TITLE: THE EFFECT OF VOLATILE ANESTHETICS ON THE PH DEPENDENCE OF CALCIUM UPTAKE BY CARDIAC SARCOPLASMIC RETICULUM.

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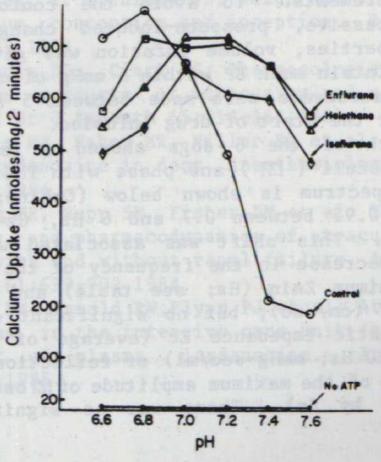
INTRODUCTION: The sarcoplasmic reticulum (SR) is the organelle in the myocardial cell which controls the amount of calcium (Ca) available for contraction. Our previous investigations have demonstrated that calcium uptake (CU) by isolated cardiac SR (CSR) is markedly effected by halothane (H) and that the potency of a given concentration of H to modify CU is pH dependent (1). That study was performed at pH 6.9 and 7.4. CU by SR, in the control state (no anesthetic), is known to have a strong pH dependence. A recent study has demonstrated that oxalate-supported, ATP-dependent CU by CSR has a maximal uptake at pH 6.6-6.8 and is markedly inhibited (90-95%) at pH 7.4-7.6 (2). Since there is a strong pH dependence of both the CU reaction and of H alteration of CU, and known interaction between volatile anesthetics (VA) and Ca dependent processes, we examined the effect of three VA (halothane, enflurane (E) and isoflurane (I) on CU by CSR from pH 6.6-7.6.

METHODS: Experiments were carried out on rabbit CSR, prepared by a modification of the method of Harigaya and Schwartz (3). CU experiments were performed at 37°C in glass vials sealed with teflon-lined caps when VA were added. The reaction medium consisted of 25 mM HEPES, 25 mM PIPES, 100 mM KCl, 5 mM $MgCl_2$, 5 mM NaN_3, 5 mM K^+ oxalate, 5 mM ATP, 0.1 mM EGTA, 0.1 mM $CaCl_2$ with $^{45}CaCl_2$ (150,000 cpm/sample) and 25-50 mcg of SR in a total volume of 1 ml. Experiments were performed in the control state and in the presence of each VA (1.3% H, 1.8% E and 1.2% I), in triplicate at each pH (6.6-7.6) for three different SR preps. Samples were incubated for 2 min to determine the initial rate of CU and for 20 min to determine the total capacity of the CSR for Ca. The incubation was terminated by filtration of an aliquot of the reaction medium onto a glass fiber filter on the Millipore filtration apparatus and rinsing the filter with cold $CaCl_2$. CU was determined using liquid scintillation counting. Since the buffering of Ca by EGTA is known to vary with pH (4), a Ca electrode was employed with additions of $CaCl_2$ to maintain the free Ca constant at each pH. The pH dependence of CU determined by this method was identical to that found using $^{45}CaCl_2$.

RESULTS: The initial rate of CU by SR in the control state was inhibited by alkaline pH, as had been shown previously (2). All three VA significantly ($p<.001$) attenuated the inhibition of CU at at pH 7.4-7.6. As clearly shown in the figure, a considerable (60-68%) increase in CU is seen at alkaline pH in the presence of VA. At pH 6.6, there was a mild (20-32%), but significant ($p<.01$), depression of CU in the presence of VA, when compared to control. The total capacity of the CSR for Ca in the control state was decreased at alkaline pH, with a decline in CU capacity beginning at pH 7.2 resulting in a decrease of 50% at pH 7.6. In comparison to control, there is a significant ($p<.01$) decrease in CU in the presence of all three VA at all pH levels studied, with the exception of E at pH 7.6, where the difference was insignificant. Overall, there was a 48 ± 11% decrease in the total capacity of the CSR for Ca in the presence of VA at all pH levels.

DISCUSSION: Our results concur with the previous findings that the rate of oxalatesupported, ATP dependent CU by CSR is pH dependent, with maximal CU at pH 6.6-6.8 and inhibition of uptake at pH 7.4-7.6 (2). Tate et al. showed that preincubation of SR with Ca, or acidification of an alkaline reaction medium, will prevent this alkaline inhibition of the rate of CU (2). We have demonstrated that although the VA also attenuate the alkaline inhibition of the initial rate of CU by CSR, they decrease the total Ca capacity of the CSR over the physiologic pH range. Since a decrease in the total CSR capacity for Ca would result in less Ca available for release, our results support the hypothesis that the VA depress myocardial contractility by decreasing the amount of Ca available for contraction. The implications of the alteration in the initial rate of CU by the VA are more difficult to interpret. One might speculate that at the normal intracellular pH of 7.18 (5), the VA increase the rate of CU, leaving less Ca available for contraction. However, at lower pH values, the effect of the VA to mildly decrease the rate of CU by CSR may not be sufficient to significantly increase the amount of Ca available for contraction, and thus, result in a negative inotropic effect.

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