Br. J. Pharmacol. (1988), **94**, 509–514

Influence of halothane on electrical coupling in cell pairs isolated from guinea-pig ventricle

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1 The actions of halothane on electrical coupling between cells were investigated in cell pairs isolated from guinea-pig ventricular muscle.

2 Under voltage-clamp conditions a step depolarization applied to one cell caused a similar change in potential in the second. Application of halothane led to the appearance of double peaks in inward current evoked by step depolarizations. These observations were interpreted in terms of uncoupling of the cells leading to escape of the second cell from the influence of the voltage-clamp in the first cell.

3 This suggestion that uncoupling in the presence of halothane led to differences in electrical activity in the two cells was confirmed in experiments in which independent electrodes were used to measure membrane potential in the two cells.

4 The voltage responses of both cells of the pair were recorded in response to constant current pulses. Administration of halothane led to abolition of the response recorded from the second cell while that of the first was enhanced. The actions are consistent with an action of halothane on gap junctions to block electrical coupling.

5 Qualitatively similar observations, consistent with electrical uncoupling, were observed with isoflurane.

6 These findings may be significant in relation to the arrhythmogenic actions of halothane.

Introduction

Intercellular communication occurs via gap junctions which provide a low resistance pathway that is important for the propagation and synchronization of electrical activity in the heart (Weidmann, 1966; De Mello, 1982). Alkanols such as octanol electrically uncouple mammalian ventricle cell pairs (Spray *et al.*, 1985; Maurer & Weingart, 1986). The effect of halothane upon intercellular communication in the heart is of interest because this anaesthetic predisposes to catecholamine-induced arrhythmias during surgery. Halothane is thought to reduce cell-to-cell coupling in sheep Purkinje fibres (Hauswirth, 1969) and cat papillary muscle fibres (Wojtczak, 1984) since in both studies internal longitudinal resistance of the preparation was increased by the anaesthetic. The advent of enzymatic techniques to isolate cardiac cells (Powell *et al.*, 1980) allows a more direct investigation of cell to cell coupling as this technique yields some cell pairs for study. The purpose of the experiments described in this paper was to examine the effects of halothane upon coupling between ventricular cell pairs of guinea-pig. Preliminary observations have been communicated to IUPHAR (Victory & Terrar, 1987).

Methods

Basic methods are described in the accompanying paper (Terrar & Victory, 1988). All experiments were performed at 37°C.

Ventricular cell pairs were identified by their characteristic morphology and electrical properties. One cell of the cell pair was impaled with a glass microelectrode containing 0.5 M K_2SO_4 (resistance 15–25 MΩ) and voltage clamped using a single electrode system (Axoclamp 2). The use of low resistance microelectrodes facilitated voltage-clamping. The function of the electrode was switched rapidly between current passing and voltage recording. The other cell was impaled with a microelectrode containing 3 M KCl (resistance 30–50 MΩ) from a second headstage: membrane potentials were recorded from this cell while the first cell was voltage clamped, and

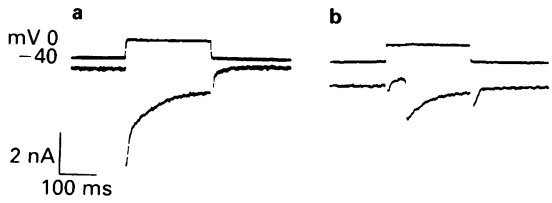

Figure 1 Second inward currents (lower trace) obtained in a cell pair impaled with a single microelectrode by depolarization from −40 mV to 0 mV for 200 ms (upper trace). Records in the absence (a) and 30 s after administration of 2% halothane (b). Stimulation frequency 0.3 Hz.

voltage signals were fed to a pre-amplifier incorporating a bridge circuit (Axoclamp 2). Membrane potential could be changed by applying a constant current through the recording electrode.

In some experiments both cells of the cell pair were unclamped and allowed to assume their resting potentials which were normally in the range −70 to −80 mV. Hyperpolarizing current pulses (0.5 to 2 nA, 200 ms, 1 Hz) were injected into the first cell and the resulting voltage responses in both cells recorded.

These experiments were performed during an eighteen month period and include observations on 34 cell pairs obtained from 27 guinea-pigs. Quantitative data are displayed as mean ± s.e. mean. Values before and after administration of anaesthetic to each cell pair were compared and Student's paired *t* test applied to the data.

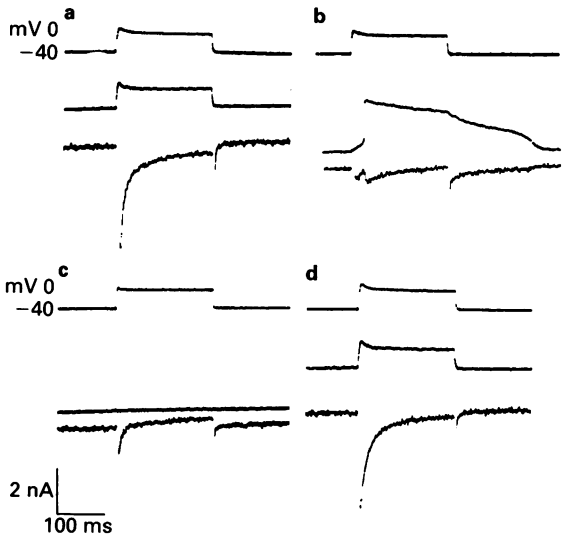

Figure 2 Records obtained by use of a double electrode technique. Second inward currents (lower traces) recorded from the first cell of a pair. Membrane potential of the first cell (upper traces). Voltage response of the second cell impaled with an independent microelectrode (middle traces). (a) In the absence of halothane (membrane potential in the second cell approximates that in the first). (b) After 30 s exposure to 2% halothane (membrane potential in the second cell is −78 mV at the start of the pulse and has a peak value during the pulse of +30 mV). (c) Continued exposure to halothane uncouples the two cells (membrane potential in the second cell is −78 mV). (d) Full recovery from the effects of halothane. Stimulation frequency 0.3 Hz.

Results

Halothane has been found to reduce the amplitude of the second inward current in response to a step depolarization in voltage-clamped ventricular cells (Terrar & Victory, 1986; 1988). In experiments where pairs of cells were impaled and voltage-clamped, application of halothane (2% vol/vol, 1.16 mM) not only reduced the amplitude of the second inward current, but caused the appearance of a double peak in the current waveform (Figure 1); continued exposure to halothane led to the suppression of the second peak, leaving a current with a single reduced peak. This effect was consistently seen in 34 cell pairs, at doses of halothane from 1.2% (0.75 mM) to 2.4% (1.57 mM). These actions of halothane were reversed on washing out the drug.

One possibility to account for these observations is that halothane reduced electrical coupling between the two cells, leading to poor control of membrane potential in the second cell (that which does not contain the electrode passing current and recording potential); this might lead to 'escape' of the second cell from the clamp, allowing an 'action potential' (or similar response) to fire, and this action potential could give rise to the second peak of inward current recorded in the first cell during uncoupling. This possibility was investigated by impaling the second cell with an additional microelectrode so that membrane potential could be recorded in this cell while the first cell was being voltage-clamped. An experiment of this kind is illustrated in Figure 2. Before halothane was applied (Figure 2a), the membrane potential in the second cell approximated that in the first voltage-clamped cell, although the effectiveness of the clamp was less in the second cell than that in the first; the second inward current showed a single large peak. When halothane was applied, the steady potential of the second cell started to become more negative. When the second peak of current appeared in the first cell, the initiation of this second peak of current corresponded with a rapid depolarization in the second cell (Figure 2b). Two other features of these complex current and voltage waveforms seen during uncoupling must be mentioned. The first

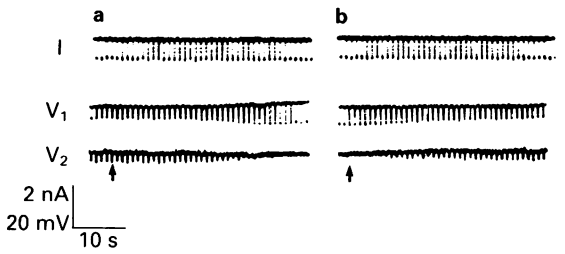

Figure 3 Current injection experiments. Both cells are unclamped at their resting potentials (First cell: −74 mV; Second cell: −76 mV). Current (1 nA) is injected into the first cell (upper trace). Voltage responses of the first cell (middle trace) and second cell (lower trace) are recorded. (a) Onset of effects of 2% halothane after application (arrow). (b) Recovery after washout of anaesthetic (arrow). Stimulation frequency 1 Hz; pulse duration 200 ms.

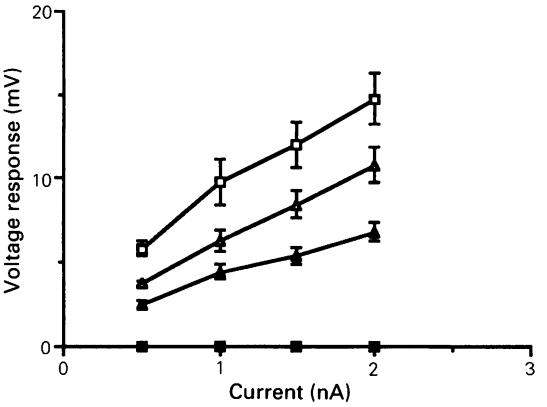

Figure 4 Voltage response for each cell of a pair plotted against current injected. Mean values for 6 cell pairs: (△, ▲) show data points in the absence, and (□, ■) show results obtained in the presence of 2% halothane. Mean values are shown with vertical lines indicating s.e. mean. Response of first cell is represented by (△, □), and that of the second cell by (▲, ■). The voltage response of the first cell was significantly increased in the presence of halothane with 1, 1.5 and 2 nA injected current ($P < 0.01$). The response of the second cell was significantly reduced by halothane at all levels of injected current ($P < 0.001$).

peak of inward current in the first cell corresponded to a slow depolarization in the second. Secondly upon repolarization to −40 mV, in the first cell a slow tail of decaying inward current was seen. As the exposure to halothane was continued, the appearance of the second peak was delayed and finally disappeared (Figure 2c). At this time the steady membrane potential in the second cell had hyperpolarized towards its resting level, and only a small residual depolarization occurred in response to the voltage-clamp step in the first cell. This sequence of events was reversed on washing out the halothane: re-appearance of the double peak occurred, with a corresponding potential change in the second cell (as if the cells were partially recoupled), and Figure 2d shows complete recovery. Similar effects were observed in ten cell pairs.

The possible effect of halothane on electrical coupling between cells was further investigated in cell pairs in which hyperpolarizing current pulses were injected and the potential changes evoked in both cells were recorded. Records from an experiment of this kind are shown in Figure 3. Halothane caused an attenuation of the voltage response in the second cell, whereas the voltage response in the first cell was enhanced; these effects gradually reversed on washout of halothane. Quantitative analysis of data obtained from 6 cell pairs after the halothane effects had reached a steady state is shown in Figure 4, where the amplitude of the hyperpolarizations is plotted as a function of injected current. Squares show the responses in the presence of halothane and circles in the absence; open symbols show the responses of the first cell and closed symbols those of the second. Again it is clear that halothane reduced the voltage response in the second cell to undetectable levels ($P < 0.001$) while increasing that of the first cell ($P < 0.01$ at 1, 1.5, and 2 nA).

These actions of halothane could result from modification of the level of intracellular calcium. However, in 8 cell pairs loaded with EGTA to buffer the cytosolic calcium at a low level, 2% halothane still caused uncoupling.

Experiments were also performed where 6 cell pairs were exposed to 3% (1.2 mM) isoflurane. This anaesthetic produced complex current waveforms as observed with halothane, consistent with the hypothesis that isoflurane also reduced the coupling in cell pairs.

During the course of these experiments it was noticed that the decay of the second inward current was slower ($P < 0.001$) in coupled cell pairs than had been previously recorded in single cells. This is illustrated in Figure 5a which shows the mean half-times of decay of 31 single (6.2 ± 0.2 ms) and 32 double cells (13.2 ± 1.1 ms). The half-time is a convenient index of decay but it is not intended to suggest that decay is governed by a single exponential process. Figure 5b shows a more detailed analysis in a representative cell pair and on a single cell; it is clear from the semilogarithmic plot that, as suggested by the measurements of half-time, decay was slower in the cell pair.

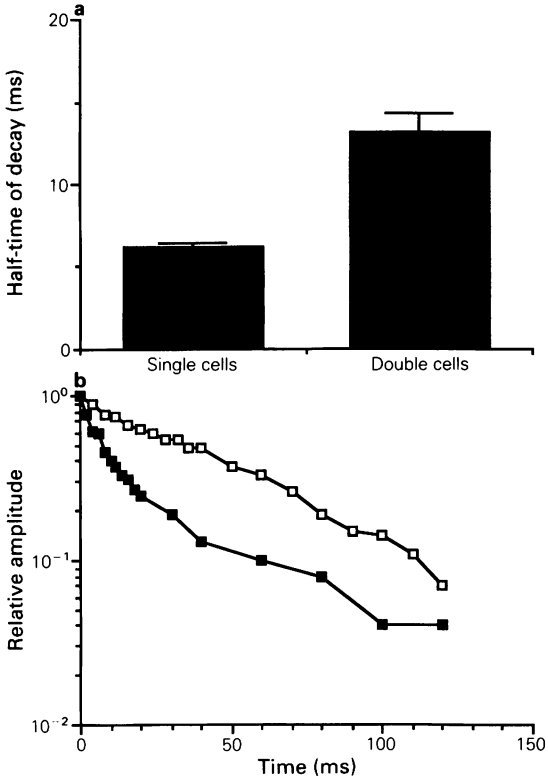

Figure 5 (a) Half-times of decay of second inward current recorded from 31 single cells and 32 double cells. Data represented on the bar graph as mean with s.e. mean shown by vertical lines. (b) Decay of second inward current (as a fraction of the peak value) plotted semilogarithmically against time (ms): (□) show the decay of current recorded from a cell pair; (■) represent the decay of current recorded from a single cell. Stimulation frequency 0.3 Hz.

Discussion

The main finding of this paper is that halothane reduced electrical coupling between myocytes of cell pairs isolated from guinea-pig ventricle. A blockade of gap junctions could allow the second cell to 'escape' from the voltage-clamp in the first cell. Activation of depolarizing inward currents (including that carried by sodium) could generate the observed depolarizing spike and in turn lead to the complex changes observed in the first cell. On further exposure to halothane the second cell may escape fully from the clamp and assume its normal resting potential. This hypothesis is supported by the observation that halothane reduced the response of the second cell to hyperpolarizing current injected in the first. In contrast the voltage response in the first cell is increased. This enhancement might be due to an increased fraction of membrane current passing through the first cell. Alternatively halothane may block potassium conductances in this preparation and the increased voltage response in the first cell could be the result of an increase in input resistance secondary to this blockade. The coupling coefficient is often used as a quantitative index of junctional conductance in electrically coupled systems. In the current injection experiments described, the coupling coefficient is the ratio of the voltage response of the second cell to that of the first. It is clear from Figure 4 that halothane reduced the coefficient of coupling between the cells of the pairs. However, the use of coupling coefficient as an index of junctional conductance has been questioned. Socolar (1977) suggests that the coefficient is only qualitatively useful in systems with weak coupling and well defined topology of cell connection. He suggests that at high values of junctional conductance the coupling ratio is rather insensitive to changes in junctional conductance. This is not applicable in our studies as we observed a dramatic reduction in coupling with halothane. This occurs in the range where coupling ratio provides a good index of junctional conductance.

Vassort *et al.* (1986) has observed that alkanols increase internal calcium in squid axons. He suggested that the uncoupling effect of general anaesthetics in cardiac cells could be explained by an increased intracellular calcium concentration as the level of cytosolic calcium is a major factor controlling gap junctional conductance. Procedures that elevate cytosolic calcium such as calcium or sodium injection and ouabain administration caused cell uncoupling in Purkinje cells (Weingart, 1977; De Mello, 1972; 1975; 1976). The action of halothane in our experiments appears to be independent of any changes in cytosolic calcium as uncoupling occurred in cells loaded with EGTA to buffer cytosolic calcium at a low level.

Acidification of the cytosol can cause cell uncoupling (Reber & Weingart, 1982). However, this effect of acidity might be caused by a rise in cytosolic calcium (De Mello, 1983) and Noma & Tsuboi (1987) propose that although proton concentration does affect the gap junction conductance the threshold for the pH effect is higher than that for calcium and its physiological role is unclear. While we cannot rule out an effect of halothane upon intracellular pH, nuclear magnetic resonance (n.m.r.) studies of Murray *et al.* (1983) in isolated rabbit heart suggest that halothane does not modify intracellular pH in cardiac muscle.

The other main finding of these studies is that the decay of second inward current is slowed in double cells compared to single cells. More rapid kinetics of

the second inward current have been observed in single cardiac cells than appeared to be the case from previous multicellular recording (see e.g. Discussion in Mitchell *et al.*, 1983). One proposal is that an increase in series resistance between the cell membrane and the recording electrode may be important in multicellular recording (Siegelbaum & Tsien, 1980). However the significance of this effect has been questioned (Noble & Powell, 1983). The presence of contaminating outward currents has been suggested by Marban & Tsien (1982) who saw speeded kinetics of the second inward current in Purkinje fibres when these outward currents were blocked with Cs. In guinea-pig ventricle there seems to be little contamination of I_{si} by transient outward currents. We have observed that kinetics of the second inward current are slowed even by the transition from single to double cell recording. The reason for this is unclear but may be due to a failure to voltage-clamp the entire surface membrane of both cells.

The implications of the uncoupling action of halothane are important as this anaesthetic can cause arrhythmias during surgery. Halothane has been shown to interfere with conduction in the A–V node (Atlee & Craighead, 1977) and in Purkinje fibres (Hauswirth, 1969). An action of halothane to reduce gap junctional conductance might explain the impairment of conduction and contribute to the genesis of re-entrant arrhythmias. One problem with this suggestion is that isoflurane is not a potent arrhythmogenic agent but still caused uncoupling of the cardiac double cells in our studies. However, the concentration of isoflurane used was high (3%, 1.2 mM) and the action of this anaesthetic upon electrical coupling at lower doses may be different. Further studies will be necessary to investigate these possibilities.

This work was supported by the M.R.C. J.G.G.V. is in receipt of an M.R.C. studentship. We thank Mr Clive Garnham for expert assistance in the preparation of isolated ventricular cells.

References

ATLEE, J.L. & ALEXANDER, S.C. (1977). Halothane effects on conductivity of the AV node and His-Purkinje system in the dog. *Anesth. Analg.*, **56**, 378–386.

DE MELLO, W.C. (1972). The healing over process in cardiac and other muscle fibres. In *Electrical Phenomena in the Heart*. ed. De Mello, W.C. pp. 323–351. New York: Academic Press.

DE MELLO, W.C. (1975). Effect of intracellular injection of calcium and strontium on cell communication in the heart. *J. Physiol.*, **250**, 231–245.

DE MELLO, W.C. (1976). Influence of the sodium pump on intracellular communication in heart fibres: Effect of intracellular injection of sodium ion on electrical coupling. *J. Physiol.*, **263**, 171–197.

DE MELLO, W.C. (1982). Intercellular communication in cardiac muscle. *Circ. Res.*, **51**, 1–9.

DE MELLO, W.C. (1983). Influence of pH on the healing over process of mammalian cardiac muscle. *J. Physiol.*, **339**, 299–307.

HAUSWIRTH, O. (1969). The influence of halothane on the electrical properties of cardiac purkinje fibres. *J. Physiol.*, **201**, 42P.

MARBAN, E. & TSIEN, R.W. (1982). Effects of nystatin-mediated intracellular ion substitution on membrane currents in calf purkinje fibres. *J. Physiol.*, **329**, 569–587.

MAURER, P. & WEINGART, R. (1986). Cell pairs isolated from adult guinea-pig heart: interventions aimed at modifying the nexal membrane resistance. *J. Physiol.*, **378**, 57P.

MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1983). Characteristics of the second inward current in cells isolated from rat ventricular muscle. *Proc. R. Soc. B*, **219**, 447–469.

MURRAY, P.A., BLANCK, T.J.J., ROGERS, M.C. & JACOBUS, W.E. (1983). Halothane-induced abnormalities of myocardial high-energy phosphate metabolism. *Anesthesiology*, **59**, A63.

NOBLE, D. & POWELL, T. (1983). The effects of series resistance on second inward current recorded under voltage-clamp in ventricular muscle. *J. Physiol.*, **345**, 7P.

NOMA, A. & TSUBOI, N. (1987). Dependence of junctional conductance on proton, calcium and magnesium ions in cardiac paired cells of guinea-pig. *J. Physiol.*, **382**, 193–211.

POWELL, T., TERRAR, D.A. & TWIST, V.W. (1980). Electrical properties of individual cells isolated from rat ventricular myocardium. *J. Physiol.*, **302**, 131–153.

REBER, W.R. & WEINGART, R. (1982). Ungulate purkinje fibres: the influence of intracellular pH on the electrical cell-to-cell coupling. *J. Physiol.*, **328**, 87–104.

SOCOLAR, S.J. (1977). The coupling coefficient as an index of junctional conductance. *J. Memb. Biol.*, **34**, 29–37.

SIEGELBAUM, S.A. & TSIEN, R.W. (1980). Calcium-activated transient outward currents in calf purkinje fibres. *J. Physiol.*, **299**, 485–506.

SPRAY, D.C., WHITE, R.L., MAZET, F. & BENNETT, M.V.L. (1985). Regulation of gap junctional conductance. *Am. J. Physiol.*, **248**, H753–H764.

TERRAR, D.A. & VICTORY, J.G.G. (1986). Influence of halothane on calcium currents in single cells isolated from guinea-pig ventricle. *J. Physiol.*, **381**, 122P.

TERRAR, D.A. & VICTORY, J.G.G. (1988). Effects of halothane on membrane currents associated with contraction in single myocytes isolated from guinea-pig ventricle. *Br. J. Pharmacol.*, **94**, 500–508.

VASSORT, G., WHITTEMBURY, J. & MULLINS, L.J. (1986).

Increases in internal Ca and decreases in internal H are induced by general anaesthetics in squid axons. *Biophys. J.*, **50**, 11–19.

VICTORY, J.G.G. & TERRAR, D.A. (1987). Influence of halothane on electrical coupling between ventricular cell pairs isolated from guinea-pig heart. *IUPHAR Proceedings*, Sydney, 1987.

WEIDMANN, S. (1966). The diffusion of radiopotassium across intercalated disks of mammalian cardiac muscle. *J. Physiol.*, **187**, 323–342.

WEINGART, R. (1977). The actions of ouabain on intercellular coupling and conduction velocity in mammalian ventricular muscle. *J. Physiol.*, **264**, 341–365.

WEINGART, R. (1986). Electrical properties of the nexal membrane in rat ventricular cell pairs. *J. Physiol.*, **370**, 267–284.

WOJTCZAK, J.A. (1984). Effects of general and local anaesthetics on intercellular coupling in the heart muscle. *Biophys. J.*, **45**, 22a.

(Received August 6, 1987
Revised November 26, 1987
Accepted January 11, 1988)