ELSEVIER

European Journal of Pharmacology 390 (2000) 95–101

ejp

www.elsevier.nl/locate/ejphar

Opposite effects of halothane on guinea-pig ventricular action potential duration

Cécile Terrenoire [a], Vincent Piriou [b], Robert Bonvallet [a], Christophe Chouabe [a], Leon Espinosa [a], Oger Rougier [a], Yves Tourneur [a,*]

[a] *CNRS UMR5578, Laboratoire de Physiologie des Eléments Excitables, Université Claude Bernard-Lyon1, F-69622 Villeurbanne Cedex France*
[b] *Département d'Anesthésie-Réanimation, Hôpital Cardio-Vasculaire, EA 1896, 28 Av Doyen Lépine, F-69500 Bron, France*

Received 29 December 1999; accepted 4 January 2000

Abstract

Halothane protects the heart against the reperfusion injury observed after an ischemia. In ischemic or anoxic conditions, a large ATP-sensitive K^+ (K_{ATP}) conductance is supposed to provide an endogenous protection to the myocardium. In this study, we tested the possibility that halothane acted by modulating this conductance. Isolated guinea-pig cardiomyocytes were successively studied in current clamp and in voltage-clamp conditions. Action potentials regulation by halothane was tested in control conditions and in situations where the K_{ATP} channels were activated. In control conditions, halothane decreased action potential duration of myocytes but did not significantly alter the inward rectifying K^+ current. Conversely, halothane lengthened action potential of cells in which the K_{ATP} conductance was activated, by inhibiting the K_{ATP} current. In ischemic conditions, simultaneous shortening of long action potentials and lengthening of shortened ones would be expected to homogenize the absolute refractory period at the border between normoxic and anoxic zones. This effect, together with a decrease in calcium load, could protect the myocardium against re-entrant arrhythmias.

Keywords: Anaesthetic volatile; Action potential; Arrhythmia; K_{ATP} channel; Myocyte; Patch clamp

1. Introduction

For years, halogenated anaesthetics used in cardiac surgery have been reported to exert cardioprotective effects (Cope et al., 1997). Halothane was shown to reduce the occurrence of dysrhythmias after ischemic events (for a review, see Nader-Djalal and Knight, 1998). The mechanisms responsible for this protection remain mostly unknown. In particular, it is not clear whether or not halothane acts differently in control and ischemic preparations.

Halothane effects on control preparations have been well characterized. At the cellular level, the anaesthetic is known to depress action potential duration in human atrium (Luk et al., 1988) and guinea-pig ventricle (Hirota et al., 1989), whereas it increases it in frog atrium (Hirota et al., 1986). These effects can be explained by a decrease of different ionic currents: halothane inhibits the fast Na^+ current (I_{Na}) (Weigt et al., 1997) and the L-type Ca^{2+} current (I_{CaL}) (Pancrazio, 1996) in guinea-pig ventricles. The delayed rectifier K^+ current (I_K) is diminished by the anaesthetic in frog atrial cells (Hirota et al., 1986) as in guinea-pig atrial and ventricular myocytes (Hirota et al., 1989). Conversely, small changes or no effects were reported on the inward rectifier (I_{K1}) in frog atrial cells (Pancrazio et al., 1993) and in guinea-pig atrial and ventricular cells (Hirota et al., 1989).

In the presence of the metabolic blocker dinitrophenol, Kwok et al. (1996) showed that the ATP-sensitive K^+ (K_{ATP}) current was reduced by halothane in guinea-pig ventricular myocytes. Such an inhibition would be expected to prolong the action potential duration.

In the present study, we examined the effects of halothane on action potentials of ventricular cells in which the K_{ATP} conductance had been activated and compared them to myocytes in control conditions.

* Corresponding author. Tel.: +33-4-72-43-10-32; fax: +33-4-78-94-68-20.
E-mail address: tourneur@physioreg0.univ-lyon1.fr (Y. Tourneur).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00019-4

2. Materials and methods

2.1. Preparation of single cells

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male guinea-pigs (200–300 g) were killed by cervical disruption. Heart dissociation was performed on a Langendorff column with well-oxygenated solution (95% O_2/5% CO_2) maintained at 37°C, as described elsewhere (Tourneur et al., 1994). At the end of the process, ventricles were separated from atria and cut into small pieces that were placed in a trypsinator and gently agitated in the modified healing solution. The supernatant solution was filtered on a 50 μm mesh filter and stored at 4°C in plastic tubes. When needed, a few drops of this solution were dispersed into Tyrode's solution. All cells were used within 12 h.

2.2. Halothane administration

Halothane was dispensed by a vaporiser (Fluotec, Cyprane, UK) in an air flow (2 l/min) provided by an air pump and controlled by a flow-meter. An infrared analyzer (Ohmeda 5250, Liberty Corner, NJ, USA) continuously monitored the gas mixture. It started bubbling into the recording solution at least 15 min before every experiment. The dilution of 2% halothane approximately corresponds to 1.2 mM at room temperature (Pancrazio, 1996).

2.3. Solutions (in mM)

Tyrode: NaCl 137; KCl 5.4; $CaCl_2$ 1; $MgCl_2$ 1; glucose 10; pyruvic acid 5; thiamine 0.2; HEPES 10 (pH adjusted to 7.2 with NaOH). (used as bath solution).

Ca^{2+}-free Tyrode: same as previous without $CaCl_2$.

Healing solution, modified from Isenberg and Klöckner (1982): K-glutamate 70; KCl 20; KH_2PO_4 10; $MgCl_2$ 1; oxalic acid 10; taurine 10; glucose 10; pyruvic acid 5; HEPES 10 (pH adjusted to 7.2 with KOH).

Pipette solution: KCl 140; $MgCl_2$ 2; EGTA 1; glucose 10; pyruvic acid 5; HEPES 10 (pH adjusted to 7.2 with KOH).

A high dose of Glibenclamide (20 μM) was used to inhibit K_{ATP} channels. The final solution was freshly prepared from a 100 mM stock solution in dimethyl sulfoxide (DMSO).

SR47063 [4-(2-cyanimino-1,2-dihydropyrid-1-yl)-2,2 dimethyl-6-nitrochromene] stock solution (10 mM) was obtained by sonication in ethanol. This solution was diluted in Tyrode before each experiment, at a lower concentration in current clamp (2 μM) than in voltage-clamp (3 μM) to ensure cell excitability.

Control experiments permitted to verify that SR47063 effects were completely reversed on washout and that the proper effect of ethanol and DMSO on the action potential could be neglected (not shown).

2.4. Source of products

Halothane was used as the clinical solution (Fluothane, ZENECA Pharma, UK). SR47063 was kindly provided by Dr. Gautier, Sanofi Research (Montpellier, France). Other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.5. Recording techniques

Cells were placed in an experimental chamber (2 ml) mounted on an inverted microscope (Leica DM-IL, Germany) and studied at room temperature (20–23°C). A 300 μm-wide capillary tube was brought up to 100–200 μm of the cell which was perfused at a constant rate (10–50 μl/min) with a dead volume of less than 500 nl. Hard glass microelectrodes (1–2 MΩ) were pulled on an horizontal puller (DMZ, Zeitz Instr., Germany). A digital patch clamp amplifier (VP500 Bio-Logic, France) ensured stimulation and data storage. Voltage and current signals, filtered at 10 kHz, were digitized at 1 and 2 kHz, respectively.

2.6. Stimulations

A standard experiment started up with current clamp measurements and was followed by the voltage-clamp study of the same cell.

In current clamp, action potentials were elicited by 3 ms current steps (0.4 Hz).

The voltage-clamp protocol (0.25 Hz) was composed of a series of voltage steps from the holding potential of −80 mV (Fig. 1). The two first steps aimed at inactivating the inward currents I_{Na} (step to −40 mV, 50 ms) and I_{CaL}

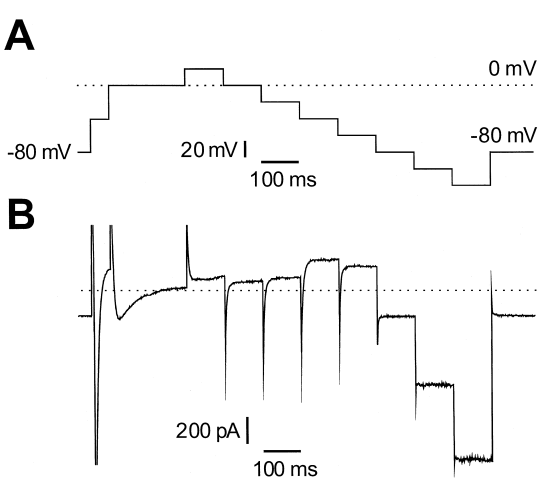

Fig. 1. Standard voltage-clamp experiments. (A) Voltage-clamp stimulation (see Section 2). (B) Current trace recorded in control conditions. The Na^+ current trace is truncated. Dotted line represents the zero current.

(step to 0 mV, 300 ms), respectively. The steady-state current, I_{ss}, was recorded at each of the eight following 20-mV steps (100 ms) from +20 to −120 mV. The time-dependent K^+ current (I_K), which is very sensitive to temperature (Kiyosue et al., 1993), was not recorded in our experimental conditions.

In every mode, recordings were carried out following the sequence (eventually repeated): control, halothane, washout.

2.7. Analysis and display of results

In current clamp experiments: the resting potential, E_m, was averaged over 20 ms before every stimulation. The action potential duration was measured at 10, 20, 50 and 90% of repolarization (respectively APD10, APD20, APD50, and APD90).

In voltage-clamp experiments: an estimation of I_{CaL} was given by the difference between the negative peak and the steady-state current at 0 mV (arrow in Fig. 2C). The steady-state current, I_{ss}, was averaged over the last 20 ms of each of the eight final steps of the protocol. Analysis was performed by custom routines written under LabView software (National Instruments, USA) and results were plotted with Origin (Microcal, Northampton, MA USA).

2.8. Statistics

Data corresponded to the average of 10 (current clamp) or 20 (voltage-clamp) successive recordings and were expressed as mean ± S.E.M., n indicating the number of experiments. We used non-parametric Fisher tests to evaluate the effect of each drug on a single preparation and Mann–Whitney tests to compare results obtained on different preparations.

3. Results

3.1. Halothane effects on myocytes in control conditions

The effects of halothane (2%) on a typical action potential (APD90 = 480 ± 82 ms, $n = 8$) are illustrated in Fig. 2A. Action potential amplitude and duration were depressed in the presence of the anaesthetic (2) and returned to their initial level after washout of halothane (3). Fig. 2B shows that halothane rapidly decreased APD20 (208 ± 12 vs. 315 ± 63 ms, $n = 8$, $P \leq 0.05$) and APD90 (344 ± 16 vs. 479 ± 82 ms, $n = 8$, $P \leq 0.05$). APD10 (181 ± 38 ms, $n = 8$) and APD50 (442 ± 81 ms, $n = 8$) were also reduced (by 50 ± 7 and 135 ± 15 ms, respectively, $n = 8$, $P \leq 0.05$). These effects were reproducible (two applications in Fig. 2B). Em measured in control conditions (−72 ± 1 mV, $n = 8$) was not altered (−72.6 ± 0.5 mV, $n = 8$, $P \leq 0.05$), which suggested that I_{ss} was not affected.

Indeed, voltage-clamp experiments revealed that halothane (2%) did not significantly modify inward nor outward I_{ss} ($n = 5$, $P \leq 0.05$, not illustrated). The zero current potential under halothane was −73 ± 2 mV ($n = 4$,

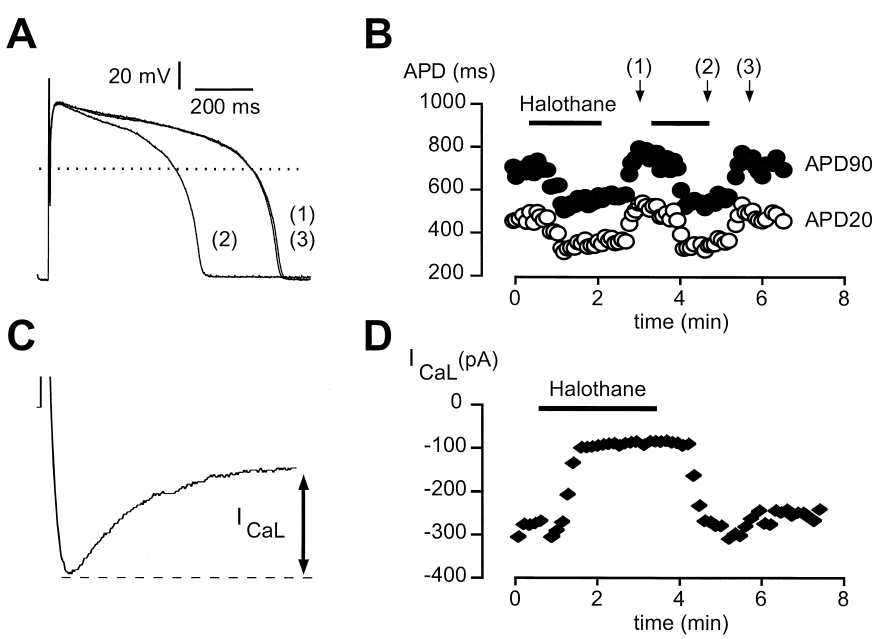

Fig. 2. Effect of halothane on a cell in control conditions. (A) Superimposition of action potentials in control (1), in the presence of 2% halothane (2) and after washout (3). Dotted line represents 0 mV. (B) Time course of the action potential duration at 20% (APD20, open circles) and 90% (APD90, solid circles) of repolarization, showing the result of two successive applications of halothane (2%, horizontal bars). Arrows labelled (1), (2), (3) refer to the corresponding traces in (A). (C) Principle of I_{CaL} estimation. This current was measured as the difference between peak and steady-state current at the end of the pulse (arrow). (D) Time course of I_{CaL} recorded at 0 mV during one application of halothane (horizontal bar).

$P \leq 0.06$), not different from its level in control conditions (-71 ± 1 mV, $n = 17$). As shown in Fig. 2D, the anaesthetic strongly reduced I_{CaL} at 0 mV (by $40 \pm 11\%$, $n = 4$, $P \leq 0.06$). These effects were rapid and could be reproduced several times on a same cell.

3.2. Evolution of some myocytes

We observed rod-shaped cells with clear-cut striations that were characterized by abnormally short action potentials (APD90 $= 132 \pm 21$ ms, $n = 5$) despite a normal resting potential (-75.3 ± 0.5 mV, $n = 5$). These myocytes initially displayed ''normal'' action potentials ((1) in Fig. 3A) that progressively evolved, during the course of the experiment, towards a state of short duration ((2) in Fig. 3A). This spectacular change is illustrated in Fig. 3B, where the dramatic decrease in APD90 took place within a few minutes, after 30–40 min spent at a ''normal'' level. The voltage-clamp study of these cells (Fig. 3C and D) revealed a marked increase in the outward part of I_{ss} (at $+20$ mV: 1781 ± 552 pA, $n = 13$, vs. 54 ± 15 pA, $n = 17$, $P \leq 0.0001$) whereas the inward part of this current did not evolve. Glibenclamide (20 μM) instantly decreased this current (Fig. 3D) so that a strong inward rectification was restored ($n = 3$). This pharmacological property together with the loss of inward rectification are characteristics of the K_{ATP} current. Interestingly, in the same time, I_{CaL} recorded at 0 mV became twice smaller than the current measured in control conditions (289 ± 48 pA, $n = 13$, vs. 499 ± 59 pA, $n = 17$, $P \leq 0.05$). After this spontaneous evolution, the electrical properties of the cells remained stable for 30 min. These particular cells exhibited electrophysiological properties similar to those of myocytes subjected to a complete metabolic blockade (Nichols et al., 1991).

3.3. Halothane effects on cells displaying short action potential

Fig. 4A shows the surprising effect of halothane in a cell with short action potential (1). Halothane slightly decreased the amplitude (2) and particularly increased the action potential duration (APD90 $= 201 \pm 19$ ms, $n = 5$, vs. 132 ± 21 ms, $n = 5$, $P \leq 0.05$). These modifications were rapid (Fig. 4B) and affected all levels of repolarization (increase of 18 ± 8 ms, 30 ± 14 ms and 57 ± 17 ms for APD10, APD20 and APD50 respectively, $n = 5$, $P \leq 0.05$). The resting potential was not altered by halothane (-73.4 ± 0.7 mV, $n = 5$, $P \leq 0.05$). One can notice that, under halothane, action potential did not recover the typical long plateau characteristic of guinea-pig ventricular myocytes.

The voltage-clamp recordings obtained on these cells (Fig. 4C) showed that I_{ss} initially exhibited a small inward rectification that considerably intensified under halothane (I_{ss} inhibited by $82 \pm 8\%$ at $+20$ mV, $n = 5$, $P \leq 0.05$) (without any change of the inward current), recalling the

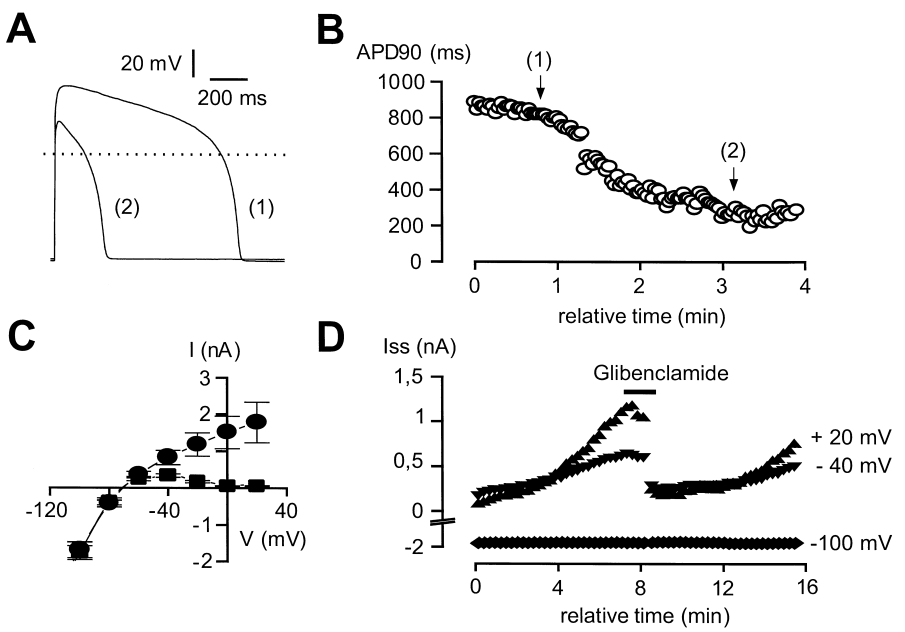

Fig. 3. Spontaneous evolution of the electrical properties of some myocytes. (A) Superimposition of action potentials recorded at the beginning of a standard experiment (1) and after cell evolution (2). Dotted line corresponds to 0 mV. (B) Time course of action potential duration at 90% of repolarization (APD90): evolution from a state of ''normal'' action potential towards a state of abnormally short action potential. The dramatic shortening spontaneously occurred but then stabilized for 30 min. The arrows labelled (1) and (2) refer to the corresponding traces in (A). (C) Current–voltage curves of I_{ss} obtained from cells displaying normal action potentials (squares, $n = 17$) vs. short action potentials (circles, $n = 13$). For each curve, standard error of the mean (S.E.M.) is represented as error bars. (D) Time course of I_{ss} at -100 mV (diamonds), -40 mV (down triangles) and $+20$ mV (up triangles). These two last traces crossed over, indicating the progressive loss of inward rectification. Glibenclamide (20 μM, horizontal bar) strongly decreased the outward current without affecting the inward current.

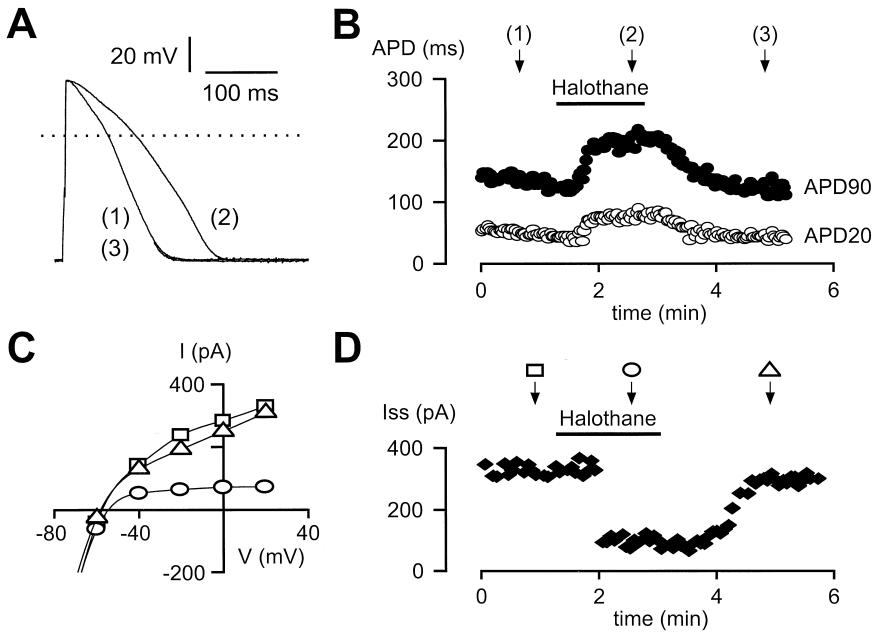

Fig. 4. Effect of halothane on a cell displaying abnormally short action potentials. (A) Typical short action potential recorded after cell evolution in control conditions (1), in the presence of halothane (2) and after washout (3). Dotted line indicates the level of 0 mV. (B) Time course of action potential duration at 20% (APD20, open circles) and 90% (APD90, solid circles) of repolarization. Application of halothane (2%) is indicated by a horizontal bar. Arrows labelled (1), (2), (3) refer to the corresponding action potential in (A). (C) Current–voltage curves of I_{ss} recorded in the same cell in control conditions (squares), under 2% halothane (circles) and after washout (triangles). (D) Time course of I_{ss} at +20 mV. Arrows labelled with open symbols (square, circle and triangle) refer to the corresponding curves in C.

effect of glibenclamide. Halothane effects were rapid as illustrated by the time course of I_{ss} at +20 mV in Fig. 4D. Simultaneously, the anaesthetic reduced the small Ca^{2+} current at 0 mV (not shown).

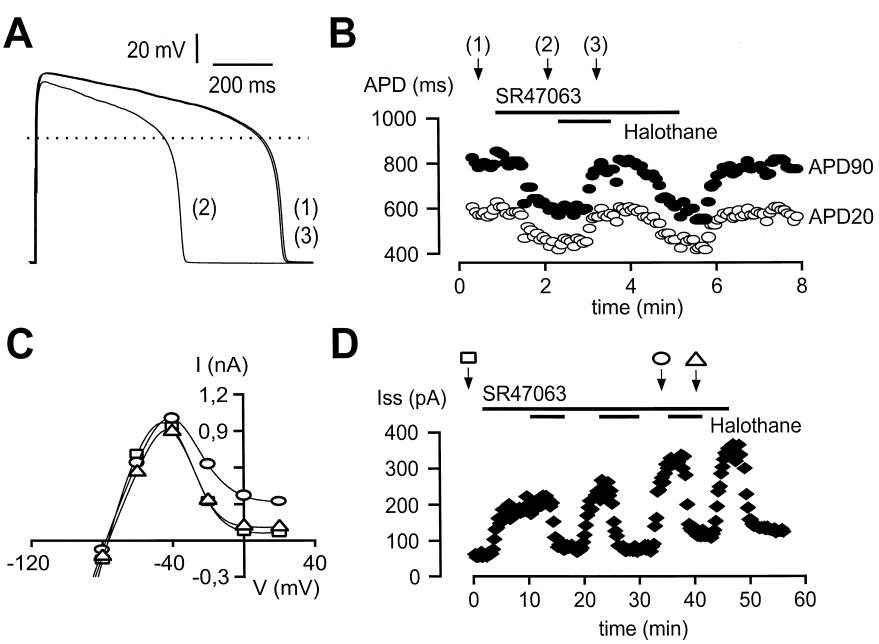

Fig. 5. Effect of halothane in the presence of SR47063, a specific K_{ATP} opener. (A) Superimposition of an action potential in control (1), under application of 2 μM SR47063 (2) and under 2% halothane in the presence of 2 μM SR47063 (3). Dotted line corresponds to 0 mV. (B) Monitoring of the action potential duration at 20% (APD20, open circles) and 90% (APD90, solid circles) of repolarization, under application of 2 μM SR47063 (upper horizontal bar) and 2% halothane (lower horizontal bar). Arrows labelled (1), (2) and (3) refer to the corresponding traces in (A). (C) Current–voltage curves obtained on the same cell in control (squares), under 3 μM SR47063 (circles) and under application of 2% halothane simultaneously with 3 μM SR47063 (triangles). (D) Steady-state current at +20 mV (I_{ss}) under applications of 3 μM SR47063 (upper horizontal bar) and 2% halothane (lower horizontal bars). Arrows labelled with open symbols (square, circle and triangle) refer to the current–voltage curves in (C).

3.4. Halothane effects on cells pre-treated with SR47063, a K_{ATP} opener

To evaluate the contribution of K_{ATP} channels to such a regulation of action potential duration, we applied a specific K_{ATP} opener (SR47063) on myocytes, which displayed long action potentials and tested the effects of halothane on these cells.

Fig. 5A shows the opposite effects of halothane (2%) and SR47063 (2 μM) on an action potential recorded in control conditions (APD90 = 823 ± 69 ms, $n = 4$). In the presence of the low dose of SR47063 (2), the action potential amplitude and duration were decreased (APD10 = 288 ± 20 ms, APD20 = 487 ± 47 ms, APD50 = 661 ± 76 ms, APD90 = 695 ± 78 ms, $n = 4$, $P \leq 0.05$) without any noticeable effect on the resting potential (−76.3 ± 0.1 mV vs. −76.1 ± 0.5 mV in controlled conditions, $n = 4$, $P \leq 0.06$). In these conditions, halothane restored the action potential amplitude and duration (Fig. 5B) at all levels of repolarization (APD10 = 296 ± 21 ms, APD20 = 531 ± 21 ms, APD50 = 733 ± 22 ms, APD90 = 770 ± 23 ms, $n = 4$, $P \leq 0.05$) while the resting potential was not markedly modified (−77.1 ± 0.1 mV, $n = 4$, $P \leq 0.06$) ((3) in Fig. 5B). Halothane and SR47063 effects were rapid (Fig. 5B) and could be reproduced several times. These results suggested that K_{ATP} channels were involved in halothane effects on action potentials. This hypothesis was confirmed by the voltage-clamp study of these myocytes.

SR47063 (3 μM) activated an outward current reversing close to −80 mV (Fig. 5C), suppressed by glibenclamide (Tourneur et al., 1994). Altogether, these properties recalled those of K_{ATP} channels. Halothane inhibited the outward current (by 61 ± 9% at +20 mV, $n = 5$, $P \leq 0.05$) and, like SR47063, did not exert any significant effect on the inward steady-state current nor on the reversal potential. Even with the progressive increase in the time-independent outward current induced by SR47063, halothane could rapidly suppress the current recorded at +20 mV (Fig. 5D). Halothane effects were reproducible as illustrated by the three successive applications in this experiment.

4. Discussion

In the present study, we compared the effects of halothane on the electrical activity of guinea-pig ventricular myocytes in control conditions and in situations where the K_{ATP} channels were activated.

Halothane reduced action potential duration at all levels of repolarization in control conditions. The resting potential was 10 mV more positive than the K^+ equilibrium potential E_K (−82 mV) in agreement with other studies (Spindler et al., 1998). This value was not significantly affected by halothane, as already reported by Hirota et al. (1989). Voltage-clamp experiments confirmed that halothane did not modify the inward rectifier K^+ current (I_{K1}), in opposition to its analogue sevoflurane (Stadnicka et al., 1997). In our conditions, halothane did not activate K_{ATP} channels. As a result, the prevailing effect of halothane on action potentials probably came from I_{CaL} inhibition, as already demonstrated by Hirota et al. (1989).

During our experiments, we observed myocytes that were apparently healthy (clear-cut striations, normal resting potential) but which displayed very short action potential duration for tens of minutes. Compared to control myocytes, the inward rectification was almost suppressed as a consequence of the development of a large glibenclamide-sensitive current reversing near E_K, thus likely flowing through K_{ATP} channels. Moreover, the Ca^{2+} current measured at 0 mV was decreased by 58%. Similar changes had been described by Belles et al. (1987) on particularly long whole-cell recordings realized at 34–36°C and using oxygenated solutions. The spontaneous evolution of the inward Ca^{2+} current and of the large K^+ current is probably not due to anoxia (Friedrich et al., 1990), since oxygen is much more soluble in water at lower temperature. It could be due to the loss of metabolites during the cell perfusion, or to the production of free radicals (Tokube et al., 1998). The reason why some cells exhibited abnormal electrical properties is not clear and might be linked to the dissociation process, the probability of such an evolution to occur being different from day to day in similar conditions.

Halothane prolonged action potential duration of cells that had spontaneously evolved (Fig. 4B), despite a depressing effect on the residual Ca^{2+} current. To determine the role of K_{ATP} channels in this regulation, we applied SR47063, a specific K_{ATP} opener (Tourneur et al., 1994), on myocytes displaying long action potentials. The consecutive shortening of action potentials was partly abolished by halothane (Fig. 5B). Indeed, in the presence of the opener, K_{ATP} channels were not fully activated and the anaesthetic still decreased the relatively large Ca^{2+} current (not shown).

In summary, when K_{ATP} channels were activated (spontaneously or chemically), action potential duration was lengthened because of halothane-induced inhibition of these channels.

Activation of K_{ATP} channels (Isomoto and Kurachi, 1997; Babenko et al., 1998; Kersten et al., 1998 for reviews) provides an endogenous protection by decreasing the Ca^{2+} load during anoxia or ischemia (Cole, 1993; Grover, 1997). However, K^+ accumulation favours the occurrence of ischemia-induced spontaneous arrhythmias and repetitive response arising around the border zone between normal and anoxic tissue (Janse and Wit, 1989; Picard et al., 1999). This could explain the anti-arrhythmic efficacy of K_{ATP} channels blockers to impair arrhythmias on pig (Wirth et al., 1999), dog (Billman et al., 1998) or guinea pig (Tosaki and Hellegouarch, 1994).

Halogenated anaesthetics are known to reduce the occurrence of ventricular dysrhythmias following ischemic events (Nader-Djalal and Knight, 1998). By analogy with the beneficial effects of K^+ channel blockers, our results suggest that halothane may act by restoring the homogeneity of the refractory period in border zones, in addition to a decrease in Ca^{2+} load, and other effects including vasodilation and prevention of free radicals production (Glantz et al., 1997).

Acknowledgements

The authors indebted to Dr. Gautier, from Sanofi, for providing us with SR47063. A preliminary form of this work has already been published: Terrenoire C, Piriou V, Tourneur Y. 1997. Effects of halothane on ionic currents of guinea-pig myocytes. *J. Mol. Cel. Cardiol.* 29 (5): A113. (Abstract).

References

Babenko, A.P., Aguilar-Bryan, L., Bryan, J., 1998. A view of SUR/KIR6.X, K_{ATP} channels. Annu. Rev. Physiol. 60, 667–687.

Belles, B., Hescheler, J., Trube, G., 1987. Changes of membrane currents in cardiac cells induced by long whole cell recordings and tolbutamide. Pflügers Arch. 409, 582–588.

Billman, G.E., Englert, H.C., Scholkens, B.A., 1998. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part II: effects on susceptibility to ventricular fibrillation induced by myocardial ischemia in conscious dogs. J. Pharmacol. Exp. Ther. 286, 1465–1473.

Cole, W.C., 1993. ATP-sensitive K^+ channels in cardiac ischemia: an endogenous mechanism for protection of the heart. Cardiovasc. Drugs Ther. 3, 527–537.

Cope, D.K., Impastato, W.K., Cohen, M.V., Downey, J.M., 1997. Volatile anesthetics protect the ischemic rabbit myocardium from infarction. Anesthesiology 86, 699–709.

Friedrich, M., Benndorf, K., Schwalb, M., Hirche, H.J., 1990. Effects of anoxia on K and Ca currents in isolated guinea pig cardiocytes. Pflügers Arch. 416, 207–209.

Glantz, L., Ginosar, Y., Chevion, M., Gozal, Y., Elami, A., Navot, N., Kitrossky, N., Drenger, B., 1997. Halothane prevents postischemic production of hydroxyl radicals in the canine heart. Anesthesiology 86, 440–447.

Grover, G.J., 1997. Pharmacology of ATP-sensitive potassium channel (K_{ATP}) openers in models of myocardial ischemia and reperfusion. Can. J. Physiol. Pharmacol. 75, 309–315.

Hirota, K., Ito, Y., Matsuda, A., Momose, Y., 1989. Effects of halothane on membrane ionic currents in guinea-pig atrial and ventricular myocytes. Acta Anaesthesiol. Scand. 33, 239–244.

Hirota, K., Momose, Y., Takeda, R., Nakanishi, S., Ito, Y., 1986. Prolongation of the action potential and reduction of the delayed outward K+ current by halothane in single frog atrial cells. Eur. J. Pharmacol. 126, 293–295.

Isenberg, G., Klöckner, U., 1982. Ca^{2+} tolerant ventricular myocytes prepared by preincubation in a «KB Medium». Pflügers Arch. 395, 6–18.

Isomoto, S., Kurachi, Y., 1997. Function, regulation, pharmacology, and molecular structure of ATP-sensitive K^+ channels in the cardiovascular system. J. Cardiovasc. Electrophysiol. 8, 1431–1446.

Janse, M.J., Wit, A.L., 1989. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. Phys. Rev. 69, 1049–1169.

Kersten, J.R., Gross, G.J., Pagel, P.S., Warltier, D.C., 1998. Activation of adenosine triphosphate-regulated potassium channels. Mediation of cellular and organ protection. Anesthesiology 88, 495–513.

Kiyosue, T., Arita, M., Muramatsu, H., Spindler, A.J., Noble, D., 1993. Ionic mechanisms of action potential prolongation at low temperature in guinea-pig ventricular myocytes. J. Physiol. (London) 468, 85–106.

Kwok, W.M., Martinelli, A.T., Stadkicka, A., Bosjnak, Z.J., 1996. Differential effects of volatile anesthetics on the cardiac ATP-sensitive K^+ channel. Anesthesiology 65, A515.

Luk, H.N., Lin, C.I., Wei, J., Chang, C.L., 1988. Depressant effects of isoflurane and halothane on isolated human atrial fibers. Anesthesiology 69, 667–676.

Nader-Djalal, N., Knight, P.R., 1998. Volatile anesthetic effects on ischemic myocardium. Curr. Opin. Anaesthesiol. 11, 403–406.

Nichols, C.G., Ripoll, C., Lederer, W.J., 1991. ATP-sensitive potassium channel modulation of the guinea pig ventricular action potential and contraction. Circ. Res. 68, 280–287.

Pancrazio, J.J., 1996. Halothane and isoflurane preferentially depress a slowly inactivating component of Ca^{2+} channel current in guinea-pig myocytes. J. Physiol. (London) 491, 91–103.

Pancrazio, J.J., Frazer, M.J., Lynch, C., 1993. Barbiturate anesthetics depress the resting K^+ conductance of myocardium. J. Pharmacol. Exp. Ther. 265, 358–365.

Picard, S., Rouet, R., Ducouret, P., Puddu, P.E.N., Flais, F., Criniti, A., Monti, F., Gerard, J.L., 1999. K_{ATP} channels and 'border zone' arrhythmias: role of the repolarization dispersion between normal and ischaemic ventricular regions. Br. J. Pharmacol. 127, 1687–1695.

Spindler, A.J., Noble, S.J., Noble, D., LeGuennec, J.Y., 1998. The effects of sodium substitution on currents determining the resting potential in guinea-pig ventricular cells. Exp. Physiol. 83, 121–136.

Stadnicka, A., Bosnjak, Z.J., Kampine, J.P., Kwok, W.M., 1997. Effects of sevoflurane on inward rectifier K^+ current in guinea-pig ventricular cardiomyocytes. Am. J. Physiol. 273, H324–H332.

Tokube, K., Kiyosue, T., Arita, M., 1998. Effects of hydroxyl radicals on K_{ATP} channels in guinea-pig ventricular myocytes. Pflügers Arch. 437, 155–157.

Tosaki, A., Hellegouarch, A., 1994. Adenosine triphosphate-sensitive potassium channel blocking agent ameliorates, but the opening agent aggravates, ischemia/reperfusion-induced injury. Heart function studies in non fibrillating isolated hearts. J. Am. Coll. Cardiol. 23, 487–496.

Tourneur, Y., Marion, A., Gautier, P., 1994. SR47063, a potent channel opener, activates K_{ATP} and a time-dependent current likely due to potassium accumulation. J. Membr. Biol. 142, 337–347.

Weigt, H.U., Kwok, W.M., Rehmert, G.C., Turner, L.A., Bosnjak, Z.J., 1997. Voltage dependent effects of volatile anesthetics on cardiac sodium current. Anesth. Analg. 84, 285–293.

Wirth, K.J., Rosenstein, B., Uhde, J., Englert, H.C., Busch, A.E., Scholkens, B.A., 1999. ATP-sensitive K^+ channel blocker HMR 1883 reduces mortality and ischemia-associated electrocardiographic changes in pigs with coronary occlusion. J. Pharmacol. Exp. Ther. 291, 474–481.