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TITLE: VOLATILE ANESTHETICS AFFECT TIME COURSE AND AMPLITUDE OF Ca²⁺ TRANSIENTS IN ELECTRICALLY-STIMULATED CARDIAC MYOCYTES.

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Halothane (HAL), isoflurane (ISO) and enflurane (ENF) are negative inotropes which limit the availability of Ca²⁺ for cardiac cell contraction. HAL and ISO appear to deplete SR stores of Ca²⁺ in a dose related manner.^{1,2} However, these data reflect only anesthetic effects on amounts of Ca²⁺ released for contraction following cell stimulation. We have developed a method of recording fast Ca²⁺ transients in single rat ventricular myocytes allowing detailed computer analysis of anesthetics effects on the time course of Ca²⁺ release and reuptake-extrusion.

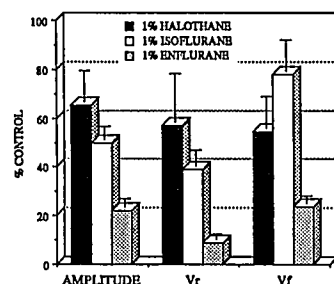
Single rat ventricular myocytes were enzymatically isolated from adult Sprague-Dawley rats, under HAL anesthesia. Ca²⁺ tolerant, quiescent cells were loaded with FURA-2 AM at 4 μM concentration for 10 minutes at 22°C. Cells were placed in a controlled temperature-atmosphere chamber mounted on the stage of a Leitz inverted fluorescence microscope. The 1.5 ml bath was superfused by oxygenated HEPES-Tyrodex solution at 35°C at 3.5 ml/min. Cells were stimulated by 3 msec depolarizing square wave, constant current pulses via suction pipette. Fluorescence emissions at 340 nm or 380 nm excitation wavelengths were captured by photomultiplier and digitized at 10 kHz by a Macintosh IIx computer. [Ca²⁺]_i was determined according to standard methods.³ Stimulated transients were recorded before or after cell exposure to anesthetic, delivered by vaporizer to the superfusate and to the chamber atmosphere. Ca²⁺ transients were analyzed by a program written in LabVIEW 2.0 to obtain values for transient amplitude, maximum rate of rise (V_r), maximum rate of fall (V_f), and the locations of the V_r and V_f values.

HAL, ISO and ENF significantly reduced the amplitude and the rate of rise and fall of the electrically-stimulated Ca²⁺ transients (see figure). 1% HAL reduced the transient amplitude from a control value of 221 nM to 145 nM, V_r from 28577 nM/sec to 16342 nM/sec, and V_f from 831 nM/sec to 454 nM/sec. 1% HAL also shifted the location of V_f from 321 msec into the transient to 708 msec (221% of control). 1% ISO had a similar effect on the location of V_f (252% of control). 1% ENF was ineffective against this parameter. All differences from control values were significant at p<0.05 using the unpaired Student's t-test.

We have demonstrated that HAL, ISO and ENF depress the amplitude and alter the time course of electrically-stimulated Ca²⁺ transients. The results infer a change in the kinetics of the Ca²⁺ release-reuptake-extrusion mechanisms in isolated cardiac myocytes. Studies are underway to isolate these mechanisms for more detailed analysis. We hypothesize that HAL and possibly ISO and ENF alter SR Ca²⁺ reuptake and may effect changes in membrane Ca²⁺ pumping or Na⁺/Ca²⁺ exchange.

References

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2. *Mechanisms of Anesthetic Action in Muscle*, in press.
3. *J Biological Chem* 260:3440-3450, 1985.



Comparison of HAL, ISO and ENF effects on Ca²⁺ transient amplitude, max. rate of rise (V_r) and max. rate of fall (V_f) in electrically-stimulated cardiac myocytes.

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TITLE: ISOFLURANE ELIMINATION FROM A MEMBRANE OXYGENATOR DURING CARDIOPULMONARY BYPASS

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Recent evidence indicates that 95% of isoflurane (ISF) can be eliminated from a bubble oxygenator in 5.8 minutes (time constant=1.94 min.).¹ This suggests that isoflurane can be used closer to the termination of CPB than previously recommended. This may not apply to membrane oxygenators whose time constants for elimination have not been previously reported. We measured the elimination of ISF from a membrane oxygenator and determined the time constant for ISF elimination.

Following approval of the institutional review board, 5 consecutive adult cardiac surgery patients undergoing CPB with moderate hypothermia (27-30°C) and hemodilution (hematocrit 18%-29%) were studied. All patients were male, and received a combination volatile (ISF) and narcotic (fentanyl, 50mcg/kg) anesthetic technique. ISF was administered via a calibrated vaporizer (Forane Vaporizer, Ohio Medical Products) into a membrane oxygenator (Bard HF-5400) with a crystalloid prime volume of 1950 ml. A fresh air:oxygen gas flow of 2 to 2.5 L/min. (FIO₂=.40) and cardiac index of 2.2 L/min. were utilized during rewarming. Oxygenator exhaust gases were sampled via the oxygenator exhaust port. ISF partial pressures were measured by mass spectrometry (System of Anesthesia and Respiratory Analysis, SARA, Allegheny International Medical Technology) at one minute intervals from the time that ISF was discontinued until weaning from CPB. The ratio of F_A/F_{AO} was used to define the washout of ISF from the oxygenator. F_A was the exhaust concentration of ISF at each measurement interval; F_{AO} was the exhaust concentration of ISF immediately prior to discontinuing ISF. A time constant was defined as the time (in minutes) to an F_A/F_{AO} of .33 (67% washout of ISF).

The mean MAC-hours before and during CPB were .56 hours and .34 hours respectively, with the total mean MAC-hours before washout of .9 hours. Oxygenator exhaust concentrations of ISF were less than .10% in 45 min.±8.9 min. The mean time constant for ISF elimination was 19 min.±2.8 min.

Table 1. Individual Patient Values of ISF Concentrations and F_A/F_{AO} Ratios at 4 minute Intervals after ISF Discontinued

Patient Number	F _A /F _{AO} Ratio (minutes)						
	FAO	4	8	12	16	20	24
1	.47	.64	.55	.45	.38	.32	.30
2	.63	.71	.71	.40	.32	.29	.27
3	.50	.42	.38	.38	.36	.35	.32
4	.50	.68	.58	.44	.44	.40	.32
5	.51	.70	.51	.41	.35	.27	.26

This study demonstrates that during hypothermic, hemodiluted CPB in adult cardiac surgical patients, ISF washout from this particular membrane oxygenator occurs more slowly than that from a reported bubble oxygenator.² ISF washout from the membrane oxygenator to < 0.10% occurs after 45±8.9 min. (8.8±2.5 min. for bubble oxygenator²). Using the time constant of 19 min., 95% washout from this membrane oxygenator would require 57 min., suggesting that considerably more time is required for the washout of ISF from a membrane oxygenator than from a bubble oxygenator.

References

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2. *Journal of Cardiothoracic Anesthesia* 2:41-44, 1988