Effects of Isoflurane and Halothane on the Calcium Ion-tension Curve in Rat Myocardium

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In order to clarify the interaction of volatile anesthetics and extracellular calcium ion on the myocardial contraction, effects of both isoflurane (1.0%) and halothane (0.5%) on the extracellular calcium ion concentration ($[Ca^{2+}]_o$)-tension curve were studied. Increasing $[Ca^{2+}]_o$ enhanced the myocardial contraction response, and the maximal response was obtained at $[Ca^{2+}]_o$ of 3.0 mM. Halothane depressed the maximal value of the tension development in response to increasing $[Ca^{2+}]_o$, while isoflurane did not ($P< 0.01$). The probit response of the developed tension to the changes in $[Ca^{2+}]_o$ indicated that isoflurane increased the median effective concentration (EC_{50}) of $[Ca^{2+}]_o$ significantly from 0.484 ± 0.051 (mean ± SEM) to 0.870 ± 0.056 mM (P=0.001), but halothane did not (P=0.018). Therefore, 1.0% isoflurane was concluded to move the $[Ca^{2+}]_o$-tension curve to the right, while a downwards shift occurred with 0.5% halothane. (Key words: extracellular calcium, halothane, isoflurane, myocardial contraction)

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Both isoflurane and halothane depress the vigor of cardiac muscle contraction, which is primarily related to the calcium ion (Ca^{2+}) loading in the myocardium. In the isolated cardiac muscle preparation, the Ca^{2+} loading can be augmented by an increase in extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), and the resultant enhancement of the muscle contraction is expressed as S-shaped $[Ca^{2+}]_o$-tension curve[1]. Therefore, we studied the effects of the two anesthetics on the myocardial contractile response to increasing $[Ca^{2+}]_o$.

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Materials and Methods

The left ventricular trabeculae carneae were excised from twelve male Wistar rats (3 mos old), anesthetized with diethyl-ether. Muscles were stimulated at 0.4 Hz at Lmax (the length where muscles produce the maximal tension), and perfused with modified Krebs-Ringer solution (Na^+ 145, K^+ 4.2, Ca^{2+} 2.0, Mg^{2+} 1.2, Cl^- 127, HCO_3^- 25.0, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, and glucose 16.0 mM). The modified Krebs-Ringer solution was bubbled with a 95% O_2–5% CO_2 gas mixture, which produced a PO_2 range

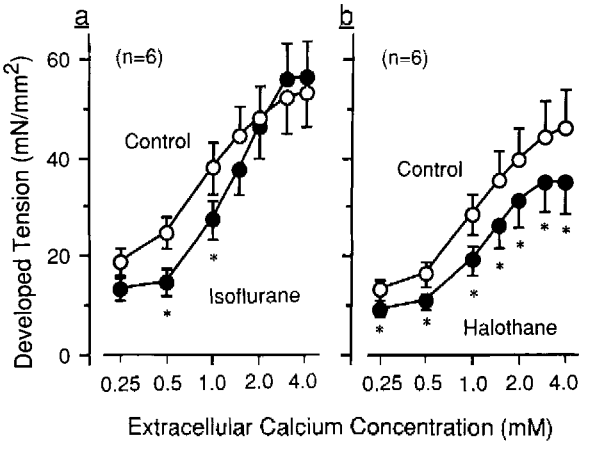

Fig. 1. Increasing extracellular calcium concentration (horizontal axis) augmented the maximal developed tension: Tm (vertical axis) in milli Newton (mN). Asterisks indicate that Tm was reduced significantly by either 1.0% isoflurane (a) or 0.5% halothane (b) at given extracellular calcium concentration ($P < 0.01$). Values are expressed as mean ± SEM. "n" is the number of muscles studied.

of 400–500 mmHg. It was warmed to 30°C before perfusing the muscle bath. Either the muscle length or the tension was controlled by the servo-system consisting of a tension transducer (Cambridge Technology, 408A), a displacement transducer (Trans-Tek, 283-00), a linear vibration motor (Ling, 420) and a servocontroller (Mead Instrument). Electric signals from both tension and length transducers were recorded on oscilloscope (Tektronic, 5523) and on multi-channel recorder (Linear, 1800). Basically muscles were contracting isotonically, and measurements were made on the isometric twitch which imposed on the isotonic contractions.

Control measurements of the maximal developed tension (Tm) and the maximal rate of tension development (dT/dt) were made at $[Ca^{2+}]_o$ of 0.25, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mM. Measurements were repeated following administration of either 1.0% isoflurane or 0.5% halothane (n=6 for each). The anesthetics were added to a 95% O_2–5% CO_2 gas mixture, using Ohio Calibrated Vaporizer (Ohio Medical Products). The anesthetic concentration in the muscle bath was determined by gas chromatography at each $[Ca^{2+}]_o$. At the end of experiment, both Tm and dT/dt were measured as back controls at $[Ca^{2+}]_o$ of 2.0 mM following anesthetic washout.

Both Tm and dT/dt were normalized by the value of the peak tension attained by increasing $[Ca^{2+}]_o$, and the percent values between 5% and 95% of controls were converted into probit in order to determine the median effective concentration (EC_{50}) of $[Ca^{2+}]_o$ (defined as $[Ca^{2+}]_o$ where either Tm or dT/dt becomes 50% of the maximal response to $[Ca^{2+}]_o$)[2]. Linear regression method was used to determine the slope and EC_{50} value of the probit response of Tm or dT/dt to the changes in $[Ca^{2+}]_o$ in each muscle preparation. We found that the EC_{50} values calculated from six different observations showed no changes for two sequential measurements.

Statistical analysis was made by repeated measurement analysis of variance. Newman-Keuls test was used for multiple comparison. The effects of either isoflurane or halothane at each $[Ca^{2+}]_o$ were analyzed by paired *t*-test. *P*-values less than 0.01 were considered statistically significant.

Results

During the control state, the mean value of Tm was augmented by increasing $[Ca^{2+}]_o$ ($P < 0.001$), and approached to the maximal value at 3.0 mM of $[Ca^{2+}]_o$. There was no significant difference in the $[Ca^{2+}]_o$-Tm re-

Table 1. Effects of anesthetics on EC_{50} value for Tm and dT/dt

		Control	Anesthetic	*P*-value
Isoflurane	Tm	0.484 ± 0.051	0.870 ± 0.056	0.001
(n=6)	dT/dt	0.470 ± 0.056	0.842 ± 0.066	0.001
Halothane	Tm	0.626 ± 0.069	0.743 ± 0.090	0.018
(n=6)	dT/dt	0.638 ± 0.080	0.681 ± 0.102	0.532

Values are mean ± SEM expressed by mM, "n" is the number of muscles studied.

lationship between two muscle groups exposed to either 1.0% isoflurane or 0.5% halothane (P=0.288). Isoflurane depressed Tm only at $[Ca^{2+}]_o$ of 0.5 and 1.0 mM, and halothane depressed at all $[Ca^{2+}]_o$ studied ($P < 0.01$) (fig. 1). The mean values of EC_{50} were increased in the presence of anesthetics (table 1), but only changes by isoflurane were statistically significant (P=0.001). Neither isoflurane nor halothane changed the slope of the probit response of Tm to $[Ca^{2+}]_o$ significantly ($P \geq 0.354$). Changes in dT/dt were similar to those in Tm. Values of back controls were not significantly different from controls ($P \geq 0.056$).

The anesthetic concentration of perfusate, which was calculated by assuming the modified Krebs-Ringer solution/gas partition coefficient to be same as water/gas partition coefficient, did not change significantly for each $[Ca^{2+}]_o$ (P=0.139). The mean value of the anesthetic concentration was 0.65 ± 0.03% (mean ± SEM) for isoflurane and 0.38 ± 0.02% for halothane.

Discussion

Both isoflurane and halothane reduce the Ca^{2+} influx during the slow inward current[3]. But the effects of the two anesthetics on the $[Ca^{2+}]_o$-tension curve were different from each other at the concentration we used in this study: A parallel shift to the right occurred with isoflurane, while a downwards shift occurred with halothane (fig. 1). An increase in $[Ca^{2+}]_o$ enhances the net influx of Ca^{2+} across the sarcolemma. The inflowing Ca^{2+} does not activate the contractile proteins directly in the rat myocardium. It may improve the filling of the internal store with Ca^{2+} that can be released in subsequent contraction[4]. The different effects of the two anesthetics on the $[Ca^{2+}]_o$-tension curve may be caused by their individual effects on the internal store of Ca^{2+}.

DeTraglia et al.[5] used the potentiated state contraction of the rabbit heart muscle, and studied the effects of isoflurane on the tension development that related to Ca^{2+} released from internal store (activator Ca from internal source). They concluded that isoflurane does not depress the activator Ca from internal source. However, the parallel shift of the $[Ca^{2+}]_o$-tension curve caused by isoflurane indicated that the depressant effect of isoflurane on the activator Ca from internal source was $[Ca^{2+}]_o$ dependent. An increase in $[Ca^{2+}]_o$ attenuated the depressant effects of isoflurane. In the potentiated state contraction, large amount of Ca^{2+} is loaded in the myocardium during foregoing stimulations. Therefore, the depressant effect of isoflurane may not occur in the potentiated state contraction.

Effects of 0.5% halothane on the activator Ca from internal source did not depend on $[Ca^{2+}]_o$. However, Price[6] reported that increasing $[Ca^{2+}]_o$ counteracts the negative inotropic ef-

fects of halothane (0.5%) completely. This could be explained on the basis of species differences in calcium metabolism, because Price used the cat myocardium, in which the tension development may not depend so much on Ca^{2+} released from the internal store as that of the rat myocardium[4]. Therefore, the downwards shift of the $[Ca^{2+}]_o$-tension curve occurred with halothane, as observed in this study, may indicate that the mechanism(s) of the negative inotropic effects of 0.5% halothane was different from that of 1.0% isoflurane.

In conclusion, our study with rat myocardium showed that increasing $[Ca^{2+}]_o$ counteracted the depressant effects of isoflurane on the tension development which is related to the Ca^{2+} release from the internal store, but this effect was absent in the presence of halothane. Isoflurane may depress the internal store of Ca^{2+} to be loaded by the extracellular Ca^{2+}. Different mechanism for the negative inotropy of halothane may act in rat heart muscle.

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