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Sites and Mechanisms of Action of Halothane on Skeletal Muscle Function in Vitro

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In isolated rat diaphragm strips, halothane augments the tension produced during caffeine-induced contractures in a dose-related manner. Potassium-induced contracture tension is augmented in the presence of halothane to a concentration of 0.75 per cent, and decreased at halothane concentrations of more than 1 per cent. The time to peak tension for potassium-induced contractures is diminished by all halothane concentrations. T-tubular disruption by hypertonic glycerol does not alter anesthetic-induced augmentation of caffeine-induced contractures. It is postulated that halothane augments calcium-release processes in sarcoplasmic reticulum. Membrane events or excitation–contraction coupling steps may be also altered by halothane. (Key words: Anesthetics, volatile: halothane. Muscle, skeletal: diaphragm, contractility.)

CONTRACTION of skeletal muscle involves a highly coordinated series of electrical, chemical and physical changes. The stimulus for muscle activation arises with depolarization of the muscle membrane, which eventually leads to a relative change in the positions of thin and thick filaments. Inhalational anesthetics interfere with events taking place in skeletal muscle subsequent to the combination of acetylcholine with receptors at the postjunctional membrane.[1,2] However, the sites involved and the way such changes affect muscle function are not well understood.

The present studies have examined the effects of halothane on skeletal muscle contraction induced by pharmacologic agents having well-defined sites of action. Based on these findings, inferences have been drawn regarding the sites and mechanisms of action of halothane in normal skeletal muscle. Halothane appears to augment calcium release from intracellular stores, while depressing excitation–contraction coupling mechanisms.

Materials and Methods

Male Wistar rats were decapitated and the right hemidiaphragms rapidly clamped so as to maintain resting length. Muscle strips approximately 12 mm long and 2 mm wide were dissected at 23 C and placed in 5 ml Krebs–Ringer solution of the following composition: NaCl 118 mM, KCl 3.3 mM, $MgSO_4$ 0.9 mM, KH_2PO_4 1.1 mM, glucose 11.1 mM, $NaHCO_3$ 24.9 mM, $CaCl_2$ 2.5 mM, *p*H 7.40. The solution was kept at 37 C *d*-Tubocurarine, .02 mM, was added to eliminate the possibility of indirect stimulation of the muscle. The solution was constantly aerated with oxygen, 95 per cent–carbon dioxide, 5 per cent. One end of the strip was attached to a glass rod, the other to a FT03C Grass force transducer. After a minimum of half an hour of aeration, exposure to halothane was begun by vaporizing the anesthetic with the inflowing gas. Halothane concentrations were determined by gas chromatography prior to every experiment by sampling the gas flowing into the muscle bath.† After 20 min, isometric muscle contraction was elicited by rapid (<3 sec) replacement of Krebs–Ringer solution with solutions containing caffeine or KCl previously equilibrated to 37 C and the appropriate halothane concentrations. Tension response was recorded on a Grass polygraph.

The concentrations of caffeine employed in these experiments were 0.125, 0.25, 0.5, 1, 2, 8, and 16 mM. Each muscle strip was exposed to the lowest caffeine concentration for 1 min per concentration, after which the next higher concentration was added. Maximum tension achieved during that minute was recorded. Other muscle strips were made to contract by 3-min exposure to 80 mM KCl in the absence of halothane. Twenty minutes after addition of halothane, the strips were exposed to increasing concentrations of KCl (10, 20, 40, 50, 70, 80, and 200 mM). Each exposure lasted 3 min and was followed by a 7-min interval of rest. KCl solutions were made up in Krebs–Ringer solution, without modification of constituents. Maximum tension and time to maximum tension were recorded during exposure to KCl. Relaxation after a KCl-induced contracture was characterized by a rapid relaxation phase followed by a slower relaxation

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† Complete equilibration of the Krebs–Ringer solution with inflowing gas was accomplished by employing a high gas flow rate (50 ml/min) relative to the small volume of solution (5 ml), and allowing 20 min to an hour for equilibration. In preliminary experiments, the concentration of halothane in the liquid phase was found to match predictions based on halothane concentration in the gas phase and the liquid/gas partition coefficient.

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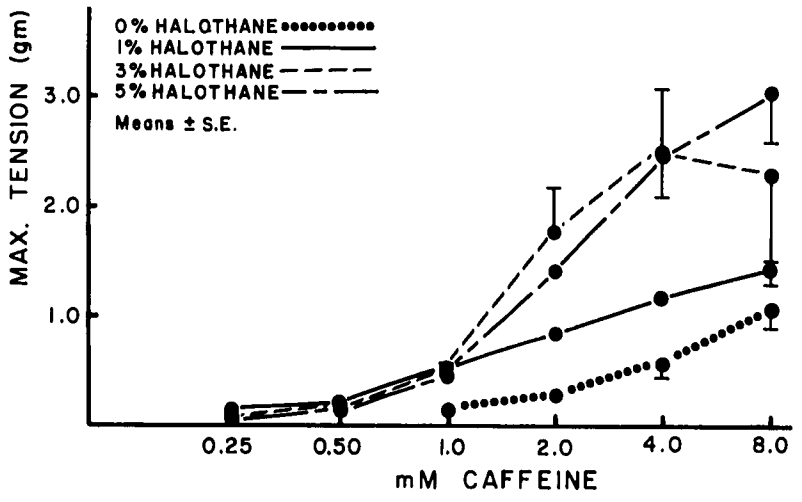

FIG. 1. Contractures produced with caffeine in the presence of various halothane concentrations. n ≥ 6 for each data point.

phase. The time of the rapid relaxation phase was determined. Muscle strips were used for exposure to either KCl or caffeine at one halothane concentration only.

Disruption of t-tubules by glycerol[3] was carried out as follows: after exposure to 200 mM KCl, Krebs–Ringer solution was replaced by 400 mM glycerol–Krebs–Ringer solution. Forty minutes later this glycerol solution was replaced with Krebs–Ringer solution and a second 3-min 200 mM KCl-induced depolarization produced. Reexposure to 400 mM glycerol–Krebs–Ringer solution for 20 min was followed by rapid washout and replacement with Krebs–Ringer solution. The tension response to 200 mM KCl was decreased by 75–85 per cent by this technique. Twenty minutes following glycerol treatment, a caffeine dose–response profile was obtained as described above in the absence of halothane. After a 20-min equilibration with halothane, a second caffeine dose–response profile was determined (figs. 3 and 4).

Wet weights for the muscle strips ranged from 8 to 20 mg in all experimental groups. The Student *t* test was employed to assess the significance of the difference between tension production (g) before and after exposure to halothane in all experiments.

TABLE 1. Potentiation by Halothane of Caffeine-induced Contractures*

Caffeine (mM)	Per Cent Halothane (v/v)			
	0.75	1	2	3
0.25	1.3	1.3	1.2	1.2
0.50	1.3	1.2	1.2	1.2
1.0	1.3	1.3	1.3	2.1
2.0	1.1	1.1	1.4	2.8
4.0	1.3	1.3	1.2	2.9
8.0	2.1	2.0	3.2	2.8
16.0	1.5	1.5	—	2.0

* Expressed as the ratio:tension with halothane/tension without halothane.

Results

Caffeine-induced contractures were potentiated by halothane in a dose-related manner (fig. 1). Maximum potentiation by halothane occurred in the range of 2–8 mM caffeine (table 1). Exposure to KCl elicited contractures with strengths that were directly related to the concentration of KCl (fig. 2 and table 2). In the presence of halothane, 0.5 or 0.75 per cent, the KCl-induced contracture tension was greater than that obtained in the absence of halothane. Halothane, 3 or 5 per cent, diminished KCl-induced contracture tension. One per cent halothane increased contracture strength upon exposure to 20 or 200 mM KCl, and was without effect at other KCl concentrations. The threshold for tension development was altered in a similar manner. In contrast, the time to peak tension subsequent to KCl depolarization was decreased by halothane in a dose-related fashion (table 3). The time of the rapid relaxation phase was not changed by halothane (not shown).

Exposure of muscle to 400 mM glycerol either greatly decreased or eliminated the tension response to 200 mM KCl (fig. 3). However, as expected, caffeine-induced contractures were still produced (fig. 4).

In other experiments (not shown) it was found that utilizing the exposure regimen employed here, the tension response to caffeine, produced identical contracture responses when repeated once after a 20-min interval. In the absence of halothane, a difference was seen between caffeine-induced contracture tension produced after exposure to glycerol compared with no exposure to glycerol (fig. 4). Failure to increase the $[Ca^{++}]$ and $[Mg^{++}]$ concentrations to 5 mM after exposure to glycerol may have accounted for this difference.[4]

Discussion

Although halothane and other inhalational anesthetics are known to influence skeletal muscle function

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and metabolism,[5] the nature of this interaction and the sites involved require further clarification. Previous studies have delineated some of the ways that volatile anesthetics may affect skeletal muscle function. Waud and Waud[2] have shown that anesthetics depress depolarization of the muscle end-plate by mechanisms not involving transmitter attachment to receptor sites. Other experiments have demonstrated both stimulant and depressant effects of anesthetics on the guinea-pig lumbrical muscle twitch response *in vitro*.[6] In general, all anesthetics are capable of augmenting or depressing the twitch response of skeletal muscle to direct electrical stimulation.[6–9] Augmentation is seen in the presence of low anesthetic concentrations, depression at higher concentrations. The concentrations relative to MACs associated with potentiation or depression vary among agents.[6] Variables that make comparison among different studies difficult, however, are concentration of anesthetic used, type of muscle studied, and temperature of the bathing solution.

In order to explain potentiation of the muscle twitch by anesthetic agents, Nelson and Denborough[10] have postulated that halothane potentiates steps involved in excitation–contraction coupling. These conclusions are based on the twitch responses of isolated skeletal muscle to halothane, caffeine, and dantrolene.

Studies utilizing isolated organelles prepared from skeletal muscle have not provided clear answers as to

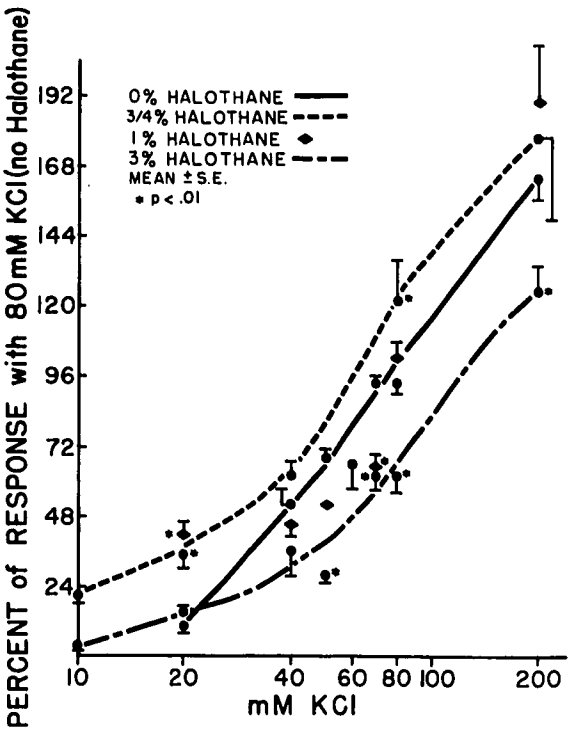

FIG. 2. Tensions produced in muscle strips by exposure to potassium in the presence of various halothane concentrations. Tension is expressed as percentage of tension produced by exposure to 80 mM potassium in the absence of halothane for each strip. Curves drawn as best fit by eye. Statistical comparison is made with contractures produced in the absence of halothane.

TABLE 2. Maximum Tension (g, Mean ± SE) Developed during KCl Depolarization

KCl (mM)	Per Cent Halothane (v/v)					
	0	0.5	0.75	1	3	5
10	—	.12 ± .02	.32 ± .06	.21 ± .06	.06 ± .02	.05 ± .02
20	0.124 ± .03	.50 ± .01*	.52 ± .09*	.71 ± .09*	.23 ± .06*	.13 ± .02
40	0.724 ± .06	.83 ± .14	.95 ± .12*	.87 ± .12	.36 ± .08*	.33 ± .05*
50	0.944 ± .12	—	—	.94 ± .13	.48 ± .06*	.68 ± .13*
70	1.33 ± .09	—	—	1.16 ± .16	1.16 ± .08	1.32 ± .24*
80	1.40 ± .08	2.05 ± .17*	1.83 ± .18	1.84 ± .16	.96 ± .10*	.64 ± .13*
200	2.36 ± .15	3.40 ± .19*	2.71 ± .29	3.48 ± .21*	1.86 ± .14*	1.48 ± .16*

* $P < 0.01$ compared with value obtained in the absence of halothane.

TABLE 3. Time (Seconds) to Peak Tension with Potassium-induced Contractures

Potassium (mM)	Per Cent Halothane (v/v)					
	0	0.5	0.75	1	3	5
10	—	124 ± 25	132 ± 22	165 ± 8	170 ± 10	149 ± 31
20	167 ± 4	130 ± 13	119 ± 16*	147 ± 7*	158 ± 13	158 ± 16
40	73 ± 11	23 ± 2*	20 ± 1*	61 ± 32	87 ± 24	36 ± 16
50	54 ± 14	—	—	81 ± 22	33 ± 12	16 ± 1*
70	16 ± 11	—	—	19 ± 2	13 ± 1*	10 ± 1*
80	19 ± 1	17 ± 1	11 ± 2*	15 ± 2*	11 ± 1*	9 ± 2*
200	12 ± 1	12 ± 1	11 ± 2	15 ± 6	7 ± 1*	6 ± 1*
Initial 80	23 ± 2	21 ± 1	19 ± 1	27 ± 1	21 ± 1	26 ± 4

* $P < 0.01$ compared with value obtained in the absence of halothane.

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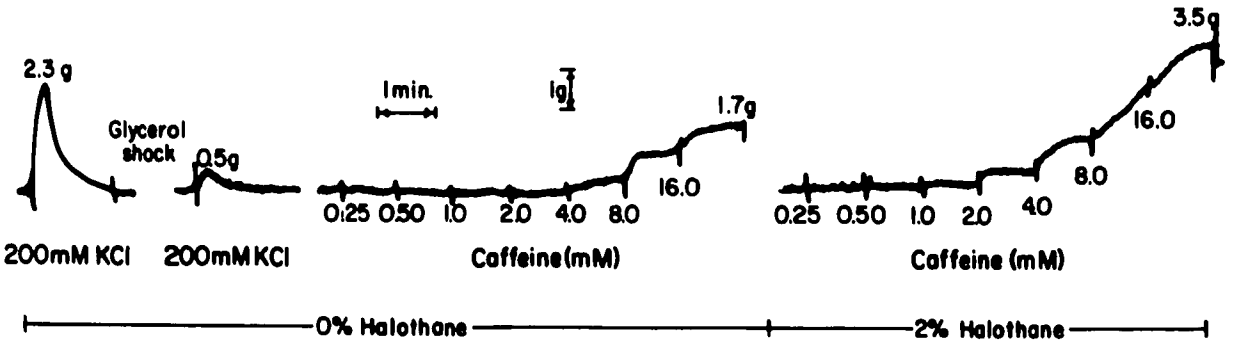

FIG. 3. Hypertonic glycerol exposure regimen in a typical muscle strip. In this case, the second exposure to caffeine took place in the presence of halothane, 2 per cent.

how and where anesthetics affect muscle function. For example, the effect of halothane on the rate of calcium uptake in isolated sarcoplasmic reticulum has been reported to be a dose-related depression,[11] an augmentation of uptake,[12] and a biphasic response (based on halothane concentration).[13] In a recent study, halothane has been found to enhance calcium

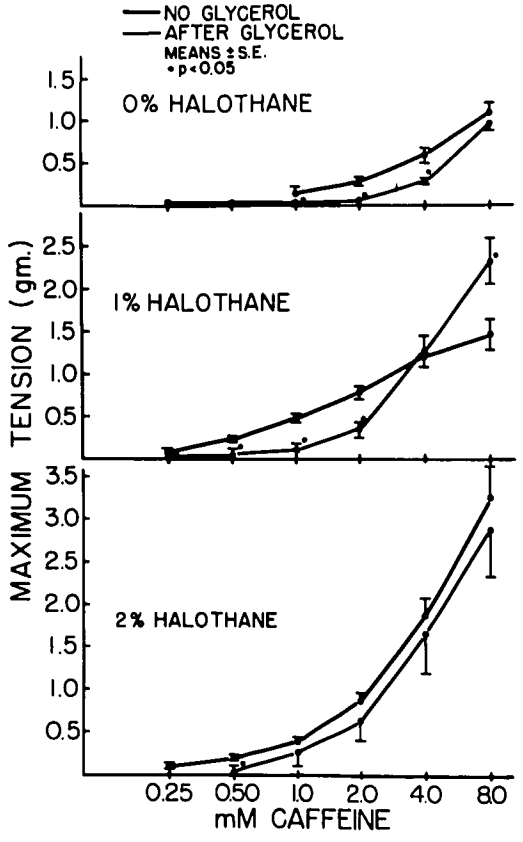

FIG. 4. Caffeine-induced contractures before and after glycerol treatment. Statistics compare tensions developed in different strips—those without glycerol exposure and those exposed to glycerol—for each halothane concentration. N ≥ 6 for each data point.

release from the sarcoplasmic reticulum of skinned muscle fibers.[14] Experiments determining the effects of clinically useful concentrations of anesthetic agents on actin, myosin, troponin, and tropomyosin functions have yet to be performed.

Several studies have shown that anesthetics may alter the manner in which pharmacologic agents affect muscle function. The pattern of these changes may help elucidate the sites of action of anesthetics on skeletal muscle. Halothane potentiation of caffeine-induced contractures is well known.[15–17] The dose-related augmentation of caffeine-induced contractures by halothane has been shown clearly in this study. Caffeine induces muscle contracture by causing an increase in intramyoplasmic calcium concentration. At caffeine concentrations of 2–5 mM, an increased calcium efflux occurs from the sarcoplasmic reticulum.[18,19] This may be related to potentiation of steps in excitation–contraction coupling, or to direct effects on the sarcoplasmic reticulum itself by decreasing the ability of this organelle to store calcium. Increased calcium efflux results in increased twitch tension in response to electrical stimulation. At the higher caffeine concentrations, calcium uptake by sarcoplasmic reticulum is blocked, and muscle contracture is produced even in the absence of depolarization of the sarcolemma.

Reed and Strobel[15] have shown that, in frog muscle, maximum potentiation of caffeine-induced contractures by halothane occurs at caffeine concentrations of 2–8 mM. The same has been demonstrated in this study. This would suggest that the primary action of halothane is to increase the amount of calcium released from the sarcoplasmic reticulum in response to membrane depolarization.

The t-tubular system communicates sarcolemmal events to the sarcoplasmic reticulum. Depolarization of the sarcolemma produces calcium release from the sarcoplasmic reticulum. Disruption of the t-tubular

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system by exposure to hypertonic glycerol blocks tension development from electrical or chemical depolarization of the sarcolemma, but does not decrease caffeine-induced contracture tension.[3,4] Therefore, augmentation of caffeine-induced contractures by halothane after t-tubular disruption is in agreement with the concept that a primary site of anesthetic action is at the sarcoplasmic reticulum.

Potassium-induced contractures occur by means of depolarization of the entire sarcolemma, thus mimicking, in some ways, electrical stimulation of muscle. Potassium-induced contracture tension and mechanical threshold are depressed by the local anesthetics, tetracaine and procaine.[20] However, unlike halothane, local anesthetics *inhibit* caffeine-induced contracture tension. It is, therefore, noteworthy that concentrations of halothane that are uniformly associated with augmentation of caffeine-induced contractures may be associated with either potentiation or depression of contractures elicited by exposure to potassium (figs. 1 and 2).

The results of these experiments may be reconciled by envisioning the following series of events: 1) Halothane, at all concentrations, enhances the rate and the amount of calcium release from the sarcoplasmic reticulum. 2) At less than 1 per cent halothane, excitation–contraction coupling steps may be either unaffected or amplified, leading to increased production of tension following membrane depolarization. 3) Concentrations of halothane exceeding about 1 per cent are associated with a significant *depression* of membrane depolarization or steps involved in excitation–contraction coupling. Therefore, less total calcium is released by the sarcoplasmic reticulum, but the rate of calcium release is still augmented. This would result in a diminution of twitch strength (or membrane depolarization-induced tension, fig. 2), while the time to reach this tension would be decreased (table 3). The failure to observe changes in the rapid phase of relaxation after KCl-induced contractures suggests that halothane has minimal effects on calcium uptake by sarcoplasmic reticulum.

In muscle biopsy specimens from individuals susceptible to malignant hyperthermia there is an unusually large augmentation of tension upon exposure to either caffeine or KCl in the presence and absence of halothane.[16] This study underscores the importance of the concentration of halothane in the interpretation of the contracture response of isolated muscle to KCl and caffeine in the diagnosis of susceptibility to malignant hyperthermia.

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