Frequency- and Length-Dependent Tension Development in Rat Heart Muscles Exposed to Isoflurane and Halothane

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Using 22 isolated rat ventricular muscle preparations, we investigated whether or not the increase in preload and/or contraction frequency may counteract the negative inotropy of both isoflurane (2.0%) and halothane (1.0%). Increases in preload from 94% of Lmax (the length where muscles produce the maximal tension) to Lmax did not alter significantly the percent decrements in tension development caused by either isoflurane or halothane. The increases in contraction frequency from 0.1 to 0.6 Hz augmented the depressant effect of isoflurane significantly ($P < 0.001$), while the depressant effect of halothane was not altered at these contraction frequencies. Small but significant counteraction occurred in the depressant effects of halothane at 0.8 and 1.6 Hz ($P = 0.002$). These changes in intracellular mechanism(s), resulted from the increase in contraction frequency, interacted with the two anesthetics on tension development, while these may not be the case for the increase in preload. (Key words: isoflurane, halothane, ryanodine, rat ventricular muscle, length-tension relationship, frequency-tension relationship)

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In the isolated heart muscle, the length of muscle fiber prior to contraction (preload) and the frequency of contraction are two major factors which determine the strength of developed tension[1]. Recently, one of these factors, an increase in contraction frequency, has been shown to counteract the negative inotropic effects of isoflurane[2–4] and halothane[4]. However, it remains to be investigated whether the augmentation of preload counteracts the negative inotropic effects of these anesthetics or not.

The augmentation of tension development by means of increases in preload and contraction frequency may involve the different intracellular mechanism(s)[5,6]. Thus, we studied whether or not each of these two factors do counteract the negative inotropic effects of either isoflurane, halothane or both.

Methods

Twenty-two male Wistar rats (3 mos old) were used in this study. Animals were divided into 3 groups. Two groups (n = 8 for each) were used to study the negative inotropic effects of anesthetics, and the other group (n = 6) was used to study the depressant effects of ryanodine on the muscle contraction. All experiments were approved by the University of Iowa School of Medicine Animal Use Committee.

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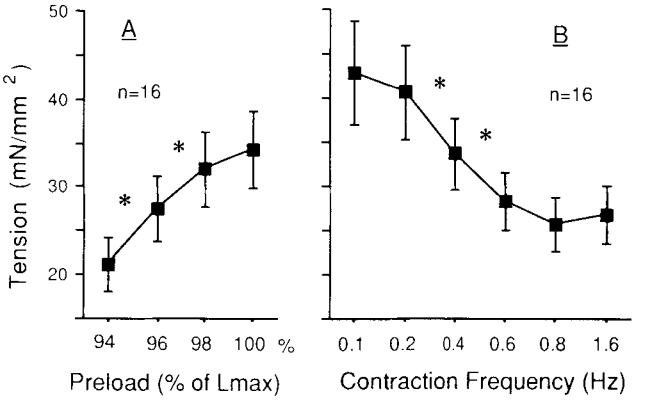

Fig. 1. The length-tension relationship (A) and the frequency-tension relationship (B) in the control state. Preload level is normalized by Lmax. Asterisks indicate that the maximal developed tension in mili Newton (mN) was significantly different from the next lower preload level or contraction frequency. "n" is the number of the muscles studied.

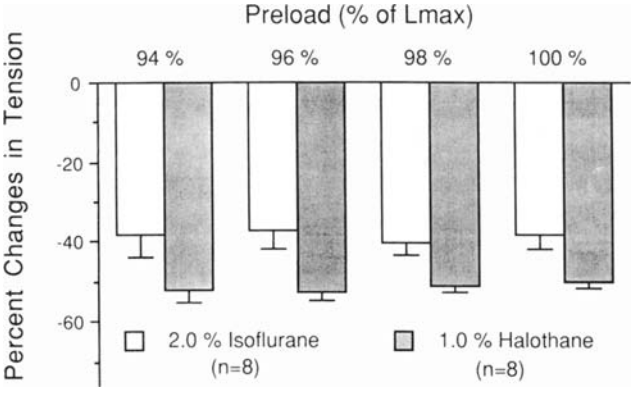

Fig. 2. The percent changes in the maximal developed tension caused by either 2.0% isoflurane (open squares) or 1.0% halothane (dark squares) at each preload level. There were no significant difference in the percent changes with the increases in preload level. "n" is the number of the muscles studied.

The trabeculae carneae was excised rapidly from the left ventricle under light anesthesia with diethyl-ether. Muscles were stimulated at 0.4 Hz at Lmax (i.e., the length where muscles produce the maximal tension), and superfused 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) at 30°C, equilibrated to pH = 7.4 by constant bubbling with a 95% O_2 – 5% CO_2 gas mixture, producing P_{O_2} range of 400–500 mmHg. Both muscle length and tension were controlled by the Biodyne system[7], consisting of a tension transducer (Cambridge Technology, 408A), a displacement transducer (Trans-Tek, 28300), a linear vibration motor (Ling, 420), and a servo-controller (Mead Instrument). Electric signals from both transducers were recorded on oscilloscope (Tektronic, 5523) and multi-channel recorder (Linear, 1800). Basically, muscles were contracting isotonically, and all measurements were made on the isometric twitch, superimposed on the isotonic contraction. Previous study in our laboratory revealed that the preparations were stable and isometric twitch had been reproducible for 4 hrs.

Following either changes in the muscle length (94%, 96%, 98% and 100% of Lmax) or those in the contraction frequency (0.1, 0.2, 0.4, 0.6, 0.8 and 1.6 Hz), the maximal developed tension (Tm) and the maximal rate of tension development (max dT/dt) were measured during the control state. Measurements were repeated following administration of 2.0% isoflurane in one group and 1.0% halothane for the other. The anesthetic concentration in the muscle bath was determined by gas chromatography. Back controls were measured at 0.4 Hz and at Lmax, after

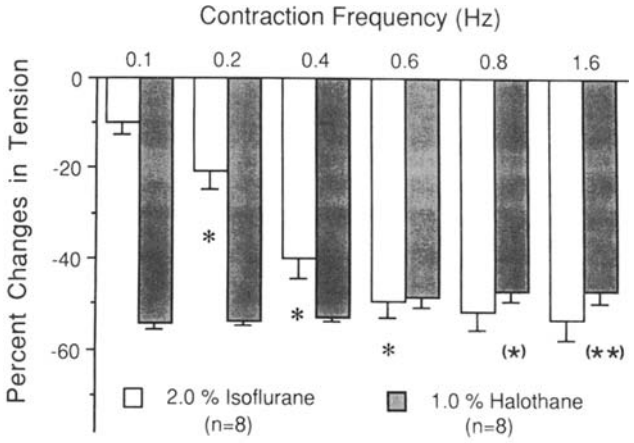

Fig. 3. The percent changes in the maximal developed tension caused by either 2.0% isoflurane or 1.0% halothane at each contraction frequency in Hz. Increases in the contraction frequency from 0.1 to 0.6 Hz augmented the percent changes in the maximal developed tension evoked by 2.0% isoflurane: $^*P < 0.01$ v.s. the next lower frequency. However, increases in contraction frequency reduced the percent decrements in tension development caused by halothane, indicating small but statistically significant counteraction ($P = 0.002$). "n" is the number of the muscles studied.

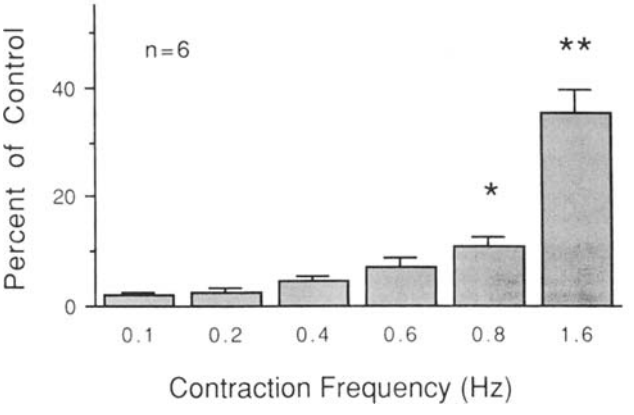

Fig. 4. The effect of ryanodine on the maximal developed tension (Tm) at each contraction frequency. Tm was normalized by the control value obtained in the absence of ryanodine. The depressant effect of ryanodine decreased at higher contraction frequency. "n" is the number of the muscles studied. $^*P < 0.01$ v.s. 0.1 and 0.2 Hz. $^{**}P < 0.01$ v.s. 0.1–0.8 Hz.

the complete washout of the anesthetics.

The additional 6 muscles were studied in order to determine the tension development that was solely related to Ca^{2+} release from the internal storage, using ryanodine to reduce the availability of Ca^{2+} from the storage[8]. Following the control measurements of Tm at 0.1, 0.2, 0.4, 0.6, 0.8 and 1.6 Hz, at Lmax, a 1.0 μM ryanodine was administered and, then, the measurements were repeated.

Each muscle served as its own control. Values are expressed as mean ± SEM, and statistical analysis was made by repeated measurement analysis of variance[9]. Newman–Keuls test was used for multiple comparison. P-values less than 0.01 were considered statistically significant.

Results

During the control state, the mean value of Tm increased significantly when preload was augmented (fig. 1A), and decreased when the contraction frequency was augmented (fig. 1B). There were no significant differences in the length-tension and frequency-tension relationships between the two muscle groups exposed to isoflurane and halothane. Increases in preload from 94 to 100% of Lmax did not modify significantly percent decrements in Tm caused by either isoflurane or halothane (fig. 2). On the other hand, changes in contraction frequency did modify the depressant effects of the two anesthetics (fig. 3): enhancement occurred with isoflurane at high contrac-

Table 1. Percent Decrements in max dT/dt Caused by Isoflurane and Halothane

Frequency (Hz)	0.1	0.2	0.4	0.6	0.8	1.6
Isoflurane (n=8)	11.0 ± 2.45	19.2 ± 3.26*	34.0 ± 2.99*	39.7 ± 2.79	44.2 ± 3.87	43.5 ± 3.57
Halothane (n=8)	48.2 ± 1.60	48.5 ± 1.36	45.9 ± 1.75	42.6 ± 2.21	37.1 ± 3.31#	39.9 ± 4.01

"n" is the number of the muscles studied.
*$P < 0.01$ v.s. next lower frequency.
#$P < 0.01$ v.s. 0.1 and 0.2 Hz.

tion frequency ($P < 0.001$), whereas small but statistically significant counteraction occurred with halothane ($P = 0.002$). However, any differences in the mean values of percent decrements caused by halothane were not statistically significant when Newman–Keuls test[9] was used. Increase in the number of the observations may result in the statistical significance with Newman–Keuls test. Changes in max dT/dt were similar to those in Tm (table 1).

Mean values of Tm and max dT/dt of the back controls were not significantly different from the controls. The mean anesthetic concentration in the muscle bath, expressed by MAC, were 1.02 ± 0.04 (mean ± SEM) for isoflurane, and 1.28 ± 0.11 for halothane. They did not differ significantly from each other.

When ryanodine, in the concentration of 1.0 μM, was administered, the mean value of Tm decreased significantly at any given contraction frequency ($P < 0.001$). Figure 4 shows the normalized developed tension (by the control value) at each contraction frequency in the presence of ryanodine, showing at below 0.6 Hz the depressant effect of ryanodine did not differ significantly but at higher contraction frequency it decreased significantly ($P < 0.001$).

Discussion

It has been shown that both increases in preload and contraction frequency result in augmentation of the developed tension through the different mechanism(s)[5,6]. The increases in preload alter not only the physical overlap of the contractile proteins but also their affinity to the intracellular Ca^{2+} [5]. The myoplasmic Ca^{2+} concentration itself is also augmented by the increases in preload[5]. But other study indicated this may not play a significant role when preload is increased[10]. On the other hand, the augmentation of the myoplasmic Ca^{2+} concentration is the primary mechanism of frequency-dependent tension development[6].

Sutko et al. identified pharmacologically the two kinds of internally released Ca^{2+} (activator calcium) using ryanodine: activator calcium released from internal source and that from external source[8]. Although it is not clear as to the two kinds of the activator calcium are independent or not, Stemmer and Akera demonstrated that the increases in contraction frequency augment the relative contribution of the external source of the activator calcium to the tension development[11]. It has been shown that the rat myocardium is especially activated by the internal source of the activator calcium more than that from the external source[12]. Therefore, the increase in contraction frequency, which may reduce the Ca^{2+} availability of internal storage site in the rat myocyte, results in the decrement of the tension development in the intact heart muscle[13]. Our findings with ryanodine study suggested that more than 90% of the activator calcium may be released from the internal source at the contraction frequency from 0.1 Hz to 0.6 Hz (fig. 4). Therefore, the activator calcium in our experiments may be released only from the internal source at these contraction frequencies.

Findings in our study may not provide direct evidence as to the reasons why increases in contraction frequency modified the negative inotropy of both isoflurane and halothane, and why increases in preload did

not. However, the most plausible explanation may be that the mechanism which reduced the amount of activator calcium with increases in contraction frequency may augment the negative inotropy of isoflurane and counteract the negative inotropy of halothane. This may not occur for the mechanism involved by the increases in preload. It is interesting to note that increases in the contraction frequency from 0.1 to 0.6 Hz did not modify the negative inotropic effects of halothane, while they counteracted the depressant effect of isoflurane (fig. 3). At these contraction frequencies, the cardiac muscle may be activated by the Ca^{2+} released from the internal storage. Thus, isoflurane may depress the activator calcium from the internal source at a different degree as the Ca^{2+} loading of internal storage decreases. But this may not be the cause for halothane. Rather, the negative inotropy of halothane might be due to the difference in the depressant effect of halothane on the two sources of the activator calcium; namely the relative contribution of the external source to the activator calcium could be increased at the contraction frequencies where the negative inotropy of halothane was counteracted.

In contrast to our findings, other investigators have reported that the increase in the contraction frequency counteracts the negative inotropic effects of isoflurane in the guinea pig[2], rabbit[3] and cat heart muscles[4]. The discrepancy between these findings and ours may be due to the species difference in the myocardial calcium metabolism[12]. Unlike the rat myocardium, the activator calcium from the internal source in these animals is augmented by the increases in contraction frequency[14]. But like rat myocardium, the activator calcium from the external source is increased in the cardiac muscle from these animals when contraction frequency is increased[11].

In conclusion, we found that increases in contraction frequency augmented the negative inotropic effects of isoflurane and attenuated those of halothane, while increases in the preload did not modify the negative inotropy of both isoflurane and halothane. The depressant effect of isoflurane on the Ca^{2+} release from the internal source may be related to the filling of the internal store with Ca^{2+}, but the depressant effect of halothane may not.

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