Effects of Halothane and Isoflurane on Isolated Human Ventricular Myocardium

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Segments of viable human left ventricular trabeculae were obtained at the time of endocardial resection for intractable ventricular ectopy. Muscle segments which showed suitable and reproducible contractions in 26 mM K Tyrode solution with 1 μM isoproterenol were electrically stimulated after rest, and at frequencies of 0.1, 0.25, 0.5, and 1 Hz. Effects of 0.75% halothane and 1.3% isoflurane on peak tension, maximum rate of tension development (dT/dt max), and on slow (calcium dependent) action potential (AP) characteristics were studied. Halothane depressed peak tension, dT/dt max, and slow AP maximum rate of depolarization (Vmax) at all frequencies, and caused a significantly greater depression of peak tension and dT/dt max at 0.5–1 Hz than after rest and at 0.1–0.25 Hz. Isoflurane did not significantly depress slow AP Vmax, showed no frequency dependent contractile depression, and depressed dT/dt max less than halothane at 0.5 and 1 Hz. Halothane and isoflurane caused differing depression in the pattern of developed tension. The differential depression by halothane and isoflurane of human ventricular myocardium was similar to that previously observed in isolated animal ventricular tissue. (Key words: Anesthetics, volatile; halothane; isoflurane. Heart; action potential; contractility.)

A VARIETY OF STUDIES in isolated animal tissues have suggested that volatile anesthetics depress myocardial contractility in part by depressing Ca2+ influx through the sarcolemma into the myocardial cell.4-6 Other studies have demonstrated that volatile anesthetics alter uptake and release of Ca2+ by the sarcoplasmic reticulum of the myocyte, which may contribute to depression of contractility.7-9 The present study was undertaken to verify that electrophysiologic observations made in animal tissues, primarily papillary muscle from guinea pig and rabbit, are reflected by similar changes in isolated human ventricular myocardium.

Materials and Methods

Surgical specimens of ventricular myocardium were obtained from the hearts of patients undergoing endocardial mapping and resection for intractable ventricular dysrhythmias, usually subsequent to myocardial infarction. Although primarily scar tissue was resected, small regions of non-infarcted ventricular margins and trabeculae were occasionally present. Segments of trabeculae which appeared to be intact and viable were dissected from the specimen and freed of scar tissue to permit exposure of cells for intracellular impalement. Muscles were superfused at 8 ml·min−1 with Tyrode solution (concentrations in mM: Na, 149; K, 4.7; Cl, 128; Ca, 2.5; Mg, 2.0; SO4, 2.0; HCO3, 25; glucose, 11; EDTA, 0.1) at 37°C. One end of the muscle segment was pinned to the base of a chamber (volume = 3 ml) and the other end was connected to a Grass FT03 force transducer. Resting tension was adjusted to that which produced the greatest developed tension in response to electrical stimulation. Muscle segments were stimulated following rest and then sequentially at frequencies of 0.1, 0.25, 0.5, and 1 Hz with 1–2 msec impulses (Grass S44 Stimulator) via stainless steel electrodes lying on either side of the muscle. Muscles which gave reproducible contractile responses to electrical stimulation over a 30-min equilibration period were employed for study. Of tissue obtained from 12 patients, six specimens yielded nine muscle segments suitable for study.

To qualitatively assess the affect of anesthetics on Ca2+ entry, muscles were partially depolarized in 26 mM K Tyrode solution (isosmotic substitution of K+ for Na+) with 1 μM isoproterenol. In this superfusate, muscles were partially depolarized and propagating calcium dependent slow action potentials (slow APs) were observed by conventional microelectrode techniques employing 3 M KCl filled 10–20 MΩ glass microelectrodes. Action potentials, contractions, and the first derivative of each signal were recorded as previously described,4 employing the maximum rate of depolarization (Vmax) of these slow action potentials as a qualitative measure of Ca2+ entry.

Volatile anesthetics were administered by passage of the 95% O2-5% CO2 gas through a calibrated anesthetic vaporizer. Vigorous bubbling of the perfuse solutions by anesthetic-containing gas has been previously shown in this system to give appropriate anesthetic concentrations assuming gas solution equilibrium.1,4 Approximately 1 MAC halothane (0.75%) and isoflurane (1.3%) were studied; in three instances, in the same muscle with an intervening washout period. Anesthetic effects on peak tension and maximum rate of tension develop-

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FIG. 1. Effects of halothane and isoflurane on slow action potential maximum rate of depolarization (Vmax) and simultaneous contractile behavior (peak tension and dT/dt-max) of human ventricular tissue. Muscles were studied in the rested state (RS) and at the indicated frequencies, while superfused with 20 mM K Tyrode solution containing 1 μM isoproterenol. Anesthetic-induced changes were calculated as a per cent of 1/2(control + recovery) value. Points with error bars represent the mean ± SEM of n experiments, except at 1 Hz where one less sample was obtained. Significance is indicated for P < 0.05. A. Effects of approximately 1 MAC halothane. All values plotted are significantly depressed below the 1/2(control + recovery) value. *Indicates significantly greater depression of the value at the indicated frequency than at RS-0.25 Hz. $Indicates significantly less depression of Vmax compared to dT/dt-max (also less than peak tension at 0.5 Hz). B. Effects of approximately 1 MAC isoflurane. Vmax was not significantly depressed at any frequency. Peak tension and dT/dt-max were significantly less than control, except at those frequencies denoted by ns. †Indicates significantly less depression compared to 0.75% halothane at the same frequency.

ment (dT/dt-max), as well as on slow AP Vmax, amplitude, and duration, were tabulated. To compensate for any systematic changes in or deterioration of the preparations, the value observed in the presence of anesthetic at each frequency was expressed as a per cent of the mean of the control and recovery measurements (1/2(control + recovery)) at each frequency. Results from one preparation which showed marked deterioration were not included.

Significant differences were determined by the following statistical comparisons: anesthetic effect (vs. control-recovery) at each frequency employed a one-sample Student’s t test; comparison between anesthetics at each frequency employed both unpaired or paired (n = 3) Student’s t test; comparison of effects among the stimulation frequencies for each anesthetic, and comparison of the per cent depression between peak tension, dT/dt-max, and Vmax at each frequency for each anesthetic employed ANOVA with Duncan’s test.

Results

In normal Tyrode solution, these muscles had a resting potential of −92 mV and showed normal fast action potentials with Vmax of approximately 250 V/sec and amplitudes of 133–135 mV. The fast AP duration was rate dependent, being 610 msec after rest and decreasing to 430 msec at 1 Hz. In normal Tyrode, there was a modest increase in peak developed tension with the increase in stimulation frequency from 0.1 to 1 Hz ("positive frequency staircase"). Stimulation rates above 1 Hz were difficult to achieve uniformly, resulted in increased resting tension, and appeared to cause muscles to rapidly fatigue when sustained; therefore, rates above 1 Hz were not employed.

In 26 mM K Tyrode, there was also a modest increase in peak tension and dT/dt-max with increasing stimulation frequency. In six muscles studied in which the cross-sectional area (CSA) of viable muscle could be accurately estimated (mean CSA = 0.90 ± 0.18 mm², range 0.25–1.44 mm²), the peak developed tension at 1 Hz averaged (±SEM) 7.5 ± 2.2 mN/mm². Slow AP Vmax declined with increased frequency: the average (±SEM) control-recovery Vmax values at RS, 0.1, 0.25, 0.5, and 1 Hz were 9.0 ± 1.9, 8.8 ± 2.2, 8.3 ± 1.9, 7.0 ± 1.8, and 6.1 ± 1.9 V/sec, respectively. Figure 1A and B shows the depression as per cent of control-recovery value of peak tension, dT/dt-max, and slow AP Vmax caused by approximately 1 MAC halothane or isoflurane. Halothane caused significant depression of peak tension, dT/dt-max, and Vmax at all frequencies. The depression of tension and dT/dt-max at 0.5 and 1 Hz was significantly greater than that observed after rest or at 0.1–0.25 Hz. The depression of Vmax was not frequency dependent, and Vmax was significantly less depressed than dT/dt-max at 0.5–1 Hz, and less than peak tension at 0.5 Hz. In contrast to halothane, isoflurane caused no frequency-dependent depression of tension development, and did not significantly depress dT/dt-max or peak tension at 1 Hz. Statistical comparison of halothane and isoflurane showed that halothane depressed dT/dt-max significantly more than isoflurane at 0.1, 0.5, and 1 Hz, and halothane caused greater depression than isoflurane of slow AP Vmax at 0.5 Hz.

Figure 2 shows the pattern of tension development and the slow APs observed with 1 μM isoproterenol in 26 K Tyrode solution in one muscle (stimulation at 1 Hz not performed). Following rest and up to 0.25 Hz, there is an early peak tension development with a small late component of tension. The responses observed in isoflurane and halothane are superimposed on the control responses. At 0.25–0.5 Hz halothane decreased the rate of tension development (dT/dt) more than isoflurane, while isoflurane appeared to shorten the duration of the contraction. In the six muscles studied with halothane, dT/dt-max was uniformly and significantly more depressed than peak tension at 0.25–1 Hz. No such difference was seen with isoflurane, and, in fact, at 0.1 Hz, isoflurane depressed peak tension more than dT/dt-
max. In two experiments performed in normal Tyrode solution, distinctly different patterns of contractile depression by halothane and isoflurane were also observed, similar to the differing effects observed in 26 mM Tyrode.

Discussion

Human ventricular muscle obtained in this study showed tension development which was reasonably appropriate for the cross-sectional area of viable muscle. Normal and slow action potentials induced in these tissues had appropriate rates of depolarization and were similar in configuration to those observed in a variety of animal tissues. However, a higher concentration of isoproterenol (1 μM) was required in this tissue than was previously employed in guinea pig papillary muscles (0.1 μM), to produce the equivalent slow APs. Unlike animal tissue, these human tissues were subjected to some preoperative ischemic damage and also required substantially greater dissection from adherent connective tissue and scar, which was necessary to permit intracellular impalements but caused more serious tissue disruption. Preoperative ischemic changes as well as acute damage incurred during dissection may have altered the sensitivity to β-adrenergic stimulation, and may also have affected the cellular mechanisms controlling tension development. Two of six patients had received amiodarone, which had been discontinued at least 10 days prior to surgery. The long-term effects of this drug would still persist and possibly blunt of the intronic effect of isoproterenol. However, there was no apparent difference in response between tissues from patients who had or had not received the drug.

The present results in human ventricular muscle are similar in certain aspects to those previously described for guinea pig papillary muscle. Halothane significantly depressed slow AP Vmax, which is a qualitative measure of slow channel calcium entry. However, at 0.5–1 Hz, the depression of slow AP Vmax by halothane was less than the degree of contractile depression as assessed by dT/dt-max. Halothane has been clearly shown to depress the intracellular Ca²⁺ transient associated with tension development. This intracellular Ca²⁺ transient, which precedes tension development, has been shown to correlate closely with dT/dt. Therefore, halothane’s significantly greater depression of dT/dt-max than of slow AP Vmax (at 0.5–1 Hz), suggests that, in addition to slow channel Ca²⁺ entry, other mechanisms of Ca²⁺ delivery to the myofibrils are depressed by halothane. Similar speculations accompanied previous work in guinea pig and rabbit papillary muscle, and multiple studies have demonstrated or suggested alteration in sarcoplasmic reticulum Ca²⁺ release caused by anesthetics.

Isoflurane did not significantly depress slow AP Vmax, but this lack of significant effect may have in part been due to the smaller sample size. Nevertheless, a significant difference in effect on Vmax between isoflurane and halothane did exist at 0.5 Hz, as previously demonstrated in guinea pig at 0.3 Hz. Likewise, isoflurane caused significantly less depression of dT/dt-max at 0.5–1 Hz, compared to halothane. This difference was also observed in guinea pig muscle at equi-anesthetic concentrations and at all frequencies. Since halothane depressed both Vmax and dT/dt-max more than isoflurane at 0.5–1 Hz, it is tempting to speculate that decreased Ca²⁺ entry may be responsible for the greater contractile depression by halothane. However, this explanation may be too simplistic. The mechanism of halothane depression of electrogenic (slow AP) Ca²⁺ entry is still uncertain. Halothane may directly inhibit slow channel function, or it may alter feedback control of Ca²⁺ entry through the slow channel by altering sarcoplasmic reticulum control of intracellular [Ca²⁺].

Unlike guinea pig papillary muscle, which develops a prominent late peak tension with β-adrenergic stimulation and partial depolarization, these segments of human ventricular muscle developed only a small late component. With regard to anesthetic effects, halothane caused a similar degree of depression at all frequencies in guinea pig muscle, while in this human...
muscle, halothane was increasingly depressant as physiologic frequencies were approached. These differences may represent an intrinsic difference in excitation-contraction coupling between human and guinea pig myocardium at various stimulation rates, and, therefore, consequent difference in anesthetic depression between species. Also complicating the comparison may be changes in the human muscle secondary to pre-surgical ischemia or drug therapy, and acute dissection injury.

There were other subtle, but consistent, differences between isoflurane and halothane. Halothane depressed $dI/dt_{-max}$, an apparently accurate measure of the Ca$^{2+}$ transient, even more than it depressed peak tension (0.25–1 Hz). In contrast, isoflurane depressed $dI/dt_{-max}$ to the same extent or less than peak tension, but tended to decrease the duration of the contraction, an effect observed in isolated guinea pig muscle.

In conclusion, effects of halothane and isoflurane in human ventricular tissue demonstrate results similar to those previously noted in isolated animal ventricle. Specifically, halothane significantly depressed slow AP rate of depolarization, more than isoflurane. At near physiologic heart rates, halothane caused greater depression of the rate of tension development of slow AP contractions. Consequently, observations of anesthetic action made in papillary muscle from other species appear to reflect similar actions observable in human myocardium.

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References