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Calcium Reverses Myocardial Depression Caused by Halothane:

Site of Action

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Increasing [Ca^{++}] in the external bathing medium antagonized the depressant effect of 0.5 per cent halothane on the contractility of cat papillary muscle. This effect, when analyzed by Lineweaver-Burk plots, appeared specific, since the maximal force attainable was the same with or without the anesthetic provided [Ca^{++}] could be increased sufficiently. These results suggest that the depression of myocardial contractility produced by clinically useful concentrations of halothane is not of metabolic origin and that it reflects, instead, an ability of halothane either to restrict the availability of Ca^{++} to the contractile proteins or to inhibit the reaction between Ca^{++} and these proteins. Experiments in which the heart was tetanized, thus permitting free access of Ca^{++} to the contractile proteins, have suggested that halothane both limits the availability of Ca^{++} to the contractile proteins and interferes with the response of these proteins to Ca^{++} after their arrival at the contractile elements. (Key words: Anesthetics, volatile: halothane; Ions: calcium; Heart: contractility.)

ALTHOUGH IT HAS BEEN KNOWN for 20 years that general anesthetic agents in ordinary doses severely reduce the contractile force of the heart,[1] the mode of action involved has not been demonstrated. Brown and Crout observed in 1971 that various anesthetics depressed myocardial contractile force in a dose-related manner which depended upon the oil–water solubility of the individual agents.[2] They postulated, therefore, that the mechanisms of action of these agents in the central nervous system and myocardium were probably identical, and that cardiac actions could be used as measures of general anesthetic potency, and possibly also as models for study of the anesthetic process. However, their own findings—namely, twofold greater cardiac than CNS activities of certain agents—cast some doubt on the unitary hypothesis they had erected. It has since seemed more likely that anesthetics act in at least two separate ways in the heart, to say nothing of the CNS.

We observed in earlier studies (unpublished) that an acute reduction in the calcium ionic strength [Ca^{++}] of the external medium led to a reduction in the isometric contractile force of electrically stimulated kitten papillary muscle which was more marked in the presence than in the absence of halothane. In the present study, we have explored the general relation between increased [Ca^{++}] in the muscle bath and isometric contractile force both in the presence and in the absence of halothane. In addition, we have explored the effects of halothane at two separate sites where the anesthetic could affect myocardial contractility, namely at the cell surface and at the intracellular contractile proteins.

Methods

The thinnest available (maximum width 0.7 mm) active papillary muscles were excised from the right ventricles of 3–4-week-old kittens (anesthetized with halothane), suspended in 25 C Krebs-Henseleit solution† gassed with 3 per cent CO_2 in O_2, and stimulated electrically between 1-cm square platinum electrodes 1 cm distant from the muscle at 12-second intervals. Square-wave pulses of 5-msec duration

† Standard Krebs-Henseleit solution

NaCl	130	mM
KCl	4.0	mM
$CaCl_2$	5.0	mM
$NaHCO_3$	10.0	mM
NaH_2PO_4	0.035	mM
$MgCl_2$	1.0	mM
Dextrose	100	mg/100 ml

The *p*H of the solution is 7.40 when equilibrated with 3 per cent CO_2 in O_2.

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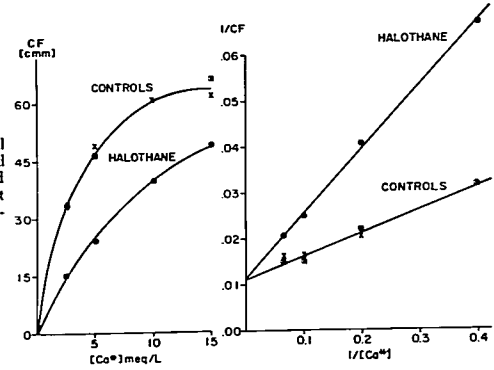

FIG. 1. Relation of maximal isometric force developed and [Ca^{++}] in the presence (•) and absence (○, ×), of 0.5 per cent halothane. 1 g = 40 chart millimeters (cmm).

and 1.2-threshold voltage were delivered from an AEL laboratory stimulator. Isometric contractile force was transduced by a Statham universal transducer and recorded on a Grass model 7 polygraph, together with the first time derivative of contractile force. Halothane‡ was supplied by a Dräger vaporizer; effluent gas samples were analyzed for halothane using a Hewlett-Packard model 700 gas chromatograph. The effect of changes in [Ca^{++}] in the bathing medium was explored in random order over the range 2.5–15 mM/l, first without halothane, then after equilibration with 0.4–0.5 per cent halothane, and finally following halothane washout. Equilibrium under each condition was assumed to be complete when there was no change in contractile force during a 15-minute period. The results are not corrected for a 40 per cent reduction in measured (Orion Ca^{++} electrode) Ca^{++} activity caused by the presence of other ions (principally phosphate and bicarbonate) in the bathing medium. (The same reduction was found for all [Ca^{++}] from 2.5–15 mM.) In some experiments the cell membranes were depolarized by tetanizing the muscle with 5 impulses/sec stimuli in the presence of caffeine and high (10 mM/l) Ca^{++} concentrations.[3]

Results

A typical result is shown at the left side of figure 1, where the peak isometric force developed is plotted against [Ca^{++}] in the external medium. On the right side of figure 1 is shown a reciprocal plot of force and [Ca^{++}] which suggests that the same force could be developed with or without halothane if [Ca^{++}] could be increased without limit (since the lines relating 1/CF and 1/[Ca^{++}] intersect at 1/[Ca^{++}] = 0, *i.e.*, at infinite [Ca^{++}]).

The results of this kind of analysis were also obtained in the ten other muscles examined; data from all 11 experiments are displayed in table 1. It can be seen from this that there was no statistically significant difference between the estimated maximal

TABLE 1. Maximal Isometric Contractile Force (grams) without and with Halothane*

Date	Control	Halothane
Feb. 1	1.01	1.05
Feb. 10	2.00	1.92
Feb. 11	1.42	1.77
Mar. 10	2.14	1.57
Mar. 13	3.58	5.55
Mar. 14	2.00	2.00
Mar. 31	0.59	0.55
Apr. 7	1.71	1.70
Apr. 10	4.33	4.50
Apr. 18	2.20	2.20
Apr. 20	3.58	3.26
Mean	2.24	2.37

* Contractile force of cat papillary muscles during exposure to 0.5 per cent halothane compared with control (average of observations prior to and following exposure to halothane). Dates identify individual muscles.

‡ Halothane was kindly supplied by Ayerst Laboratories.

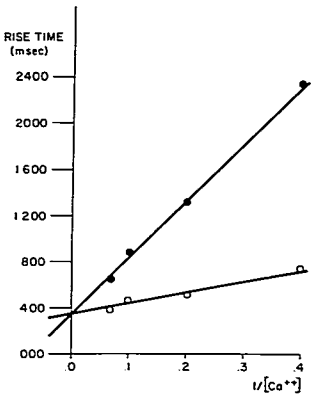

FIG. 2. Relation of maximal rate of force development and [Ca^{++}] in the presence (•) and absence (○) of 0.5 per cent halothane. Rise time = time needed to generate 1 g of force.

force levels which could be developed in the presence and in the absence of halothane.

Not only the total force developed but also the maximal rate of development of that force (df/dt_{max}) was reduced by halothane, as is shown in figure 2, where lines relating reciprocals of df/dt_{max} and [Ca^{++}] were constructed, showing intersection of these lines at the force axis, as was the case with the total isometric force developed.

TABLE 2. Rise Time (msec) without and with Halothane*

Date	Control	Halothane
Feb. 1	835	710
Feb. 10	400	375
Feb. 11	350	340
Mar. 10	340	560
Mar. 13	120	100
Apr. 7	260	345
Apr. 10	270	265
Apr. 18	230	260
Apr. 20	265	290
Mean	342	361

* Time needed to generate 1 gram of force at the maximal rate of force generation in each of nine papillary muscles during exposure to 0.5 per cent halothane compared with control (average of observations prior to and following exposure to halothane). Dates identify individual muscles.

Not all of the data usable for the computation of total force (table 1) were equally valuable in the estimation of rate of force development, since in two cases a straight line determined by at least three points could not be drawn, and for this reason data from only nine muscles are included in table 2, which again indicates that the same maximal df/dt was available at infinite [Ca^{++}] in the presence and in the absence of halothane.

In table 3 are shown data comparing the isometric contractile force generated by 3/min twitches with the tetanic contracture caused by stimulating at five impulses/sec for 3 seconds.[3] Observations made during exposure to 0.5 per cent halothane were compared with the average of control determinations made prior to exposure to halothane and following its elimination. The results are expressed as a ratio of halothane-affected to control responses, and indicate that, in the muscles studied, 0.5 per cent halothane reduced twitch force by 63 per cent and force of contracture by 41 per cent.

Discussion

Both metabolic and physicochemical explanations for the myocardial depression caused by anesthetics have been proposed.[1,5] While neither has convincing support, the weight of evidence favors the latter. However, efforts to identify a particular site of action have been unrewarding.[6]

TABLE 3. Depression Produced by 0.5 Per Cent Halothane: Twitch vs. Contracture*

Date	Twitch	Contracture
Oct. 15	0.59	0.80
Oct. 22	0.25	0.53
Nov. 8	0.19	0.44
Nov. 9	0.38	0.54
Nov. 15	0.28	0.69
Nov. 21	0.23	0.38
Nov. 28	0.53	0.66
Nov. 29	0.51	0.67
Mean	0.37	0.59

* Comparison of depression (as fraction of control peak isometric contractile force) produced by 0.5 per cent halothane when contraction is elicited by single electrical depolarization (twitch) and by tetanization (contracture). Dates identify individual muscles.

Present knowledge of the contractile process in cardiac muscle indicates that it is fundamentally similar to that in skeletal muscle, with one important difference relating to Ca^{++} storage and availability. In both skeletal and cardiac muscle, the combination of Ca^{++} with the contractile proteins effects contraction; however, in skeletal muscle Ca^{++} is readily available from intracellular sources, while in cardiac muscle the intracellular Ca^{++} stores are relatively small and these ions must be continuously supplied from extracellular sources in order to permit the contractions to continue.[7]

Interpreted against this background, our experiments with increasing $[Ca^{++}]$ in the bathing medium indicate that exposure to halothane produces an increased dependence of contractility on Ca^{++} sources in the extracellular fluid, thus favoring a physical rather than a metabolic explanation for the depression caused by this anesthetic. However, the apparent interference with the effectiveness of externally supplied Ca^{++} could represent either an inhibition of Ca^{++} transport from the exterior into the cytoplasm or an interference with the actions of Ca^{++} on the contractile proteins following their arrival at these sites.

Our studies with cardiac tetanization were designed to discriminate between these possibilities, since prolonged membrane depolarization could be expected to eliminate any limitations of access of extracellular Ca^{++} to the contractile proteins. The results of these studies (table 3) show that the response of the contractile proteins is deficient in the presence of halothane even when Ca^{++} is permitted free access. Therefore, halothane must interfere with the interaction of Ca^{++} with the contractile proteins.

On the other hand, the effect of halothane on the ("twitch") response to brief electrical stimulation is much more pronounced than that exerted on the contracture, indicating that the contractile proteins are not the only site of action. Our data are consistent with the existence of two equally important sites of action: cell membrane and contractile proteins.

Although we have no compelling evidence that enzymic effects are involved, the Lineweaver-Burke type of analysis employed in reaching our conclusions permits an estimate of the extent of the interference with Ca^{++} utilization, since the x axis (infinite force) intercepts of the back-extrapolated lines relating $1/[Ca^{++}]$ and 1/force (or velocity) are equivalent to $-1/K_m$, and K_m in turn is the $[Ca^{++}]$ at which the system attains half-maximal activation. From this analysis we estimate that 0.5 per cent halothane reduces the effectiveness of Ca^{++} in generating myocardial contractile force by roughly 75 per cent. An independent calculation, not assuming enzyme kinetics and based on analysis of tangents drawn to the original force—$[Ca^{++}]$ curves at 1 mM free Ca^{++}—suggests a reduction of Ca^{++} effectiveness by 68 per cent (see fig. 1, *left*). Since the concentration of halothane employed was less than that needed to produce general anesthesia, it is apparent that inhibition of the effectiveness of Ca^{++} by halothane is an important phenomenon which could account for the remarkable myocardial depression that this agent produces.

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