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Halothane Inhibits Contraction and Action Potential Duration to a Greater Extent in Subendocardial than Subepicardial Myocytes from the Rat Left Ventricle

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Background: **Halothane inhibits the 4-aminopyridine–sensitive transient outward K^+ current (I_{to}), which in many species, including humans, plays an important role in determining action potential duration. As I_{to} is greater in the ventricular subepicardium than subendocardium, halothane may have differential effects on action potential duration and, therefore, contraction in cells isolated from these two regions.**

Methods: **Myocytes were isolated from the subendocardium and subepicardium of the rat left ventricle. Myocytes from each region were electrically stimulated at 1 Hz to measure contractions and action potentials and exposed to 0.6 mM halothane (approximately 2 × minimum alveolar concentration$_{50}$ for the rat) for 1 min. The time from the peak of the action potential to repolarization at 0 and −50 mV was measured to assess the effects of halothane on action potential duration.**

Results: **Halothane inhibited contraction to a significantly ($P = 0.002$) greater extent in subendocardial myocytes than in subepicardial myocytes: the amplitude of contraction during control conditions was 3.6 ± 0.4 μm and 3.2 ± 0.7 μm in subendocardial and subepicardial cells, respectively, and this was reduced to 1.1 ± 0.2 μm (29 ± 2% of control, $P < 0.0001$, n = 10) and 1.4 ± 0.3 μm (46 ± 3% of control, $P = 0.007$, n = 7), respectively, after a 1-min exposure to 0.6 mM halothane. Control action potential duration (at −50 mV) was 67 ± 10 and 28 ± 4 ms in subendocardial and subepicardial myocytes, respectively, and these values were reduced to 39 ± 6 ms (58 ± 3% of control, $P < 0.001$) and 20 ± 3 ms (73 ± 5% of control, $P = 0.009$) by halothane, respectively.**

Conclusions: **Action potential duration was reduced to a greater extent in subendocardial than subepicardial myocytes, which would contribute to the greater negative inotropic effect of halothane in the subendocardium. Furthermore, the transmural difference in action potential duration was reduced by halothane, which could contribute to its arrhythmogenic properties.**

THE volatile anesthetic halothane has both positive and negative inotropic effects on isolated ventricular tissue.[1–8] It has been proposed that the initial transient positive inotropic effect observed on application of halothane results from a potentiation of the Ca^{2+}-induced Ca^{2+}-release process,[7–10] whereas the sustained negative inotropic effect in intact cells is caused by a decrease in myofilament Ca^{2+} sensitivity[8,11,12] in conjunction with an inhibition of the electrically evoked Ca^{2+} transient[8,11,13,14] (resulting primarily from a decrease in sarcoplasmic reticulum Ca^{2+} content[9,12,15]). The inhibitory action of halothane on the L-type Ca^{2+} current (I_{Ca})[16–20] and consequent decrease in action potential duration[8] would contribute to the decrease in the Ca^{2+} transient (and sarcoplasmic reticulum Ca^{2+} content[12]).

Recently, Davies *et al.*[21] reported that halothane inhibited the 4-aminopyridine sensitive transient outward K^+ current (I_{to}), which in many species (including humans) is involved in early repolarization of the ventricular action potential. I_{to} density is greater in ventricular subepicardium than subendocardium.[22–27] As a consequence of the transmural gradient of I_{to} expression and its blockade by halothane, halothane may affect action potential duration differentially across the ventricular wall. As contractility is modulated by action potential duration,[28] the negative inotropic effect of halothane may be expected to differ in cells isolated from the ventricular subendocardium compared with the subepicardium.

Furthermore, because of the greater expression of I_{to} in the subepicardium, the action potential duration is shorter than in the subendocardium. This transmural gradient in action potential duration (and therefore refractoriness) is important for the normal spread of repolarization throughout the ventricle (*i.e.*, repolarization proceeds from subepicardium to subendocardium). As previously alluded to, inhibition of I_{to} by halothane is likely to affect action potential duration differentially across the ventricular wall, which could result in disturbances in normal transmural action potential duration gradients (with consequent changes in transmural refractoriness). Agents that modify the normal passage of repolarization and refractoriness are potentially arrhythmogenic,[29] and it is interesting to note that, in the clinical situation, halothane anesthesia is associated with an increased incidence of arrhythmia.[30,31]

The aim of the current experiments was to test the hypothesis that halothane has differential effects on action potential duration and contractility in cells isolated from the ventricular subendocardium and subepicardium, and that halothane affects the transmural gradient of action potential duration, which could contribute to its arrhythmogenic properties.

Materials and Methods

Cell Isolation

The experiments described were conducted on Wistar rats (weight, approximately 250 g; provided by Biomed-

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ical Services, University of Leeds, Leeds, United Kingdom) that were given access to food and water *ad libitum* and maintained under a 12-h light–dark cycle. Animals were killed by a blow to the head followed by cervical dislocation (schedule 1 procedure sanctioned by the UK government Home Office, London, United Kingdom, under project license 60/02087), and the heart was rapidly excised into an "isolation solution" (see below for composition), supplemented with 750 μM $CaCl_2$ and equilibrated with 100% O_2. The heart was flushed of blood by retrograde perfusion *via* the coronary arteries with the above solution and then perfused for 4 min with the isolation solution, to which 100 μM Na_2 EGTA was added.[32] The heart was then perfused for 7 min with the isolation solution supplemented with 1 mg/ml collagenase (type 1; Worthington Biochemical Corp., Lakewood, NJ) and 0.1 mg/ml protease (type XIV; Sigma, Poole, Dorset, United Kingdom), after which the ventricles were cut from the heart. The left ventricular free wall was then separated from the septum, and tissue was dissected from the subendocardium and subepicardium. Samples from both regions were then finely chopped and shaken in the collected enzyme solution (to which 1% bovine serum albumin was added) for 5-min intervals. Dissociated myocytes were collected by filtration at the end of each 5-min digestion, and any remaining tissue was returned for further enzyme treatment. The dissociated myocytes from each region were centrifuged at 30*g* for 1 min, resuspended in the Ca^{2+}-containing isolation solution, and stored at room temperature until required.

Single ventricular myocytes can be readily isolated from different regions of the heart and therefore represent an ideal preparation to investigate regional variations in myocyte properties. Furthermore, individual myocytes are small enough that equilibration of halothane is rapid throughout the entire preparation, which minimizes problems associated with diffusion delays that could occur in multicellular preparations. In addition, single cells appear to respond to inotropic interventions in qualitatively the same way as multicellular preparations.[33,34]

Solutions

The isolation solution was composed of 130 mM NaCl, 5.4 mM KCl, 1.4 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 5 mM HEPES, 10 mM glucose, 20 mM taurine, 10 mM creatine, pH 7.1 (NaOH) at 37°C. After dissociation, myocytes were perfused with a physiologic salt solution of the following composition: 140 mM NaCl, 5.4 mM KCl, 1.2 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 5 mM HEPES, 10 mM glucose, 1 mM $CaCl_2$, pH 7.4 (NaOH) at 37°C (contraction experiments) or 30°C (action potential experiments). Halothane (0.6 mM) was added to the above solution from a 0.5-M stock solution made up of dimethyl sulfoxide. Halothane-containing experimental solutions contained 0.12% dimethyl sulfoxide, a concentration that had no significant effect on contractions (not shown).

The tip of each patch pipette was filled with a solution of the following composition: 140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.1 at 30°C. The pipette was then back-filled with the above solution supplemented with 400 μg/ml amphotericin B. Unless stated otherwise, all solution constituents were obtained from Sigma (Poole, Dorset, United Kingdom).

Recording Cell Length

Freshly dissociated myocytes were transferred to a tissue chamber (volume, 0.1 ml) attached to the stage of an inverted microscope (Nikon Diaphot; Nikon UK, Kingston-Upon-Thames, Surrey, United Kingdom). The cells were allowed to settle for several minutes onto the glass bottom of the chamber before being superfused at a rate of approximately 3 ml/min with the physiologic salt solution. Solutions were delivered to the experimental chamber by magnetic drive gear metering pumps (Micropump, Concord, CA), and solution concentration and temperature (37°C) were maintained by feedback circuits.[35]

Myocytes were field stimulated at a frequency of 1 Hz (stimulus duration, 2 ms) *via* two platinum electrodes situated in the sides of the chamber. A charge coupled device camera attached to the side port of the microscope captured the cell image, and cell length was monitored continuously using a system developed by IonOptix (IonOptix Inc., Milton, MA), which is capable of discriminating a 0.01-μm change in cell length. Contractions were digitized at 200 Hz, and their time course was analyzed with IonWizard software (IonOptix Inc.). Contraction amplitude was measured from the baseline to peak and expressed as an absolute change in length and as a percentage of the control contraction amplitude before halothane exposure. Once contractions were stable during control conditions, 0.6 mM halothane was applied for 1 min. After the removal of halothane, if contraction did not return to within ± 10% of control, data were excluded from analysis.

Recording Action Potentials

Action potentials were recorded using the perforated patch clamp technique in current clamp mode (Axoclamp 200A; Axon Instruments, Inc., Foster City, CA). After the formation of a gigaseal, cells were left for up to 15 min until access resistance had decreased to less than 30 MΩ. Action potentials were evoked in response to current pulses of 1 nA (2 ms in duration) at a frequency of 1 Hz. Voltage traces were filtered at 1 kHz, digitized at 5 kHz, and the time course of action potentials was analyzed using PClamp software (Axon Instruments Inc.). Because of the delay associated with establishing electrical access, these experiments were protracted. To increase the success rate of these experiments, they

were conducted at 30°C where the cells were more stable and could tolerate the experimental protocols. However, it should be noted that the resulting action potential configurations recorded at 30°C would differ slightly from those recorded at 37°C because of the temperature sensitivity of ion channel conductance.

Statistical Analysis

Data are presented as mean ± SEM, and statistical comparisons were conducted with the Student paired or unpaired *t* tests as appropriate or analysis of variance followed by corrected *t* tests (Tukey) for multiple comparisons, using either SigmaPlot 2000 or SigmaStat 2.0 (Jandel Scientific, Erkrath, Germany). All graphs were prepared using SigmaPlot 2000 (Jandel Scientific).

Results

Effects of Halothane on Contraction of Subendocardial and Subepicardial Cells

Figure 1 shows slow time base records of contraction of two representative cells isolated from the subendocardium (fig. 1A) and subepicardium (fig. 1B) of the rat left ventricle. During control conditions, contraction amplitude during 1 Hz stimulation was 3.6 ± 0.4 and 3.2 ± 0.7 μm in subendocardial and subepicardial cells, respectively. In both cell types, application of 0.6 mM halothane led to a similar ($P = 0.618$) initial increase in contraction amplitude (to 145 ± 7 and 153 ± 16% of control in subendocardial [n = 10] and subepicardial cells [n = 7], respectively), followed by a sustained inhibition of contraction as observed previously.[7,8] At the end of a 1-min exposure to halothane, contraction was reduced to 1.1 ± 0.2 μm ($P < 0.0001$ for control *vs.* halothane, n = 10; to 29 ± 2% of control) in subendocardial cells, a degree of inhibition that was significantly greater ($P = 0.002$, analysis of variance) than observed in subepicardial cells (contraction reduced to 1.4 ± 0.3 μm, $P = 0.007$ for control *vs.* halothane, n = 7; to 46 ± 3% of control; fig. 1C). On removal of halothane, contractions returned to control values (to 101 ± 2 and 102 ± 1% of control in subendocardial and subepicardial cells, respectively).

The transmural difference in the sustained negative inotropic effect of halothane was qualitatively the same at 23 and 37°C. For example, at 23°C, after a 1-min exposure to 0.6 mM halothane, contractions were inhibited to a greater extent in subendocardial myocytes (to 14 ± 4% of control, n = 5) than in subepicardial myocytes (to 30 ± 6% of control, n = 3).

Figure 2 shows fast time base recordings of contractions during control conditions and after a 1-min exposure to 0.6 mM halothane. Neither the time to peak nor

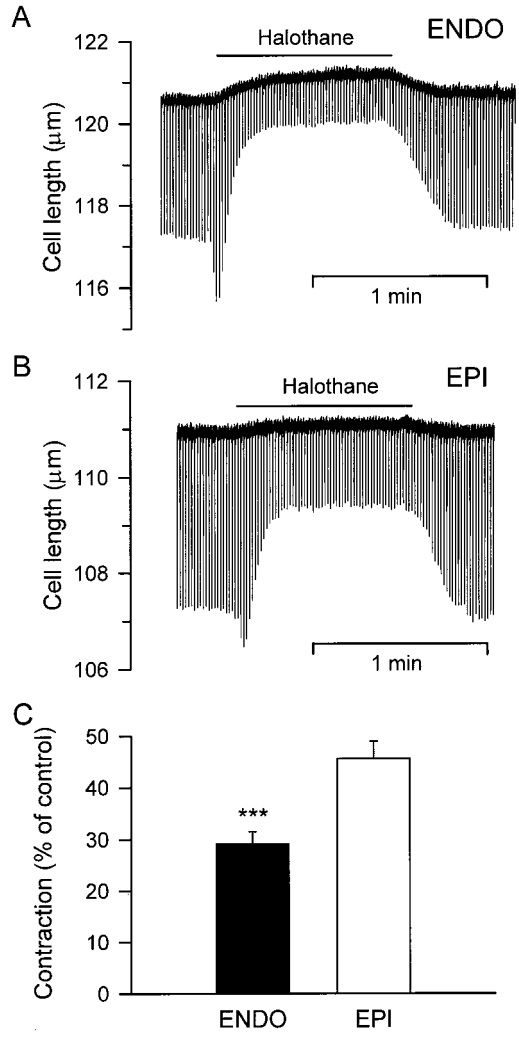

Fig. 1. Effects of 0.6 mM halothane on contraction in a representative (*A*) subendocardial (ENDO) and (*B*) subepicardial (EPI) myocyte. (*C*) Mean data (± SEM) describing the extent of inhibition of contraction during the sustained negative inotropic effect in subendocardial and subepicardial myocytes (n = 10 and 7, respectively). ***$P < 0.001$, sustained negative inotropic effect in subendocardial *versus* subepicardial myocytes.

the time to half relaxation of contractions differed between subendocardial and subepicardial myocytes during control conditions. However, in subendocardial myocytes, compared with control, halothane significantly prolonged the time to peak of contraction during the initial positive inotropic effect, abbreviated the time to peak of contraction during the sustained negative inotropic effect, and prolonged the time to half relaxation (table 1). These results are similar to those reported by Harrison *et al.*,[8] although those experiments were conducted on cells isolated from general ventricular tissue. In subepicardial myocytes, the only significant difference in time course associated with halothane exposure was prolongation of the time to peak of contraction during the initial positive inotropic effect (table 1).

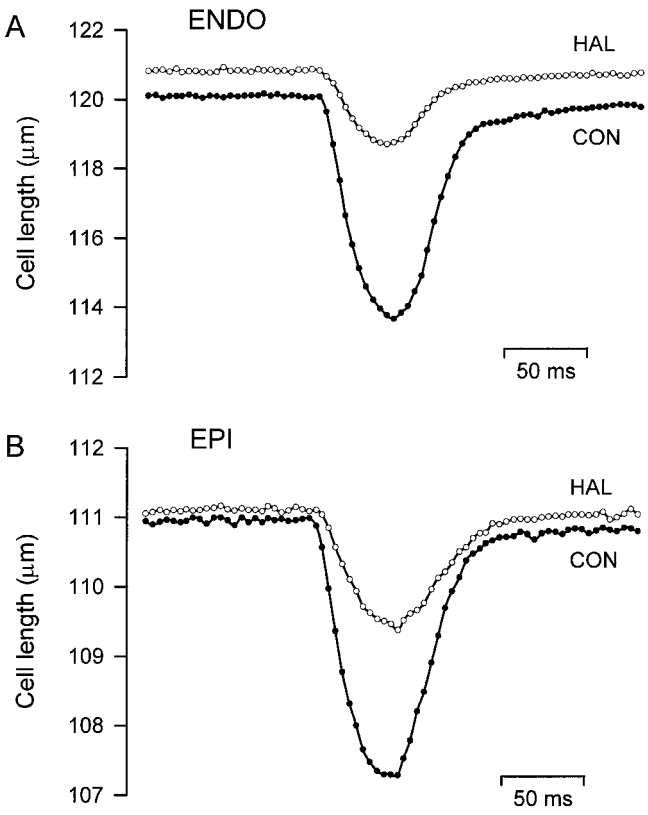

Fig. 2. Fast time base records of contraction during control conditions and after a 1-min exposure to 0.6 mM halothane in a (*A*) subendocardial (ENDO) and (*B*) subepicardial (EPI) myocyte. The traces shown are an average of three consecutive contractions. HAL = halothane; CON = control.

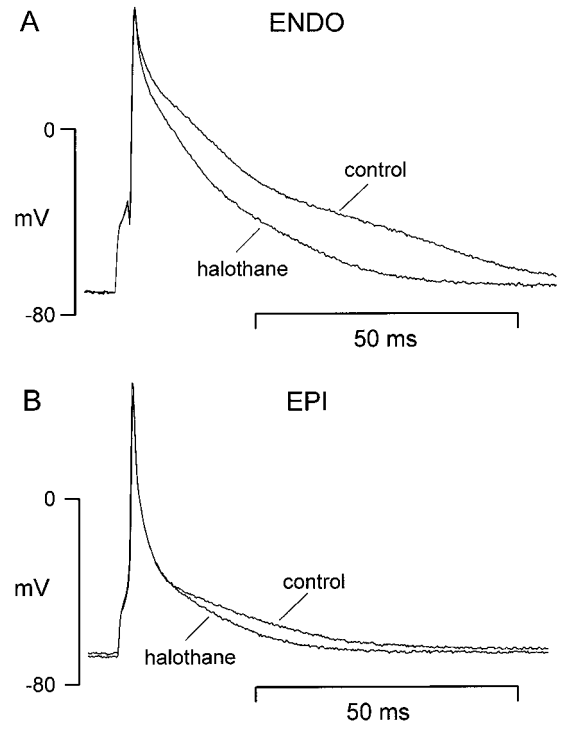

Fig. 3. Representative action potential records during control conditions and after a 1-min exposure to 0.6 mM halothane in a (*A*) subendocardial (ENDO) and (*B*) subepicardial (EPI) myocyte. The traces shown are an average of 15 consecutive action potentials.

Effects of Halothane on Action Potentials in Subendocardial and Subepicardial Cells

Figures 3A and B show representative action potentials recorded from subendocardial and subepicardial myocytes during control conditions and after a 1-min exposure to 0.6 mM halothane. During control conditions, action potential duration (time from the peak to repolarization at −50 mV) was 67 ± 10 ms in subendocardial myocytes but was significantly shorter ($P = 0.005$, unpaired *t* test) in subepicardial myocytes (28 ± 4 ms). At the end of a 1-min exposure to halothane, action potential duration (at −50 mV) was significantly reduced in both cell types: to 58 ± 3% of control in subendocardial myocytes (*i.e.*, to 39 ± 6 ms, $P < 0.001$ *vs.* control) and to 73 ± 5% of control in subepicardial myocytes (*i.e.*, to 20 ± 3 ms, $P = 0.009$ *vs.* control), with halothane having a greater abbreviating effect in the subendocardium than the subepicardium ($P < 0.05$, analysis of variance).

Figure 4 shows mean data for action potential duration at 0 mV (fig. 4A) and at −50 mV (fig. 4B) in both cell types during control conditions and after a 1-min exposure to halothane. These data illustrate that halothane led to significant reductions in action potential duration during both early and late phases of repolarization. However, it is also important to consider the transmural difference in action potential duration during control conditions and with halothane. Figure 4B illustrates that mean action potential duration (at −50 mV) differed by 39 ms during control conditions between the subendocardium and subepicardium, but this difference was reduced by half (to 19 ms) in the presence of halothane.

Table 1. Time Course of Contractions in Subendocardial and Subepicardial Myocytes during Halothane Exposure

Time to Peak of Contraction (ms)			Time from Peak to Half Relaxation (ms)		
Control	Transient Positive Inotropy	Sustained Negative Inotropy	Control	Transient Positive Inotropy	Sustained Negative Inotropy
Subendocardial myocytes					
40 ± 2	44 ± 2*	36 ± 2†	35 ± 4	35 ± 3	41 ± 4†
Subepicardial myocytes					
35 ± 2	39 ± 2*	33 ± 3	31 ± 4	33 ± 4	34 ± 3

* $P < 0.01$. † $P < 0.05$.

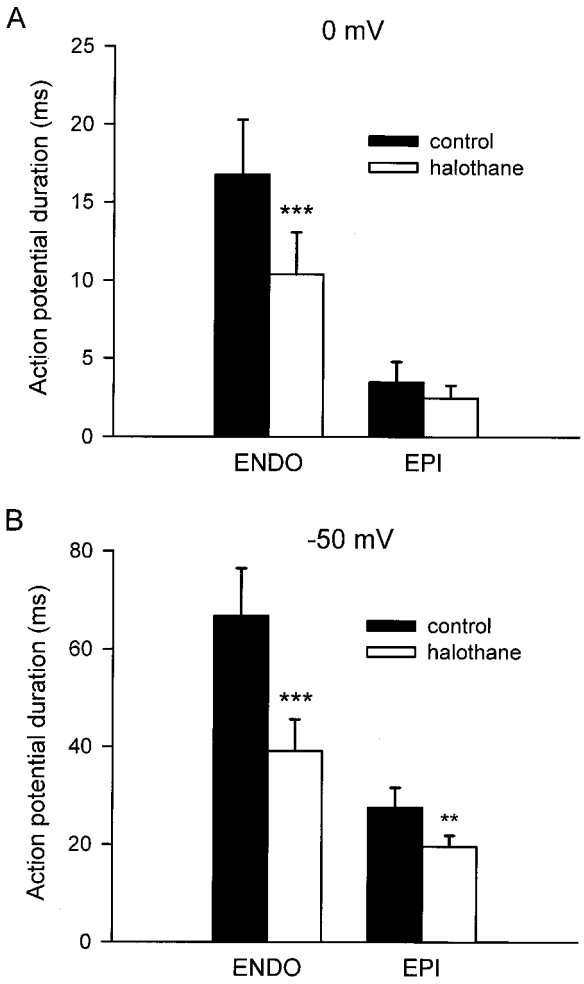

Fig. 4. Mean data (± SEM) describing action potential duration measured at 0 mV (*A*) and −50 mV (*B*) in subendocardial (ENDO; n = 7) and subepicardial (EPI; n = 6) myocytes during control conditions and after a 1-min exposure to 0.6 mM halothane. **$P < 0.01$, ***$P < 0.001$ *versus* control.

Discussion

Regional Differences in the Negative Inotropic Effect of Halothane

Figure 1 illustrates that a clinically relevant dose of halothane (0.6 mM, approximately 2 × minimum alveolar concentration$_{50}$) reduced contraction to a significantly greater extent in subendocardial than subepicardial myocytes. Halothane is known to affect several targets in cardiac myocytes that could contribute to the observed regional effects of halothane on contraction (see Introduction). For example, halothane reduces the L-type Ca^{2+} current (which results in abbreviation of the action potential), myofilament Ca^{2+} sensitivity, sarcoplasmic reticulum Ca^{2+} content, and I_{to}, but sensitizes Ca^{2+}-induced Ca^{2+} release (which is likely to contribute to the reduction in sarcoplasmic reticulum Ca^{2+} content). The data presented here suggest that there must be transmural differences in the sensitivity of one or more of these targets to halothane. Cardiac contractility depends crucially on action potential duration,[28] and the greater shortening of action potential duration induced by halothane in subendocardial cells would contribute to the greater negative inotropic effect of halothane compared with subepicardial cells (see below).

One interesting additional possibility is that the extent of halothane-induced reduction in myofilament Ca^{2+} sensitivity may differ between the subendocardium and subepicardium, which could contribute to the greater negative inotropic effect of halothane in the subendocardium. Figures 1 and 2 show that, during halothane exposure, diastolic cell length increases in both cell types, and the effect appears more marked in subendocardial than subepicardial myocytes. Such an increase in diastolic cell length is indicative of a decrease in myofilament Ca^{2+} sensitivity as it occurs in the absence of a change in diastolic Ca^{2+}.[12] Analysis of the extent to which diastolic cell length increased during an exposure to halothane showed that, in subendocardial myocytes, cell length increased significantly from 108. 2 ± 4.8 to 108.9 ± 4.8 μm ($P = 0.0016$ *vs.* control, paired *t* test; by 0.6 ± 0.1% of control cell length) but increased to a lesser extent (from 118.4 ± 9.2 to 118.8 ± 9.3 μm ($P = 0.026$ *vs.* control, paired *t* test; by 0.3 ± 0.1% of control cell length) in subepicardial myocytes. Although there was no significant difference in the magnitude of the increase in cell length between the two cell types, these data suggest that myofilament Ca^{2+} sensitivity may be inhibited to a greater extent in cells from the ventricular subendocardium than the subepicardium, although further experimentation would be required to verify this.

Regional Differences in the Effect of Halothane on the Action Potential

Figure 3 illustrates that, during control conditions, the subepicardial action potential is shorter than the subendocardial action potential because of the greater density of I_{to} in subepicardium *versus* subendocardium.[22–27] In the presence of halothane, action potential duration (at −50 mV) is shortened to a greater extent in the subendocardium than the subepicardium. As previously discussed, this would contribute to the greater negative inotropic effect of halothane observed in the subendocardium *versus* subepicardium.

The regional difference in the effects of halothane on action potential duration can be explained in terms of the inhibitory effects of halothane on I_{Ca} and I_{to}. Considering the expected electrophysiologic effects of halothane on I_{Ca} in isolation, inhibition of an inward current (*e.g.*, I_{Ca} by halothane) would induce a shortening of the ventricular action potential. It is clear that there is no difference in the density of I_{Ca} across the ventricular wall,[23,27,36,37] and one would therefore expect similar action potential–abbreviating effects resulting from halothane-induced inhibition of I_{Ca} in the subendocardium and subepicardium.

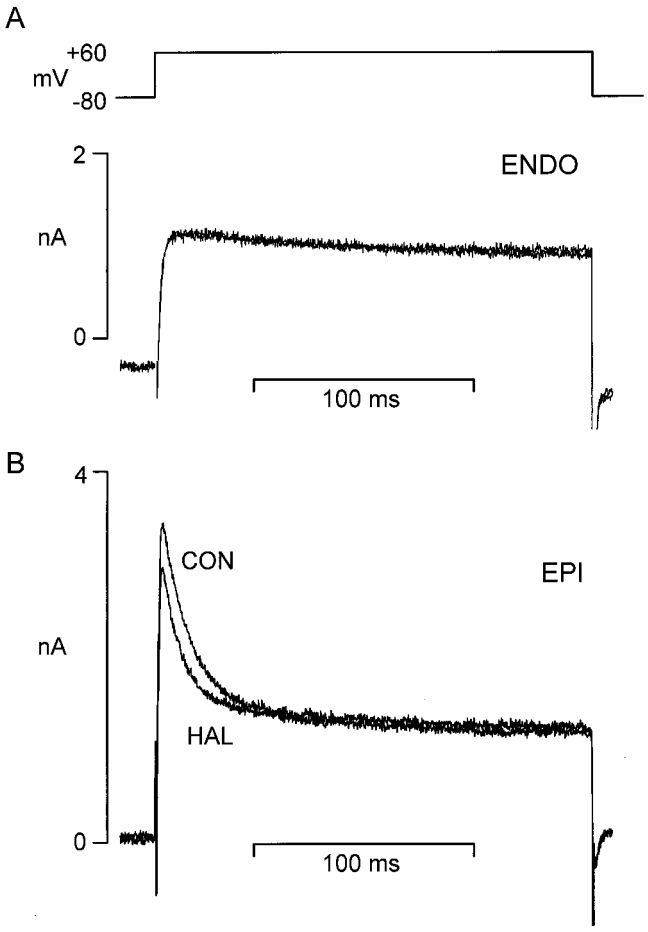

Fig. 5. Representative recordings of I_{to} in (*A*) subendocardial (ENDO) and (*B*) subepicardial (EPI) cells during control conditions and after a 1-min exposure to 0.6 mM halothane. Currents were measured with the whole cell patch clamp technique as described by Davies *et al.*,[21] with 20 μM $CdCl_2$ used to block I_{Ca}. I_{to} was evoked by 200-ms voltage clamp pulses to +60 mV from a holding potential of −80 mV.

For illustrative purposes, figure 5 shows representative records of I_{to} from subendocardial and subepicardial myocytes (measured during conditions to block I_{Ca}[21]) in the absence and presence of 0.6 mM halothane. I_{to} displays rapid activation (and thus overlaps in time with the activation of I_{Ca}) and is greater in subepicardial than subendocardial myocytes. Halothane induced a robust inhibition of I_{to} in subepicardial myocytes but had little effect on subendocardial I_{to}. Inhibition of this outward current alone by halothane[21] would be expected to prolong action potential duration and, as I_{to} is expressed to a greater extent in the subepicardium than subendocardium,[22–27] then the effect of blocking this current on the action potential duration would be expected to be greater in the subepicardium than the subendocardium. However, the net effect of halothane on action potential duration will be the result of the combined effects of inhibition of both I_{Ca} and I_{to}. In subepicardial myocytes, where I_{to} density is high (fig. 5B), simultaneous inhibition of an outward current (I_{to}) and an inward current (I_{Ca}) would result in only a small net change in the balance of inward and outward currents, and consequently only a modest effect on the duration of the action potential. That action potential duration is reduced in subepicardial cells suggests that inward current is inhibited to a greater extent than outward current (*i.e.*, the inhibitory effect of 0.6 mM halothane on I_{Ca} is greater than I_{to} during these experimental conditions).

Considering subendocardial myocytes, where I_{to} density is much lower (fig. 5A), exposure to halothane would lead to only a small inhibition of outward current (because of the limited blockade of I_{to}), whereas the inward I_{Ca} would be inhibited to the same degree as in subendocardial cells. As such, inward current (I_{Ca}) would be inhibited to a greater degree than outward current (I_{to}), with the result that action potential duration would be abbreviated to a greater extent (figs. 3 and 4).

Transmural Gradient in Action Potential Duration Is Reduced by Halothane

One consequence of the regional effects of halothane is that the transmural difference in action potential duration is reduced. Figure 4 illustrates that, during control conditions, the difference between the mean subendocardial and subepicardial action potential duration (at −50 mV) is 39 ms, but this difference is reduced to 19 ms by halothane. It should be noted, however, that action potential duration was measured in isolated cells and that, *in vivo*, the transmural gradient in action potential duration will be smaller because of the electronic coupling of cells (see Wolk *et al.*[38] for review). The small transmural gradient in action potential duration that exists *in vivo* is important for normal transmural dispersion of repolarization–refractoriness, which helps prevent reentrant arrhythmias. However, agents that affect the normal passage of repolarization or transmural dispersion of refractoriness are known to predispose to reentrant arrhythmias.[29] Our data suggest that, *in vivo*, the transmural dispersion of repolarization is reduced by halothane, which generally would be considered to be antiarrhythmic, not proarrhythmic. However, it is possible that *in vivo*, where the transmural gradient in action potential duration is smaller than recorded in isolated cells (see above), that the greater action potential–shortening effect of halothane on the subendocardium could potentially reduce the normal transmural gradient of repolarization. Given the complex architecture of the heart in terms of fiber orientation and impulse propagation, a potential consequence of altered repolarization would be an enhanced prospect of collision of repolarization wavefronts that would induce local conduction block.[38] Therefore, the inhibitory influence of halothane on membrane currents, by altering normal repolarization, may modulate the vulnerability of the heart to arrhythmias induced by additional stimuli such as a premature ventricular excitation[39] and could contribute to

the increased incidence of arrhythmias observed in the clinical situation.[30,31]

These data show for the first time that halothane has differential effects on contractility and action potential duration in subendocardial and subepicardial myocytes. The greater action potential shortening observed in the subendocardium *versus* the subepicardium may contribute to the greater negative inotropic effect of halothane observed in the subendocardium. However, further experiments examining halothane-induced changes in myofilament Ca^{2+} sensitivity and cytosolic Ca^{2+} regulation in the two cell types are required to further elucidate the differential effects of halothane on the subendocardium and subepicardium.

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