The influence of halothane on calcium uptake by isolated cell membranes

The depression of cardiac contractile force is a substantial hazard in the use of halothane in general anaesthesia (see review by Krebs & Kersting, 1972). However, few attempts have been made to elucidate the mechanism underlying the negative inotropic action of this volatile anaesthetic agent. The interference of halothane with various stages of glycolysis in rat isolated atria was described by Ko & Paradise (1969, 1970, 1971). But it remains doubtful whether the results obtained by these authors can satisfactorily explain the reduction of cardiac amplitude. Drugs that influence cardiac contractility owe their effect to their modification of calcium ion migration in heart muscle. To our knowledge, the influence of halothane on calcium ion migration has not been adequately studied. In the experiments described by Lain, Hess & others (1968) the influence of halothane on isolated cardiac sarcoplasmic reticulum was studied, but the purity of the preparation was uncertain. Moreover, the suspension medium contained oxalate which gives rise to an unphysiological situation. For this reason we have reinvestigated the action of halothane on the uptake of Ca^{2+}- ions by an isolated cell membrane preparation obtained from guinea-pig ventricular muscle.

A homogenate of ventricular muscle was centrifuged against a sucrose gradient and the fraction containing the cell membrane was isolated and recentrifuged at 200 000 *g*. The pellet was resuspended and appropriately diluted (protein concn 4·8 mg ml^{-1}). The preparation* consisted of almost pure cell membranes including transverse tubuli. The uptake of ^{45}Ca was studied in a medium composed as follows: KCl 100; $MgCl_2$ 5; imidazole 10; NaH_2PO_4 23 mM and $CaCl_2$ $3 \cdot 10^{-5}$M. A sample of 10 ml of this medium was transferred to a small flask containing 300 μl of a $^{45}CaCl_2$ solution (1 ml = $3 \cdot 10^5$ counts min^{-1}). The final Ca^{2+} concentration in the medium was $2 \cdot 7 \times 10^{-5}$ M. ATP-Na_2 (0·5 ml of a 0·1 M solution) was added, the pH was adjusted to 7·3 and 50 μl of the membrane homogenate rapidly mixed with the solution thus obtained. After periods of incubation up to 60 min, samples (1 ml) were each rapidly filtered (Millipore filter, pores 0·45 μm). The radioactivity of the protein fraction on the filter and that of the filtrate were determined by means of a liquid scintillation spectrometer. The amount of $^{45}Ca^{2+}$ bound to the protein fraction was calculated and expressed as a percentage of the maximum amount that could be taken up by the protein (theoretically if all the $^{45}Ca^{2+}$ had been transferred from the medium to the protein fraction, the concentration would be 1·24 m mol Ca^{2+} mg^{-1} protein). In a parallel series of experiments a given amount of liquid halothane was dissolved in the medium just before the homogenate of the call membranes was added. The flask was kept closed but the halothane concentration of the medium slowly decreased. This was confirmed in separate control experiments where concentration was checked by gas chromatography. It was impossible to keep the halothane concentration constant by bubbling it together with nitrogen through the suspension since then precipitation of protein occurred. Table 1 shows the increase in $^{45}Ca^{+}$ uptake in the cell membrane fraction as a function of time in control experiments and in the presence of halothane. The decrease in halothane concentration is also shown.

Halothane obviously increases the rate of $^{45}Ca^{2+}$ uptake by the isolated membrane preparation. Moreover, the total amount of calcium that can be bound is also increased by halothane since at prolonged periods of incubation (up to 60 min) the

* We are greatly indebted to Dr. R. Lüllmann-Rauch for electronmicroscopic control of the preparation. A detailed description of the procedure of isolation etc., will be submitted for publication shortly (Porsius & van Zwieten).

Table 1. *Uptake of ^{45}Ca by isolated cell membrane, expressed as percentage of maximum uptake and the influence of different halothane concentration.* The control values represent the mean (± s.e.) for 8–9 ^{45}Ca experiments, while for halothane experiments n = 3. All values are significantly different ($P < 0.01$) from the controls, except those marked.

	0 min	6 min	12 min	18 min	24 min	42 min	60 min
^{45}Ca-uptake (%) control	0	14·5 ± 0·5	24·2 ± 1·0	33·0 ± 1·0	40·6 ± 1·6	55·5 ± 1·9	59·5 ± 1·5
^{45}Ca-uptake (%)	0	15·8 ± 0·4	26·9 ± 1·2	35·6 ± 1·4	42·8 ± 2·2*	55·0 ± 2·5*	59·7 ± 2·9*
Halothane $\times 10^{-4}$M	4·4 ± 0·1	3·5 ± 0·1	3·2 ± 0·1	3·0 ± 0·1	2·7 ± 0·1	2·2 ± 0·1	1·8 ± 0·1
^{45}Ca-uptake (%)	0	17·4 ± 1·0	30·2 ± 1·9	41·0 ± 2·5	52·4 ± 3·2	62·5 ± 2·6	66·3 ± 1·8
Halothane $\times 10^{-4}$M	8·2 ± 0·2	7·2 ± 0·2	6·9 ± 0·2	6·5 ± 0·3	5·7 ± 0·3	4·4 ± 0·1	3·4 ± 0·2
^{45}Ca-uptake (%)	0	16·8 ± 0·5	29·7 ± 1·0	39·8 ± 1·5	50·0 ± 2·2	63·3 ± 3·6	64·4 ± 2·4
Halothane $\times 10^{-4}$M	23 ± 0·9	22·2 ± 0·5	19·7 ± 1·0	17·8 ± 1·2	17·0 ± 0·5	13·7 ± 0·9	10·6 ± 1·0
^{45}Ca-uptake (%)	0	18·1 ± 1·1	30·3 ± 1·1	41·7 ± 1·4	49·0 ± 1·7	not	63·6 ± 2·4
Halothane $\times 10^{-4}$M	50·8 ± 0·7	46·8 ± 2·0	45·3 ± 1·3	44·0 ± 0·5	42·4 ± 1·5	detd.	28·5 ± 1·2

difference in $^{45}Ca^{2+}$ uptake was significant between halothane and control experiments. In separate experiments, the rate of wash-out of $^{45}Ca^{2+}$ initially accumulated by cell membranes was not influenced by halothane in the concentrations in Table 1. The initial accumulation amounted to 0·74 nmol Ca^{2+} mg^{-1} protein.

The accelerated and increased uptake of calcium ions by isolated membrane preparations as a result of exposure to halothane would imply that less ionized intracellular calcium is available for excitation-contraction coupling. Concomitantly, the effect of halothane on calcium migration as described in the present communication may contribute substantially to the negative inotropic action of this anaesthetic agent. It may be added that on electrically driven guinea-pig isolated atria (frequency 180 min^{-1}) a steep dose response curve in the range 10^{-4}–10^{-3}M halothane is obtained (Porsius & van Zwieten to be published).

Thus, the effective halothane concentrations used in the experiments with cell membranes are within the range of those that will give rise to a negative inotropic response.

We are greatly indebted to Prof. Dr. H. Lüllmann and Dr. Th. Peters for their hospitality and support at the Department of Pharmacology, Christian Albrechts-University, Kiel (W. Germany).

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March 27, 1973

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