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Effects of halothane on the membrane potential in skeletal muscle of the frog

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1 Halothane has many effects on the resting membrane potential (V_m) of excitable cells and exerts numerous effects on skeletal muscle one of which is the enhancement of Ca^{2+} release by the sarcoplasmic reticulum (SR) resulting in a sustained contracture. The aim of this study was to analyse the effects of clinical doses of halothane on V_m, recorded using intracellular microelectrodes on cleaned and non stimulated sartorius muscle which was freshly isolated from the leg of the frog *Rana esculenta*.

2 We assessed the mechanism of effects of superfused halothane on V_m by the administration of selective antagonists of membrane bound Na^+, K^+ and Cl^- channels and by inhibition of SR Ca^{2+} release.

3 Halothane (3%) induced an early and transient depolarization (4.5 mV within 7 min) and a delayed and sustained hyperpolarization (about 11 mV within 15 min) of V_m.

4 The halothane-induced transient depolarization was sensitive to ryanodine (10 μM) and to 4-acetamido-4′-isothiocyanatostilbene 2,2′ disulphonic acid (SITS, 1 mM).

5 The hyperpolarization of V_m induced by halothane (0.1–3%) was dose-dependent and reversible. It was insensitive to SITS (1 mM), tetrodotoxin (0.6 μM), and tetraethylammonium (10 mM) but was blocked and/or prevented by ryanodine (10 μM), charybdotoxin (CTX, 1 μM), and glibenclamide (10 nM).

6 Our observations revealed that the effects of halothane on V_m may be related to the increase in intracellular Ca^{2+} concentration produced by the ryanodine-sensitive Ca^{2+} release from the SR induced by the anaesthetic. The depolarization may be attributed to the activation of Ca^{2+}-dependent Cl^- (blocked by SITS) channels and the hyperpolarization to the activation of large conductance Ca^{2+}-dependent K^+ channels, blocked by CTX, and to the opening of ATP-sensitive K^+ channels, inhibited by glibenclamide.

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Abbreviations: Ag/AgCl, silver/silver chloride; BKCa, large conductance Ca^{2+}-dependent K^+ channels; Ca^{2+}-ATPase, adenosine triphosphatase-dependent Ca^{2+} pump; $[Ca^{2+}]_i$, internal Ca^{2+} concentration; CTX, charybdotoxin; h.p.l.c., high performance liquid chromatography; i.d, internal diameter; K_{ATP}, ATP-dependent K^+ channels; o.d, outer diameter; SITS, 4-acetamido-4′-isothiocyanatostilbene 2,2′disulphonic acid; SR, sarcoplasmic reticulum; TEA, tetraethylammonium; TTX, tetrodotoxin; u.v., ultraviolet; V_m, membrane resting potential; $\mu V\ h^{-1}$, microvolt per hour

Introduction

Volatile anaesthetic agents have been reported to exert numerous effects on the central nervous system. Synaptic transmission is more sensitive to general anaesthetics than axonal conduction (Pocock & Richards, 1993). Voltage-gated Na^+ and K^+ channels are generally thought to be very insensitive to general anaesthetics; voltage-gated fast transient K^+ currents are quite insensitive to volatile agents while voltage gated Ca^{2+} channels are thought to be probably the most anaesthetic-sensitive of all voltage-gated channels (Franks & Lieb, 1998). The general anaesthetic halothane is a well known inhalational anaesthetic. Electrophysiological reports have shown that halothane blocks the Na^+ current without inducing a shift in the voltage-dependence of its steady-state inactivation (Haydon *et al*., 1984) and reduces voltage-activated K^+ currents at least as much, if not more, than Na^+ current in the squid giant axon (Urban & Haydon, 1987). Halothane depresses the delayed outward K^+ current and slightly reduces the inward rectifier K^+ current in cardiac myocytes (Pancrazio *et al*., 1993). Halothane also exerts varied effects on the resting membrane potential (V_m) of excitable cells. It does not affect V_m in mouse diaphragm (Gage & Hamill, 1976), crayfish axon (Bean *et al*., 1981), hyperpolarizes the membrane in frog motoneurons and in central neurons (Nicoll & Madison, 1982), and in hippocampal CA1 neurons (Maclver & Kendig, 1991), produces various effects (depolarization or hyperpolarization) on cat respiratory neurons (Takeda & Haji, 1992), tends to depolarize the membrane in squid axon (Haydon *et al*., 1984; 1988; Urban & Haydon, 1987; Haydon & Simon, 1988). The sarcoplasmic reticulum (SR) is one of the intracellular sites of action of halothane which has been shown to increase Ca^{2+} release from the SR in skeletal muscle (Palade *et al*., 1989). Halothane prolonged the opening of isolated ryanodine receptor Ca^{2+} release channels

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from the SR (Bull & Marengo, 1994; Connelly & Coronado, 1994). The ryanodine receptor Ca^{2+} release channel or the α_1-subunit of the dihydropyridine receptor are thought to be involved in malignant hyperthermia, a pharmacogenetic disorder. The effects of halothane on muscle membrane protein regulatory Ca^{2+}-homeostasis have shown that halothane influenced Ca^{2+}-ATPases (Karon *et al*., 1995; Lopez & Kosk-Kosicka, 1995); induced oligomerization of the terminal cisternae Ca^{2+}-binding protein calsequestrin, the junctional ryanodine receptor Ca^{2+}-release channel and the transverse-tubular α_1-dihydropyridine receptor of rabbit skeletal muscle (Froemming *et al*., 1999).

Anaesthetic effects on ion channels are modulated by the electrolyte environment and may depend on membrane potential (Urban & Friederich, 1998). The aim of the present work was to study the effects of clinically effective concentrations of halothane on the resting membrane potential of frog skeletal muscle using conventional microelectrodes and to determine the mechanism by which halothane altered the membrane potential. We report that, in frog skeletal muscle, halothane induced a ryanodine-sensitive hyperpolarization which was also sensitive to charybdotoxin and to glibenclamide, and suggests that Ca^{2+}-dependent and ATP-sensitive K^+ channels are involved in the hyperpolarizing effect induced by the anaesthetic.

Methods

Experiments were performed at 20°C on sartorius muscle isolated from the frog (*Rana esculenta*) leg. After double pithing the frog, sartorius muscles were rapidly isolated and pinned at the bottom (filled with sylgard) of a dissection chamber filled with a Ringer solution. The internal surface of the muscle was carefully cleaned from the connective tissue. Then the muscle was transported in the experimental chamber, pinned at the bottom (filled with sylgard) and perfused with a Ringer solution at a rate of 0.015 l min^{-1}. Muscles were equilibrated for at least 30 min before recordings were obtained.

The composition of the Ringer solution was (mM): NaCl 110.5; KCl 1.5; KH_2PO_4 1; $CaCl_2$ 2; $NaHCO_3$ 2.4; bubbled with 95% O_2-5% CO_2 (pH = 7.4). In some experiments, the following inhibitors were used: 0.6 μM tetrodotoxin (TTX, Sankyo, Tokyo, Japan) to entirely inhibit the Na^+ current (Pater & Sauviat, 1987), 10 mM tetraethylammonium (TEA, Sigma Aldricht, Saint Quentin Fallavier, France) to completely suppress the delayed outward K^+ current (Pater & Sauviat, 1987), 1 mM 4-acetamido-4′-isothiocyanatostilbene 2,2′disulphonic acid (SITS, Sigma) to suppress Cl^- currents, 10 μM ryanodine (Sigma) to inhibit the Ca^{2+} release from the SR (Nasri-Sebdani *et al*., 1990), 10 nM charybdotoxin (CTX, Alomone Labs, Jerusalem, Israel) to block large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (Garcia *et al*., 1995; Miller, 1995); 10 μM glibenclamide (Hoechst Pharmaceuticals, Hounslow, U.K.) to inhibit the ATP-dependent K^+ (K_{ATP}) channels (Sauviat *et al*., 1991).

Halothane (Belamont, Paris, France) was delivered by an agent-specific vaporizer line (Halothan Vapor 19.1, Drager Werk AG, Lübeck, Germany) with the air gas 95% O_2-5% CO_2 aerating the solution. After vaporization, the anaesthetic gas was passed through the physiological solution. Each solution was equilibrated with halothane for at least 15 min before introduction to the experimental chamber. Concentration of halothane was expressed in per cent of gas delivered. Halothane concentration in the control solution was assayed using h.p.l.c. (Horn *et al*., 1995). Briefly, 0.2 ml Ringer solution was sampled from the experimental chamber at the muscle level during the perfusion with solutions containing various halothane doses, using a gas tight syringe (Hamilton, model 1725LT, Touzart et Matignon, Les Ulis, France) connected to a gas tight stop cock (Hamilton, model HVP). To establish calibration curves, a solution containing 1 mM halothane was prepared in normal saline by adding halothane (0.105 ml) in a 1 liter glass bottle containing saline with glass marbles. The bottle was immediately sealed with a gas tight stopper (less than 0.05 ml air remained in the bottle) and gently stirred for 30 min. Serial dilutions were made using the same technique in order to draw a calibration curve with at least four points. Chromatographic analysis was performed under isocratic conditions at room temperature. The separation was performed on a Beckman Ultrasphere ODS 5 μm C18, 4.6 × 150 mm column (Beckman, Gagny, France). The mobile phase consisted of 50% methanol and 50% water (v v^{-1}) set at a flow rate of 0.0015 l min^{-1}. Detection was made by a variable wave-length u.v.-vis detector (model 1050, Hewlett-Packard, Les Ulis, France) set at 210 nanometers. Twenty-five microliters of the Ringer solution (or standard solution) were injected in the system directly from the Hamilton syringe. No internal standard was used because preliminary experiments have shown that adding standard led to increased variability due to loss of halothane. The intra-day coefficient of variation was less than 8% at concentrations ranging from 0.05 −1 mM. Halothane was delivered by the vaporizer in the clinical dose range 0.1 − 3%. The relation between the anaesthetic concentration (mM) measured in the bathing solution and the concentration (volume percentage) delivered by the gas vaporizer was linear (Figure 1).

The resting membrane potential (V_m) of frog skeletal muscle was recorded using intracellular conventional glass (o.d. 1.5 mm; i.d. 0.86 mm, with inner filament; Clark Electromedical Instruments; Reading, U.K.) microelectrodes (resistance: 20 − 25 megaohms; tip potential less than ± 3 mV) filled with saturated KCl 3 M (pH 2.5). The recording apparatus was similar to that described by Sauviat (1981). Microelectrodes were connected to a positive input stage of a differential

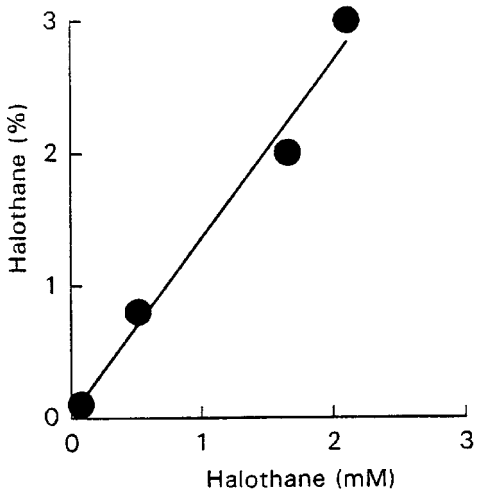

Figure 1 Relationships between the halothane percentage (ordinates) delivered at 20°C by the vapor pressure system in the experimental chamber at the muscle level and the concentration (abscissa) of halothane (mM) measured by chromatography. The data were linearly fitted with a first order regression line. All values are mean ± s.e.mean of three experiments.

voltage follower through an Ag/AgCl electrode (type E200); the bath solution was connected to the second positive input stage of the voltage follower by means of an Ag/AgCl electrode (type E203). The drift between these electrodes (Clark Electromedical Instruments, Reading, U.K.) was given as low as 25 μV h^{-1}. The zero potential, corresponding to the potential of the bath, was checked before and after each impalement and balanced if necessary. V_m was either recorded continuously during the same impalement or obtained by successive cross impalements from one side to the other side of the muscle. The criteria taken into account to evaluate the quality of V_m recording were the following: the impalement of the electrode into the cell must occur without any positive or negative rebound and remain stable for at least 15 s (Figure 2a). Due to the possible difference between animals, in the present study, 15 successive impalements were recorded in each solution tested, and their mean value was taken as V_m value of the muscle. V_m was displayed on an oscilloscope (Nicolet 310, Madison, WI, U.S.A.), its value was read on a digital display (DPM, Metrix, France) and entered on the mass storage of a desk computer (Venex System AT80486DX 33, Paris, France) for further statistical analysis.

Data are expressed as mean values ± s.e.mean.; (n) corresponded to the number of muscles tested. Comparison between groups were made using paired Student's t-test delivered by the software Sigmaplot (Jandel Scientific GmbH, Erkrath, Germany), a P value <0.05 was considered as statistically significant.

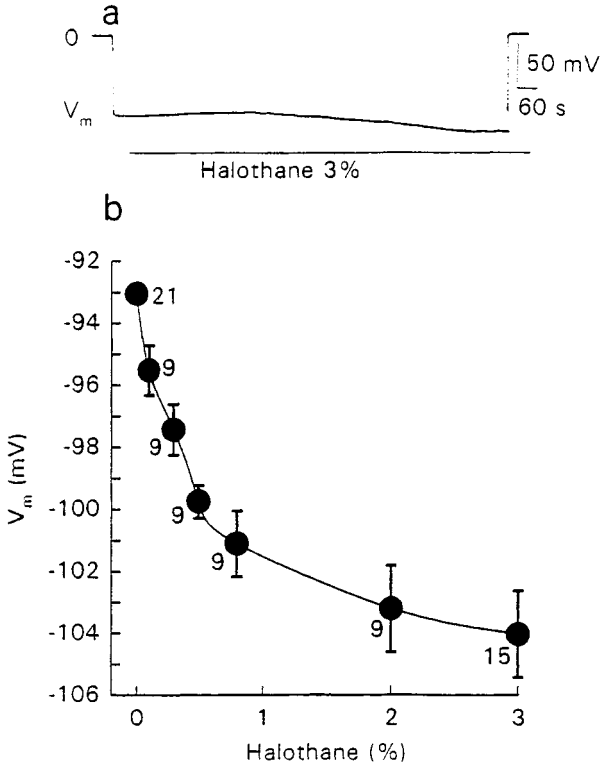

Figure 2 Effect of halothane on the resting membrane potential (V_m) of frog sartorius muscle recorded using intracellular microelectrode. (a) Continuous recording of V_m obtained during the same impalement in the Ringer solution before and during halothane (3%) application. (b) Dose-response curve for the variations of V_m amplitude (ordinate) induced by increasing the concentration of halothane in the Ringer solution (abscissa). The points and bars represent the mean and s.e.mean. of data obtained from (n) different muscles. The number associated to each point represents n.

Results

The application of a Ringer solution containing halothane (3%) to frog sartorius muscle lead to the development of an early transient depolarization followed by a delayed sustained hyperpolarization of V_m which reached an apparent steady state within 15 min (Figure 2a). The amplitude of the depolarization reached 4.5 mV within 7 min ($V_{m\ control} = -93.2 \pm 1.1$ mV; $V_{m\ halothane\ 7\ min} = -88.7 \pm 1.0$ mV, $P<0.05$, $n=7$). It did not develop when muscles were previously bathed in a Ringer solution containing 10 μM ryanodine ($V_{m\ ryanodine} = -94.8 \pm 1.1$ mV; $V_{m\ ryanodine+halothane\ 7\ min} = -96.1 \pm 1.3$ mV, $n=3$) or 1 mM SITS ($V_{m\ SITS} = -93.3 \pm 0.8$ mV; $V_{m\ SITS+halothane\ 7\ min} = -92.2 \pm 1.1$ mV, $n=6$). The hyperpolarization ($V_{m\ halothane\ 15\ min} = -104.3 \pm 1.0$ mV, $n=6$) developed in the presence of SITS ($V_{m\ SITS\ 15\ min} = -102.0 \pm 1.3$ mV, $n=6$) but not in the presence of ryanodine. It was dependent on the anaesthetic concentration present in the solution (Figure 2b). The amplitude of the proportional increase of V_m reached about 8 and 16% of the control value in the presence of 0.5 and 3% halothane respectively. The hyperpolarizing effect of halothane 3% was reversible and fell to approximately 6% after 30 min washout ($V_{m\ 30\ min\ washout} = -98.7 \pm 0.6$ mV, $n=6$).

To determine the origin of the hyperpolarization induced by halothane, we used the halothane concentration (3%) which produced the maximal effect, and successively tested several possibilities including blockade of an inward Na^+ current; blockade of Ca^{2+} release by the SR; an increase in outward K^+ currents which participate in generation of V_m.

The halothane (3%)-induced hyperpolarization of V_m was not prevented when muscles were previously treated by the addition of 0.6 μM TTX or 10 mM TEA to the control solution. Table 1 shows that TTX application hyperpolarized the membrane but did not prevent the extra increase of V_m induced by halothane. Table 1 also reveals that TEA did not markedly alter V_m but did not prevent the hyperpolarizing effect of halothane on V_m while subsequent addition of ryanodine (10 μM) to the solution containing TEA markedly suppressed the hyperpolarization. As shown in Table 2, the addition of 10 μM ryanodine to the Ringer solution did not

Table 1 Effect of halothane (3%) on frog sartorius muscle resting membrane potential (V_m) after prior tetrodotoxin (TTX) or tetraethylammonium (TEA)-treatment

Treatment (*X*)	n	V_m (mV) *Control*	(*X*)	*X* + *Halothane*	*Ryanodine*
TTX	3	-94.6 ± 0.7	-97.2 ± 0.6*	$-103.5 \pm 0.7^+$	
TEA	3	-91.6 ± 0.3	-93.4 ± 0.4	$-101.5 \pm 0.4^+$	$-96.1 \pm 0.5^\times$

(Control) Ringer solution; (X) Ringer solution containing TTX (0.6 μM) or TEA (10 nM), (X + halothane) Ringer solution containing X and halothane (3%); (ryanodine) Ringer solution containing TEA halothane (3%) and ryanodine (10 μM). V_m was recorded 15 min after each solution change. Mean values ± s.e.mean. of n muscles studied. $P<0.05$, paired values were compared as follows.* (X) versus control; $^+$(X + halothane) versus (X); $^\times$(X + halothane + ryanodine) versus (X + halothane).

change V_m and subsequent application of 3% halothane to muscles previously treated with ryanodine did not modify V_m. The application of ryanodine, after halothane treatment, restored V_m close to the values recorded in the control solution (Table 3).

The addition of 10 nM CTX to the standard solution did not modify V_m (Table 2). CTX application prevented development of the hyperpolarization of V_m induced by halothane (Table 2). Subsequent application of CTX after halothane treatment reversed the effect of the anaesthetic on V_m (Table 3).

Glibenclamide (10 μM) applied to the standard solution significantly increased V_m (Table 2). After glibenclamide treatment, halothane significantly increased V_m (Table 2). Conversely, the application of glibenclamide to muscles pretreated with halothane 3% significantly reversed the hyperpolarizing effect of the anaesthetic (Table 3).

Discussion

Present data show that halothane has complex effects. It reveals, for the first time that, in frog skeletal muscle, the effects of halothane application on V_m are the result of a balance between a depolarization, sensitive to ryanodine and SITS and an hyperpolarization, sensitive to ryanodine CTX and glibenclamide.

Table 2 Effect of ryanodine, charybdotoxin (CTX) or glibenclamide on frog sartorius muscle resting membrane potential (V_m) after prior halothane 3%-treatment

Treatment (X)	n	*V_m (mV) Control*	*Halothane*	*Halothane + (X)*
Ryanodine	6	-93.4 ± 0.7	-101.9 ± 0.9*	$-95.0 \pm 0.5^{+}$
CTX	6	-93.3 ± 0.5	-100.8 ± 0.6*	$-96.1 \pm 0.5^{+}$
Glibenclamide	6	-93.0 ± 0.2	-101.6 ± 1.0*	$-96.7 \pm 0.8^{+}$

(Control) Ringer solution; (halothane) Ringer solution containing halothane (3%); (halothane + X) Ringer solution containing halothane (3%) ryanodine (10 μM), CTX (10 μM) or glibenclamide (10 μM). V_m was recorded 15 min after each solution change. Mean values ± s.e.mean of n muscles studied. $P < 0.05$, paired value were compared as follows. *(halothane) versus (control); +(halothane + X) versus (halothane).

Table 3 Effect of halothane 3% on the resting membrane potential (V_m) of frog sartorius muscle after prior ryanodine, charybdotoxin (CTX) or glibenclamide treatment

Treatment (X)	n	*V_m (mV) Control*	*(X)*	*X + Halothane 3%*
Ryanodine	6	-94.6 ± 0.7	-95.2 ± 0.6	-94.9 ± 0.7
CTX	6	-93.8 ± 0.5	-93.5 ± 0.7	-92.9 ± 0.5
Glibenclamide	6	-93.6 ± 0.3	-95.4 ± 0.4*	$-96.6 \pm 0.3^{+}$

(Control) Ringer solution; (X) Ringer solution containing ryanodine (10 μM), CTX (10 μM) or glibenclamide (10 μM); (X + halothane) Ringer solution containing ryanodine (10 μM), charybdotoxin (10 μM) or glibenclamide (10 μM) and halothane (3%). V_m was recorded 15 min after each solution change. Mean values ± s.e.mean of n muscles studied. $P < 0.05$, paired value were compared as follows. *(X) versus (control); $^{+}$(X + halothane) versus (X).

This study shows that both, the depolarization and the hyperpolarization induced by halothane treatment, are ryanodine-sensitive. Ryanodine inhibits the Ca^{2+} release from the SR in various tissues including skeletal muscle (Lynch & Frazer, 1994), ventricular myocytes (Pancrazio & Lynch, 1994), vascular smooth muscle (Akata & Boyle, 1996). In frog skeletal muscle, ryanodine did not modify V_m but prevented or reversed the effect of halothane on V_m. This suggests that the release of Ca^{2+} from the SR was involved. Halothane increased the Ca^{2+} release from the SR in frog skeletal muscle at low pH and at resting internal Ca^{2+} concentration ($[Ca^{2+}]_i$) indicating that it circumvents the closing of the channels produced by these conditions (Beltran *et al*., 1996). It also increased the basal Ca^{2+} level when Ca^{2+} influx and efflux were blocked in porcine tracheal smooth muscle cells (Pabelick *et al*., 1999). Ryanodine receptors mainly control the excitation-contraction coupling in skeletal muscle, i.e. the increase in SR Ca^{2+} permeability triggered by cell depolarization (Lüttgau & Moicescu, 1978; Ashley *et al*., 1991; Coronado *et al*., 1994). The activation of the Ca^{2+} release from the SR has been attributed to dissociation of the Ca^{2+}-ATPase by halothane which is thought to dissociate the Ca^{2+}-ATPase and to activate the enzyme (Karon *et al*., 1995). The Ca^{2+}-ATPase of skeletal muscle SR is thought to exchange Ca^{2+} while ATP was hydrolyzed (MacLennan, 1990).

The depolarization induced by halothane on frog skeletal muscle was prevented by SITS, a Cl^{-} channel blocker. This suggests that a resting Cl^{-} influx is associated with halothane application. Halothane has been reported to open a Cl^{-} current in postnatal rat hyppocampal neurons (Yang *et al*., 1992) and to activate a high conductance anion channel in cultured neocortical astrocytes (Felisberti *et al*., 1997). The observation that ryanodine prevented the development of the depolarization induced by halothane suggests that the Cl^{-} current is triggered by the rise in $[Ca^{2+}]_i$ produced by the Ca^{2+} release from the SR induced by halothane. Therefore, this suggests that Ca^{2+}-activated Cl^{-} channels are involved in the depolarization which develops during halothane application.

The pharmacological characteristics of the delayed hyperpolarization induced by halothane on frog skeletal muscle membrane revealed that it was not prevented by TTX, TEA, or SITS but, it was blocked and prevented by ryanodine and CTX. The inefficiency of TTX, TEA, or SITS in preventing this effect of halothane on V_m suggested that it cannot be attributed to the blockade of a TTX-sensitive Na^{+}, TEA-sensitive delayed outward K^{+}, or Cl^{-} resting currents. However, the observation that ryanodine blocked or prevented the hyperpolarizing effect of halothane suggests that the Ca^{2+} release from the SR induced by halothane might be responsible for this effect. In the present experiments the hyperpolarization of V_m induced by halothane was blocked or prevented by CTX, a blocker of BK_{Ca} channels. Therefore, this suggests that the rise in $[Ca^{2+}]_i$ produced by the ryanodine-sensitive Ca^{2+} release induced by halothane triggers the activation of BK_{Ca} channels since, in frog skeletal muscle, an increase in internal free Ca^{2+} initiates a high K^{+} conductance (Lüttgau & Wettwer, 1983). CTX-sensitive BK_{Ca} channels have been reported in many tissues including skeletal muscle (Marty, 1989). In addition, in frog skeletal muscle, the release of Ca^{2+} from the SR induced by caffeine promotes a rise in cytosolic Ca^{2+} and an increase in K^{+} efflux rate coefficients which was reduced by CTX, a blocker of BK_{Ca} channels; insensitive to apamin, a blocker of small conductance Ca^{2+}-dependent K^{+} channels; reduced by tolbutamide, an inhibitor of K_{ATP} channels; and insensitive to barium, a blocker of most K^{+} channels (Venosa & Hoya, 1999). Present observations suggest

that halothane mimicked the effect of caffeine on skeletal muscle and led to a rise in $[Ca^{2+}]_i$ and to the activation of BK_{Ca} channels. Our observation that the activation of BK_{Ca} channels was blocked by ryanodine agree with previous results reported on guinea-pig dorsal motor nucleus of the vague in which ryanodine has been found to inhibit the slow Ca^{2+}-dependent K^+ current underlying the prolonged after hyperpolarization (Sah, 1996). Nevertheless, it was different from the observation that high conductance Ca^{2+}-dependent K^+ channels were almost insensitive to high concentrations of halothane in cultured rat hippocampal neurons (McLarnon & Sawyer, 1998).

An increase in Ca^{2+} release from the SR enhanced $[Ca^{2+}]_i$ and stimulated the ATP-dependent Ca^{2+} reuptake of the SR which produces a more important hydrolysis of ATP and in turn an increase in the metabolic rate (Rasmussen *et al*., 1990). The observation that the hyperpolarizing effect of halothane on V_m was sensitive to glibenclamide, a blocker of K_{ATP} channels, reveals that halothane opened the K_{ATP} channel. A decrease in sarcolemmal ATP concentration favoured the opening of K_{ATP} channels which may be the most common K^+ channels of the surface membrane of skeletal muscle (Spruce *et al*., 1985).

Present results suggest that the varied effects of halothane on V_m reported in excitable cells depend on the balance between Ca^{2+}-sensitive inward and outward currents triggered by the rise in $[Ca^{2+}]_i$ following the halothane-induced Ca^{2+} release from the SR, in addition to the effect of the anaesthetic on the other voltage-dependent conductances.

In conclusion, our results show that, in frog skeletal muscle, halothane successively depolarizes and then hyperpolarizes V_m. Both effects, blocked or prevented by ryanodine may be a consequence of the Ca^{2+} release from the SR induced by the anaesthetic. Our data suggest that a SITS-sensitive Ca^{2+}-activated Cl^- current participates in the depolarization while CTX-sensitive BK_{Ca} channels, and glibenclamide-sensitive K_{ATP} channels participate in the hyperpolarization induced by the anaesthetic.

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