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The Effect of Volatile Anesthetics on the pH Dependence of Calcium Uptake by Cardiac Sarcoplasmic Reticulum

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The effect of volatile anesthetics (VA) on the *p*H dependence of calcium uptake by cardiac sarcoplasmic reticulum (SR) was studied. SR was incubated at 37° C with $^{45}CaCl_2$ in the control state (no anesthetic) and in the presence of each of the VA from *p*H 6.6–7.6. The VA used were: halothane, 1.3%; enflurane, 1.8%; and isoflurane, 1.2%. In the control state, the initial rate of calcium uptake, measured after a 2-min incubation, was maximal at *p*H 6.8 (mean ± SEM: 665 ± 37 nmoles/mg) and markedly inhibited at *p*H 7.6 (107 ± 9 nmoles/mg). In the presence of the VA, the calcium uptake rate was mildly depressed (7–32%) at *p*H 6.6–6.8, unchanged at *p*H 7.0, and greatly enhanced (52–78%) at *p*H 7.2–7.6, when compared to control. The maximal uptake of calcium by the SR at a calcium concentration of 10^{-6}M, measured by a 20-min incubation, had a similar *p*H dependence in the control state, with a decline first evident at *p*H 7.2 and a 50% drop in the maximal uptake of calcium from *p*H 7.0–7.6. The presence of the VA was associated with a uniform depression of the maximal uptake of calcium by the SR at all *p*H levels measured. In view of these findings, it appears that *p*H does affect SR function in the presence of VA. This alteration of the *p*H effect by VA may be a factor responsible for discrepancies in results previously reported by investigators studying the effects of VA on the uptake of calcium by the SR. (Key words: Acid-base equilibrium: acidosis; alkalosis. Anesthestics, volatile: enflurane; halothane; isoflurane. Heart: contractility. Ion, calcium: transport; uptake. Muscle, cardiac: sarcoplasmic reticulum.)

THE SARCOPLASMIC RETICULUM (SR) is one of the organelles involved in the regulation of calcium concentration in the myocardial cell.[1–3] The SR is involved in the release and reuptake of myoplasmic calcium during contraction, and is one of the possible sites at which the volatile anesthetics might act to depress cardiac contractility. Previous investigations of the effects of volatile anesthetics on the uptake of calcium by the SR have produced varying results, ranging from increased calcium uptake,[4,5] to decreased calcium uptake,[4,6] and, in some cases, little to no effect on calcium uptake.[7] This is probably due to the fact that the experimental conditions vary in terms of SR preparations, *p*H, incubation temperature, and concentrations of adenosine 5'-triphosphate (ATP) and calcium. Since the uptake of calcium by cardiac SR in the control state (absence of anesthetic) is known to be *p*H dependent, with increased uptake at acidic *p*H and inhibition at alkaline *p*H,[8–12] we felt that different *p*H conditions may affect SR function in the presence of anesthetics. Knowledge of the effect of different *p*H conditions on calcium uptake in the presence of the volatile anesthetics may help sort out the discrepancies of previous reports. Therefore, we examined the effect of the volatile anesthetics, halothane, enflurane, and isoflurane, on calcium uptake by cardiac SR from *p*H 6.6–7.6.

Materials and Methods

Cardiac SR was prepared from adult albino rabbit hearts using a modification of the method of Harigaya and Schwartz.[13] We utilized rabbit cardiac SR because we wanted a species in which we could perform complimentary biochemical and physiological experiments, and compare them to previous studies.[6,10,11,14] Protein concentration was determined by the Coomasie binding method using serum albumin as the standard.[15] Water used for buffers was deionized and glass distilled.

Calcium uptake experiments were performed in 31-ml glass vials. The reaction medium consisted of 100 mM KCl, 5 mM $MgCl_2$, 5 mM sodium azide, 5 mM potassium oxalate, 5 mM ATP, 25 mM piperazine-N,N'-bis-2-ethane-sulfonic acid (PIPES), 25 mM 4-(2-hydroxyethyl)-1-piperazine sulfonic acid (HEPES), 0.1 mM ethylene glycol bis (beta-amino ethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 0.1 mM $CaCl_2$ with $^{45}CaCl_2$ (150,000 cpm/sample). The temperature of the reaction medium was brought to 37° C, and then the reaction was started with the addition of 25–50 micrograms of SR. The total volume was 1 milliliter.

Experiments were performed in the control state (no

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anesthetic) and in the presence of each anesthetic (1.3% halothane, 1.8% enflurane, and 1.2% isoflurane), separately. Only one concentration was used for each anesthetic because the focus was on the changes with *p*H, rather than changes with different anesthetic concentrations. Volatile anesthetic concentrations were determined by infrared spectroscopy, as previously described.[16] Experiments done in the presence of anesthetics had the volatile anesthetic added using a Hamilton microliter syringe at the same time the SR was added *via* a micropipette. Immediately after the VA and SR were added, the vials were sealed with teflon caps and incubated at 37° C. Experiments were done in triplicate at each *p*H ranging from 6.6–7.6 in increments of 0.2 *p*H units. The *p*H level was checked prior to and after each experiment, and varied less than 0.04 *p*H units during the course of the incubation.

In order to determine the initial rate of calcium uptake and the maximal uptake of calcium by the SR, timed experiments, using $^{45}CaCl_2$, were done at each *p*H with samplings of the reaction medium taken at 0, 2, 4, 6, 8, 10, 20, and 30 min. In subsequent experiments, the initial rate of calcium uptake was estimated by a 2-min incubation, and the maximal uptake of calcium by the SR was estimated by a 20-min incubation. The incubation was terminated by filtration of an aliquot of the reaction medium onto a glass fiber filter on a Millipore filtration apparatus. The filter was immediately rinsed with 15 ml of 10 mM $CaCl_2$, and counted in a scintillation vial with 5 ml of scintillation counting cocktail.

Estimations of free ions and ligands in our reaction medium at each *p*H were calculated using the computer program of Fabiato and Fabiato.[17] The buffering of the calcium concentration by EGTA is known to vary with *p*H, with greater binding of calcium by EGTA at more alkaline *p*H.[18] To account for the differences in calcium ion concentration at different *p*H levels, we employed a calcium electrode,[19] and added calcium chloride to maintain a constant free calcium concentration at 5×10^{-6} M for each *p*H in the control state. Calcium uptake by cardiac SR, as measured by the calcium electrode, was performed at *p*H 6.6–7.6 to verify that anesthetic effects were not due to the small change in free calcium in the reaction mixture. Additional $CaCl_2$ was added at *p*H 7.4 and 7.6 to keep the free calcium concentration constant, since there is greater binding of calcium to the ligands (EGTA and ATP) at the higher *p*H. The *p*H dependence of calcium uptake, determined by this method, was identical to that found using $^{45}CaCl_2$. In the $^{45}CaCl_2$ experiments, the free calcium concentration ranged from 0.8×10^{-6} M to 8×10^{-6} M.

All reagents used were analytical grade. HEPES and PIPES, nonionic buffers, and ATP were purchased from Calbiochem-Behring Corp., American Hoechst Corp. $^{45}CaCl_2$, 10 mCi/ml, was purchased from New England Nuclear Co. GFF glass fiber filters were purchased from Whatman, Inc. Liquid scintillation counting was performed in a Beckman LS 2800 scintillation counter using Complete Counting Cocktail 3a70 purchased from Research Products International, Corp. Thymol-free halothane was a gift from Halocarbon Laboratories (Hackensack, NJ), enflurane was purchased from Ohio Laboratories (Madison, WI), and isoflurane from Anaquest (Madison, WI). The calcium electrode was purchased from Ionetics (Costa Mesa, CA). The *p*H meter, Model 4500, was purchased from Beckman Instruments, Inc. (Fullerton, CA), and the *p*H electrode was purchased from Markson Science, Inc. (Del Mar, CA).

Statistical analysis of the data was done using three-way analysis of variance, the factors being *p*H, SR preparation, and experimental condition (control *vs.* anesthetic). This was done to determine the significance of the difference in calcium uptake in the control state and in the presence of anesthetics and the effect of *p*H on calcium uptake in the presence and absence of anesthetic. Testing of both between and within groups was done using paired *t* tests to determine which of the group means differed. Significance was determined at $P < .05$.

Results

Figure 1 is an example of the time dependence of calcium uptake at *p*H 6.6, 6.8, and 7.4. As is shown in figure 1, the uptake of calcium is linear up to about 3 min. The reaction reaches a plateau at 20 min. With this information, we approximated the rate of calcium uptake from a 2-min incubation, and maximal calcium uptake (at a calcium concentration of 10^{-6}M) from a 20-min incubation.

Two-minute Incubation

Figure 2 shows the effect of volatile anesthetics on the rate of calcium uptake by cardiac SR after a 2-min incubation as a function of *p*H. A three-way analysis of variance of these data shows a highly significant ($P < .0001$) difference in the rate of calcium uptake in the control state when compared to calcium uptake in the presence of anesthetics. It also shows that the changes in calcium uptake as a function of *p*H are highly significant ($P < .0001$). The interaction of experimental condition (control *vs.* anesthetic) and *p*H is also highly significant ($P < .0001$), indicating that the control SR responds differently to *p*H than the SR in the presence of anesthetics.

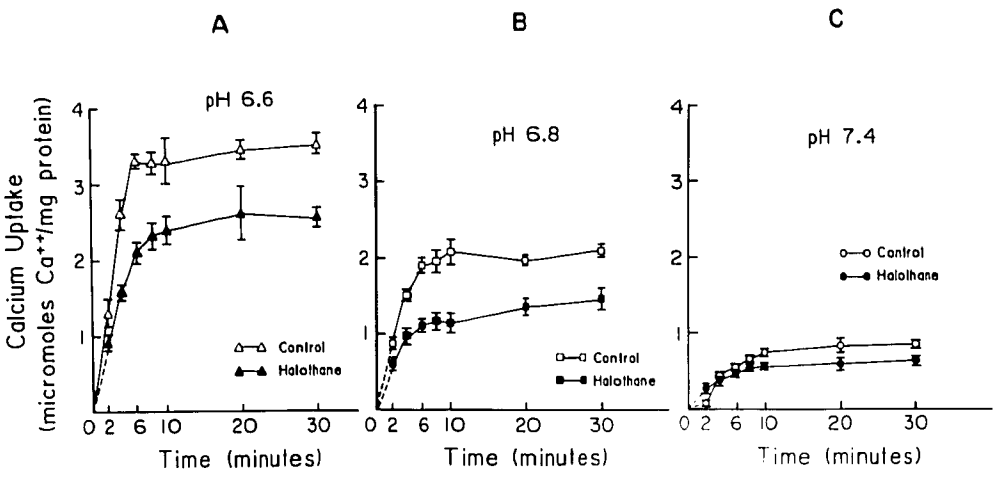

FIG. 1. Time dependence of calcium uptake using $^{45}CaCl_2$ at *p*H 6.6 (*A*), *p*H 6.8 (*B*), and *p*H 7.4 (*C*), in the control state (open symbols) and in the presence of 1.3% halothane (blackened symbols). Experimental conditions are as stated in "Materials and Methods." The initial uptake of calcium is linear up to 3 min, and the reaction reaches a plateau at 20 min. Halothane decreases both the calcium uptake rate and plateau at *p*H 6.6–6.8. At *p*H 7.4, halothane increases the calcium uptake rate, but decreases the plateau. The plateau represents the maximal uptake of calcium by the SR. Each data point is the mean of three experimental determinations. Error bars represent standard error of the mean. Significant differences are as noted in "Results."

The analysis of variance supports the results of paired *t* tests (table 1), which show that all three anesthetics significantly ($P < .001$) attenuate the inhibition of calcium uptake seen in the control state at *p*H 7.2–7.6. The anesthetics produce a 52–78% increase in the rate of calcium uptake, when compared to the control state (fig. 2).

The effect of the anesthetics at *p*H 6.6–6.8, in contrast to the higher *p*H, is to decrease the rate of calcium uptake, when compared to control. At *p*H 6.6, all three anesthetics significantly decrease the rate of calcium uptake, when compared to the control state. At *p*H 6.8, both halothane and isoflurane significantly decrease the rate of calcium uptake, while enflurane has no significant effect when compared to control. There is little change in calcium uptake at *p*H 7.0 in the presence of any of the three volatile anesthetics, although the small change in the presence of halothane is statistically significant ($P < .02$).

When the control state is removed from the analysis of variance, the response of the SR to all three anesthetics is similar with regard to changes in *p*H. This is supported by the results of paired *t* tests in table 1, which show that the differences between the anesthetics themselves are not significant, except at *p*H 6.8 (enflurane), *p*H 7.2 (isoflurane), and *p*H 7.4 (isoflurane *vs.* enflurane).

A separate set of paired *t* tests were done to compare the differences in calcium uptake between each *p*H under the different experimental conditions (control, halothane, enflurane, isoflurane). In the control state, the difference in calcium uptake with the various hydrogen ion concentrations is highly significant ($P < .001$), except between *p*H 6.6 and 6.8, where the difference is not statistically significant. In the presence

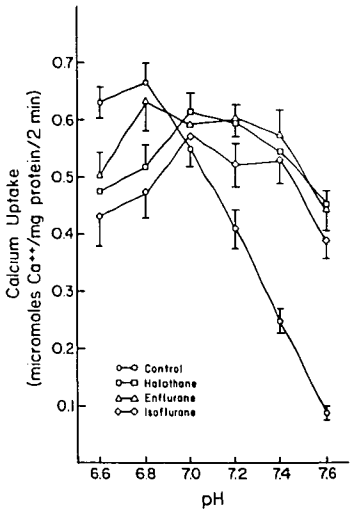

FIG. 2. The *p*H dependence of the rate of calcium uptake (2 min incubation) in the control state (circles) and in the presence of the volatile anesthetics, 1.3% halothane (squares), 1.7% enflurane (triangles), and 1.2% isoflurane (diamonds). Calcium uptake was measured using $^{45}CaCl_2$. Experimental conditions are as stated in "Materials and Methods." Each point is the mean of nine experimental determinations performed in duplicate (n = 18). Error bars represent standard error of the mean. Significant differences are as noted in "Results" and in table 1.

TABLE 1. The Values that *P* is "Less Than," Determined From Paired *t* tests Comparing Differences between Calcium Uptake in the Control State with Each of the Volatile Anesthetics, and Comparing Differences between the Volatile Anesthetics Themselves, After a 2-Min Incubation.

*p*H	C *Vs.* H	C *Vs.* E	C *Vs.* I	H *Vs.* E	H *Vs.* I	E *Vs.* I
6.6	.001	.01	.001	n.s	n.s.	n.s.
6.8	.01	n.s.	.001	.01	n.s.	.001
7.0	.02	n.s.	n.s.	n.s.	n.s.	n.s.
7.2	.001	.001	.01	n.s.	.01	.05
7.4	.001	.001	.001	n.s.	n.s.	.05
7.6	.001	.001	.001	n.s.	n.s.	n.s.

C = control; H = halothane; E = enflurane; I = isoflurane; n.s. = not significant.

of each of the volatile anesthetics, there is no significant difference between pH 6.6 and 7.6. However, the differences between the pH 7.0–7.2 range and the two extreme hydrogen ion concentrations (pH 6.6 and 7.6) is significant ($P < .001$) for all three anesthetics.

Twenty-minute Incubation

Figure 3 shows the effect of volatile anesthetics on the maximal calcium uptake by the SR after a 20-min incubation as a function of pH. A three-way analysis of variance shows a highly significant ($P < .0001$) difference in calcium uptake in the control state when compared to calcium uptake in the presence of anesthetic. The changes in calcium uptake as a function of pH are also highly significant ($P < .0001$). However, the interaction of experimental condition and pH was not significant, indicating that the SR responded to hydrogen ion concentration in a similar fashion under all conditions. This is seen in figure 3, where calcium uptake decreases with decreasing hydrogen ion concentration (increasing pH) in the control state and in the presence of each anesthetic. Therefore, although the amount of calcium uptake by the SR after a 20-min incubation was quantitatively different from calcium uptake in the presence of anesthetics, the SR responded qualitatively in a similar fashion to changes in hydrogen ion concentration, both in the control state and in the presence of anesthetic. This finding was supported by paired t tests, done to compare changes in calcium uptake as a function of pH under each condition, which showed a significant ($P < .05$) decrease in calcium uptake occurring at pH 7.2, both in the control state and in the presence of anesthetic.

Table 2 lists the results of t tests done to determine specific differences between the control and each of the anesthetics, and between the anesthetics themselves, at each pH. These comparisons indicate that the amount of calcium uptake by the control SR is significantly decreased by the presence of anesthetic, except for enflurane at pH 7.6. In the presence of halothane and isoflurane, the SR responds identically to changes in hydrogen ion concentration. However, the presence of enflurane produces significantly less depression of calcium uptake than halothane or isoflurane, even though the pattern of response to pH is similar. This difference in the SR response to enflurane is seen in figure 3.

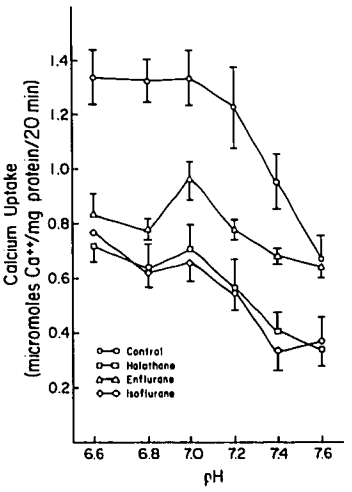

FIG. 3. The pH dependence of the maximal uptake of calcium by the SR (20 min incubation) in the control state (circles) and in the presence of the volatile anesthetics, 1.3% halothane (squares), 1.7% enflurane (triangles), and 1.2% isoflurane (diamonds). Calcium uptake is measured using $^{45}CaCl_2$. Experimental conditions are as stated in "Materials and Methods." Each point is the mean of nine experimental determinations performed in duplicate (n = 18). Error bars represent standard error of the mean. Significant differences are as noted in "Results" and table 2.

Discussion

The rate of oxalate-supported, ATP-dependent calcium uptake by cardiac SR of several species in the absence of volatile anesthetics is known to be pH dependent.[4,8–12] The highest initial calcium uptake rate occurs at pH 6.2–6.8, and inhibition of uptake occurs at pH 7.2–7.6. Our results for the rate of calcium uptake by cardiac SR in the control state concur with these findings.

Tate *et al.* have shown that preincubation of cardiac SR with calcium prevents the inhibition of calcium uptake at pH 7.2–7.6.[10] The results of our experiments show that the volatile anesthetics can also prevent the inhibition of the initial rate of calcium uptake at these pH levels. These data suggest that the volatile anesthetics, similar to calcium preincubation, remove the requirement of hydrogen ion for the uptake of calcium. It is possible that the anesthetics alter the environment of the calcium pump protein, perhaps by changing its catalytic properties.

The results of our experiments concur with previous results measuring calcium uptake by canine cardiac SR at 5mM ATP and 37° C, which showed increased calcium uptake at pH 7.3 in the presence of 1–1.13% halothane,[4] as well as in the presence of .64–2.82% enflur-

TABLE 2. The Values P is "Less Than" Determined from t tests Comparing Differences between Calcium Uptake in the Control State with Each of the Volatile Anesthetics and Comparing Differences between the Volatile Anesthetics Themselves, After a 20-Min Incubation.

pH	C Vs. H	C Vs. E	C Vs. I	H Vs. E	H Vs. I	E Vs. I
6.6	.001	.001	.001	.05	.02	n.s.
6.8	.001	.001	.001	.05	n.s.	.01
7.0	.001	.001	.001	.05	n.s.	.01
7.2	.001	.01	.001	n.s.	n.s.	.05
7.4	.001	.01	.001	.01	.01	.001
7.6	.001	n.s.	.001	.001	n.s	.01

C = control; H = halothane; E = enflurane; I = isoflurane; n.s. = not significant.

ane and .82–1.99% isoflurane.[5] The findings of a decrease in the maximal uptake of calcium by the SR in the presence of enflurane at *p*H 7.0 and halothane at *p*H 6.9 are also supported by previous studies, even though the concentrations used in those studies (2.5–7.5% enflurane and halothane 1.75%) are higher than those used in this study.[4,6]

The intramyocardial *p*H of the perfused rabbit heart has been shown to be 7.18 by two different methods.[20–22] It appears that, at the normal intramyocardial *p*H, the volatile anesthetics cause an increase in the velocity of calcium uptake and a decrease in the maximal calcium uptake when compared to control. These findings at the anesthetic concentrations studied do not necessarily pertain to lower or higher concentrations. It is interesting that all three anesthetics altered calcium uptake in a similar fashion, despite the fact that approximately 1 MAC halothane was compared to approximately .6 MAC isoflurane and enflurane.[23]

In comparing the effects of the volatile anesthetics on the SR, the results show that halothane and isoflurane effect the SR in an almost identical fashion. Enflurane, on the other hand, effects the SR somewhat differently in that it has less of a depressant effect on the maximal calcium uptake by the SR at all hydrogen ion concentrations. Enflurane also has less of a depressant effect on the rate of calcium uptake by the SR, most notably at *p*H 6.8.

In summary, the volatile anesthetics decrease the rate of calcium uptake by cardiac SR at *p*H 6.6–6.8, have little effect at *p*H 7.0, and significantly increase the rate of calcium uptake at *p*H 7.2–7.6. Overall, the volatile anesthetics depress the maximal amount of calcium uptake by the SR from *p*H 6.6–7.6, when compared to the control state.

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