterdam: Elsevier.

TSUKADA, Y., NAGATA, Y., HIRANO, S. & MATSUTANI, T. (1963). Active transport of amino acid into cerebral cortex slices. *J. Neurochem.* **10**, 241–256.

WING, D.R. & PATON, W.D.M. (1980). [^{14}C]-Serine incorporation into phospholipid in the guinea-pig ileum: effects of halothane. *Biochem. Soc. Trans.*, **8**, 124–125.

WUTHIER, R.E. (1976). Chromatography of phospholipids and glycolipids on Whatman SG-81 silica-gel loaded paper. In *Lipid Chromatographic Analysis*. ed, Marinetti, G.V. pp. 59–110. New York: Marcel Dekker.

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