ATP-dependent effects of halothane on SR Ca\(^{2+}\) regulation in permeabilized atrial myocytes

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Abstract

Objective: Previous work suggests that modification of sarcoplasmic reticulum (SR) function may contribute to the cardioprotective effect of halothane during ischaemia and reperfusion. The aim of this study was to investigate the effects of halothane on spontaneous Ca\(^{2+}\) release from the sarcoplasmic reticulum (Ca\(^{2+}\) sparks and waves).

Methods: Rat atrial myocytes were permeabilized with saponin and perfused with solutions approximating to the intracellular milieu and containing fluo-3. SR Ca\(^{2+}\) release was detected using confocal microscopy.

Results: In the presence of 5 mM ATP, halothane (0.25–2 mM) had no significant effect on the amplitude or frequency of spontaneous Ca\(^{2+}\) waves. However, in the presence of 0.05 mM ATP, halothane (0.25–2 mM) induced a concentration-dependent decrease in the amplitude and an increase in the frequency of spontaneous Ca\(^{2+}\) waves, e.g., 1 mM halothane decreased the amplitude by 34.7 ± 3.5% (n = 9) and increased the frequency by 67 ± 19.9% (n = 7). In the presence of 5 mM ATP, 1 mM halothane had no significant effect on the amplitude or frequency of Ca\(^{2+}\) sparks. When [ATP] was reduced to 0.05 mM, Ca\(^{2+}\) spark frequency decreased by 67.9 ± 14% and the amplitude increased by 27.5 ± 4.9% (n = 13). Subsequent introduction of halothane (0.5–1 mM) induced a transient burst of Ca\(^{2+}\) sparks, consistent with ryanodine receptor (RyR) activation. Further experiments showed that the decrease in Ca\(^{2+}\) spark frequency following ATP depletion was associated with a progressive increase in the SR Ca\(^{2+}\) content over 1–2 min. This rise in SR Ca\(^{2+}\) content did not occur when 1 mM halothane was present during ATP depletion.

Conclusions: These data suggest that the sensitivity of the RyR to activation by halothane increases at low [ATP]. In metabolically impaired cells, halothane would be expected to lessen any rise in SR Ca\(^{2+}\) content and to reduce the amplitude of spontaneous Ca\(^{2+}\) release. These effects of halothane are considered in relation to the events that occur during ischaemia and reperfusion.

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1. Introduction

The onset of myocardial ischaemia is followed by profound changes in metabolite levels and a rapid decline in contractile force [1]. Intracellular levels of H\(^{+}\), Pi and ADP increase, while ATP and phosphocreatine (PCr) are progressively depleted. On reperfusion, Ca\(^{2+}\) rapidly enters the cell via the Na/Ca exchanger, facilitated by raised levels of intracellular Na\(^{+}\). During this phase, Ca\(^{2+}\) overload results in the cyclic uptake and release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) [2], which spreads to neighbouring cells via gap junctions [3]. Spontaneous Ca\(^{2+}\) release activates a transient inward current (\(I_{\text{Ca}}\)), which is associated with after-depolarisations and triggered arrhythmias [4]. The prolonged activation of Ca\(^{2+}\) -
dependent ATPases compromises metabolic recovery and may ultimately induce hypercontracture and myocardial injury [5].

Previous studies have shown that halogenated anaesthetics reduce myocardial injury resulting from ischaemia and subsequent reperfusion [6]. The underlying mechanisms are uncertain, and multiple sites of action may be involved, e.g., it has been shown that halogenated anaesthetics influence the activity of KATP channels on both the sarcolemmal [7] and mitochondrial membranes [8], inhibit myofilament force production [9] and reduce Ca2+ influx via Ica [10]. There is also evidence that the cardioprotective effect of halogenated anaesthetics may reflect a direct action on the SR. Halothane in particular has been shown to abolish spontaneous Ca2+ oscillations and to reduce hypercontracture following reoxygenation: effects mimicked by structurally unrelated inhibitors of SR function [5]. However, although halogenated anaesthetics have been shown to modify SR Ca2+ release triggered by sarcolemmal Ca2+ influx [11], their influence on Ca2+ sparks or spontaneous Ca2+ release has not been characterised in detail. This is of interest because recent work has shown that cytosolic changes associated with ischaemia inhibit Ca2+ sparks and spontaneous Ca2+ release [12–14]. This decrease in SR Ca2+ efflux results in a marked increase in the SR Ca2+ content, which may exacerbate spontaneous Ca2+ release on reperfusion [13]. Hence, any influence of halogenated anaesthetics on Ca2+ sparks or spontaneous Ca2+ release could have important consequences for the events that occur during ischaemia and reperfusion.

The aim of the present study was to characterise the effects of the volatile anaesthetic halothane on the properties of Ca2+ sparks and spontaneous Ca2+ release. Halothane was used in preference to other halogenated anaesthetics, as it has been reported to be the most potent activator of the ryanodine receptor (RyR) [15]. Cells were permeabilized to allow the direct effects of halothane on SR Ca2+ regulation to be studied independently from sarcolemmal ion fluxes [4]. The results suggest that clinically relevant levels of halothane have little influence on spontaneous forms of Ca2+ release under normal cytosolic conditions. However, in the presence of reduced levels of cytosolic ATP, halothane has marked effects on both Ca2+ sparks and spontaneous Ca2+ release. The potential importance of these effects is discussed in relation to the events that occur during ischaemia and reperfusion.

2. Methods

2.1. Myocyte isolation and permeabilization

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996). Adult Wistar rats (220–250 g) were sacrificed and atrial myocytes isolated by collagenase digestion as described previously [16]. The isolated cells were permeabilized by exposure to saponin (10 μg/ml) in a mock intracellular solution for 6 min, before centrifugation and resuspension. Unless otherwise stated, chemicals were obtained from Sigma. Permeabilized cells were perfused with weakly Ca2+-buffered solutions approximating to the intracellular milieu, and SR Ca2+ release was detected using fluo-3. The basic solution contained (mM): KCl, 100; HEPES, 25; EGTA, 0.05–0.36; phosphocreatine 10; ATP, 0–5 and fluo-3, 0.002, pH 7.0, 22 °C. MgCl2 was added (from 1 M stock solution) to produce a free concentration of 1.0 mM. The free [Ca2+] was adjusted to 220 nM by addition of CaCl2. Solutions containing ATP concentrations less than 5 mM were prepared as previously described [14]. In most experiments, 5 mM sodium azide was included in the solutions to inhibit mitochondrial activity. Halothane was added from a stock solution prepared in dimethyl sulphoxide (DMSO) prior to each experiment. This concentration of DMSO did not exceed 0.4%, which did not influence SR Ca2+ release (not shown).

2.2. Confocal Ca2+ measurement

The apparatus used for [Ca2+] measurement has been described previously [16]. Briefly, the cells were placed in a cylindrical bath (5-mm diameter) in a Perspex block. The bottom of the bath was formed by attaching a coverslip to the underside of the block using epoxy resin. A drop of solution containing cells was placed at the bottom of the bath and a tightly fitting Perspex column inserted into the well until the lower surface was close to myocytes, which had come to rest on the coverslip. Perfusion was achieved by pumping solution (0.3 ml/min) down a narrow bore running longitudinally through the column.

The chamber was placed on the stage of a Nikon Diaphot Eclipse TE2000 inverted microscope, and the cells were viewed using a 60× water immersion lens (Plan Apo, NA 1.2). A confocal laser-scanning unit (Bio-Ap, Micro-radiance 2000, Herts, UK) was attached to the side port of the microscope. The dye was excited at 488 nm, and emitted fluorescence was measured at >515 nm. Image processing and analysis were done using IDL (Research Systems, Boulder, USA) and Laserpix (Bio-Rad) and ImageJ (http://rsb.info.nih.gov/ij/) software. Curves were fitted using Origin (Microcal, MA, USA).

2.3. Data analysis and statistics

Data are presented as mean values±S.E.M. Where necessary, statistical significance was determined using a paired or unpaired t-test as appropriate, using Origin software. P<0.05 was considered significant.
3. Results

3.1. ATP-dependent effects of halothane on spontaneous Ca\(^{2+}\) release

In the presence of 220 nM Ca\(^{2+}\), permeabilized atrial myocytes exhibited spontaneous increases in fluo-3 fluorescence (Ca\(^{2+}\) transients) due to the cyclic uptake and release of Ca\(^{2+}\) from the SR. In the example shown in Fig. 1A (upper), the perfusate contained 5 mM ATP, and spontaneous Ca\(^{2+}\) release occurred at approximately 12-s intervals. Under these conditions, introduction of 1 mM halothane had no significant effect on the amplitude or frequency of spontaneous Ca\(^{2+}\) release. Fig. 1A (lower) shows results obtained from the same myocyte following a decrease in the ATP concentration ([ATP]) to 0.05 mM. As previously reported in ventricular cells [16], ATP depletion was associated with a maintained increase in the amplitude and a decrease in the frequency of spontaneous Ca\(^{2+}\) release.

Accumulated data illustrating the effect of 1 mM halothane on spontaneous Ca\(^{2+}\) release at 5 and 0.05 mM ATP are shown in Fig. 1B. Previous studies suggest that during anaesthesia, the halothane concentration ([halothane]) in arterialised blood is 0.1–0.7 mM, but can rise to 1.2 mM during induction [17,18]. The shaded region in each graph indicates the clinically relevant [halothane]. These data show that 1 mM halothane had no effect on spontaneous Ca\(^{2+}\) release in the presence of 5 mM ATP. However, in the presence of 0.05 mM ATP, levels of halothane within the clinically relevant range induced a concentration-dependent increase in the frequency and a decrease in the amplitude of spontaneous Ca\(^{2+}\) release.

Fig. 1. (A) Fluo-3 fluorescence records from a saponin-permeabilized atrial myocyte perfused with a solution approximating to the intracellular milieu, at a free [Ca\(^{2+}\)] of 220 nM. Effects of 1 mM halothane on spontaneous Ca\(^{2+}\) release at 5 mM or 0.05 mM ATP are shown. (B) Accumulated data showing the relative changes in the frequency (upper) and amplitude (lower) of spontaneous Ca\(^{2+}\) release. Values differing significantly from control (in the absence of halothane) are indicated (*p<0.05, n=5–10).
Higher levels of halothane (>2 mM) influenced both the amplitude and frequency of spontaneous Ca\(^{2+}\) release in the presence of 5 mM ATP. However, these effects are unlikely to be of clinical relevance given the high levels of halothane required.

3.2. Effects of halothane on the SR Ca\(^{2+}\) content at the point of spontaneous Ca\(^{2+}\) release

Under the conditions shown in Fig. 1, the SR Ca\(^{2+}\) content is maximal at the point of spontaneous Ca\(^{2+}\) release. This is because the rise in SR Ca\(^{2+}\) content due to Ca\(^{2+}\) uptake via SERCA is terminated by each spontaneous event. Therefore, further experiments were carried out to investigate whether halothane influences the ‘threshold’ SR Ca\(^{2+}\) content at which spontaneous Ca\(^{2+}\) release occurs. Fig. 2A (left) shows the last in a series of spontaneous Ca\(^{2+}\) transients obtained in the presence of 0.05 mM ATP. This is followed by a response induced by 20 mM caffeine, applied when the next spontaneous Ca\(^{2+}\) release would otherwise have occurred. In this example, the amplitude of the caffeine-induced Ca\(^{2+}\) transient was approximately 20% greater than the amplitude of the spontaneous event. This protocol was then repeated in the same cell following introduction of 1 mM halothane (right). As shown in Fig. 1A, 1 mM halothane increased the frequency and decreased the amplitude of spontaneous Ca\(^{2+}\) release. Rapid application of 20 mM caffeine at the point of spontaneous Ca\(^{2+}\) release revealed that the maximum SR Ca\(^{2+}\) content was significantly smaller than that obtained in the absence of halothane.

This protocol was repeated at a range of [halothane] in the presence of 0.05 or 5 mM ATP. The accumulated data (Fig. 2B) demonstrate that in the presence of 0.05 mM ATP, increasing [halothane] over the range 0.25–1 mM was associated with a concentration-dependent decrease in the SR Ca\(^{2+}\) content at the point of spontaneous Ca\(^{2+}\) release. In the presence of 5 mM ATP, halothane had no significant effect on the SR Ca\(^{2+}\) content at concentrations ≤1 mM. However, a progressive decrease in the maximum SR Ca\(^{2+}\) content did occur when [halothane] was increased above 2 mM. Further accumulated data showing the effects of 1 mM halothane on the SR Ca\(^{2+}\) content at the point of spontaneous Ca\(^{2+}\) release, as a function of [ATP], are shown in Fig. 2C. These data show that 1 mM halothane lessens the increase in the SR content at the point of spontaneous release, which occurs as [ATP] decreases.

3.3. Effects of adenosine on the halothane-induced changes in spontaneous Ca\(^{2+}\) release

ATP depletion has a number of effects on SR Ca\(^{2+}\) regulation including (i) a decrease in the rate of SR Ca\(^{2+}\) accumulation, (ii) a decrease in the open probability (Po) of the RyR, which leads to an increase in the SR Ca\(^{2+}\) content and (iii) reduced occupancy of the adenine nucleotide binding site on the RyR [14]. Further experiments were carried out in an attempt to distinguish which of these effects leads to an increase in halothane sensitivity. Most yielded inconclusive or negative results and will be considered only briefly. As shown previously, partial inhibition of SERCA with cyclopiazonic acid reduces the

![Fig. 2](image-url)
frequency of spontaneous Ca$^{2+}$ release without affecting the amplitude [16]. However, following partial inhibition of SERCA with 20 μM cyclopiazonic acid, 1 mM halothane did not modify spontaneous Ca$^{2+}$ release in the presence of 5 mM ATP (n=4, not shown). Introduction of 0.2 mM tetracaine [16] decreased the frequency of spontaneous Ca$^{2+}$ release by 49±4.8% (n=8) and increased the amplitude by 27.8±4.2% (n=8). As with ATP depletion, this is believed to reflect a decrease in the Po of the RyR (see Discussion). However, in the presence of tetracaine, 1 mM halothane did not modify spontaneous Ca$^{2+}$ release at normal levels of ATP (n=4, not shown).

The possibility that the increased sensitivity to halothane reflects reduced occupancy of the ATP-binding site on the RyR was investigated using adenosine, which has been shown to bind to the adenine nucleotide site with a similar affinity to ATP, but with a much lower efficacy [19]. In Fig. 3A, a cell was initially exposed to a solution containing 5 mM ATP under conditions which precipitated spontaneous Ca$^{2+}$ release from the SR. The solution was then changed to one containing 0.05 mM ATP and 4.95 mM adenosine. This resulted in a decrease in the frequency of spontaneous Ca$^{2+}$ release and a marked increase in amplitude. However, in the presence of adenosine, the effect of 1 mM halothane on the amplitude and frequency of spontaneous Ca$^{2+}$ release was markedly reduced. The accumulated data (Fig. 3B) show that in the presence of 0.05 mM ATP and 4.95 mM adenosine, 1 mM halothane decreased the amplitude of the spontaneous Ca$^{2+}$ transient by 7.6±3% (n=10), while the frequency increased by 23.4±10.4% (n=10). This compares with a decrease in amplitude of 34.8±3% (n=10) and an increase in frequency of 67.7±19% (n=10) when 1 mM halothane was added in the presence of 0.05 mM ATP, zero adenosine.

3.4. ATP-dependent effects of halothane on spontaneous Ca$^{2+}$ sparks

Previous work suggests that the balance between the Ca$^{2+}$ leak associated with Ca$^{2+}$ sparks and uptake via SERCA is an important determinant of the SR Ca$^{2+}$ content [14,20]. Under the conditions shown in Fig. 1, the SR Ca$^{2+}$ content is changing constantly, and sparks are only apparent during the period before each spontaneous Ca$^{2+}$ release (not shown). Therefore, further experiments were carried out in the presence of a higher level of EGTA (0.36 mM), which prevents propagation of Ca$^{2+}$ release between localised Ca$^{2+}$ release sites [14]. This allowed the effects of halothane to be studied under conditions, where the SR Ca$^{2+}$ content and frequency of spontaneous Ca$^{2+}$ sparks were relatively constant under control conditions.

In Fig. 4A, a myocyte was perfused with a solution containing 220 nM Ca$^{2+}$ and 5 mM ATP. Control line scan images revealed the presence of spontaneous Ca$^{2+}$ sparks (i,ii). Halothane (1 mM) was then introduced into the perfusate, and further line scan images collected over the subsequent 2-min period. However, in the presence of 5 mM ATP, 1.0 mM halothane had no significant effect on frequency or amplitude of Ca$^{2+}$ sparks (iii–vii). In Fig. 4B, a cell was equilibrated for 2 min with a solution containing 5

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**Fig. 3.** (A) An atrial myocyte perfused with a solution containing 220 nM Ca$^{2+}$ and 5 mM ATP. The solution was then changed to one containing 0.05 mM ATP and 4.95 mM adenosine, before introduction of 1 mM halothane as indicated. (B) Accumulated data showing the effects of 1 mM halothane on the steady-state amplitude and frequency of spontaneous Ca$^{2+}$ release in the presence of 0.05 mM ATP or 0.05 mM ATP and 4.95 mM adenosine. *Significantly different from control values in the presence of 0.05 mM ATP (p<0.05, n=10).
mM ATP and a control line scan image obtained (i). The perfusing solution was then changed to one of similar composition, but with 0.05 mM ATP. Consistent with previous findings [14], this resulted in a decrease in the frequency of spontaneous Ca\textsuperscript{2+} sparks and an increase in the amplitude (ii). After 2 min in the 0.05 mM ATP solution, 0.5 mM halothane was added to the perfusate. This resulted in a burst of large amplitude Ca\textsuperscript{2+} sparks (iii). The amplitude and frequency of halothane-induced Ca\textsuperscript{2+} sparks then declined progressively over 1–2 min (iv–vii). Fig. 4C shows cumulative data illustrating the halothane-induced changes in Ca\textsuperscript{2+} spark properties obtained using the protocols shown in A and B. In the presence of 5 mM ATP, 1 mM halothane had no significant influence on the frequency or amplitude of spontaneous Ca\textsuperscript{2+} sparks (left). However, decreasing the [ATP] from 5 to 0.05 mM was associated with a 67.9±14%
decrease in the frequency and a $27.5\pm 4.9\% (n=13)$ increase in the amplitude of spontaneous Ca$^{2+}$ sparks. On introduction of 0.5 or 1.0 mM halothane, the Ca$^{2+}$ spark frequency transiently increased above that observed under control conditions, in the presence of 5 mM ATP (Fig. 4D). With 1 mM halothane, the increase in spark frequency was greater and the subsequent decline in both frequency and amplitude more rapid than with 0.5 mM halothane. This suggests that the initial Ca$^{2+}$ efflux is greater on application of 1 mM halothane, resulting in more rapid depletion of SR Ca$^{2+}$.

3.5. Changes in SR Ca$^{2+}$ content associated with modulation of Ca$^{2+}$ spark properties

Further experiments were carried out to assess how changes in the properties of Ca$^{2+}$ sparks shown in Fig. 4 influence the SR Ca$^{2+}$ content. In Fig. 5A, a cell was equilibrated for 4 min with a solution containing 5 mM ATP and 220 nM Ca$^{2+}$. Caffeine (20 mM) was rapidly applied, and the amplitude of the fluorescence transient was used as an index of the steady-state SR Ca$^{2+}$ content. Both the line scan images (upper) and the integrated responses (lower) obtained during caffeine application are shown. After a further 1-min perfusion, [ATP] was decreased to 0.02 mM for 2 min before reapplication of caffeine. As previously reported [16], the amplitude of the caffeine-induced response increased markedly following exposure to 0.02 mM ATP. This protocol was repeated in the same cell, but with 1 mM halothane introduced 45 s before ATP depletion (right). In the presence of halothane, there was no significant increase in the amplitude of the caffeine response following exposure to 0.02 mM ATP. The cumulative data (Fig. 5B) show that the amplitude of the caffeine-induced fluorescence transient increased by $39.8\pm 6.8\% (n=6)$ following a decrease in [ATP] from 5 to 0.02 mM. However, no significant increase occurred when ATP depletion occurred in the presence of 1 mM halothane.

4. Discussion

4.1. Properties of spontaneous Ca$^{2+}$ release in cardiac cells

Under normal physiological conditions, Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels located within the t-tubules triggers Ca$^{2+}$ release from closely opposed RyRs located in the junctional SR. During repetitive stimulation, the SR Ca$^{2+}$ content is submaximal, and propagation between Ca$^{2+}$ release sites does not occur [21]. In contrast, spontaneous Ca$^{2+}$ release occurs during Ca$^{2+}$ overload, when the SR

![Fig. 5. (A) Cells were equilibrated for 4 min with solutions containing 5 mM ATP and 220 nM Ca$^{2+}$ (left) before caffeine (20 mM) was rapidly applied. Both the line scan images and the integrated responses during caffeine application are shown. After a further 1-min perfusion, [ATP] was decreased to 0.02 mM for 2 min and caffeine then reapplied. This protocol was repeated in the same cell, but with 1 mM halothane introduced 45 s before introduction of 0.02 mM ATP (right). (B) Accumulated data showing the relative change in the amplitude of the caffeine-induced Ca$^{2+}$ transient obtained using the protocols shown in (A). *Significantly different from control ($p<0.05$, $n=6$).]
Ca\textsuperscript{2+} content increases to a ‘threshold’ level [22]. The initiation of spontaneous Ca\textsuperscript{2+} release is believed to involve an increase in Po of the RyR due to the binding of Ca\textsuperscript{2+} to regulatory sites within the SR lumen [23,24]. As the SR Ca\textsuperscript{2+} content rises above ~80% of the maximum level, the influence of luminal Ca\textsuperscript{2+} on the RyR and the frequency of spontaneous Ca\textsuperscript{2+} sparks increase markedly [14,20,25]. Spontaneous Ca\textsuperscript{2+} release occurs when the gain of the Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) mechanism increases to such an extent that propagation between SR Ca\textsuperscript{2+} release sites can occur.

Previous work has shown that factors, which influence Ca\textsuperscript{2+} uptake via SERCA or Ca\textsuperscript{2+} release via the RyR, have distinct effects on the properties of spontaneous Ca\textsuperscript{2+} release in skinned cells. Inhibition of SERCA slows the rise in luminal [Ca\textsuperscript{2+}], thereby increasing the time required to reach the threshold for spontaneous Ca\textsuperscript{2+} release. This results in a decrease in release frequency without any significant change in amplitude [16]. However, substances which modify RyR function alter both the amplitude and frequency of spontaneous Ca\textsuperscript{2+} release, e.g., tetracaine-induced inhibition of the RyR increases the amplitude of the spontaneous Ca\textsuperscript{2+} transient and reduces the release frequency [16]. This can be explained if RyR inhibition allows the SR Ca\textsuperscript{2+} content to reach a higher level before luminal feedback enables propagation of Ca\textsuperscript{2+} release; in effect, the luminal ‘threshold’ is shifted to a higher SR Ca\textsuperscript{2+} content. In this study, the increase in frequency and the decrease in amplitude of spontaneous Ca\textsuperscript{2+} release, which occurred in the presence of halothane (Fig. 1A), are consistent with an activating effect on the RyR, such that the threshold for spontaneous Ca\textsuperscript{2+} release occurs at a lower SR Ca\textsuperscript{2+} content (Fig. 2A).

4.2. The concentration dependence of halothane’s action on the SR

In intact rat myocytes, low levels of halothane (0.1–0.5 mM) induce a transient increase in the amplitude of the electrically stimulated Ca\textsuperscript{2+} transient [11]. This is believed to reflect ‘sensitisation’ of the CICR mechanism. In this study, higher levels of halothane (>2 mM) were required to influence the properties of Ca\textsuperscript{2+} sparks or spontaneous Ca\textsuperscript{2+} release under control conditions (Figs. 1 and 4). This apparent difference in the potency of halothane’s action may reflect a number of factors, e.g., when the cytosolic [Ca\textsuperscript{2+}] is increased to the point where spontaneous Ca\textsuperscript{2+} release occurs, the high rate of Ca\textsuperscript{2+} uptake via SERCA may facilitate reaccumulation of any halothane-induced Ca\textsuperscript{2+} leak. However, this seems unlikely because halothane had no effect on spontaneous Ca\textsuperscript{2+} sparks under control conditions, as would be expected with increased RyR activation (Fig. 4). Another possibility is that halothane may have a greater effect on the physiological Ca\textsuperscript{2+} release process (triggered by sarcolemmal Ca\textsuperscript{2+} influx) than spontaneous Ca\textsuperscript{2+} release induced by luminal feedback on the RyR under Ca\textsuperscript{2+} overload conditions. Consistent with this, previous studies on skinned cells have shown that triggered and spontaneous Ca\textsuperscript{2+} release differ in sensitivity to endogenous modulators of RyR function [16,22].

4.3. The ATP dependence of halothane’s action of the SR

The effects of ATP depletion on Ca\textsuperscript{2+} sparks and spontaneous Ca\textsuperscript{2+} release have been studied in detail elsewhere [14]. Briefly, the influence of ATP on spontaneous Ca\textsuperscript{2+} release (Fig. 1) is consistent with its reported action on the RyR. In isolated channels, ATP binding to a specific site on the RyR increased the Po of the channel and facilitated activation by Ca\textsuperscript{2+} [26]. This suggests that ATP depletion and the associated inhibition of RyR gating allow the SR Ca\textsuperscript{2+} content to reach a higher level before propagated Ca\textsuperscript{2+} release occurs (Fig. 1A). The effect of ATP depletion on RyR function can also explain the reduced frequency of spontaneous Ca\textsuperscript{2+} sparks and the increase in SR Ca\textsuperscript{2+} content (Fig. 4B).

Experiments on isolated RyRs have shown that halothane can increase the channel Po [27]. However, it is not clear from previous work why the effect of halothane should exhibit a strong ATP dependence (Figs. 1 and 4). One possibility is that halothane interacts with the adenine nucleotide site on the RyR. This is supported by the fact that halothane’s action is ameliorated by adenosine (Fig. 3), which has been shown to act as a competitive ATP antagonist [19]. Furthermore, halothane influences a number of other ATP-dependent processes, e.g., halothane can induce activation of sarcolemmal adenine receptors and modify the gating properties of K\textsubscript{ATP} channels on the sarcolemmal and mitochondrial membranes [7,8]. In sea urchin eggs, halothane potentiates Ca\textsuperscript{2+} release induced by cyclic ADP ribose [28], which acts by binding to the adenine nucleotide site on the RyR [29]. Finally, cooperative binding of halothane and ATP to firefly luciferase has recently been demonstrated [28]. Taken together, these data suggest that the effects of halothane observed at low levels of ATP may reflect increased occupancy of the adenine nucleotide-binding site on the RyR, either by halothane itself or due to a cooperative interaction between halothane and ATP.

4.4. Possible clinical significance of halothane’s action on the SR

While most of the existing work on ischaemia has focused on ventricular muscle, there is increasing interest in the possibility that ischaemia of the atrium may also have important functional consequences. Atrial fibrillation often occurs in patients presenting with acute myocardial infarction, and it has been suggested that atrial ischaemia may be a contributory factor [30]. Furthermore, atrial fibrillation is the most common sustained arrhythmia resulting directly from coronary artery bypass graft. Recent studies suggest...
that atrial ischaemia following cardioplegic arrest during coronary artery bypass graft is a major causal factor [31,32]. The ATP-dependent effects of halothane reported in this study might be expected to have a number of beneficial effects in the context of ischaemia and reperfusion, e.g., the balance between SR Ca\(^{2+}\) leak (due to spontaneous Ca\(^{2+}\) sparks) and Ca\(^{2+}\) uptake (via SERCA) has an important influence on the SR Ca\(^{2+}\) content [33]. Recent work suggests that the cytosolic changes associated with ischaemia (particularly ATP depletion) inhibit RyR activation, resulting in a pronounced rise in SR Ca\(^{2+}\) content above the normal maximum level [13]. This led to the proposal that an increase in SR Ca\(^{2+}\) content during ischaemia might exacerbate spontaneous Ca\(^{2+}\) release when Ca\(^{2+}\) enters the cell on reperfusion. This study suggests that halothane will maintain the open probability of the RyR in circumstances where ATP depletion occurs, thereby limiting any potential rise in SR Ca\(^{2+}\) content (Figs. 2 and 5). When spontaneous Ca\(^{2+}\) release does occur in the presence of halothane, the frequency is higher, but the amplitude is significantly lower (Fig. 1A). A smaller rise in Ca\(^{2+}\) will induce a smaller transient inward current, which will be less likely to depolarize the cell to the threshold level needed to trigger an action potential [4]. This may be of importance because action potentials triggered by spontaneous Ca\(^{2+}\) release are known to be a major cause of delayed afterdepolarisations and associated arrhythmias [4].

5. Conclusions

In the presence of physiological levels of ATP, halothane has little direct effect on SR Ca\(^{2+}\) regulation. However, in the presence of micromolar levels of [ATP], the sensitivity of the RyR to halothane increases markedly. Following the onset of ischaemia, halothane would be expected to lessen the rise in SR Ca\(^{2+}\) content and to reduce the amplitude of spontaneous Ca\(^{2+}\) release. These effects may contribute to the cardioprotective effects of halothane by reducing the severity of spontaneous Ca\(^{2+}\) release during reperfusion.

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