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Effects of propofol and enflurane on action potentials, membrane currents and contraction of guinea-pig isolated ventricular myocytes

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1 The effects of two general anaesthetics, propofol and enflurane, on electrical activity and contractions were investigated in single myocytes isolated from guinea-pig ventricles.

2 Propofol and enflurane depressed the plateau and shortened the duration of action potentials.

3 Under voltage-clamp conditions, propofol and enflurane reduced the amplitude of inward calcium current and of additional inward current activated by cytosolic calcium.

4 Contractions (measured with an optical technique) accompanying either action potentials or second inward currents (in response to depolarizations to 0 mV) were reduced by both anaesthetics. The mechanisms for calcium entry during contractions accompanying pulses to positive potentials such as + 60 mV are thought to differ from those accompanying second inward currents which are evoked by pulses from − 40 to 0 mV. Enflurane enhanced the amplitudes of contractions accompanying pulses to positive potentials; in contrast these contractions were depressed by propofol.

5 In experiments where recovery processes were investigated by use of pairs of voltage-clamp pulses with a variable interval between them, enflurane but not propofol slowed the recovery of contractions and calcium-activated 'tail' currents. These observations are consistent with the hypothesis that enflurane may impair calcium handling by the sarcoplasmic reticulum whereas propofol has little, if any, effect at this site.

6 In conclusion, the actions of propofol and enflurane on second inward currents contribute to their effects on action potentials and contraction. The negative inotropic effect of both anaesthetics may result partly from reduced calcium influx to trigger contraction, and for enflurane, partly from an impairment of calcium handling by the sarcoplasmic reticulum.

Keywords: Propofol; enflurane; calcium currents; calcium-activated currents; isolated ventricular myocytes

Introduction

Certain general anaesthetics depress myocardial contractility, an effect which may, in certain circumstances, prove harmful. Anaesthetics may depress contractility either (i) by reducing the amount of calcium available to the contractile apparatus and/or (ii) by a direct effect on the contractile proteins.

Enflurane is a halogenated inhalational anaesthetic which has negative inotropic actions in man (Calverley *et al.*, 1978), experimental animals (Cutfield, 1983) and isolated atria and ventricles of various mammalian species (Shimosato *et al.*, 1969; Brown & Crout, 1971; DeTraglia *et al.*, 1988). Several studies of the possible cellular mechanisms underlying these cardiodepressant actions have indicated that anaesthetics such as enflurane inhibit calcium influx into cardiac cells and so reduce the transient increase in cytosolic calcium concentration that triggers and controls contraction (Lynch *et al.*, 1982; Nakao *et al.*, 1988; Bosnjak *et al.*, 1991). Triggered release of calcium from the sarcoplasmic reticulum (SR) contributes to this calcium transient and thus to activation of contractile filaments. Evidence suggests that halogenated anaesthetics may inhibit calcium uptake by the SR (Su & Kerrick, 1980) and/or cause calcium to leak from the SR (Su & Kerrick, 1980; Wheeler *et al.*, 1988; 1990; Katsuoka *et al.*, 1989) via the SR calcium release channel (Herland *et al.*, 1990). These actions would also impair cardiac contractility.

In addition, it has been suggested that anaesthetics reduce the calcium sensitivity of contractile proteins (Merin *et al.*, 1974; Pask *et al.*, 1981; Murat *et al.*, 1988; 1990), however, these studies indicate that if these agents do depress contractile proteins, they do so at high anaesthetic concentrations and the effects are small compared to their overall negative inotropic effects. Despite the extensive research in this field it is still not clear what cellular actions of enflurane underlie the negative inotropic effects of this agent.

There has been several reports on the effects of the intravenously-administered anaesthetic propofol (2,4-diisopropylphenol) on the circulation, the most consistently reported effects both in man and in experimental animals being depressed systolic and diastolic arterial pressures and reduced cardiac output (Al-Khudhairi *et al.*, 1982; Claeys *et al.*, 1983; Stephan *et al.*, 1986; Coetzee *et al.*, 1989; Goodchild & Serrao, 1989). It appears that the haemodynamic depression induced by propofol arises, at least in part, from a direct depression of myocardial contractility (Puttick *et al.*, 1992). To date, there are no published studies on the effects of propofol on the cellular mechanisms underlying this contractile depression.

The experiments described in this paper have examined and compared the effects of the halogenated inhalation anaesthetic enflurane and the intravenous anaesthetic propofol on membrane currents thought to underlie cardiac contraction, and the possible effects of these agents on the SR. To achieve this, action potentials, membrane currents and contraction were recorded from guinea-pig isolated ventricular myocytes. Preliminary results have been reported to the British Pharmacological Society (Puttick & Terrar, 1989).

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Methods

Cells were isolated from guinea-pig ventricles by collagenase digestion (Powell *et al.*, 1980; Mitchell *et al.*, 1983). Aliquots of cell suspension were mounted on the surface of an agar-coated coverslip in a perspex organ bath. The superfusing solution contained (mM): NaCl 118.5, KCl 4.2, $NaHCO_3$ 14.5, KH_2PO_4 1.18, $MgSO_4$ 1.18, glucose 11.1 and $CaCl_2$ 2.5. All solutions were bubbled with 95% O_2:5% CO_2 (pH 7.4) and maintained at 37°C. A tap at the inflow to the bath permitted rapid changeover to the anaesthetic-containing solution. Propofol was prepared at a concentration of 50 mM, in a vehicle containing 50:50 polyethyleneglycol:dimethylsulphoxide. This stock solution was applied to the reservoir to give a final concentration of 25, 50 or 100 μM. The vehicle when applied alone to the isolated cells had no apparent effect on the action potentials, membrane currents and contractions. In other experiments the superfusing solution was pre-equilibrated for at least 45 min with enflurane (Abbott) from an Enfluratec vapouriser. The enflurane concentration in the bath was assayed by infra-red spectrophotometry (Speden, 1963). The concentrations determined were: 2%, 0.90 ± 0.02 mM: 3%, 1.46 ± 0.09 mM: 4%, 2.76 ± 0.14 mM.

Heart cells were impaled with glass microelectrodes containing either 0.5 M K_2SO_4 and 10 mM KCl or 3 M KCl (resistance 18–30 MΩ). Electrodes containing 3 M $CsCl_2$ were used in a few experiments, to suppress outward potassium current.

Membrane potentials were recorded and voltage signals were fed to a preamplifier incorporating a bridge circuit (Axoclamp 2). Action potentials could be elicited by brief (2 ms) depolarizing pulses. Cells could also be voltage-clamped with a single electrode system in which the function of the electrode was switched rapidly between current passing and voltage recording (Wilson & Goldner, 1975). The Axoclamp 2 system was used at a switching rate of 2–8 kHz. Cells were clamped at a holding potential of – 40 mV to inactivate sodium current and voltage signals were displayed on a digital storage oscilloscope (Gould 4020) and recorded on magnetic tape for later analysis. The second inward current was evoked by a step depolarization from – 40 mV to 0 mV. The magnitude of this current was measured as the difference between the peak current and the steady current at the end of the pulse (Mitchell *et al.*, 1983; 1987a). In most experiments, voltage-clamp and action potential records were obtained at a steady-state stimulation rate of 0.3 Hz; however, for the purpose of the double-pulse experiments, stimulation frequency was 0.2 Hz.

Contraction was monitored by a photodiode which was mounted in the eyepiece of the microscope (Mitchell *et al.*, 1983; 1987a). Light in the field of view was restricted to the edge of the cell under study. Changes in the output of the photodiode correspond to the extent of contraction which was measured in arbitrary units.

These experiments were performed over 2 years and observations made in over 250 cells from 87 guinea-pigs. Results are displayed and either quoted as mean ± s.e.mean for both control and anaesthetic or as the mean percentage reduction ± s.e.mean evoked by the anaesthetic. Values before and after anaesthetic administration were compared (Student's paired *t* test).

Results

Both propofol and enflurane, applied at clinically relevant concentrations, in the solution bathing cardiac cells depressed the magnitude of contraction. In addition there was a depression in the plateau and a shortening of the duration of the accompanying action potential. The dose-dependence of the effects of propofol and enflurane on contraction and the time taken for 20 and 90% repolarization of the action potential (APD_{20} and APD_{90} respectively) is shown in Table 1.

Table 1 Percentage reduction of the action potential duration and accompanying contraction induced by propofol and enflurane

	Concentration (μM)		
Propofol	25	50	100
n	10	10	14
APD_{-20} (% reduction)	7 ± 1	16 ± 5*	35 ± 3**
APD_{-90} (% reduction)	7 ± 1*	15 ± 3**	21 ± 3**
Contraction (% reduction)	19 ± 6*	34 ± 9**	52 ± 6**
	Concentration (% vol)		
Enflurane	2	3	4
n	6	7	11
APD_{-20} (% reduction)	21 ± 5*	39 ± 7*	47 ± 5**
APD_{-90} (% reduction)	15 ± 3*	18 ± 3**	26 ± 3**
Contraction (% reduction)	21 ± 2*	—	42 ± 7**

APD_{-20} and APD_{-90} are the times taken for 20 and 90% repolarization of the action potential respectively. Each value represents the mean ± s.e.mean, *n* is the number of guinea-pig isolated ventricular cells.
*$P<0.05$ and **$P<0.005$ compared with control, Student's paired *t* test. Mean basal absolute APD_{-20} and APD_{-90} was 128 ± 11 ms and 253 ± 13 ms ($n = 34$), respectively, for cells exposed to propofol and 116 ± 10 ms and 240 ± 12 ms ($n = 24$), respectively, for cells exposed to enflurane.

Second inward currents were evoked by applying 200 ms step depolarizations from the holding potential of – 40 mV to 0 mV. Both propofol and enflurane, applied in the solution bathing cardiac cells depressed the second inward current and reduced the amplitude of the concomitant contraction. Table 2 shows the dose-dependence of the effects of propofol and enflurane on second inward currents and accompanying contractions. The effects of these anaesthetics on second inward currents was a consistent observation in all cells studied and would be expected to contribute to the

Table 2 Percentage depression of membrane currents and contraction induced by propofol and enflurane

	Concentration (μM)		
Propofol	25	50	100
Second inward current	5 ± 3* (23)	25 ± 3** (22)	47 ± 3* (19)
Calcium-activated 'tail' current	22 ± 3* (12)	29 ± 4** (12)	50 ± 9* (12)
Contraction	27 ± 3* (23)	31 ± 5** (22)	60 ± 3** (19)
	Concentration (% vol)		
Enflurane	2	3	4
Second inward current	22 ± 3* (9)	31 ± 3* (21)	41 ± 4** (13)
Calcium-activated 'tail' current	30 ± 4* (6)	43 ± 5** (13)	53 ± 5** (10)
Contraction	31 ± 4* (6)	42 ± 4** (14)	53 ± 5** (13)

Each value shows mean percentage reduction ± s.e.mean, the number of guinea-pig isolated ventricular myocytes is in parentheses.
*$P<0.05$/**$P<0.005$ compared with control values: Student's paired *t* test. Mean basal absolute amplitude of second inward current and calcium activated 'tail' current was – 2.2 ± 0.1 nA ($n = 64$) and – 0.6 ± 0.05 nA ($n = 36$), respectively, for cells exposed to propofol and – 2.1 ± 0.1 nA ($n = 43$) and – 0.5 ± 0.07 nA ($n = 29$), respectively, for cells exposed to enflurane.

negative inotropic effects and to the modification of the action potential configuration described above.

Voltage clamp pulses to different membrane potentials were applied and current-voltage curves constructed (Figure 1). The currents at the end of the step depolarizations were plotted as triangles and peak inward or outward currents plotted as squares. The difference between these two curves provides a measure of the amplitude of the second inward current. However, this estimate of second inward current is complicated, particularly at positive potentials, by contaminating outward currents. This problem was minimized in the cells used for this experiment by the intracellular application of the potassium channel blocker, caesium (Matsuda & Noma, 1984). Under these conditions, enflurane and propofol reduced the amplitude of the second inward current without substantial effect on the shape of the current-voltage relation (Figures 1a and 1b respectively).

On repolarization to the holding potential of − 40 mV after a 200 ms step depolarization to + 60 mV a slow 'tail' of decaying outward current can be seen. This 'tail' is thought to reflect deactivation of the delayed rectifier potassium current. Propofol (50 μM) and enflurane (3%) reduced this current by 29 ± 7% ($P<0.05$; $n = 8$) and 32 ± 5% ($P<0.05$; $n = 7$) respectively. A reduction of outward current might be expected to lengthen the action potential but as described above, both anaesthetics shorten action potentials. This indicates that anaesthetic effects on the counterbalancing inward currents predominate.

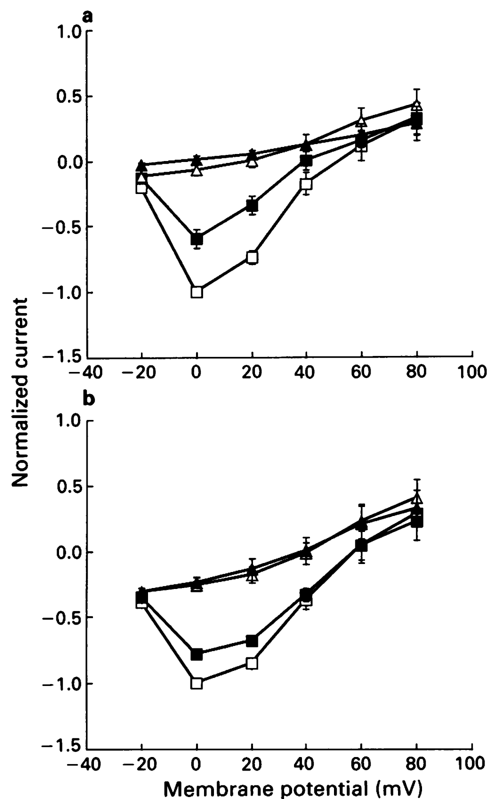

Figure 1 Normalized current as a function of membrane potential during voltage-clamp pulses measured from isolated cells loaded with Cs. Cells were voltage-clamped at − 40 mV. For each depolarization the current was measured at the end of the 200 ms depolarization (triangles) and at the peak inward or outward current (squares). All currents were normalized with respect to the peak inward current at 0 mV. Mean basal absolute current amplitude at 0 mV was − 2.3 ± 0.2 nA for cells subsequently exposed to enflurane and − 2.0 ± 0.2 nA for cells subsequently exposed to propofol. Data in the absence of anaesthetic are shown by open squares. Data in the presence of anaesthetic when a steady state was reached are shown by solid symbols. (a) Effects of 3% enflurane ($n = 5$ cells) and (b) effects of 50 μM propofol ($n = 8$ cells) on the current-voltage relation.

Current carried by calcium is thought to contribute the major fraction of peak second inward current. However, additional inward current appears to be activated by the increase in cytosolic calcium that accompanies the second inward current (Mitchell *et al.*, 1987b; Fedida *et al.*, 1987). This calcium-activated current may be recorded on repolarization to − 40 mV after brief (20 ms) step depolarizations to 0 mV as a slow 'tail' of decaying inward current. The percentage depression induced by various concentrations of propofol and enflurane on the calcium-activated 'tail' currents is shown in Table 2. A representative record from a single cell illustrating the effect of enflurane on this 'tail' current is shown in Figure 2.

The possibility that enflurane and propofol have actions additional to the depression in second inward current that may contribute to the contractile depression was investigated in experiments in which a paired pulse protocol was used. At various intervals (0.2−1 s) after the start of a conditioning depolarization (200 ms, from a holding potential of − 40 mV to 0 mV) to evoke the second inward current and accompanying contraction, a 'test' depolarization (to 0 mV for 20 or 200 ms) was applied to assess the recovery of second inward currents, calcium-activated 'tail' currents and contraction. The conditioning depolarizations led to substantial inactivation of calcium channels and perhaps to a depletion of calcium available for release from intracellular stores. The recovery rate of calcium-activated 'tail' current and contraction is dependent to an extent on the rate of recovery of calcium uptake/release from intracellular stores. The rationale for these experiments was to assess the extent of repriming of the mechanisms responsible for contraction and calcium-activated 'tail' currents after the conditioning depolarization. The effects of propofol and enflurane on this repriming could thus be investigated. At brief intervals between these pulses the amplitudes of second inward current, calcium-activated tail current and contractions were all depressed. The recovery of these amplitudes as the pulse interval increased is illustrated in Figures 3 and 4. In these graphs the amplitudes of the second inward current, calcium-activated 'tail' current

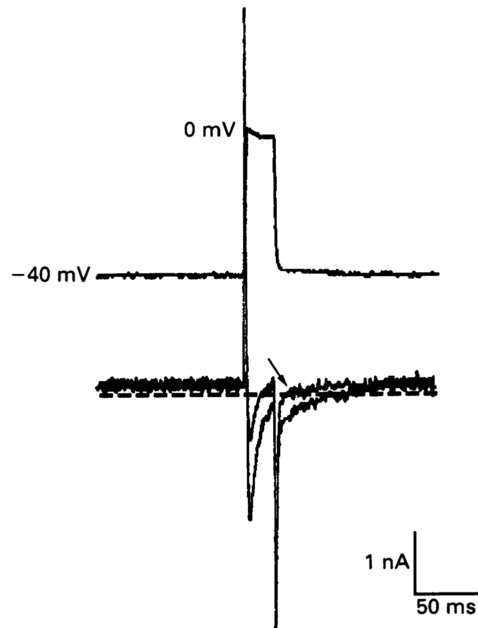

Figure 2 Membrane potential (upper trace) and current (lower trace) during 20 ms step depolarizations to 0 mV from the holding potential of − 40 mV. This protocol was used to obtain calcium-activated 'tail' currents which are seen on repolarization to − 40 mV. The arrow indicates the record obtained in the presence of 3% enflurane when a steady-state had been reached. The zero current level is shown by a dashed line.

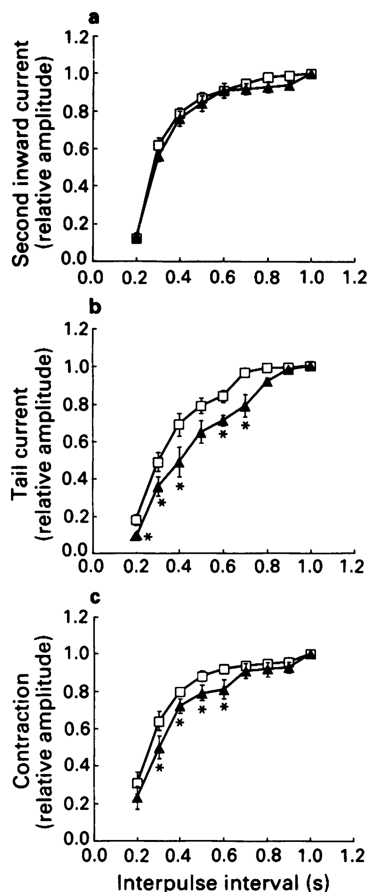

Figure 3 The effect of 3% enflurane (▲) on the recovery of second inward current (a), calcium activated 'tail' current (b) and contraction (c), in paired pulse protocols. The test pulses for (a) and (c) were 200 ms while for (b) the duration was reduced to 20 ms. The time scales show delay between the test pulse and the start of the conditioning pulse of 200 ms duration. Data are expressed as a fraction of their values at an interpulse interval of 1 s. Data obtained in the absence of anaesthetic are shown by (□). Points represent the means from 7 guinea-pig isolated ventricular myocytes; s.e.mean shown by vertical lines.

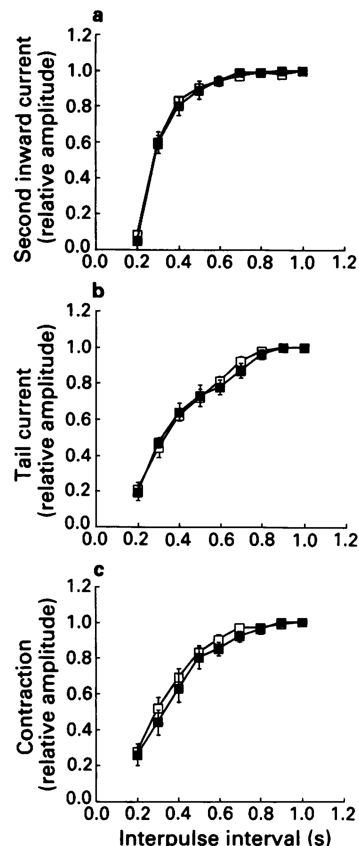

Figure 4 The effect of 50 μM propofol (■) on the recovery of second inward current (a), calcium activated 'tail' current (b) and contraction (c), in paired pulse protocols. The test pulses for (a) and (c) were 200 ms while for (b) the duration was reduced to 20 ms. The time scales show delay between the test pulse and the start of the conditioning pulse of 200 ms duration. Data are expressed as a fraction of their values at an interpulse interval of 1 s. Data obtained in the absence of anaesthetic are shown by (□). Points represent the means from 8 guinea-pig isolated ventricular myocytes; s.e.mean shown by vertical lines.

and contraction were plotted as a fraction of their value at an interpulse interval of 1 s when recovery may still have been incomplete. Propofol had no apparent effect on the recovery rate of second inward currents, calcium-activated tail currents and contraction (Figure 4). Although there was no detectable effect of enflurane (3%) on the recovery rate of the second inward current, the rate of recovery of calcium-activated tail currents and contraction was delayed (Figure 3).

Calcium channel inactivation is thought to be controlled by both calcium- and voltage-dependent mechanisms (Lee *et al.*, 1985). A possible effect of propofol and enflurane on the steady-state inactivation of calcium channels was investigated (data not shown). Propofol (50 μM) had no apparent effect on calcium channel inactivation. Enflurane (3%; 1.45 mM), however, appeared to enhance calcium channel inactivation, an effect which was significant ($P<0.05$; $n = 8$) at negative membrane potentials where, in the absence of anaesthetic, inactivation was incomplete.

Contractions accompanying pulses to positive potentials, under certain conditions, are thought to be triggered by a different source of calcium from those accompanying pulses to 0 mV, possibly calcium entry through sodium-calcium exchange (Terrar & White, 1989). Calcium entering via this route may also trigger calcium release from the SR. To assess the effects of propofol and enflurane on contractions accompanying pulses to positive potentials, contractions were monitored accompanying 200 ms step depolarizations to + 60 mV from a holding potential of − 40 mV. The pulses to + 60 mV were immediately preceded by pulses to 0 mV to inactivate calcium channels and thus ensure no calcium entry via this route during the subsequent pulse to + 60 mV. Figure 5a and b shows the effects of 3% enflurane and 50 μM propofol, respectively, on currents and contractions accompanying pulses to 0 mV and + 60 mV. In the presence of propofol (Figure 5b) the amplitudes of the second inward current which accompanies the pulse to + 0 mV and the slowly developing outward current which accompanies the depolarization to + 60 mV were both reduced, as were the concomitant contractions. Enflurane (Figure 5a) also reduced the amplitudes of the membrane currents accompanying the pulses to 0 mV and + 60 mV and depressed the amplitude of the contraction accompanying the second inward current; however, the contraction accompanying the pulse to + 60 mV was enhanced by enflurane. These observations are illustrated graphically in Figure 6, where the anaesthetic induced percentage change in the amplitude of contraction accompanying pulses to positive potentials is plotted for the individual cells studied. Although there is some overlap in the effects of both of these anaesthetics it is apparent that the predominant effect of propofol on the contractions accompanying pulses to + 60 mV is to cause little change or a reduction in amplitude, whereas the tendency with enflurane is to cause no change or an increase in amplitude. To clarify

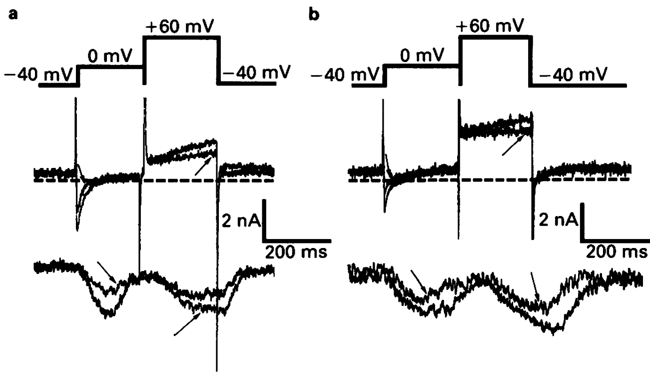

Figure 5 Membrane current (middle trace) and contraction (lower trace) evoked in response to the voltage protocol shown in the upper trace. The cells were depolarized from the holding potential of − 40 mV to + 60 mV after a 200 ms prepulse to 0 mV. Records obtained in the presence of (a) 3% enflurane and (b) 50 μM propofol when a steady state had been reached are indicated by arrows. Dashed line indicates the zero current level.

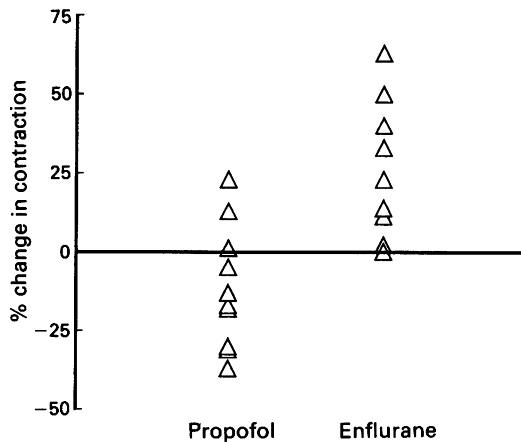

Figure 6 The effects of 50 μM propofol and 3% enflurane on contractions accompanying a 100 mV step depolarization (200 ms) from the holding potential of − 40 mV. Cells were depolarized to + 60 mV from a holding potential of − 40 mV 10 ms after a 200 ms prepulse to 0 mV. Data are expressed as percentage change compared to corresponding values obtained in the absence of anaesthetic. Each point represents data obtained from a single cell.

the events underlying this observation with propofol, experiments were performed in which the cells were perfused throughout the whole procedure i.e. during both control periods and periods of anaesthetic exposure, with either ryanodine (1 μM) or caffeine (10 mM) and the effects of propofol on contractions accompanying pulses to + 60 mV observed. The range and distribution of values from individual cells for the percentage change induced by propofol in the amplitude of contractions accompanying pulses to + 60 mV (see Figure 6), was the same in the absence or presence of ryanodine or caffeine in the solution bathing the cells ($n = 10$; $P > 0.05$ and $n = 8$; $P > 0.05$ respectively: Student's unpaired t test).

Discussion

Calcium entry during the plateau of the action potential is thought to activate and control contraction (Reuter, 1973; Fozzard, 1980). Both propofol and enflurane decreased the duration and depressed the plateau of action potentials and reduced the amplitude of the accompanying contraction. These effects would indicate that the anaesthetics reduce calcium influx into the cells. Calcium which enters the cell via 'L'-type calcium channels (Nilius *et al.*, 1985) carries the major part of the second inward current (Lee & Tsien, 1982; Mitchell *et al.*, 1983; Matsuda & Noma, 1984). Both propofol and enflurane reduced the second inward current. A reduction of the second inward current would decrease the magnitude of the cytosolic calcium transient and this may account, at least in part, for the observed depression in the amplitude of the accompanying contraction.

Calcium-activated 'tail'currents which are activated by the cytosolic calcium transient were reduced by propofol and enflurane. This implies that these anaesthetics either: (i) directly inhibited the membrane processes thought to be responsible for these tail currents, namely electrogenic sodium:calcium exchange (Mullins, 1979; Kimura *et al.*, 1986; Mechmann & Pott, 1986) and current through non-selective cation channels (Colquhoun *et al.*, 1981) or (ii) they attenuated the calcium transient. The reduction in calcium influx found with propofol and enflurane would be expected to reduce the increase in cytosolic calcium levels both directly and indirectly by reducing calcium-induced calcium release from the SR. These effects may be sufficient to account for the reduction in tail current and the depression of contractility. However, propofol and enflurane may also have additional effects on SR calcium release which could contribute to the negative inotropic actions.

Under very similar experimental conditions to those used in the experiments described in this paper, the effects of low doses of the calcium channel blockers, verapamil and nifedipine, on the percentage depression of second inward current and contraction have been studied. These studies indicate that a relatively large reduction in second inward current was required for a relatively small depression of contraction (Terrar & Victory, 1988a). In contrast, propofol and enflurane produced a greater depression of contraction than verapamil for a given reduction in second inward current. This indicates that both the anaesthetics may have actions additional to that of second inward current inhibition to account for their negative inotropic effects.

The possibility that propofol and enflurane have a direct effect on SR calcium release was investigated by use of double-pulse voltage clamp protocols. The magnitudes of the calcium-activated tail current and contraction at a given interpulse interval were taken as an index of the calcium transient, which, in turn depends on the extent of refilling of the SR. Thus, any delay in the recovery of the tail current and contraction induced by propofol when compared to the recovery of the second inward current may be explained in terms of a delay in the uptake/release of calcium from the SR. Propofol had no apparent effect on the recovery rate of second inward current, calcium-activated 'tail' current and contraction. In contrast, enflurane delayed the recovery rate of the calcium-activated 'tail' current and contraction while having little effect on the recovery rate of second inward current. In similar experiments, halothane (Terrar & Victory, 1988a) and isoflurane (Terrar & Victory, 1988b) also have been shown to delay the recovery rate of tail currents and contraction. The results with enflurane may be explained in terms of enflurane inhibiting the refilling of the SR calcium store. This may result from enflurane either inhibiting calcium uptake and/or causing calcium 'leak' from the SR. These results are consistent with those of Su & Kerrick (1980) who showed that enflurane reduced calcium uptake capacity of the SR in functionally skinned myocardial fibres from the rabbit. In addition, results of Katsuoka & Ohnishi (1989) have shown that enflurane reduces SR calcium content and that this reduction has a high degree of correlation to the negative inotropic effect of enflurane. In contrast propofol does not appear to affect calcium uptake into or release from SR calcium stores.

In guinea-pig ventricular muscle, calcium entry through

nifedipine-sensitive 'L' type channels and calcium release from intracellular stores are essential for triggering and controlling contraction (Reuter, 1979; Fozzard, 1980; Fabiato, 1983). Calcium current with these characteristics can be activated in guinea-pig ventricular myocytes at membrane potentials in the range − 40 to 0 mV (Mitchell *et al*., 1983). As membrane potential is made more positive (to + 60 mV), contraction amplitude in these cells is not consistently blocked by nifedipine and increases even though it would be expected that calcium entry through 'L' channels would decrease (Mitchell *et al*., 1987b). Contraction at positive potentials is thought to be via a route other than calcium channels, possibly sodium:calcium exchange (Brill *et al*., 1987; Barcenas-Ruiz & Weir, 1987; Terrar & White, 1989).

Contractions accompanying depolarizing pulses to 0 mV were consistently depressed by both enflurane and propofol. However, there was a distinct difference between the effects of propofol and enflurane on contractions accompanying pulses to positive potentials. Enflurane enhanced (8 cells) or had no affect (1 cell) on contractions accompanying pulses to positive potentials. In contrast, most of the cells exposed to propofol exhibited a reduction (6 cells) of these contractions, with just three cells exhibiting a small increase in contraction amplitude. The effects of enflurane may be explained in terms of an inhibition of calcium uptake into the SR or a 'leak' of calcium from the SR. As sodium:calcium exchange is thought to mediate influx of calcium at positive potentials and would not be able to extrude calcium, the effects of enflurane on the SR may leave more cytosolic calcium available for contraction. Halothane has been reported to have similar effects to enflurane with regards to contraction accompanying pulses to positive potentials (Terrar & Victory, 1989).

If the effects of propofol on contractions accompanying pulses to + 60 mV were mediated via an effect on the SR one would have expected that the range and distribution of values representing percentage change in the amplitude of contractions accompanying pulses to + 60 mV would have been affected by the presence both before and during propofol administration of the inhibitors of SR function, ryanodine and caffeine. As this was not the case it may be concluded that propofol does not induce its effects on contractions accompanying pulses to positive potentials via an effect on the SR. Possible alternative explanations for the effects of propofol on contractions accompanying pulses to positive potentials include (i) a direct inhibition by propofol of calcium influx via sodium:calcium exchange, resulting in reduced calcium influx during the pulse to positive potentials or (ii) a direct reduction by propofol of the sensitivity of the contractile proteins.

In conclusion, both enflurane and propofol depress the amplitude of second inward currents and calcium-activated inward currents. There seems, however, to be a clear cut difference between propofol and halogenated anaesthetics such as enflurane (this study), halothane (Terrar & Victory, 1988a) and isoflurane (Terrar & Victory, 1988b) concerning actions which have been interpreted as resulting from a major effect of halogenated anaesthetics on the SR (double-pulse experiments and experiments using depolarizations to + 60 mV). Inhibition of calcium release from the SR by enflurane may occur via either an inhibition of calcium uptake and/or an increased leak of calcium from the SR. These actions would impair the ability of the SR to sequester calcium and would result in a reduction of the amount of calcium available for release. Both the reduction in calcium influx via second inward current and an inhibition of calcium release from intracellular stores could account for the negative inotropic effects of enflurane. In contrast, propofol does not have a major effect on SR function. Thus propofol seems to depress contractility by a reduction of second inward current and a possible depression of myofilament sensitivity.

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