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Effects of halothane on the transient outward K^+ current in rat ventricular myocytes

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1 Halothane has been shown to affect several membrane currents in cardiac tissue including the L-type calcium current (I_{Ca}), sodium current and a variety of potassium currents. However, little is known about the effects of halothane on the transient outward K^+ current (I_{to}).

2 Single ventricular myocytes from rat hearts were voltage clamped using the whole cell patch configuration and an EGTA-containing pipette solution to record the Ca^{2+}-independent, 4-aminopyridine sensitive component of I_{to}. 300 μM Cd^{2+} or 10 μM nifedipine was used to block I_{Ca}.

3 At +80 mV, I_{to} (peak current minus current at the end of the pulse) was 1.8 ± 0.2 nA under control conditions which was reduced to 1.3 ± 0.2 nA by 1 mM halothane ($P < 0.001$, mean $\pm$ s.e.mean, $n = 9$). The inhibition of I_{to} by halothane was concentration-dependent ($K_{0.5}$, 1.1 ± 0.2 mM). One mM halothane led to a 16 mV shift in the steady-state inactivation curve towards negative membrane potentials ($P = 0.005$, $n = 8$) but had no significant effect on the activation-voltage relationship ($P = 0.724$).

4 One mM halothane also increased the rate of inactivation of I_{to}; the dominant time constant of inactivation was reduced from 14 ± 1 to 9 ± 1 ms ($P = 0.017$, mean $\pm$ s.e.mean, $n = 6$).

5 These data show that halothane reduced I_{to}; 0.3 mM, close to the MAC_{50} value for halothane, inhibited the current by 15% and as such, the inhibition of I_{to} will be relevant to the clinical situation. Halothane induced a shift in the steady-state inactivation curve and accelerated the inactivation process of I_{to} which could be responsible for its inhibitory effect.

6 Due to the differential transmural expression of I_{to} in ventricular tissue, inhibition of I_{to} would reduce the transmural dispersion of refractoriness which could contribute to the arrhythmogenic properties of halothane.

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Abbreviations: 4-AP, 4-aminopyridine; I_{Ca}, L-type Ca^{2+} current; I_{to}, the transient outward K^+ current; MAC, minimum alveolar concentration

Introduction

Halothane has been shown to affect several transmembrane currents in cardiac tissue. For example, the L-type Ca^{2+} current (I_{Ca}) is depressed by halothane (Bosnjak *et al*., 1991; Eskinder *et al*., 1991; Ikemoto *et al*., 1985; Pancrazio, 1996; Puttick & Terrar, 1992; Terrar & Victory1988a,b). As I_{Ca} is the main trigger for the release of Ca^{2+} from the sarcoplasmic reticulum during excitation-contraction coupling, inhibition of I_{Ca} will contribute to the well described negative inotropic of halothane (Harrison *et al*., 1999; Housmans & Murat, 1988a,b; Sonntag *et al*., 1978). Halothane also inhibits the T-type Ca^{2+} channel (Eskinder *et al*., 1991), the sodium channel (Eskinder *et al*., 1993; Weigt *et al*., 1997), the cardiac (Supan *et al*., 1991) and smooth muscle delayed rectifier potassium channels (Buljubasic *et al*., 1992; Eskinder *et al*., 1995) as well as a wide range of recombinant K^+ channels (Zorn *et al*., 1993). However, little is known about the effect of halothane on the transient outward K^+ current (I_{to}) in the heart. This current activates and inactivates rapidly during the initial phase of the cardiac action potential. As such it plays an important role in determining action potential plateau amplitude, action potential duration and therefore contractility of the heart.

I_{to} is not ubiquitous in cardiac preparations but has been described in ventricular tissue in a variety of species including rat, sheep, human and rabbit (Clark *et al*., 1988; Josephson *et al*., 1984; Kenyon & Gibbons, 1979; Nabauer *et al*., 1993). In most tissues, I_{to} has two components (Coraboeuf & Carmeliet, 1982): a Ca^{2+}-independent, 4-aminopyridine (4-AP) sensitive component carried by K^+ ions (Coraboeuf & Carmeliet, 1982; Kenyon & Gibbons, 1979) and a Ca^{2+}-activated component carried by Cl^- ions (Sipido *et al*., 1993; Zygmunt & Gibbons, 1991). In rat ventricular myocytes however, the preparation chosen for the present experiments, 10 mM 4-AP induces >90% inhibition of I_{to} (Dukes & Morad, 1991) which led Dukes & Morad (1991) to conclude that only a single voltage-dependent component of I_{to} is expressed in rat ventricular tissue.

I_{to} is expressed to a greater extent in ventricular sub-epicardium than in sub-endocardium (Antzelevitch *et al*., 1991). This contributes to the shorter action potential in the sub-epicardium (cf. sub-endocardium) such that repolarization of the ventricular action potential proceeds from sub-epicardium to sub-endocardium. Inhibition of I_{to} would be expected to have a more profound effect on action potential duration in sub-epicardial cells than sub-endocardial cells and as such, transmural differences in the action potential duration would be reduced. Disturbance of the normal transmural dispersion of refractoriness is potentially arrhythmogenic and it is interesting to note that in the clinical situation, halothane anaesthesia is associated with an increased incidence of

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arrhythmia (Atlee & Bosnjak, 1990). As such it is important to determine the halothane-sensitivity of I_{to} as inhibition of this current could contribute to the arrhythmogenic characteristics of halothane.

In the present study, the effect of a range of concentrations of halothane (0.1–1 mM) on the amplitude, voltage-dependence and inactivation kinetics of the Ca^{2+}-independent component of I_{to} have been studied in rat ventricular myocytes. A preliminary account of this work was presented at the Biophysical Society meeting in 1999 (Davies *et al*., 1999).

Methods

Preparation of ventricular cells

Rats (300–350 g weight) of either sex were killed by a blow to the head and subsequent cervical dislocation (procedures covered by a U.K. Home Office licence). The heart was rapidly excised and perfused retrogradely with a series of solutions based on a nominally Ca^{2+}-free 'isolation solution' of the following composition (in mM): NaCl 130, KCl 0.4, $MgCl_2$ 1.4, NaH_2PO_4 0.4, HEPES 5, glucose 10, taurine 20, creatine 10, pH 7.1 at 37°C (NaOH). The heart was perfused initially with the isolation solution plus 750 μM $CaCl_2$ for several minutes to clear the heart of blood and then with the nominally Ca^{2+}-free isolation solution plus 100 μM EGTA for 4 min. The heart was then perfused with the isolation solution supplemented with 1 mg ml^{-1} collagenase (type 1, Worthington Biochemical Corp., Freehold, New Jersey, U.S.A.), 0.1 mg ml^{-1} protease (Sigma, type XIV) and 80 μM $CaCl_2$ for 7 min after which the ventricles were cut from the heart, finely chopped and agitated gently in enzyme solution (supplemented with 1% bovine serum albumin) for 5 min intervals. Dissociated cells were harvested at the end of each 5 min digestion and the remaining tissue subjected to further enzyme treatment.

Solutions

Solutions were delivered to the experimental chamber by magnetic drive gear metering pumps (Micropump, Cole Palmer, U.S.A.) and solution level and temperature controlled by feedback circuits (Cannell & Lederer, 1986). Following dissociation, cells were stored in and subsequently superfused with a physiological salt solution of the following composition (in mM): NaCl 140, KCl 5.4, $MgCl_2$ 1.2, NaH_2PO_4 0.4, HEPES 5.0, glucose 10.0, $CaCl_2$ 1.0, pH 7.4 at 30°C (NaOH). 10 μM nifedipine (Dukes & Morad, 1991) or 300 μM $CdCl_2$ (Stengl *et al*., 1998) were added to this solution to block I_{Ca}. The pipette solution had the following composition (in mM): K-aspartate 120, KCl 20, $MgCl_2$ 4, Na_2ATP 3, HEPES 5, EGTA 10, pH 7.1 at 30°C (KOH).

Halothane was delivered from a stock solution made up in dimethyl sulphoxide (DMSO). Following dilution of the stock solution the final concentration of DMSO in the physiological salt solution never exceeded 0.2%, a dose that had no effect on I_{to} in rat ventricular cells (data not shown). In some experiments 10 mM 4-AP was added to inhibit I_{to}. All experiments were carried out at 30°C.

Recording I_{to}

Cells were transferred to a small tissue chamber (volume, 0.1 ml) attached to the stage of an inverted microscope (Nikon Diaphot) and allowed to settle for several minutes onto the glass bottom of the chamber before being superfused at a rate of ~3 ml min^{-1} with the physiological salt solution (see above). The whole cell patch clamp technique was used to measure membrane currents (Axopatch 200A, Axon Instruments U.S.A.). Glass pipettes were fire polished and when filled with the EGTA-containing pipette solution (see above) had a resistance of 2–5 MΩ. Series resistance and capacitance were compensated for in all cells. Voltage clamp signals were low pass filtered at 2 kHz (Bessel filter), and digitized at 1 kHz using pClamp software (Axon Instruments). The holding potential in all experiments was −80 mV and I_{to} was evoked by 200 or 500 ms depolarizing voltage clamp pulses between −60 and +80 mV in 20 mV steps. The amplitude of I_{to} was measured as the difference between the peak outward current and the current at the end of the pulse.

Statistical analysis

Results are presented as the mean ± s.e.mean from *n* cells. Paired *t*-tests were used on data that were normally distributed. If paired data failed a normalization test, Wilcoxon signed rank tests were used to analyse data. A significant difference was declared if $P<0.05$.

Results

Effect of halothane on I_{to}

Figure 1A–C illustrates current records in the presence of 300 μM Cd^{2+} to block I_{Ca}. As the test potential was made progressively more positive a rapidly activating outward current was evoked (Figure 1A) which decayed to a sustained level during the 200 ms pulse. The application of 1 mM halothane reduced peak outward current (Figure 1B) and appeared to accelerate current decay during the pulse (see Figure 5). Following wash-out of halothane, peak current (and current inactivation, see Figure 5C) returned to control values. Subsequent addition of 10 mM 4-AP greatly reduced the rapidly activating component of outward current (Figure 1C) to a similar degree as observed previously (Dukes & Morad, 1991). The sensitivity of the rapidly activating current component to 4-AP and its measurement with EGTA-containing pipette solutions suggests that this current reflects activation of the Ca^{2+}-insensitive form of I_{to}. The magnitude of I_{to} (measured as peak current minus current at the end of a 200 ms pulse to +80 mV) was 1.8 ± 0.2 nA in nine cells under control conditions and this was reduced significantly to 1.3 ± 0.2 nA by 1 mM halothane ($P<0.001$, Figure 1D). Removal of halothane led to the complete restoration of the current (post control, 1.8 ± 0.2 nA; $P<0.001$ for halothane *vs* post control; Figure 1D) demonstrating that the effects of halothane on I_{to} were not contaminated by time-dependent changes in current and were fully reversible.

Figure 2A illustrates current-voltage relationships for I_{to} under control conditions and after equilibration with 1 mM halothane. 300 μM Cd^{2+} was present to inhibit I_{Ca}. Depression of I_{to} by halothane was significant ($P<0.05$) at voltages more positive than +20 mV. Cd^{2+} shifts the voltage-dependence of inactivation of I_{to} to more positive voltages (Stengl *et al*., 1998) and so experiments were repeated with 10 μM nifedipine as an alternative Ca^{2+} channel blocking agent. Figure 2B illustrates that in the presence of nifedipine, I_{to} was activated at potentials more positive than −40 mV (Dukes & Morad, 1991) and that the inhibitory action of halothane became apparent at potentials more positive than −40 mV. At positive voltages,

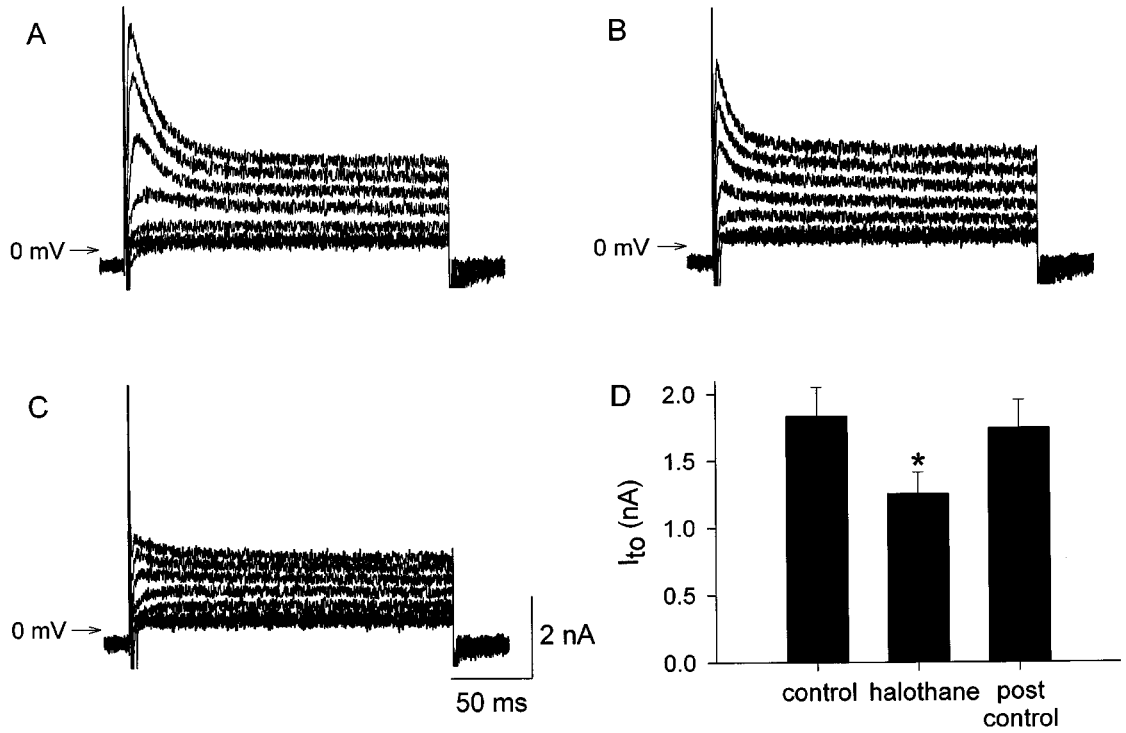

Figure 1 Records of current evoked during 200 ms test pulses from a holding potential of −80 mV. Test potentials ranged from −60 to +80 mV in 20 mV steps. Currents evoked under control conditions (A), following a 1 min exposure to 1 mM halothane (B) and in the presence of 10 mM 4-AP (C). Arrows indicate zero current level. In (A–C), 300 μM Cd^{2+} was present in all superfusion solutions to inhibit I_{Ca} and halothane was washed out before exposure to 4-AP. (D), mean data (±s.e.mean) for nine cells describing the amplitude of I_{to} at +80 mV under control conditions, following a 1 min exposure to 1 mM halothane and after wash out of halothane (post control). Current amplitudes were calculated as the peak current minus the current at the end of the 200 ms test pulse. Halothane led to a significant reduction in I_{to} (*$P<0.001$) compared to control. On removal of halothane currents returned to control values ($P<0.001$ for halothane *vs* post control).

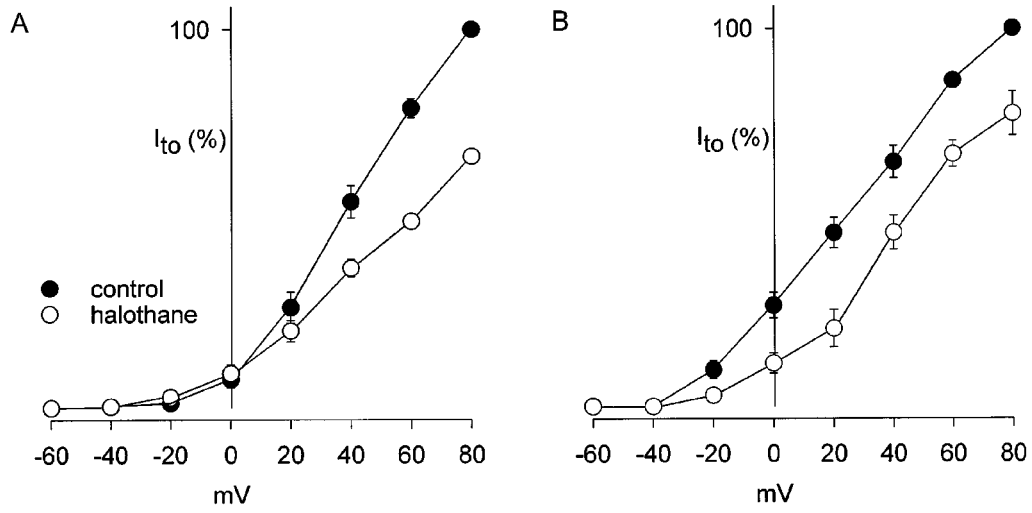

Figure 2 Current voltage relationships for I_{to} in the presence of 300 μM Cd^{2+} (A) or 10 μM nifedipine (B) to inhibit I_{Ca}. The magnitude of currents (as peak current minus current at the end of the pulse) were expressed as a percentage of that evoked at +80 mV. Mean (±s.e.mean) data are shown for control conditions and in the presence of 1 mM halothane: $n=4$ for both (A and B). At voltages more positive than +20 mV (A), and +60 mV (B) halothane significantly inhibited I_{to} ($P<0.05$).

where significant inhibition of I_{to} was observed, a constant fraction of I_{to} was blocked suggesting the effects of halothane on I_{to} were not voltage-dependent.

The effects of a range of halothane concentrations (0.1, 0.3, 1 and 3 mM) on peak I_{to} evoked by test pulses from −80 to +80 mV was studied. Peak I_{to} was reduced progressively: 0.1, 0.3 and 1 mM halothane reduced I_{to} by 3.2±6.8%, 15.4±3.1% and 34.2±3.0%, respectively ($n=3-23$ cells). At 3 mM halothane, it was difficult to separate residual I_{to} from the decay of the capacity transients and as such I_{to} appeared to be abolished completely. This might result from greater inhibition of the current in addition to accelerated current inactivation (see below). With this caveat, and assuming 100% inhibition of I_{to} by 3 mM halothane, fitting the concentration-dependence of I_{to} with a typical dose-response function generated a $K_{0.5}$ value of 1.1±0.2 mM.

Effect of halothane on the activation-voltage relationship

Activation-voltage relationships were constructed from current-voltage relationships (see Figure 2) under control conditions and in the presence of 1 mM halothane. Data with both Cd^{2+} and nifedipine as the I_{Ca} antagonist are shown in Figure 3. Currents at various test potentials were divided by the driving force for K^+ (with -85 mV as the value for the equilibrium potential for potassium, E_K) and the resulting conductance expressed as a percentage of that achieved at $+80$ mV for each condition. With Cd^{2+}, 1 mM halothane led to a small but not significant shift in the activation-voltage curve to more negative potentials (see Table 1) whereas, with nifedipine, there was a small but not significant shift towards positive membrane potentials (see Table 1). Statistical analysis of the effects of halothane on the $V_{0.5}$ values of activation curves for data recorded both in the presence of Cd^{2+} and nifedipine suggested that halothane had no significant effect on the activation-voltage relationship ($P=0.724$, $n=8$).

Effects of halothane on steady-state inactivation and kinetics of I_{to}

To determine whether the inhibitory action of halothane involved a shift of the steady-state inactivation curve, 200 ms test pulses to $+80$ mV were evoked following a 500 ms conditioning pulse to various voltages between -100 and 0 mV (voltage clamp protocol repeated every 2 s). Figure 4A shows typical data under control conditions and Figure 4B with 1 mM halothane. Experiments were carried out in the presence of both Cd^{2+} and nifedipine to inhibit I_{Ca}. Inactivation curves were obtained by plotting I_{to} during the test pulse against the membrane potential during the conditioning pulse (Figure 4C,D). I_{to} was normalized to the maximal I_{to} during the test pulse to $+80$ mV after the conditioning pulse to -100 mV. Data were fitted with the Boltzmann function to derive values of $V_{0.5}$ and slope factor (Table 1). For data with nifedipine, $V_{0.5}$ and slope factor were -50.7 ± 0.4 and -6.9 ± 0.3 mV, respectively, for control data, (Figure 4D and Table 1). 1 mM halothane led to an 18 mV shift in the $V_{0.5}$ of the steady-state inactivation curve towards negative potentials ($P=0.025$ for control *vs* halothane): $V_{0.5}$ and slope factor were -68.6 ± 0.9 and -10.3 ± 0.8 mV, respectively (Figure 4D and Table 1). On removal of halothane the steady-state inactivation curve returned to control values ($P=0.125$ between control and post control). A similar significant shift in the $V_{0.5}$ of the steady-state inactivation curve was found when Cd^{2+} was used to block I_{Ca} ($P=0.013$ for control *vs* halothane; see Figure 4C and Table 1). Statistical comparisons were made on the magnitude of the halothane-induced shifts in the steady-state inactivation curves irrespective of which agent was used to inhibit I_{Ca}. This comparison showed that halothane led to a mean shift of 16 mV towards negative membrane potentials ($P=0.005$, $n=8$). As such, at -80 mV in the presence of nifedipine, only $\sim$75% of current was available for activation and therefore this shift in the steady-state inactivation curve would contribute to the inhibitory effect of halothane on I_{to} from a holding potential of -80 mV.

Figure 1B suggested that halothane accelerated the decay of current during the pulse when compared to control (Figure 1A). To investigate the effect of halothane on the kinetics of inactivation of I_{to}, individual current records evoked by test pulses to $+80$ mV were fitted with a double exponential function. Figure 5A,B show the quality of the fit of the double exponential function to the inactivation phase of I_{to} under control conditions (Figure 5A) and in the presence of 1 mM halothane (Figure 5B). The fit was dominated by the first of the two exponentials (τ_1); in six cells under control conditions, τ_1 was 14 ± 1 ms which was significantly reduced to 9 ± 1 ms by 1 mM halothane ($P=0.017$, $n=6$; see Figure 5C). On removal of halothane, inactivation kinetics returned to control values (post control τ_1, 14 ± 1 ms). These data are consistent with a reversible acceleration of the inactivation process of I_{to} by halothane.

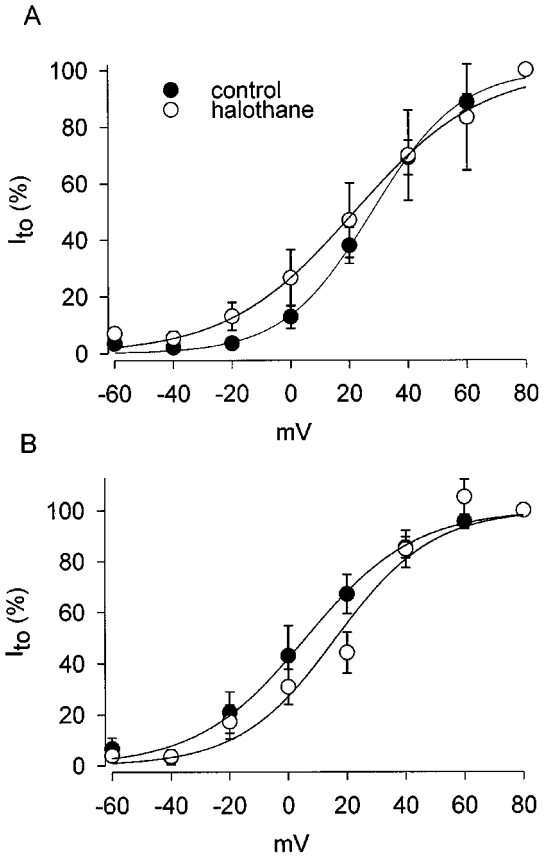

Figure 3 Activation curves for I_{to} (expressed as per cent conductance) under control conditions and in the presence of 1 mM halothane. Experiments were carried out with Cd^{2+} (A) and nifedipine (B) to block I_{Ca}. Data were calculated as described in the text and are shown as mean$\pm$s.e.mean from four cells. Values from curve fits to the Boltzmann function are shown in Table 1.

Table 1 Data shown in Figures 3 and 4 were fitted with the Boltzmann function to yield values of $V_{0.5}$ and slope factor

Activation-voltage relationship

	300 μM Cd^{2+}		*10 μM nifedipine*	
	$V_{0.5}$ (mV)	*Slope factor* (mV)	*$V_{0.5}$* (mV)	*Slope factor* (mV)
Control	$+27.7\pm0.8$	14.9 ± 0.7	$+6.0\pm1.3$	18.5 ± 1.1
Halothane	$+21.8\pm1.7$	21.5 ± 1.6	$+16.3\pm3.5$	16.5 ± 3.1

Steady-state inactivation

	300 μM Cd^{2+}		*10 μM nifedipine*	
	$V_{0.5}$ (mV)	*Slope factor* (mV)	*$V_{0.5}$* (mV)	*Slope factor* (mV)
Control	-19.7 ± 0.6	-7.0 ± 0.6	-50.7 ± 0.4	-6.9 ± 0.3
Halothane	-31.3 ± 1.1*	-9.9 ± 1.0	-68.6 ± 0.9*	-10.3 ± 0.8

*$P<0.05$ compared to control.

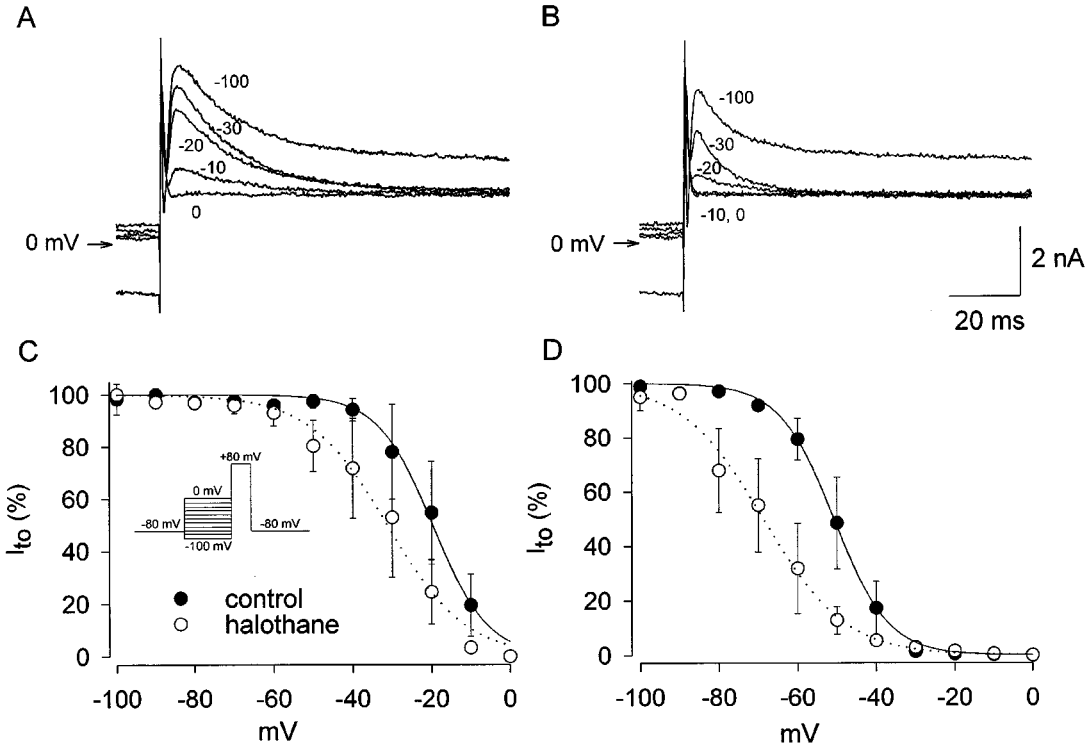

Figure 4 Steady-state inactivation of I_{to}. Cells were clamped to a range of conditioning potentials (between −100 and 0 mV in 10 mV steps) for 500 ms followed by a test pulse to +80 mV to evoke residual I_{to}. Current records following conditioning pulses from −100, −30, −20 −10 and 0 mV are shown for clarity under control conditions (A) and with 1 mM halothane (B). Arrows indicate zero current level. 300 μM Cd^{2+} was present throughout to inhibit I_{Ca}. Steady-state inactivation curves are shown under control conditions and in the presence of 1 mM halothane with 300 μM Cd^{2+} (C) and 10 μM nifedipine (D) as the Ca^{2+} channel antagonist. Currents evoked during the test pulse were expressed as a percentage of maximum I_{to} and plotted against the conditioning pulse potential. Data are presented as mean±s.e.mean for four cells and were fitted with the Boltzmann function which returned values of $V_{0.5}$ and slope factor (see Table 1).

The restitution or reactivation of I_{to} was investigated using a double pulse protocol (see inset to Figure 6C for voltage protocol). Test pulses to +80 mV were applied at various test intervals (between 10 and 100 ms in 10 ms steps) following a 200 ms conditioning pulse to +80 mV. Figure 6 illustrates the resulting currents under control conditions (Figure 6A) and in the presence of 1 mM halothane (Figure 6B). Figure 6C shows I_{to} during the test pulse (expressed as a percentage of I_{to} during the conditioning pulse) plotted against the test interval under control conditions and with 1 mM halothane. Single exponential functions were fitted to these data to derive the time constant (τ) for restitution of I_{to}. In six cells under control conditions, τ was 15.6±2.1 ms. 1 mM halothane slowed restitution of I_{to} (τ of 30.7±4.6 ms) and this effect approached significance ($P=0.057$, $n=6$).

Discussion

Effects of halothane on I_{to}

Halothane led to a decrease in the magnitude of I_{to} at concentrations likely to be relevant to general anaesthesia; for example, 0.3 mM (close to the MAC_{50} value for halothane) inhibited the current by 15%. The impact this inhibition would have on the configuration of the ventricular action potential is discussed below. From a holding potential of −80 mV, I_{to} was evoked by clamp steps more positive than −40 mV similar to that observed previously (Dukes & Morad, 1991; Stengl *et al*., 1998). 1 mM halothane led to a 16 mV shift in the steady-state inactivation curve towards negative membrane potentials such that at the normal resting potential (e.g. −80 mV) only ~75% of the control current was available for activation. This value is close to the percentage inhibition of I_{to} by the same concentration of halothane (72%, see Figure 1D). Halothane also led to an acceleration of the inactivation of I_{to} (Figure 5) as has been reported for I_{Ca} (Pancrazio, 1996). As there was no significant effect of halothane on the activation-voltage relationship (Figure 3) these data suggest that the inhibitory effect of halothane on I_{to} results primarily from a shift in the steady-state inactivation curve (Figure 4, Table 1).

Controversy has surrounded potential mechanisms for the effects of volatile anaesthetics on membrane currents. As halothane, and other general anaesthetics, inhibit a wide range of membrane channels (see Introduction), it has been suggested that their inhibitory effects are non-specific and result from disturbance of the lipid environment around membrane channels rather than from a direct effect at the level of any individual protein (see Franks & Lieb, 1982 for review). More recent data however, has pointed to volatile anaesthetics and 1-alkanols having direct effects on proteins. For example, Correa (1998) reported that halothane affected gating characteristics of *Shaker* K^+ channels and that 1-alkanols interact with the S4-S5 cytoplasmic linker of the dShaw2 K^+ channel (Harris *et al*., 2000). In light of such recent findings, it is becoming more widely accepted that anaesthetic agents have direct effects on membrane proteins which contribute to their actions.

The transient outward K^+ channels in the heart, Kv4.2, Kv4.3 and Kv1.4, display N-type inactivation which is thought

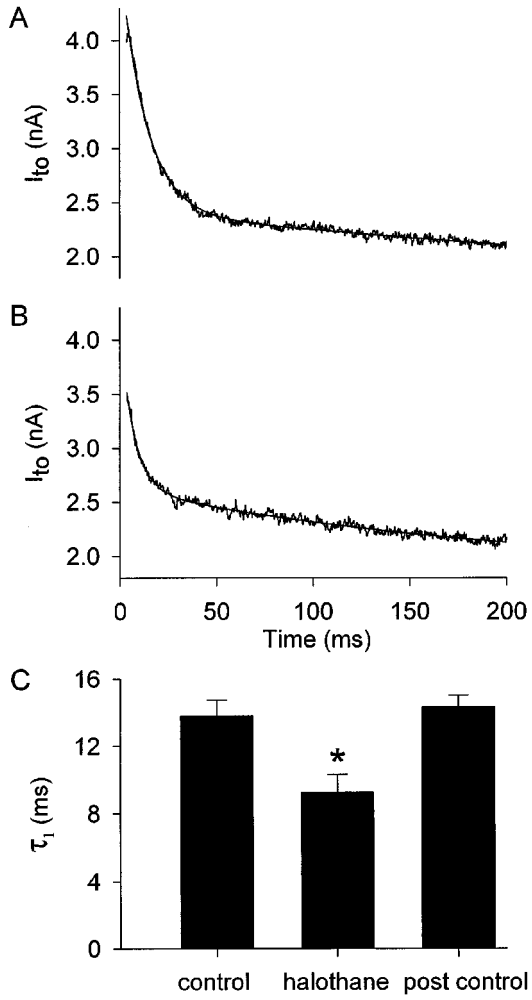

Figure 5 Effect of halothane on current inactivation. The inactivation phase of I_{to}, evoked by 200 ms test pulses from −80 to +80 mV, were best fit by a double exponential function under control conditions (A) and in the presence of 1 mM halothane (B). 300 μM Cd^{2+} was present throughout to inhibit I_{Ca}. In the examples shown, the first and dominant time constant (τ_1) was 13.3 ms under control conditions (A) and this was reduced to 7.0 ms by halothane (B). Data in (A and B) are from the same cell. (C) mean value (±s.e.mean) of τ_1 from six cells under control conditions and in the presence of 1 mM halothane. Halothane led to a significant decrease in τ_1 (*$P=0.017$ for halothane *vs* control). On wash out of halothane τ_1 returned to control values ($P=0.018$ for halothane *vs* post control).

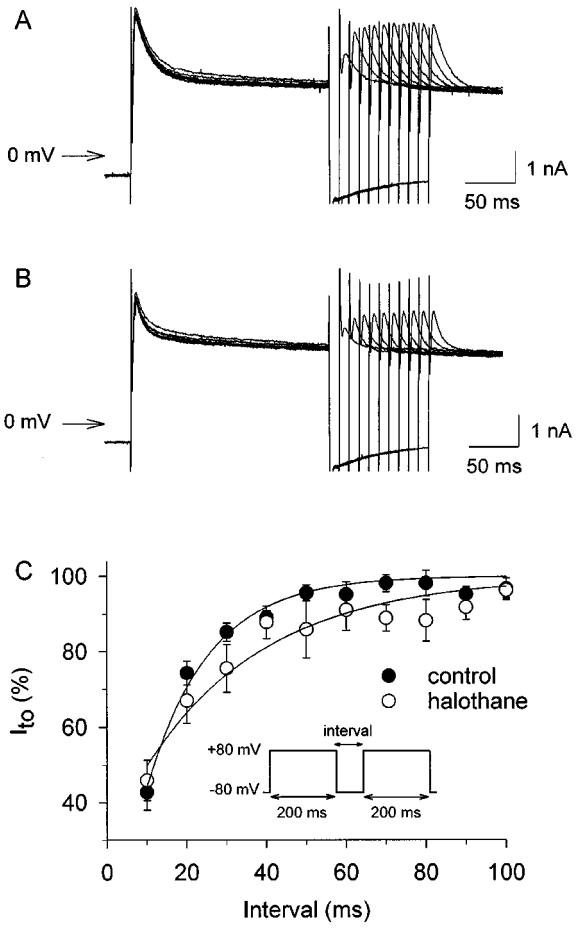

Figure 6 Effects of halothane on the restitution of I_{to}. (A) currents evoked under control conditions. (B) Currents evoked in the presence of 1 mM halothane. See inset in (C) for voltage protocol and text for further details. 300 μM Cd^{2+} was present throughout to inhibit I_{Ca}. (C) Restitution curves under control conditions and in the presence of 1 mM halothane. Data ae shown as mean±s.e.mean for six cells. Curves represent single exponential functions fitted to these data (see text for details of curve fits).

to depend on a ball and chain type mechanism located on the cytoplasmic side of the channel (Hoshi *et al.*, 1990; Zagotta *et al.*, 1990). The results of the present study (shift of the inactivation curve to negative potentials, acceleration of the onset of inactivation and possible slowing of the recovery from inactivation) are consistent with a halothane-induced increase in the affinity of the inactivating ball for its docking site (S4-S5 linker) at the channel mouth. Preliminary work by Haider *et al.* (2000) predicted the structure of the Kv4.2 channel based on the structure of the KcsA channel (Doyle *et al.*, 1998) using comparative modelling techniques. When this structure was probed with halothane molecules, Haider *et al.* (2000) showed that halothane bound to hydrophobic regions on the Kv4.2 channel near to the receptor for the inactivating ball (S4-S5 linker). Furthermore, as the inactivating particle is composed of both hydrophilic and hydrophobic regions (Antz *et al.*, 1997), it may also be a potential target for halothane interaction. The data of Haider *et al.* (2000) suggest that the effects of halothane on I_{to} could be due to a direct interaction with the protein rather than a result of non-specific disturbance of the lipid environment of the channel although the latter possibility cannot be discounted. It is intriguing that the modelling data described above implicate the receptor for the inactivating particle (the S4-S5 linker) as a potential target site for halothane interaction as this region was found to be involved in the binding of 1-alkanols to dShaw2 K^+ channels by Harris *et al.* (2000).

Concentration-dependent effects and clinical relevance

Halothane led to a concentration-dependent decrease in I_{to}, which became apparent at a concentration of 0.1 mM. 0.3 mM halothane, a concentration close to the MAC_{50} value of halothane when expressed in terms of aqueous concentration (Franks & Lieb, 1996) led to a 15% inhibition of I_{to} and, as such, I_{to} will be inhibited during normal anaesthesia. Furthermore, as the concentration of halothane is likely to exceed the MAC_{50} value during induction, considerably greater inhibition of I_{to} may occur during the course of anaesthetic procedures.

Effects of halothane on action potential configuration

I_{to} plays an important role in the early repolarization of the ventricular action potential in many species including humans and thus inhibition of this outward, repolarizing current will act to prolong the duration of the ventricular action potential. It should be borne in mind that halothane also has a potent inhibitory action on I_{Ca} (see Introduction) which would be expected to decrease action potential duration. As such the overall effect of halothane on action potential configuration will depend on the relative density of expression of the various halothane-sensitive ion channels between species and tissues. For example, exposure to halothane led to an overall reduction of action potential duration in rat ventricular myocytes (Harrison *et al*., 1999; Hayes *et al*., 1996), guinea-pig papillary muscles (Ozaki *et al*., 1989), canine Purkinje fibres (Polic *et al*., 1991) and human atrial fibres (Luk *et al*., 1988) which is consistent with a dominant inhibitory action of halothane on I_{Ca} in these preparations. However, (Bosnjak & Kampine, 1986) reported in cat papillary muscles that halothane induced a considerable negative inotropic effect but had little effect on the duration of action potentials. In this latter study action potential duration was prolonged by halothane in the presence of verapamil (Bosnjak & Kampine, 1986) which is consistent with halothane-induced blockade of outward potassium current.

As described in the Introduction, I_{to} is expressed to a greater degree in the sub-epicardium than in the sub-endocardium. As I_{to} is depressed by halothane at clinically relevant concentrations, the transmural difference in action potential duration would be reduced during halothane anaesthesia which could contribute to the arrhythmogenic characteristics of halothane under clinical conditions (Atlee & Bosnjak, 1990). Similar conclusions have been drawn previously based on the action of halothane on action potential characteristics in canine Purkinje fibres (Polic *et al*., 1991) where halothane reduced regional variations in action potential duration between distal and proximal fibres which would also alter the dispersion of refractoriness in the ventricular conduction system. Halothane induced changes in transmural repolarization of the ventricle would lead to modification of T-wave morphology of the electrocardiogram, an effect which is observed relatively frequently during halothane anaesthesia.

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