Laboratory Investigations

Contractile force and resting tension in the presence of halothane and increased extracellular potassium or decreased extracellular pH in isolated guinea pig atria

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To gain a better understanding of the direct actions of halothane on myocardial function in ischaemia, we studied the effects of increasing extracellular potassium concentration and decreasing extracellular pH (acidosis), alone or in combination with halothane, on the contractile force and resting tension in isolated atria. Guinea pig left atria were superfused with Tyrode's solution and stimulated at 1 Hz. Isometric contractile force and resting tension were measured using a force displacement transducer. Perfusate potassium concentrations were increased from 5.4 mmol·L⁻¹ to either 8.1 mmol·L⁻¹ or 10.8 mmol·L⁻¹ by adding KCl to the standard Tyrode's solution, and its pH was decreased from 7.4 to either 7.0 or 6.5 by decreasing bicarbonate. In standard Tyrode's solution (potassium 5.4 mmol·L⁻¹, pH 7.4), halothane 0.5–2% reduced contractile force in a dose-dependent manner ($P < 0.05$); the effective concentration of halothane for 50% inhibition of contractile force

(IC₅₀) was 1.3%. Both increasing extracellular potassium and decreasing extracellular pH decreased the contractile force in a potassium- or pH-dependent fashion. The negative inotropism of halothane (1%) was not altered by increasing potassium concentrations, whereas 1% halothane caused a greater decrease in contractile force at pH 6.5 than at pH 7.4. Halothane (1%) enhanced the acidosis (pH 6.5)-induced increases in resting tension. Arrhythmias were produced in one of eight preparations during acidosis, while four of eight preparations demonstrated arrhythmias during acidosis in the presence of halothane. These data suggest that acidosis and halothane may have a synergistic interaction on the contractile force and resting tension of the atria. The increase in resting tension observed during acidosis/halothane conditions suggests that an increase in cytosolic calcium is associated with these synergistic interactions between acidosis and halothane.

Key words

ACID-BASE EQUILIBRIUM: acidosis;
ANAESTHETICS, VOLATILE: halothane;
HEART: atria, contractility, resting tension;
IONS: potassium.

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Accepted for publication 26th February, 1994.

Pour mieux comprendre l'action directe de l'halothane sur la fonction myocardique pendant l'ischémie, nous avons étudié les effets de l'augmentation du potassium extracellulaire et de la diminution du pH extracellulaire (acidose), seuls ou en association avec l'halothane, sur la force contractile et la tension de repos d'oreillettes isolées. Des oreillettes gauches de cobaye furent perfusées avec une solution de Tyrode et stimulées à 1 Hz. La force contractile isométrique et la tension de repos ont été mesurées avec un transducteur de force de déplacement. Les concentrations de potassium perfusées ont été augmentées de 5,4 mmol·L⁻¹ à 8,1 mmol·L⁻¹ ou à 10,8 mmol·L⁻¹ par l'ajout de KCl à la solution standard de Tyrode, et son

pH abaissé de 7,4 à 7,0 ou 6,5 par baisse des bicarbonates. Avec la solution standard de Tyrode (potassium $5,4 \text{ mmol} \cdot \text{L}^{-1}$, pH 7,4), l'halothane (0.5–2%) diminue la force contractile proportionnellement à la dose ($P < 0,05$); la concentration efficace d'halothane requise pour produire une inhibition de 50% de la force contractile (IC_{50}) a été de 1,3%. L'augmentation du potassium extracellulaire et la diminution du pH extracellulaire réduisent toutes les deux la force contractile proportionnellement au potassium ou au pH. L'inotropisme négatif de l'halothane (1%) n'est pas modifié par l'augmentation de la concentration de potassium alors que l'halothane produit une diminution plus importante de la force contractile à un pH de 6,5 que de 7,4. L'halothane (1%) exagère l'augmentation de la tension de repos induite par l'acidose (pH 6,5). Des arythmies sont apparues sur une des huit préparations pendant l'acidose en présence d'halothane. Ces données suggèrent que l'acidose et l'halothane pourraient avoir une activité synergique sur la force contractile et la tension de repos des oreillettes. L'augmentation de la tension de repos observée pendant l'acidose combinée à l'halothane suggère l'association d'une augmentation du calcium cytosolique avec des interactions synergiques entre l'acidose et l'halothane.

There is considerable inconsistency in the results previously reported by investigators studying the effects of halothane on myocardial function in ischaemia. Kissin *et al.*¹ reported that halothane depresses contractility in the hypoperfused myocardium to a lesser degree than at a normal level of myocardial perfusion. Hoka *et al.*² reported that the increase in ^{45}Ca content in the ischaemic area was less in the presence of halothane than in its absence. In contrast, Lowenstein *et al.*³ reported that halothane produces greater dysfunction in the ischaemic region of the myocardium than in the nonischaemic region. Lieberman *et al.*⁴ revealed in patients undergoing halothane anaesthesia for coronary artery revascularization that ischaemia occurred with considerable decreases in arterial blood pressure and in coronary perfusion pressure. To explain these discrepant results, it has been suggested that an indirect result of halothane's cardiovascular effects operates through changes in coronary blood flow. That is, the beneficial effect of halothane in myocardial ischaemia is thought to be mediated by improved oxygen supply relative to oxygen demand,^{1,2,5} while myocardial dysfunction caused by halothane is thought to be mediated by decreases in coronary blood flow due to hypotension.^{3,4,6–8} However, the question remains as to whether or not these effects of halothane result only as a consequence of changes in coronary blood flow, or if the enhanced direct effect of halothane on contractility of the ischaemic myocardium is a contributing factor. To gain a better understanding of the direct actions of hal-

othane on the myocardial function in ischaemia, we studied the effects of increasing extracellular potassium ($[K]_e$) and decreasing extracellular pH (pHe), alone or in combination with halothane, on contractile force and resting tension in isolated superfused guinea pig atria. Both increased $[K]_e$ and decreased pHe are known to contribute to the electrophysiological and mechanical abnormalities induced by acute ischaemia.^{9–13}

Methods

The protocol was approved by the Animal Investigation Committee of the Hospital. Thirty-four guinea pigs of either sex, weighing 300–600 g, were anaesthetized with enflurane and the beating hearts were excised as soon as the response to tail clamp was lost. The left atrium was rapidly dissected and suspended in a 30 mL organ bath superfused with Tyrode's solution at $30 \pm 0.5^\circ\text{C}$. Tyrode's solution was equilibrated both in a reservoir and in an organ bath with 95% O_2 /5% CO_2 , providing a PO_2 of $>500 \text{ mmHg}$ and PCO_2 of about 35 mmHg . Suffusate flow rate was kept at $10 \text{ ml} \cdot \text{min}^{-1}$ with a roller perfusion pump. The composition of standard Tyrode's solution in $\text{mmol} \cdot \text{L}^{-1}$ is: NaCl 125; KCl 5.4; MgCl_2 1.05; NaHCO_3 24; NaH_2PO_4 0.42; CaCl_2 2.7; and glucose 10. One end of the preparation was fixed with a pair of punctate platinum stimulation electrodes, through which the preparations were stimulated (Nihon Kohden SEN 3201 stimulator), and the other end was connected by means of a silk thread to an isometric force displacement transducer (Nihon Kohden SB-IT) with diastolic tension adjusted to 1 g. The basic stimulation rate was 1 Hz and stimuli were rectangular pulses of 1-msec duration at 1.5 times the threshold current. Isometric contractile force (CF) and resting tension (RT) were recorded on a thermal pen strip chart recorder (Nihon Kohden WT-687G). Individual preparations were equilibrated for at least 60 min in standard Tyrode's solution before the start of experiments.

Experimental studies involved exposure of atria to halothane under five different conditions of bathing solution. In standard Tyrode's solution ($[K]_e$ $5.4 \text{ mmol} \cdot \text{L}^{-1}$, pHe 7.4), the effects of 0.5%, 1.0%, and 2.0% of halothane on CF were studied (physiological group, $n = 9$). The CF was measured before (baseline) and 40 min after addition of halothane. In subsequent experiments the bathing solution potassium ($[K]_e$) and pH (pHe) were modified to mimic four abnormal conditions. That is, $[K]_e$ was increased from $5.4 \text{ mmol} \cdot \text{L}^{-1}$ to either $8.1 \text{ mmol} \cdot \text{L}^{-1}$ or $10.8 \text{ mmol} \cdot \text{L}^{-1}$ by adding KCl to the standard Tyrode's solution (increased $[K]_e$ group, $n = 9$), and pHe was decreased from 7.4 to either 7.0 or 6.5 by decreasing NaHCO_3 from $24 \text{ mmol} \cdot \text{L}^{-1}$ to either $9 \text{ mmol} \cdot \text{L}^{-1}$ or $3 \text{ mmol} \cdot \text{L}^{-1}$, respectively (decreased pHe group, $n = 8$). In all solutions, the total sodium con-

centration was kept constant (see Table 1 of reference 10). Halothane (1%) was added after attaining steady-state [K]_e or pHe conditions; mostly 30 min after changing to one of the four abnormal conditions. The CF was measured in standard Tyrode's solution before changing [K]_e or pHe, after attaining steady state [K]_e or pHe conditions, and 40 min after addition of halothane. Since an increase in RT (contracture) was observed during acidosis or acidosis/halothane conditions, we then studied, in a separate set of experiments, the effects of halothane (1%) on acidosis (pHe 6.5)-induced contracture ($n = 8$). Isometric RT was measured 40 min after the start of acidosis and/or halothane.

Halothane was introduced into the O₂/CO₂ gas mixtures via a calibrated vaporizer at gas flow rates of 4 L · min⁻¹. The order of halothane, [K]_e, and pHe was randomized at each group to avoid a possible systematic time-dependent effect. At the end of experimental period a 3-ml sample of bathing solution was taken for the analysis of halothane concentrations (Shimadzu GC4 gas chromatography) and pHe (Radiometer ABL 300 blood gas analyzer).

Statistical analyses of the effects of halothane, [K]_e, and pHe were accomplished by Mann-Whitney test or Kruskal-Wallis test. Significance was considered at P value of < 0.05 .

Results

There were no differences between groups in the baseline CF obtained in standard Tyrode's solution before the start of experiments; baseline CF was 1.24 ± 0.24 g, 1.16 ± 0.32 g, and 1.21 ± 0.25 g (mean $\pm$ SD), respectively, in physiological, increased [K]_e, and decreased pHe groups. Effects of halothane, increasing [K]_e, and decreasing pHe, alone or in combination, on the CF of the atria are summarized in Figures 1, 2, 3, 4 and 5. In Figures 1, 2, and 4, CF was normalized to the baseline CF. To facilitate comparisons between the negative inotropism of halothane at different [K]_e and pHe, the data are expressed as the percentage of the CF measured before halothane exposure at each [K]_e and pHe in Figures 3 and 5.

In standard Tyrode's solution, CF of the atria was reduced in a dose-dependent fashion with increasing halothane concentrations from 0.5% to 1.0% and 2.0% ($P < 0.05$, Figure 1). The effective concentration of halothane for 50% inhibition of CF (IC₅₀) was 1.3%. Assuming that the MAC value for guinea-pig is equal to that of rat (no MAC value is available for guinea pig), halothane 1.3% corresponds to 1.1 – 1.2 MAC.¹⁴ Compared with the baseline CF (100%) obtained at [K]_e 5.4 mmol · L⁻¹, increasing [K]_e from 5.4 mmol · L⁻¹ to either 8.1 mmol · L⁻¹ or 10.8 mmol · L⁻¹ decreased the

FIGURE 1 Effects of halothane concentrations on the contractile force (CF) of the atria in standard Tyrode's solution. The CF was normalized to the baseline CF obtained before halothane exposure. Bars represent mean $\pm$ SD. $P < 0.05$; different from baseline CF.

FIGURE 2 Effects of increasing perfusate potassium concentrations on the contractile force (CF) of the atria. The CF was normalized to the baseline CF obtained in standard Tyrode's solution before changing potassium concentrations. Bars represent mean $\pm$ SD. $P < 0.05$; different from baseline CF.

CF in a [K]_e-dependent fashion ($P < 0.05$, Figure 2). Figure 3 indicates that the negative inotropism of 1% of halothane was not altered by increasing [K]_e from 5.4 mmol · L⁻¹ to either 8.1 mmol · L⁻¹ or 10.8 mmol · L⁻¹. Compared with the baseline CF (100%) obtained at pHe 7.4, decreasing pHe from 7.4 to either 7.0

FIGURE 3 Effects of 1% halothane on the contractile force (CF) of the atria at different potassium concentrations. The data are expressed as percentage of CF measured before halothane exposure at each potassium concentration. Bars represent mean $\pm$ SD. NS; not different from the data obtained at potassium 5.4 mmol $\cdot$ L $^{-1}$.

or 6.5 reduced the CF in a pH-dependent fashion ($P < 0.05$, Figure 4). Figure 5 indicates that little difference was observed between the negative inotropism of 1% of halothane at pH 7.4 and at pH 7.0, whereas at pH 6.5 halothane (1%) caused a greater decrease in CF than the changes produced at pH 7.4.

Figure 6 shows results of typical experiments on the effects of halothane (1%) and acidosis (pH 6.5), alone or in combination, on the CF and RT. In standard Tyrode's solution, CF decreased but RT did not change 40 min after exposure to halothane 1% (upper panel). During acidosis (pH 6.5), RT increased progressively in addition to worsening of the CF (middle panel); these changes were potentiated by adding halothane 1% to acidosis (lower panel). More importantly, arrhythmias were induced during acidosis/halothane conditions (lower panel). To facilitate comparisons, the changes of RT were normalized to the baseline CF obtained in standard Tyrode's solution before the start of acidosis or acidosis/halothane conditions. Figure 7 shows the mean RT values obtained in eight preparations. The magnitude of increases in RT induced by acidosis/halothane conditions was greater than that induced by acidosis alone, suggesting that halothane (1%) enhances acidosis-induced increases in RT. Arrhythmias were produced in one of eight preparations during acidosis, while four of eight preparations demonstrated arrhythmias during acidosis/hal-

FIGURE 4 Effects of decreasing perfusate pH on the contractile force (CF) of the atria. The CF was normalized to the baseline CF obtained in standard Tyrode's solution before changing pH. Bars represent mean $\pm$ SD. $P < 0.05$; different from baseline CF.

FIGURE 5 Effects of halothane 1% on the contractile force (CF) of the atria at different pH. The data are expressed as percentage of CF measured before halothane exposure at each pH. Bars represent mean $\pm$ SD. NS; not different. $P < 0.05$; different from the data obtained at pH 7.4.

othane conditions but this difference was not statistically significant when compared with chi-square analysis.

The vaporizer setting of 0.5%, 1.0%, and 2.0% pro-

FIGURE 6 Results of typical experiments on the time course of the changes in contractile force (CF) and resting tension (RT). Halothane 1% reduced CF but did not change RT in standard Tyrode's solution (upper panel). The RT increased progressively in addition to worsening of the CF at pH 6.5 (middle panel); these changes were potentiated by adding halothane 1% to acidosis (lower panel). Note that arrhythmias were induced during acidosis/halothane conditions (lower panel). BL: base line.

duced perfusate concentrations of 3.1 ± 0.6 , 9.5 ± 0.3 , and 22.5 ± 2.2 mg · dl⁻¹ of halothane (mean $\pm$ SD, $n = 8$), respectively. The pHe of Tyrode's solution was 7.41 ± 0.06 , 7.04 ± 0.04 , and 6.58 ± 0.08 at 24 mmol · L⁻¹, 9 mmol · L⁻¹, and 3 mmol · L⁻¹ of NaHCO₃, respectively ($n = 6-8$).

Discussion

There is increasing evidence to implicate a variety of factors in the genesis of arrhythmias and contractile failure associated with myocardial ischaemia. These include hypoxia, reduced high-energy phosphate supply, acidosis, and the accumulation of potassium and lysophosphoglycerides.^{10,13} Of these various factors, many of the electrophysiological and mechanical effects of acute ischaemia are known to be mimicked by the combined effects of increased extracellular potassium ([K]_e) and decreased extracellular pH (pHe).⁹⁻¹³ Accordingly, in order to gain greater insight into the direct actions of halothane on myocardial function in ischaemia, we chose to study the

Resting Tension (%)

FIGURE 7 Effects of acidosis (pHe 6.5, left) and acidosis/halothane conditions (right) on the resting tension (RT) of the atria. The changes of RT are normalized to the baseline CF obtained in standard Tyrode's solution before the start of acidosis or acidosis/halothane conditions. Bars represent mean $\pm$ SD. $P < 0.05$; different from the data obtained during acidosis.

effects of increasing [K]_e and decreasing pHe (acidosis), alone and in combination with halothane, on the contractile force (CF) and resting tension (RT) in isolated superfused atria.

In standard Tyrode's solution, halothane caused a dose-dependent reduction of CF in guinea pig atria (Figure 1). The IC₅₀ of halothane 1.3% calculated in this study was greater than that of halothane 0.5% reported in human atrial tissues.¹⁵ This difference would be due to the differences in preparations and experimental protocol.

The most important findings of this study are that acidosis and halothane have synergistic effects on the CF and RT of the atria. Although the negative inotropism of halothane 1% was not altered by increasing [K]_e (Figure 3), halothane (1%) caused a greater decrease in CF at pHe 6.5 than at pHe 7.4 (Figure 5). These synergistic interactions of acidosis and halothane on the CF of the atria might be because the effects of acidosis both on the slow calcium channels and on the sarcoplasmic reticulum (SR) of the myocardium are quite similar to those of halothane. In the voltage-clamp experiments, Wada and Goto¹⁶ and Chesnais *et al.*¹⁷ reported that slow inward current is markedly depressed during acidosis compared with values at normal pHe conditions, while Ike-moto *et al.*¹⁸ revealed that halothane, like acidosis, reduces the slow inward current of the myocardium. In contrast, Ohnishi¹⁹ and Wheeler *et al.*²⁰ reported that halothane increases calcium release from the SR, while Lain *et al.*²¹ and Su and Kerrick²² reported that hal-

othane inhibits calcium uptake by the SR. More importantly, Blanck and Thompson,²³ and Casella *et al.*²⁴ demonstrated that these actions of halothane on the cardiac SR are pHe-dependent. Blanck and Thompson²³ revealed that the steady-state level of calcium in the SR was decreased by halothane at pHe 6.9, whereas halothane had no effect at pHe 7.3. From these results, they concluded that at pHe 6.9 halothane might induce a greater efflux of calcium from the SR compared with control values. Casella *et al.*²⁴ studied the effects of halothane on the pHe dependence of calcium uptake by cardiac SR and revealed that, during acidosis, halothane depresses calcium uptake rate by the SR, compared with the control state. These findings of Blanck and Thompson²³ and Casella *et al.*²⁴ suggest that, during acidosis, halothane may contribute to a greater decrease in SR capacity for calcium and perhaps lead to a decreased availability of contractile calcium. The reduced slow inward current, and the enhanced release and reduced uptake of calcium by SR, which are both induced by the interaction of acidosis and halothane, would quickly deplete the calcium stores and result in a greater myocardial depression.

Halothane (1%) enhanced acidosis (pHe 6.5)-induced myocardial contracture (a rise in RT, Figures 6 and 7) as well as acidosis-induced myocardial depression (Figures 5 and 6), suggesting that an increase in cytosolic calcium might be associated with these synergistic interactions between acidosis and halothane.²⁵ It has been revealed that an increase in cytosolic calcium is related to both a rise in RT and an increase in electrical resistance of gap junction,²⁶ which provides a low resistance pathway in the heart.²⁷ Orchard²⁸ and Nakanishi *et al.*²⁹ demonstrated that cytosolic calcium increases during acidosis. Gettes *et al.*¹¹ revealed that decreasing pHe to as low as 6.0 by lowering bicarbonate in the perfusate results in an increase in internal longitudinal resistance (cell-to-cell uncoupling). Terrar and Victory,²⁷ and Burt and Spray³⁰ reported that halothane, like acidosis, causes closure of the gap junction. Although the mechanisms underlying closure of the gap junction by halothane are uncertain, Vassort *et al.*³¹ suggested that the uncoupling effect of general anaesthetics could be explained by increased intracellular calcium concentrations. Therefore, it seems likely that halothane potentiates acidosis-induced increases in cytosolic calcium, leading to myocardial contracture and cell-to-cell uncoupling. As the resistance to propagation from cell to cell increases, conduction velocity decreases, synchronization of force development of the individual cells of the syncytium is delayed, and contractility is reduced.^{30,32}

Increasing $[K]_e$ from $5.4 \text{ mmol} \cdot \text{L}^{-1}$ to either $8.1 \text{ mmol} \cdot \text{L}^{-1}$ or $10.8 \text{ mmol} \cdot \text{L}^{-1}$ did not have a synergistic interaction with halothane (Figure 3). These results may

suggest that cytosolic calcium does not increase by increasing $[K]_e$. The data of our previous study¹¹ showing that increasing $[K]_e$ alone from $5.4 \text{ mmol} \cdot \text{L}^{-1}$ to $15.0 \text{ mmol} \cdot \text{L}^{-1}$ did not influence cell-to-cell coupling, but decreasing pHe to as low as 6.0 by lowering bicarbonate in the perfusate resulted in an increase in internal longitudinal resistance will support this possibility.

Arrhythmias were produced in one of eight preparations during acidosis, while four of eight preparations demonstrated arrhythmias during acidosis/halothane conditions. Although this difference did not reach statistical significance probably due to a small number of experiments, we predict that the interaction of acidosis and halothane might be important in relation to the arrhythmogenic actions of halothane. Stowe *et al.*³³ studied the combined effects of acid-base changes and halothane on action potential properties of guinea pig right ventricle, and revealed that the opposing effects of alkalosis to shorten, and of acidosis to lengthen both action potential duration and effective refractory period were attenuated at low levels and abolished at high levels of halothane. From these results, they suggested that the effects of halothane on the cardiac cell membrane during variations in pHe may contribute to arrhythmia development in the clinical setting. In addition, if the rise in RT is closely related to cell-to-cell uncoupling,²⁶ our results showing that halothane enhances acidosis-induced contracture suggest that a reduction in junctional conductance may also contribute to arrhythmia development during halothane anaesthesia. Reductions in conduction velocity predispose cardiac tissue to reentrant type arrhythmias, and it has been known that reentrant excitation rather than abnormal automaticity or triggered activity has been invoked as an electrophysiologic mechanism for arrhythmias during halothane anaesthesia.^{34,35}

Finally, the limitations of our study should be discussed. First, we did not investigate the interaction between hypoxia and halothane. It might be argued that, if the aim of our study was to investigate the direct actions of halothane on the myocardial function in ischaemia, the effects of hypoxia as well as the effects of increasing $[K]_e$ and decreasing pHe, alone or in combination with halothane, on the CF should have been studied in isolated superfused preparations because, as described above, hypoxia is one of the factors responsible for arrhythmias and contractile failure associated with myocardial ischaemia. However, in our preliminary study, the effects of hypoxia on the CF were progressive. Thus, we could not obtain a stable state of CF, i.e., a baseline CF, during hypoxia. For this reason, we did not study the interaction of hypoxia and halothane in this experiment. Second, this study was performed in atrial tissue. Because the excitation-contraction coupling processes and the me-

chanical restitution of atrial tissues are different from those of ventricular muscles,^{36,37} further experiments would be necessary to determine whether halothane induces similar effects in ventricular myocardium.

In conclusion, we found that halothane potentiates both acidosis-induced myocardial depression and acidosis-induced myocardial contracture. An increase in RT observed during acidosis in the presence of halothane suggests that an increase in cytosolic calcium is associated with these synergistic interactions between acidosis and halothane.

Acknowledgements

We thank Dr. T. Ban, Department of Pharmacology, for helpful discussion. This project was supported in part by financial assistance from the Department of Anaesthesiology, Yamaguchi University School of Medicine.

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