A POSSIBLE MECHANISM OF ANESTHETIC-INDUCED MYOCARDIAL DEPRESSION

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SUMMARY

Evidence showing that anesthetic-induced myocardial depression is caused by direct interaction of the anesthetic with the tropomyosin-troponin complex is presented. A clinical concentration (1% v/v gas) of halothane, a general anesthetic, was found to depress ATPase activity of myofibrils prepared from cat heart. Depression was significant at low [Ca^{++}], but was antagonized by increasing [Ca^{++}]. The antagonism was proved to be competitive. Halothane sensitivity of ATPase was lost after the tropomyosin-troponin complex was removed from myofibrils with mild alkaline washing, but was restored by adding the complex back to the desensitized myofibrils.

INTRODUCTION

Although general anesthetics have been recognized as myocardial depressants for twenty years, the mechanism of this action has remained obscure. During open heart surgery, it is a routine procedure today to inject Ca^{++} intravenously in order to enhance the myocardial function, yet the mechanism is again unknown. While trying to solve this biochemically and clinically important problem, Price et al. (1) found that the depression of isometric contractile force of cat papillary muscle caused by halothane (2-bromo-2-chloro-1:1:1-trifluoroethane) is antagonized competitively by increasing the concentration of calcium in the bathing solution. The contracture caused by substituting K^+ for Na^+ in the bathing solution is also reduced by halothane (2) indicating that

the anesthetic reduces the ability of the muscle to contract even when Ca^{++} have access to the contractile proteins(3,4).

Brodkin et al. (5) reported that halothane depresses myofibrillar ATPase of rat heart; however, the concentration of halothane they used was almost fifty times greater than the anesthetic concentration. Also, they did not study calcium-sensitive ATPase, which is physiologically important. There have been other similar studies, but none of them is definitive as has been summarized in an excellent recent review by Merin (6).

Upon starting a study of the effect of anesthetics on myofibrillar ATPase, we set up the following criteria and insisted on them: a) the depression must take place at an anesthetically useful concentration; and b) the depression should be antagonized by Ca^{++}. Finally we were able to show that myofibrils prepared from cat heart by a method involving a minimum amount of time after excision and care to prevent oxidation of the preparation can fulfill these criteria. From a reconstitution experiment, we also obtained evidence that halothane interacts directly with the tropomyosin-troponin complex.

MATERIALS AND METHODS

All chemicals were of reagent grade and all solutions were prepared in glass redistilled water. HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid) was purchased from Calbiochem (Los Angeles, California). ATP with low calcium content and EGTA (ethyleneglycol-bis(β-aminoethyl ether)N,N'-tetraacetic acid) were purchased from the Sigma Chemical Company (St. Louis, Missouri). Halothane was a gift from Ayerst Laboratories (New York, New York).

Myofibrils were prepared from cat heart by the method of Honig (7) with a slight modification made to purify them by using Triton X-100 (8). The extraction of native tropomyosin "crude extract" and its purification by ammonium sulfate were made by the method of Ebashi et al.(3,4) from the same heart preparations.

Ca-EGTA buffer was prepared according to the method of Ohnishi et al. (9). Free Ca^{++} concentration in the myofibrillar suspensions was measured by the murexide method developed by Ohnishi and Ebashi (10,11) using a TCS dual-wavelength spectrophotometer Model CL (P.O. Box 141, Southampton, Pennsylvania).

ATPase activity was measured by assaying liberated inorganic phosphate by using polyvinylpyrolidone as a catalyzer to form a phosphomolybdate complex (12). This method made assay simple, easy, and reliable because (i) deproteinization is not necessary, (ii) ATP splitting during assay is negligible even when the assay is done at room temperature, and (iii) unlike other methods, absorbence is not influenced by chelating substances such as EDTA or EGTA when added to the reaction medium (details will be published elsewhere). Halothane was vaporized by nitrogen gas through a Dräger vaporizer, and the concentration was measured by a Hewlett Packard Model 700 gas chromatograph. One ml of a solution containing 80 mM KCl, 2 mM $MgCl_2$, 40 mM HEPES (pH 7.0), 5 mM sodium azide, 2 mM EGTA and various amounts of $CaCl_2$ to establish the desired value of pCa was pipetted into a test tube and then bubbled with halothane gas for five minutes at 25°C. We monitored the amount of halothane dissolved in the solution by measuring UV absorption at 230 nm, and found that five minutes was more than enough to establish an equilibrium under the present experimental conditions. We also observed that in the range of

the present concentration of halothane (0 to 2% v/v gas), the amount dissolved in the solution was linearly proportional to the gas concentration.

Thus, we added 0.2 ml of myofibril suspension to the solution, and blew halothane gas into the open space in the test tube for thirty seconds. The concentration of flowing gas was reduced by the factor of 1/1.2 to match the dilution caused by the addition of myofibrils. Following this, the test tube was sealed and incubated for five minutes. We then added 24 µl of 50 mM ATP by a Hamilton syringe, blew gas into the tube and sealed it again. The tube was then shaken gently for ten minutes after which the reaction was stopped and the inorganic phosphate concentration assayed.

RESULTS AND DISCUSSION

The relation between ATPase activity of myofibrils and pCa as affected by halothane is shown in Figure 1. Shifting of the curve toward the right indicates that halothane depresses myofibrillar ATPase and that the depression is competitively antagonized by Ca^{++} (13). We can also verify this antagonism to be competitive by the method of Lineweaver and Burk (Figure 2), where A is the increase of ATPase from the value at pCa = 8.

Honig et al. have shown that β-blockers can suppress the ATPase activity of cardiac actomyosin, and that native tropomyosin plays an important role in the reaction (14). By a similar method, we studied the role of native tropomyosin in the halothane-induced ATPase depression. Myofibrils were washed eight times in 2 mM $NaHCO_3$ solution, a procedure known to remove the tropomyosin-troponin system from actomyosin (15,4). After this alkaline washing, myofibrils lose their sensitivity to

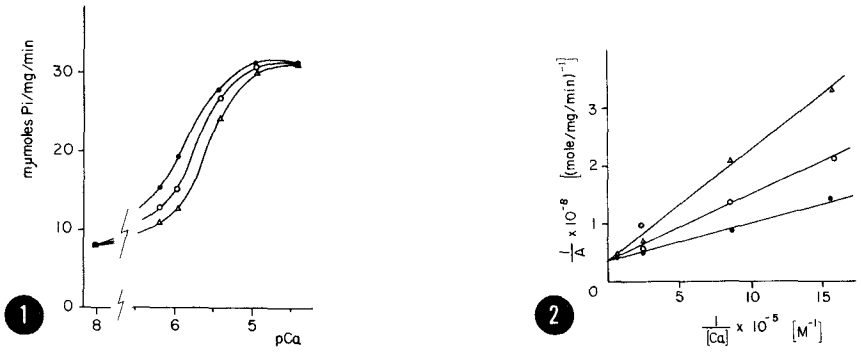

Figure 1. Effect of halothane concentration (% v/v gas) on myofibrillar ATPase. (●) no additions; (o) 1% halothane; (△) 2% halothane; conditions: 80 mM KCl, 2 mM $MgCl_2$, 40 mM HEPES buffer (pH 7.0), 1 mM ATP, 5 mM sodium azide 2 mM Ca-EGTA buffer; protein concentration, 0.5 mg/ml; 25°C.

Figure 2. Lineweaver-Burk plot of data in Figure 1. A is the increase of ATPase activity from the value at pCa = 8. Symbols are the same as in Figure 1.

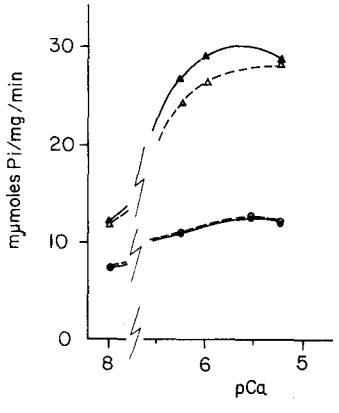

Figure 3. Restoration of Ca^{++} and halothane sensitivities of desensitized myofibrils by the addition of "crude extract" of native tropomyosin. The ordinate is expressed in terms of protein concentration of original myofibrils. (●, o) 0.3 mg/ml desensitized myofibrils; (▲, △) 0.3 mg/ml desensitized + 0.08 mg/ml "crude extract" of native tropomyosin. Solid lines, no additions; dashed lines, 2% halothane. Other experimental conditions are the same as in Figure 1 except for KCl which is 10 mM as suggested by Reddy and Honig (16).

both Ca^{++} and halothane (circles in Figure 3). However, if we add "crude extract" of native tropomyosin to the desensitized myofibrils, we can restore both the Ca^{++} sensitivity and the halothane sensitivity (triangles in Figure 3). A "purified preparation" of native tropomyosin gave essentially the same results, but the activity of the latter is less than the "crude extract" on the basis of unit protein weight. This seems to suggest the involvement of troponin in this restoration, because troponin is known to be lost easily during the purification of tropomyosin (3,4). Indeed, purification by repeated isoelectric precipitations removed the restoration capacity from our native tropomyosin preparations.

So far, the cause of anesthetic induced myocardial depression has been considered to be located in the cell membrane including the sarcoplasmic reticulum, and to take place by some kind of inhibition of Ca^{++} transport in the sarcoplasm. Although we still believe that this effect plays a part in the phenomenon, evidence given in the present paper strongly suggests that a considerable part of depression has its origin in the direct interaction of anesthetics with the tropomyosin-troponin complex. Further studies to locate the site of action on the complex are in progress.

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