ENFLURANE INCREASES THE ADRENALINE THRESHOLD FOR THE DEVELOPMENT OF SLOW RESPONSES IN ISOLATED CANINE TRABECULAE

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SUMMARY

To determine if enflurane has different effects on myocardial sensitivity to adrenaline than those previously reported for halothane, we have studied the enflurane-adrenaline interaction in isolated canine trabeculae using doses of adrenaline necessary to produce slow responses (adrenaline threshold for the development of slow responses, A TSR) as an indicator. The preparations were depolarized in Tyrode's solution containing potassium chloride 26 mmol litre\(^{-1}\), then adrenaline concentrations in the solution were increased stepwise. Enflurane 1% had no significant effect, but 2% and 4% significantly increased the A TSR two-fold and seven-fold, respectively. To investigate the influence of enflurane on the adrenaline-adrenoceptor interaction, we studied the effects on the A TSR of 2% enflurane alone or in combination with either prazosin 8 ng ml\(^{-1}\) or metoprolol 17 ng ml\(^{-1}\). Compared with the A TSR obtained with 2% enflurane alone, alpha, -block with prazosin did not alter, but beta,-block with metoprolol significantly increased the A TSR (three-fold). These effects of enflurane are qualitatively as well as quantitatively similar to those reported previously for halothane. Thus, assuming that the MAC value for enflurane is about 2-2.5 times greater than that for halothane, the effects of enflurane on slow channel conductance (antiarrhythmic effects of enflurane) might be about two to three times greater than those of halothane at an equivalent depth of anaesthesia. Such differences may explain in part the clinical observation that ventricular arrhythmias are less likely with enflurane than with halothane. (Br. J. Anaesth. 1993; 71: 253-257).

KEY WORDS


Arrhythmogenic doses of adrenaline differ for different inhalation anaesthetics [1-4]. Enflurane causes fewer arrhythmias than halothane with exogenous administration of adrenaline [1-3, 5, 6]. In addition, enflurane differs from halothane in its propensity to sustain ventricular arrhythmias. Johnston, Eger and Wilson [2] reported that, when ventricular arrhythmias appeared with enflurane, the abnormal beats were occasional, intermittent and not necessarily sustained. In contrast, when ventricular irritability appeared during halothane anaesthesia, it was often followed by frequent abnormal beats, leading to ventricular tachycardia [2]. Isoflurane is comparable to enflurane in this regard [2-4]. However, with enflurane there is a wide variation in patient response to adrenaline, and the dose-response curve for adrenaline arrhythmias with enflurane is significantly flatter than with halothane or isoflurane [2, 7]. Despite intensive study, the reason for these differences is not clear.

In isolated, partially depolarized guineapig myocardium, Ebara, Hasegawa and Mitsuiye [8] studied the effects of temperature on the myocardial sensitivity to catecholamines using the sensitivity of slow calcium (Ca\(^{2+}\)) channels to catecholamine as an indicator. In their experimental model, catecholamine-induced increases in slow channel conductance produced depolarization of the resting membrane in quiescent ventricular muscle. The depolarization was enhanced and often led to automatic activity as temperature decreased. These results suggest that the sensitivity of slow Ca\(^{2+}\) channels to catecholamines reflects, at least in part, myocardial sensitivity to catecholamines and that the sensitivity is increased at decreased temperatures. To determine if enflurane has effects on myocardial sensitivity to adrenaline which differ from those reported previously for halothane [9], we also used the sensitivity of slow Ca\(^{2+}\) channels to adrenaline—the dose of adrenaline necessary to produce slow responses (adrenaline threshold for the development of slow responses, A TSR)—as an indicator and studied the effects of enflurane, alone or in combination with either selective alpha, - (prazosin) or beta,- (metoprolol) adrenergic receptor antagonists, on the A TSR in isolated, partially depolarized canine trabeculae.

METHODS

Our study was approved by the Animal Investigation Committee of the hospital. Details of the experimental design were similar to those of our previous...
ATSR. In this study, ATSR was defined as the dose activated by adding adrenaline to the solution. Tyrode's solution, and slow Ca$^{2+}$ channels were partially depolarizing the preparations in K$^{+}$-rich solutions greater than threshold (mostly 1 mA) in standard adrenaline concentrations in the solution reached a level not significantly different from baseline (B) ($P < 0.05$).

Propagating slow responses were generated when the stimulator was recorded by a differential-input, high-impedance amplifier (Nihon Kohden AVM-10) coupled with a cathode follower (Nihon Kohden MEZ-7101) and displayed on a Nihon Kohden VC-10 oscilloscope. The extracellular voltage change was recorded by a suction apparatus that was carefully limited Harvard pump. The beating heart then was removed by sternotomy and placed immediately in oxygenated Tyrode's solution at room temperature. The trachea was intubated and the lungs ventilated via a volume-limited Harvard pump. The beating heart then was removed by sternotomy and placed immediately in oxygenated Tyrode's solution at room temperature. The trachea was intubated and the lungs ventilated via a volume-limited Harvard pump. The beating heart then was removed by sternotomy and placed immediately in oxygenated Tyrode's solution at room temperature.

Vigorous contractions caused by adrenaline frequently dislodged the intracellular electrode, therefore electrocardiograms were recorded from the surface of the preparations. In our experimental model, increased extracellular resistance was essential to obtain a reliable extracellular voltage decrease along the preparations. For this purpose, the preparations, pinned on a small silicon rubber platform, were surrounded with a uniform and very thin layer of Tyrode's solution [10] and covered by a single layer of cotton mesh. One end of the preparation was placed at the lumen of a 1-mm tube through which oxygenated Tyrode's solution at 37 °C flowed at a rate of 3 ml min$^{-1}$ to determine ATSR. Standard and K$^{+}$-rich Tyrode's solution were aerated with 95% oxygen-5% carbon dioxide through a calibrated vaporizer for at least 30 min before administration of drugs was randomized to avoid a possible systematic time-dependent effect.

FIG. 1. Adrenaline threshold for the development of slow responses (ATSR) with different concentrations of enflurane (mean, SEM, * Significantly different from baseline (B) ($P < 0.05$).

One-way analyses of variance with critical-difference testing were used to determine statistical significance between baseline values and values obtained at each concentration of each drug. $P < 0.05$ was considered statistically significant. The mean ATSR values obtained before (baseline) and 30 min after administration of each concentration of enflurane (1, 2 and 4%; $n = 7$), and after 2% enflurane alone or in combination with prazosin 8 ng ml$^{-1}$ or metoprolol 17 ng ml$^{-1}$ ($n = 7$). Enflurane was equilibrated with Tyrode's solution in the reservoir by passing 95% oxygen-5% carbon dioxide through the calibrated vaporizer for at least 30 min before administration to the preparation. The mean concentrations of 1, 2 and 4% enflurane in solution, verified by gas chromatography (Shimadzu GC-4), were 9.9 (SEM 0.6) mg dl$^{-1}$, 20.2 (0.1) mg dl$^{-1}$ and 38.2 (0.9) mg dl$^{-1}$ ($n = 7$), respectively. The order of administration of drugs was randomized to avoid a possible systematic time-dependent effect.

RESULTS

The mean ATSR values obtained before (baseline) and 30 min after administration of enflurane, alone or in combination with either prazosin or metoprolol, are shown in figures 1 and 2. Enflurane 1% had no significant effect, but 2% and 4% enflurane significantly increased the ATSR (two-fold and seven-fold, respectively) (fig. 1). In combination with 2% enflurane, alpha-1-block with prazosin did not alter the ATSR, whereas beta-1-block with metoprolol significantly increased it; compared with the ATSR...
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Fig. 2. Adrenaline threshold for the development of slow responses (ATSR) with 2% enflurane alone (E) or in combination with either prazosin 8 ng ml⁻¹ (E + P) or metoprolol 17 ng ml⁻¹ (E + M) (mean, SEM). Significant differences: * from baseline (B); † from the value obtained with 2% enflurane.

obtained with 2% enflurane alone, prazosin 8 ng ml⁻¹ did not change, but metoprolol 17 ng ml⁻¹ significantly increased the ATSR (three-fold) (fig. 2).

DISCUSSION

We studied the direct effects of enflurane on myocardial sensitivity to adrenaline in isolated, partially depolarized canine trabeculae. The myocardial cell, depolarized by K⁺, has a low degree of excitability, one factor responsible for this being an increased membrane shunting (outward K⁺) conductance [11, 12]. That is, in the K⁺-depolarized myocardium, slow inward current is affected by the presence of large outward K⁺ current that flows during the rising phase of the action potential [13]. In most such preparations, muscle excitability is restored or enhanced by addition of catecholamines, which increase slow channel conductance [14], or by addition of small concentrations of barium (Ba²⁺), which reduces membrane shunting conductance [13], to the superfusate—procedures which are thought to intensify the slow inward current.

In the present study, we used the dose of adrenaline required to produce slow responses (the ATSR) as an indicator for myocardial sensitivity to adrenaline. It could be argued that the ATSR might be affected by the changes in the membrane shunting conductance. However, as the increase in membrane shunting conductance is thought to be K⁺-dependent [15], we feel justified in assuming that membrane shunting conductance was kept almost constant throughout our experiments, in which preparations were depolarized equally in K⁺-rich Tyrode's solution containing KCl 26 mmol litre⁻¹ before (when baseline ATSR values were obtained) and during administration of enflurane. In addition, Lynch and colleagues found, in their experiment with isolated, partially depolarized guinea pig papillary muscles, that enflurane did not influence K⁺ conductance because it did not change the action potential duration of slow responses. Based on these assumptions, we believe that the ATSR reflects, at least in part, the responsiveness of slow Ca²⁺ channels to adrenaline in isolated heart preparations. As adrenaline exerts its effects on the heart by increasing slow channel conductance [14], which is triggered by adrenaline-adrenoceptor interaction, the responsiveness of slow Ca²⁺ channels to adrenaline in isolated heart preparations should reflect cardiac responses to adrenaline [8]. Activation of the slow Ca²⁺ channel initiates slow responses, which are responsible for both abnormal automaticity [17] and re-entry [18]. More importantly, a re-entrant mechanism is thought to underlie the arrhythmogenic interaction between volatile anaesthesics and adrenaline [9, 19, 20]. Therefore, we predicted that, at least in part, the threshold for adrenaline-induced arrhythmias and the sensitivity of the myocardium to adrenaline could be investigated using the ATSR as an indicator.

We found that enflurane significantly increased the ATSR in a dose-dependent manner (fig. 1). This action of enflurane, as with that of halothane [9], is closely correlated with its Ca²⁺ entry-blocking action. Bomsjø, Supan and Rusch [21] and Eskinder and colleagues [22] recently showed, using the whole-cell voltage-clamp technique, that enflurane reduces the Ca²⁺ current of isolated canine ventricular and Purkinje cells in a concentration-dependent manner. Lynch and colleagues [16] reported that enflurane depresses both the upstroke velocity and the conduction of slow responses in isolated, partially depolarized guineapig papillary muscles. In addition, in our previous study [9], verapamil, in common with enflurane, increased the ATSR in a dose-dependent manner. If these in vitro results are applicable to surgical patients, our findings are indicative of an antiarrhythmic action rather than an arrhythmia-producing action of enflurane, and several recent reports suggest that this may be the case. First, ventricular ectopic beats were suppressed during the administration and reappeared after discontinuation of enflurane in a patient undergoing vagotomy and antrectomy [23]. Second, enflurane decreased the incidence of ventricular fibrillation in a canine model of acute occlusion-reperfusion arrhythmias [24]. Third, enflurane decreased the incidence of death from ventricular fibrillation in rats with ligation of the left anterior descending coronary artery [25].

Enflurane has less potential than halothane for production of arrhythmias in the presence of exogenous adrenaline [1–3, 5, 6]. Munson and Tucker [1] reported that, in the dog, the adrenaline dosage for enflurane was 17.1 µg kg⁻¹ and the corresponding value for halothane was 4.6 µg kg⁻¹. Johnston, Eger and Wilson [2] studied patients who were undergoing trans-sphenoidal pituitary operations for which they received nasal and oral submucosal injections of adrenaline to minimize surgical bleeding, and found that the arrhythmic threshold of adrenaline was 10.9 µg kg⁻¹ for enflurane and 2.1 µg kg⁻¹ for halothane, although the dose identified was that necessary to produce a positive response in 50% of the patients (ED₅₀). Sumikawa, Ishizaka and Suzuki [3] reported that, in the dog, the mean values of the arrhythmogenic doses and the corresponding plasma concentrations of adrenaline were 11.43 µg kg⁻¹ min⁻¹ and 206.3 ng ml⁻¹ during enflurane; 2.18 µg
kg\(^{-1}\) min\(^{-1}\) and 38.7 ng ml\(^{-1}\) during halothane anaesthesia. These findings indicate that four to five times as much adrenaline is required to produce ventricular arrhythmias during enflurane as during halothane anaesthesia.

If, as postulated in the review of Atlee and Bosnjak [20] and in our previous study [9], inhalation anaesthetics have both antiarrhythmic (Ca\(^{2+}\) entry blocking actions) and arrhythmia-producing effects (effects on the refractory period, Na\(^{+}\) channel conductance and passive membrane properties), such differences between enflurane and halothane in the arrhythmogenic doses of adrenaline might exist because the antiarrhythmic effects of enflurane are greater or its arrhythmogenic effects are smaller (or both) than those of halothane. We have demonstrated in the present study that the effects of enflurane on the ATSR are similar to those reported previously for halothane in the same concentration: 2 \(^{\%}\) and 4 \(^{\%}\) enflurane increased the ATSR two-fold and seven-fold, while 2 \(^{\%}\) and 4 \(^{\%}\) halothane increased it two-fold and five-fold, respectively [9]. Thus, assuming that the MAC value for enflurane is about 2–2.5 times that of halothane [26], our results suggest that the antiarrhythmic effects of enflurane may be about 2–3 times greater than those of halothane at the equivalent depth of anaesthesia. Bosnjak, Supan and Rusch [21] and Eskinder and colleagues [22] reported recently on whole-cell voltage-clamp experiments with single isolated canine ventricular or Purkinje cells in which, quantitatively, enflurane and halothane produce a similar inhibition of Ca\(^{2+}\) channel currents at equianesthetic concentrations. These results seem to conflict with our data, but it should be noted that the aim of their studies differed from ours. They investigated the relationship between the negative inotropic and chronotropic actions of volatile anaesthetics and inhibition of Ca\(^{2+}\) channel currents, and so they needed to record isolated Ca\(^{2+}\) channel current. For this purpose (in order to eliminate Na\(^{+}\) and K\(^{+}\) currents) they substituted Ba\(^{2+}\), tetraethylammonium chloride and caesium ion for Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\). In contrast, we aimed to investigate the direct effects of enflurane on the arrhythmia-producing actions of adrenaline in isolated heart preparations and therefore we induced slow responses by adding adrenaline to the superfusate containing Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\). Slow inward current is carried by Na\(^{+}\) and by Ca\(^{2+}\) [27], and outward K\(^{+}\) current may flow through slow channels [27, 28]. Therefore, the differences between the results reported by Bosnjak, Supan and Rusch [21] and Eskinder and colleagues [22] and those of our present study might be attributable to the differences between isolated Ca\(^{2+}\) current and slow inward current. If, as described above, slow responses are responsible for re-entry type arrhythmias [18], which are thought to underlie the arrhythmogenic interaction between volatile anaesthetics and adrenaline [9, 19, 20], it should be possible to investigate the effects of enflurane on myocardial sensitivity to adrenaline (antiarrhythmic effects of enflurane in the present study) using the ATSR as an indicator. In contrast, Atlee and Roky [29, 30] and Atlee and Alexander [19] have shown that halothane prolongs A-V nodal, His-Purkinje and ventricular conduction, whereas enflurane prolongs only A-V nodal conduction in dogs. From these results, they concluded that conduction changes such as occur with halothane are necessary for ventricular arrhythmias caused by re-entry of excitation and that enflurane is less likely to cause these arrhythmias. In addition, Burt and Spray [31] studied the effects of halothane and enflurane on the passive membrane properties of the myocardium (gap junction-mediated intercellular communication) in cultured neonatal rat cardiac myocytes, and found that complete block of junctional conductance occurred with smaller concentrations of halothane than enflurane.

To investigate the influence of enflurane on adrenaline-adrenoceptor interaction, we studied the effects on the ATSR of 2 \(^{\%}\) enflurane alone or in combination with prazosin or metoprolol. In our study of the effects of halothane on adrenaline-adrenoceptor interaction [9], we reported that alpha\(_{1}\)-block with prazosin 4, 8 and 16 ng ml\(^{-1}\) or droperidol 20, 40 and 80 ng ml\(^{-1}\) did not alter the ATSR, whereas beta\(_{1}\)-block with metoprolol 8, 17 and 34 ng ml\(^{-1}\) significantly increased the ATSR (two-fold, four-fold and six-fold, respectively). In the same study [9], we reported also that the effects of prazosin and metoprolol on the ATSR were not significantly different from ours. They investigated the relationship between negative inotropic and chronotropic actions of volatile anaesthetics and inhibition of Ca\(^{2+}\) channel currents, and so they needed to record isolated Ca\(^{2+}\) channel current. For this purpose (in order to eliminate Na\(^{+}\) and K\(^{+}\) currents) they substituted Ba\(^{2+}\), tetraethylammonium chloride and caesium ion for Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\). In contrast, we aimed to investigate the direct effects of enflurane on the arrhythmia-producing actions of adrenaline in isolated heart preparations and therefore we induced slow responses by adding adrenaline to the superfusate containing Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\). Slow inward current is carried by Na\(^{+}\) and by Ca\(^{2+}\) [27], and outward K\(^{+}\) current may flow through slow channels [27, 28]. Therefore, the differences between the results reported by Bosnjak, Supan and Rusch [21] and Eskinder and colleagues [22] and those of our present study might be attributable to the differences between isolated Ca\(^{2+}\) current and slow inward current. If, as described above, slow responses are responsible for re-entry type arrhythmias [18], which are thought to underlie the arrhythmogenic interaction between volatile anaesthetics and adrenaline [9, 19, 20], it should be possible to investigate the effects of enflurane on myocardial sensitivity to adrenaline (antiarrhythmic effects of enflurane in the present study) using the ATSR as an indicator. In contrast, Atlee and Roky [29, 30] and Atlee and Alexander [19] have shown that halothane prolongs A-V nodal, His-Purkinje and ventricular conduction, whereas enflurane prolongs only A-V nodal conduction in dogs. From these results, they concluded that conduction changes such as occur with halothane are necessary for ventricular arrhythmias caused by re-entry of excitation and that enflurane is less likely to cause these arrhythmias. In addition, Burt and Spray [31] studied the effects of halothane and enflurane on the passive membrane properties of the myocardium (gap junction-mediated intercellular communication) in cultured neonatal rat cardiac myocytes, and found that complete block of junctional conductance occurred with smaller concentrations of halothane than enflurane.

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REFERENCES
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