Date:

Title: Halothane Stimulation and Inhibition of Ca^{2+} Transport

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Introduction:

The cardiac sarcoplasmic reticulum (SR) is an intracellular organelle which controls the availability of calcium ion (Ca^{2+}) for contraction and might be the site at which halothane acts to depress myocardial contractility. We have examined the effects of halothane on isolated cardiac SR and have found that halothane can both inhibit and stimulate Ca^{2+} transport depending on the functional state of the SR. Ca^{2+} transport by the SR requires ATP, Mg^{2+} and Ca^{2+}; we have investigated the interaction of halothane and each of these substrates with the Ca^{2+} transport mechanism of the SR.

Methods:

Canine ventricular muscle was homogenized and the SR isolated by differential centrifugation according to the method of Harigaya and Schwartz.[1] Ca^{2+} uptake by the SR vesicles was measured using ^{45}Ca; an aliquot of ^{45}Ca loaded SR was applied to a glass fiber filter and rinsed. ^{45}Ca retained on the filter, representing intravesicular Ca^{2+}, was measured by liquid scintillation counting. Thymol-free halothane was used for all experiments. Halothane concentration was measured by gas chromatography and ultra-violet spectroscopy.[2,3]

Results:

The ATP dependence of Ca^{2+} uptake was measured at several halothane concentrations. It was observed that halothane decreased the K_M of the Ca^{2} pump for ATP and also decreased the Vmax for Ca^{2+} transport. More interestingly at low ATP concentrations, 0.5, and 2mM, halothane stimulated Ca^{2+} transport (Table 1); at higher concentrations of ATP, 5 and 10mM, halothane inhibited Ca^{2+} transport. Similar experiments were performed while varying either $MgCl_2$ or $CaCl_2$ at several concentrations of halothane. Halothane was noted to decrease the Vmax for Ca^{2+} transport in the presence of increasing concentrations of $MgCl_2$. Significant inhibition of Ca uptake by halothane was found at 0.1, 1 and 5mM $MgCl_2$ concentrations, but at 10mM $MgCl_2$ 2.2% halothane increased Ca^{2+} transport by 181%.

Discussion:

Other investigators using either isolated cardiac sarcoplasmic reticulum or functionally skinned cardiac cells have observed that halothane decreases the rate of Ca^{2+} uptake by the sarcoplasmic reticulum.[4,5] Their experimental observations were made at only a single concentration of ATP, $MgCl_2$, and Ca^{2+}. We have noted that halothane can also stimulate Ca^{2+} transport by the isolated sarcoplasmic reticulum and that stimulation occurs at low ATP or high Mg^{2+} concentrations. Halothane is a membrane active chemical and might be acting on the lipid environment of the membrane or directly on the Ca^{2+} pump protein to unmask other active Ca^{2+} transport sites. Since we have shown that halothane can both stimulate and inhibit Ca^{2+} transport by the isolated SR, the role of the SR in halothane depression of myocardial contractility is equivocal.

Table 1. Effect of Halothane and ATP on Ca^{2+} Uptake[a,b]

Halothane (%) / ATP (mM)	0	2.2	2.9	4.8	8.2
0.5	26.0 (1.8)	48.0 (2.1)	54.9 (2.4)	47.7 (3.5)	21.2 (1.5)
1.0	30.7 (8.8)	X	104 (2.7)	96.6 (9.7)	68.4 (4.6)
2.0	149 (4.0)	195 (3.8)	182 (3.8)	181 (3.6)	87 (2.0)
5.0	278 (6.2)	260 (4.6)	249 (6.4)	185 (2.9)	106 (3.0)
10.0	161 (4.3)	92.3 (7.7)	58.3 (1.6)	53.1 (16.2)	13.5 (3.4)

a. Samples were incubated at 37°, pH 7.5 for 2 min in 50mM HEPES, 100mM KCl, 5mM $MgCl_2$, 5mM NaN_3, 5mM Koxalate, 0.1mM EGTA and 0.1mM $CaCl_2$.

b. The values in the table represent nmoles of Ca^{2+}/mg protein/2 min. Each value is the mean of four determinations and the standard error of the mean is shown in parentheses.

References:

1. Harigaya S, Schwartz A: Circ. Res. 25: 781-794, 1969.

2. Miller MS, Gandolfi AJ: Anesth. 51:542-544,1979.

3. Blanck TJJ, Thompson M : Anesth. and Analg. (in press).

4. Lain RF, Hess ML, Gertz EW, Briggs FN : Circ. Res. 23:597-604,1968.

5. Su JY, Kerrich WG: Pflugers Arch. 380:29-34, 1979.