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Effect of Halothane on Cardiac Sarcoplasmic Reticulum Ca^{2+}-ATPase at Low Calcium Concentrations

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SUMMARY

The effect of halothane on cardiac sarcoplasmic reticulum Ca^{2+}-ATPase activity was studied at low calcium concentrations (0.4–20 μM). Clinical concentrations of halothane (1%–3%, v/v) were found to depress Ca^{2+}-ATPase activity more severely at lower calcium levels than at the higher calcium levels previously reported (>0.1 mM). An increase in calcium concentration in the external medium of a preparation of isolated cardiac sarcoplasmic reticulum vesicles antagonized the halothane-induced depression of the Ca^{2+}-ATPase activity. The depression of calcium-activated ATPase activity by halothane appears to take place by a competitive-type inhibition. The Ca^{2+}-ATPase V_{max} remained constant at 0.175 μmole/min/mg of protein with an increasing K_m (0.47 μM–4.09 μM). Halothane depression of sarcoplasmic reticulum function may in part explain the ability of halothane to depress myocardial function.

SR[3] plays an important role in the relaxation of cardiac contractile proteins through its ability to accumulate calcium from the cardiac cytoplasm (1, 2). The ability of SR to take up calcium is linked to a Ca^{2+}-ATPase enzyme found in the SR membrane (1). ATP hydrolysis by SR has been shown to be depressed by anesthetic agents (halothane and chloroform) both in whole cells (3) and in SR vesicles (1, 4, 5). These previous studies were performed either at nonphysiological calcium concentrations or at halothane concentrations that far exceed clinically useful levels.

Studies by Diamond and Berman (1) using rabbit white skeletal muscle SR have shown that concentrations of halothane below 5 mM had no effect on skeletal SR activity. Only at higher halothane concentrations was there a depression of skeletal SR function. Halothane concentrations of 5 mM correspond to 16% (v/v) halothane in the gas over a liquid (6). Concentrations of halothane used clinically range from 0.4% to 2.5% (v/v) (6). Studies by Lain *et al.* (5) and Dhalla *et al.* (4) using cardiac SR showed a depression of function by lower concentrations of anesthetics. However, these studies were performed at nonphysiological calcium concentrations (>0.1 mM calcium). During cardiac cell contraction, the SR are exposed to calcium concentrations ranging from 0.1 μM to 50 μM (2). Therefore we designed our experiments to determine cardiac SR Ca^{2+}-ATPase activity at physiological calcium concentrations while the SR vesicles were exposed to clinical levels of halothane. Studies of halothane-SR interactions at physiological calcium levels (<20 μM) and clinical concentrations of halothane (1%–3%, v/v) yield more meaningful data on the cardiac muscle response to halothane than examining skeletal SR at high concentrations of calcium during exposure to nonphysiological concentrations of halothane.

The SR was prepared from 250 g of heart ventricular tissue obtained from freshly slaughtered cattle. The procedure for preparing SR vesicles was by differential centrifugation and has been described elsewhere (7). The following changes were made in the isolation procedure. Oxalate was not included in the isolation medium, and the storage buffer contained 0.3 M sucrose, 10 mM histidine-HCl, 100 mM KCl, and 5 mM $MgCl_2$, adjusted to pH 7.0. Stock solutions of SR vesicles were stored at −70° for up to 1 week at a concentration of 2.5 mg of protein per milliliter with no loss of Ca^{2+}-ATPase activity.

Contamination of the SR preparation by plasma membrane and mitochondria was determined by the presence of Na^+,K^+-ATPase activity (a sarcolemma-bound enzyme) and isocitrate dehydrogenase activity (an inner mitochondrial membrane-associated enzyme). Na^+,K^+-ATPase activity was determined by the method of Jones *et al.* (8). Mitochondrial isocitrate dehydrogenase activity was determined by the method of Plaut (9). The crude

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[3] The abbreviation used is: SR, sarcoplasmic reticulum.

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homogenate had an Na^+,K^+-ATPase activity of 0.5 nmole/min/mg of protein, and no na^+,K^+-ATPase could be detected in the purified preparation. The crude homogenate contained a total of 9200 enzyme units of isocitrate dehydrogenase (83.7 μmoles/min/mg of protein) whereas the purified preparation contained only 13.5 enzyme units (4.8 μmoles/min/mg of protein). These data, along with standard electron micrographs, indicate minimal contamination of the final SR vesicle preparation by mitochondria. More than 99% of the mitochondrial Ca^{2+}-ATPase enzyme units were removed in the purification procedure. Mitochondrial Ca^{2+}-ATPase specific activity decreased approximately 95%. Furthermore, mitochondrial Ca^{2+}-ATPase would be inactive at the low calcium concentrations used in this study. Anand *et al.* (10) found that mitochondrial Ca^{2+}-ATPase is not detectable until 0.1 mM calcium is present in the assay medium. Mitochondria accumulate calcium at a rate of 2.4 nmoles/min/mg of mitochondrial protein when in the presence of 5.0 μM calcium (11). Assuming 10% mitochondrial contamination of the SR vesicles, mitochondria would contribute 0.5 nmole/min/mg of protein or less than 0.5% of the measured Ca^{2+}-ATPase activity. Mitochondria would contribute less than 3% of the measured Ca^{2+}-ATPase activity when in the presence of 20 μM calcium (11). Also, our assay medium contains 5 mM $MgCl_2$, which has been shown to inhibit calcium accumulation by mitochondria (12). We conclude that mitochondrial Ca^{2+}-ATPase has contributed little to the results presented in the paper.

SR Ca^{2+}-ATPase activity was determined using 4.4 ml of an assay medium containing 100 mM KCl, 5 mM $MgCl_2$, and 10 mM histidine-HCl, adjusted to pH 7.0 (Buffer A). Ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid was added to this medium in order to determine the basal activity of calcium-independent ATPase activity. Calcium concentrations were varied (0.4–20 μM) by additions of 0.2 ml of calcium ion to the above assay medium. Free calcium concentrations were determined using an Orion 90-02 calcium-specific electrode. Protein at a final concentration of 0.1 mg of protein per milliliter was achieved by the addition of 0.2 ml of SR stock solution. ATP hydrolysis was begun by the addition of 0.2 ml of 125 mM disodium ATP in Buffer A. The assay had a final volume of 5.0 ml and was performed at 37°. Samples (0.5 ml) were taken at 0, 0.5, 1.0, 1.5, 2.0, and 2.5 min. The reaction was stopped in these samples by the addition of 0.5 ml of 8% trichloroacetic acid. Free phosphate was measured by the method of Fiske and Subbarow (13). The basal level of ATPase activity was subtracted from the ATPase activity in the presence of calcium to obtain the calcium-dependent ATPase activity.

Prior to the addition of cardiac SR vesicles to the assay medium, halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Abbott Laboratories, North Chicago, Ill.) was bubbled through the medium, using air as the carrier gas. The medium was exposed to 0, 1%, 2%, and 3% (v/v) [0, 0.31, 0.63, 0.94 mM (6), respectively] halothane using a Dräger halothane vaporizer. Bubbling took place for 10 min to allow the anesthetic to equilibrate with the assay medium. On addition of the SR vesicles to the assay medium, the halothane/air mixture was allowed only to flow over the surface of the assay medium.

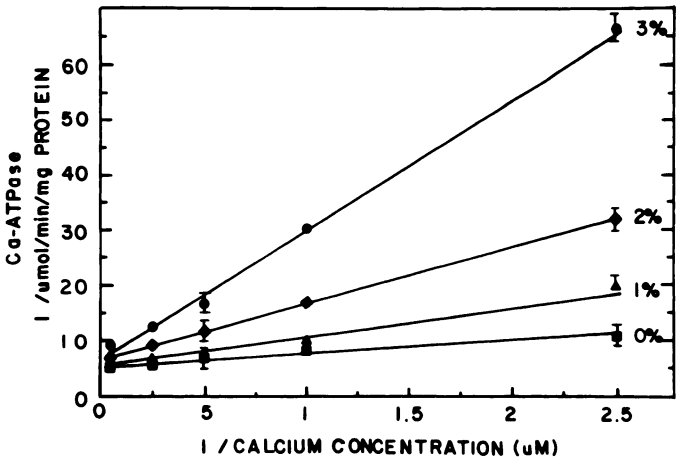

FIG. 1. *Double-reciprocal plots of cardiac SR Ca^{2+}-ATPase activity versus calcium concentration at various halothane concentrations (0–3%, v/v)*

Each data point represents the average of four experiments. Representative standard deviations are shown.

Figure 1 presents data on cardiac SR Ca^{2+}-ATPase activity versus calcium concentrations when the SR vesicles were exposed to various concentrations of halothane. The data are presented as Lineweaver-Burk plots. In the absence of halothane the rate of ATP hydrolysis in our vesicles (0.174 μmole/min/mg of protein) compares well with values previously reported [0.184 μmole/min/mg of protein (7)]. Data in this figure indicate a depression of the Ca^{2+}-ATPase activity of cardiac SR vesicles by halothane. The magnitude of halothane depression is dependent on the calcium concentration in the assay medium. Halothane was not able to depress the Ca^{2+}-ATPase activity as severely at higher calcium concentrations as at lower calcium concentrations. These data are presented in Table 1. When the calcium concentration

TABLE 1

Halothane depression of cardiac SR Ca^{2+}-ATPase activity as a function of calcium concentration

Data are presented as percentage activity remaining after halothane treatment. Values are averages of results of four experiments ± standard deviation.[a] (V_{max} for Ca^{2+}-ATPase is 0.174 μmole/min/mg of protein.)

Calcium	% Halothane (v/v)			
	0%	1%[b]	2%[c]	3%[d]
μM				
0.4	100 ± 7	56 ± 6	34 ± 6	17 ± 6
1.0	100 ± 8	67 ± 3	49 ± 8	28 ± 6
2.0	100 ± 10	73 ± 8[e]	57 ± 6	40 ± 7
4.0	100 ± 2	78 ± 5	61 ± 7	44 ± 4
20.0	100 ± 5	95 ± 3[f]	75 ± 3	65 ± 8

[a] Unless otherwise indicated, $p < 0.005$ as compared with 0% halothane.

[b] 1% Halothane = 0.32 mM.

[c] 2% Halothane = 0.63 mM.

[d] 3% Halothane = 0.94 mM.

[e] $p < 0.01$.

[f] $p > 0.1$.

TABLE 2

K_m and V_{max} values for Ca^{2+}ATPase in cardiac SR vesicles in the presence and absence of halothane

Values were calculated from the data in Fig. 1.

% Halothane (v/v)	K_m	V_{max} [a]
	μM	μmoles/min/mg protein
0%	0.44	0.184
1%	0.98	0.172
2%	1.62	0.163
3%	4.09	0.175

[a] Average V_{max} = 0.174 μmole/min/mg of SR vesicles.

was held constant, higher halothane levels caused greater loss of Ca^{2+}-ATPase activity, whereas at a constant halothane concentration the depression caused by halothane treatment was inversely related to calcium concentration.

Reversal of halothane-induced depression of contractile force and Ca^{2+}-ATPase activity by calcium ions has been seen previously in halothane-induced depression of cardiac contractile force (14) and in halothane-depressed rabbit skeletal SR Ca^{2+}-ATPase (1). However, rabbit skeletal SR Ca^{2+}-ATPase can be depressed only by high halothane concentrations (>16% or 5 mM). The cardiac SR vesicles used in this study were much more sensitive to both the halothane and calcium concentration than are skeletal SR vesicles (1).

Calcium activation of SR activity takes place with complex kinetics (15). A double-reciprocal plot of the activation process over a wide range of calcium concentrations would not yield a straight line. However, within the calcium concentrations used for this study (0.4 μM–20 μM), Pick and Racker (15) found calcium activation of the SR to be linear.

Table 2 presents data for the K_m and V_{max} of the SR Ca^{2+}-ATPase when the SR vesicles were exposed to various concentrations of halothane. These data were obtained from the double-reciprocal plots shown in Fig. 1. For increasing concentrations of halothane, the data indicate a constant V_{max} of 0.175 μmole/min/mg of protein while the K_m increases. Analysis of these data (Table 2), the data of Diamond and Berman (1), and the data of Price (14) imply that the depression of SR Ca^{2+}-ATPase activity by halothane takes place by a competitive-type inhibition. Although halothane may be competing with calcium ions for its binding site, there is no evidence to show that anesthetics act in this manner (16). Data simulating competitive-type inhibition can be explained in another manner. SR Ca^{2+}-ATPase has been shown to require 30 molecules of tightly bound phospholipids for optimal ATP hydrolytic activity (17), and recent studies indicate that this activity requires a normal lipid bilayer (18). It is known that halothane can dissolve in and disrupt the lipids of membranes (16). Consequently, halothane may be lowering the binding constant of the Ca^{2+}-ATPase for calcium by disrupting the lipids surrounding the enzyme. Price and Ohnishi (6) presented data to support this concept when they reported that halothane is capable of displacing calcium bound to membrane proteins. Such a depression of calcium binding to Ca^{2+}-ATPase by halothane treatment would yield data that would indicate a competitive-type inhibition.

In a clinical setting halothane can depress the myocardial beating rate and intensity at the halothane levels and calcium concentrations used in this study. Halothane depression of cardiac SR function may in part explain the ability of halothane to depress myocardial function.

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