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In Vitro Interaction between Halothane and Succinylcholine in Human Skeletal Muscle: Implications for Malignant Hyperthermia and Masseter Muscle Rigidity

Jeffrey E. Fletcher, Ph.D.,* and Henry Rosenberg, M.D.†

This study examines *in vitro* the contractures induced by halothane and succinylcholine in skeletal muscle taken as biopsy specimens from 42 patients referred to the authors' laboratory for diagnosis of malignant hyperthermia (MH) susceptibility. In addition, possible differences between the response of preparations from these same patients with and without a history of masseter muscle rigidity following succinylcholine (SCh) administration were determined to investigate the *in vitro* relationship of masseter muscle rigidity to MH. Halothane 3%-induced contractures in preparations from MH susceptibles were similar, whether the group had a history of masseter muscle rigidity (1.15 ± 0.18 g; $n = 10$) or not (1.02 ± 0.21 g; $n = 14$). Halothane did not induce significant contractures in those diagnosed as normals. Succinylcholine alone did not elicit contractures from preparations derived from MH susceptibles or nonsusceptibles. Succinylcholine induced significant contractures in all preparations preexposed to halothane. Preparations from MH-negative patients with a history of masseter muscle rigidity were rendered sensitive to halothane (contractures of 1.17 ± 0.30 g; $n = 4$) when SCh was present. In contrast, halothane added in the presence of SCh did not induce contractures to the same extent in preparations from MH-negative patients without a history of masseter muscle rigidity. This is the first reported *in vitro* method by which to examine the clinically troublesome interaction between SCh and halothane. This approach also may prove to be important in further investigations of the relationship between masseter muscle rigidity and MH. (Key words: Anesthetics, volatile: halothane. Hyperthermia: malignant. Muscle, skeletal: masseter. Neuromuscular relaxants: succinylcholine.)

MALIGNANT HYPERTHERMIA (MH), a potentially fatal genetic disorder, is associated with the use of halothane and other halogenated volatile anesthetic agents and succinylcholine (SCh).¹ Masseter muscle rigidity (MMR) is a relatively common problem in anesthesia following SCh administration in children.² There appears to be a relationship between MH and MMR, as about 60% of the cases of MMR are believed to signal the potential for an episode of MH.³⁻⁵ Therefore, many patients exhibiting MMR are referred for diagnosis of

MH. The most widely used means of MH diagnosis involves a muscle biopsy and *in vitro* contracture test using isolated muscle strips. Susceptibility to MH is determined by the contracture threshold of muscle strips exposed to halothane and/or caffeine.⁵⁻⁷ Despite the apparently similar etiologies of MH and MMR, no studies have demonstrated an *in vitro* response to halothane or caffeine that is peculiar to patients with a history of MMR.

The mechanisms underlying MH, MMR, and halothane-induced contractures are poorly understood. The frequency of MMR in children is greatly increased if SCh administration follows halothane induction.² In addition, increases in serum creatine phosphokinase (CPK) values normally observed postoperatively with SCh administration are potentiated when halothane is used as the induction agent.^{8,9} The interaction between SCh and halothane has not been investigated in an *in vitro* system. Since halothane-induced contractures identify MH susceptibles, we use the response of muscle strips to halothane challenge as a means to understand the biochemical processes altered in MH¹⁰ and the interaction of various drugs with halothane.^{11,12}

This pharmacologic study examines the interaction between halothane and SCh in causing contractures of skeletal muscle preparations from MH-susceptible people. In addition, the two groups resulting from the MH diagnostic tests (MH positive and MH negative) are subdivided into those patients with, and those without, a history of MMR in order to observe similarities and differences between MH and MMR.

Methods

MATERIALS

Succinylcholine chloride powder was purchased from Sigma Chemical Co. (St. Louis, Missouri). Caffeine was obtained from Eastman (Rochester, New York) and halothane from Ayerst (New York, New York).

HUMAN MUSCLE BIOPSIES

Approval by the Hahnemann University Human Studies Committee was obtained for this study. Forty-

* Senior Instructor of Anesthesiology and Pharmacology.

† Professor of Anesthesiology.

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Address reprint requests to Dr. Rosenberg.

two patients were selected for biopsy, based on suspicion of MH, as previously described.¹² Any patient history of masseter muscle rigidity was recorded. Patients were diagnosed as MH positive or MH negative, based on the halothane and caffeine contracture tests, described below. In most cases, muscle biopsies were done under femoral and lateral femoral cutaneous blocks, avoiding infiltration of the muscle with the anesthetic.¹³

HALOTHANE CONTRACTURE TEST

The muscle used in these tests was the vastus lateralis. Dimensions of the muscle strips isolated for mounting in the tissue baths were 0.5–2.5 cm (length) by 2–5 mm (width) by 1–3 mm (thickness). The preparation and stimulation of the muscle strips was essentially the same as previously described,¹² except that curare was omitted from the Krebs solution in the present study. Basically, muscle strips were stimulated at 0.2 Hz in a 5.0-ml tissue bath containing Krebs solution at 37° C bubbled with O₂:CO₂ (95:5). Preparations (usually eight fiber bundles per patient) were equilibrated for a minimum of 30 min before beginning drug additions. Halothane 3% in O₂:CO₂ (95:5) was then bubbled through the chamber. The halothane concentration in the gas phase was determined by gas chromatography. Time of halothane exposure was 5 min. Patients were judged MH susceptible if halothane caused contractures of greater than 0.7 g in any one out of eight strips. The remaining patients were diagnosed as MH negative. This is a slight departure from our previous procedure⁵ in which halothane was used at a concentration of 1–2% and a contracture of 0.5 g was considered MH positive.

CAFFEINE CONTRACTURE TEST

Usually two strips per patient were tested with caffeine. The muscle bundles were typically used for the halothane contracture test first. The preparations were then washed three times with fresh Krebs solution. Following a 30-min equilibration period in Krebs solution bubbled with 95% O₂:5% CO₂, caffeine (0.125–16 mM) was added to the bath in concentrations increasing at twofold increments, as previously described.¹² Patients were diagnosed as MH susceptible if any strip developed a greater than 0.3 g contracture to caffeine 2 mM.⁵

INTERACTION STUDIES

The muscle strips were first used for the halothane contracture test. The strips were then washed three times with fresh Krebs solution and equilibrated for 30 min in Krebs solution bubbled with 95% O₂:5% CO₂. Halothane, when used, was bubbled through the preparation, as described for the halothane contracture test.

SCh was dissolved in Krebs solution and injected into the 5-ml bath in a 0.5-ml volume for a final concentration of 50 mM. SCh 50 mM was the minimum concentration that yielded reproducible results, based on pilot studies using muscle from humans (unpublished observations) and more detailed studies using muscle from rats (manuscript in preparation). Although the Sigma SCh chloride powder was used throughout this study, we obtained identical results with Anectine Flo-Pack® sterile powder (Burroughs Wellcome). Control preparations from each patient were injected with 0.5 ml of Krebs solution (no SCh). For the succinylcholine (SCh) and halothane interaction studies the first agent (either halothane or SCh) was added to the bath. After 5 min, the other agent (SCh or halothane) was added to the bath and the baseline tension monitored for a second 5-min period. In some cases the preparations were washed, equilibrated 30 min, and reexposed to the agents, as described above. The effects were generally reproducible, unless an unusually large contracture (greater than 1.5 g) occurred to the first challenge. The 30-min equilibration period was essential for reproducibility. The interaction studies were not conducted with muscle strips from all of the patients. The number of patients used in any study is indicated in table 1. Commonly the average response of one to four muscle strips was used as a single patient value. For example, muscle bundles from two patients diagnosed as MH+/MMR+ (table 1, line B, first data column) were exposed to SCh for 5 min, and halothane subsequently was bubbled through the bath. The average response to halothane of the four strips tested from one patient (0.7, 0.5, 1.3, and 1.2 g) was 0.9 g. Only one muscle strip was tested for the second patient (1.4 g). The two values of 0.9 and 1.4 g were used to compute the $\bar{X} \pm \text{SEM}$ shown in table 1.

DATA ANALYSIS

For the analysis of the multiple unpaired comparisons in table 1, a one-way analysis of variance and Duncan's multiple range test were used. The groups in table 1 were only tested at significance levels of $P < 0.01$ and $P < 0.05$.

Results

The results of the halothane contracture test are shown in table 1. The diagnostic patient classifications of MH-susceptible (MH+) and MH negative (MH-) were subdivided into those patients with a history of masseter muscle rigidity (MMR+) and those with no known history of MMR (MMR-). Halothane induces significantly greater contractures in skeletal muscle strips from biopsies of patients diagnosed as MH+ than in those from the MH- population (table 1, line A). There

TABLE 1. Interaction between Halothane (3%) and Succinylcholine (SCh; 50 mM) on Contractures Induced in Human Skeletal Muscle

Sequence of Agent Addition		Patient Classification			
		MH+		MH-	
First	Second	MMR+	MMR-	MMR+	MMR-
		Contracture (g)			
A. None	Halothane	1.15 ± 0.18 (10)*	1.02 ± 0.21 (14)*	0.15 ± 0.06 (5)	0.09 ± 0.03 (13)
B. SCh	Halothane	1.15 ± 0.25 (2)†	1.21 ± 0.21 (5)‡	1.17 ± 0.30 (4)‡§	0.51 ± 0.11 (9)
C. None	SCh	0.00 ± 0.00 (2)†	0.00 ± 0.00 (5)	0.02 ± 0.02 (3)	0.00 ± 0.00 (6)
D. Halothane	SCh	0.96 ± 0.15 (5)¶	1.24 ± 0.22 (9)¶**	0.71 ± 0.15 (5)¶††	0.68 ± 0.11 (14)‡‡

SCh and halothane were added within 5 min of each other. The values represent the maximum contracture within 5 min of addition of the second agent. The response shown is the mean ± SEM calculated from the average response for the number of patients indicated in parentheses. See "Methods" for description of statistical analysis.

* $P < 0.01$ compared with MH-negative (rigidity or no rigidity) groups given halothane alone.

† Group sample size too small for inclusion in statistical comparisons.

‡ $P < 0.01$ compared with SCh followed by halothane for the MH-/

MMR- group.

§ $P < 0.01$ compared with respective group given halothane alone.

¶ $P < 0.01$ compared with respective group given SCh alone.

** $P < 0.05$ compared with halothane followed by SCh for MH-/MMR+ or MH-/MMR-.

†† $P < 0.05$ compared with respective group given SCh followed by halothane.

‡‡ $P < 0.05$ compared with respective group given SCh alone.

was no difference between preparations from MH+ patients, with or without a history of MMR, in response to halothane. There also was no difference between the two subclasses (MMR+ and MMR-) from MH- patients in the response of muscle strips to halothane.

The caffeine contracture test was a less sensitive indicator of MH. None of the MH- patients, as judged by the halothane contracture test, were positive by the caffeine contracture test. However, only two patients were diagnosed as MH+ by the caffeine contracture test. Neither patient had a history of MMR.

Succinylcholine (SCh), when added alone to the bath, did not elicit significant contractures in preparations from any of the four patient groups (table 1, line C). In contrast, SCh did induce significant contractures in preparations preequilibrated with halothane (fig. 1). This interaction was observed regardless of the patient classification (table 1, line D). SCh, when added in the presence of halothane, caused greater contractures in preparations from MH+/MMR- patients than in those from MH-/MMR- patients. When the order of addition was reversed, formerly unresponsive preparations

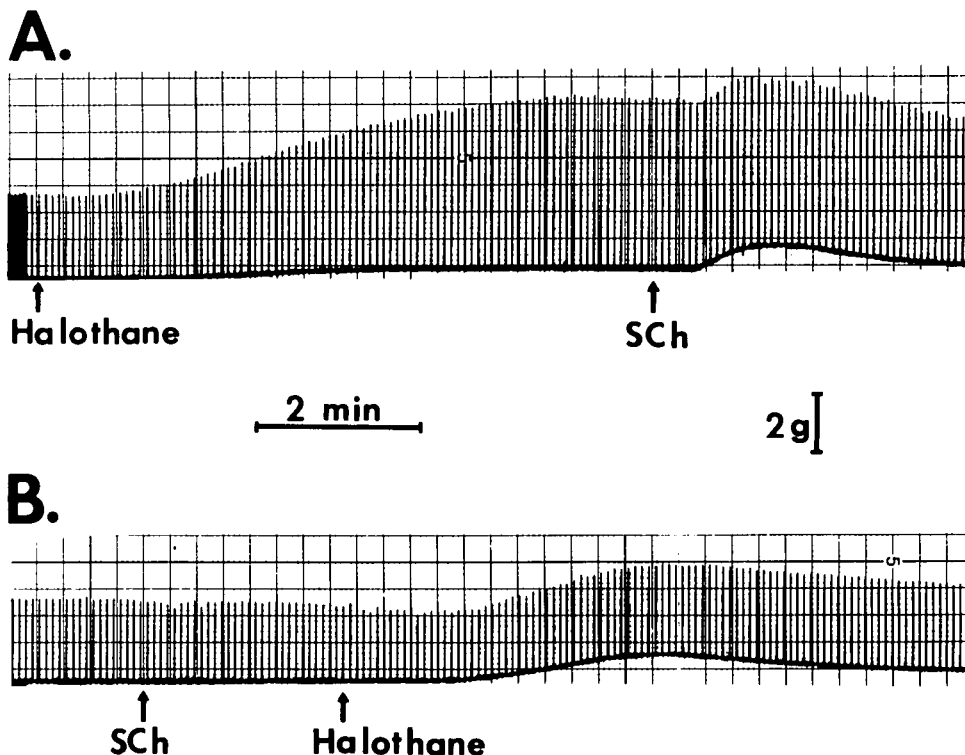


FIG. 1. Effects of halothane (3%) and succinylcholine (SCh; 50 mM) on directly stimulated human skeletal muscle. Tracings A and B are of the same muscle preparation. After the SCh response (contracture) in tracing A, the preparation was taken off halothane, washed, and equilibrated for 30 min. Tracing B shows the drug additions following the 30-min equilibration. Notice that SCh and halothane only induce significant contractures if the other agent is present. Although this strip does respond to halothane with an increased twitch height, as is normally observed for muscle from MH+ and MH- patients, the strip did not exhibit a significant contracture response (increased resting tension) to halothane alone. The muscle strip is from a MH+/MMR+ patient diagnosed by the typical reaction to halothane in other strips of muscle.

from MH⁻/MMR⁺ patients were rendered sensitive to halothane, but only if pretreated with SCh (table 1, line B). Although the muscle strips from some MH⁻/MMR⁻ patients were obviously more responsive to halothane with SCh present (range of patient contracture means 0.1 to 1.0 g), this response for the group as a whole was not significantly different from the response of the same group to halothane alone. Halothane-induced contractures of preparations from MH positives were not significantly greater in the presence than in the absence of SCh. The lack of effect of SCh in those MH positives was apparent whether the patients had a history of MMR or not. The MH⁻/MMR⁺ group was the only one in which a significant difference was observed between the response to SCh in the presence of halothane and the response to halothane in the presence of SCh. For this group, the halothane-induced contracture with SCh pretreated preparations was significantly greater than the SCh-induced contracture with halothane-pretreated preparations.

Discussion

Succinylcholine (SCh) alone did not elicit contractures of MH muscle in our study, even at a concentration of 50 mM, in contrast to the results of Moulds and Denborough.¹⁴ However, our results are in agreement with Halsall and Ellis¹⁵ and Kalow *et al.*,¹⁶ who also did not observe contractures to SCh unless a second agent was present. Although skeletal muscle preparations from humans normally do not exhibit contractures in response to SCh, our results show they can be made sensitive to SCh by preexposure to halothane. It is not appropriate to interpret these findings as suggesting that SCh will not cause MH in the absence of halothane. In fact, SCh can trigger MH in pigs in the absence of halothane.^{17,18} However, the agents used for anesthesia in these two reports (thiopentone, N₂O), although considered safe for use with MH-susceptible patients,¹ have not been examined for an *in vitro* interaction with SCh. Therefore, it is conceivable that an agent could trigger an MH episode and yet not induce contractures in skeletal muscle. Currently, the only established relationship between contractures induced in isolated skeletal muscle and MH is the positive correlation between halothane-and/or caffeine-induced contractures and MH.

Although the concentration of SCh (50 mM) required for the present investigation was unusually high for pharmacologic studies, nevertheless, this appears to be an *in vitro* model useful for studies of the mechanisms underlying MH and masseter muscle rigidity (MMR). The contractures observed with either halothane or SCh are antagonized by low concentrations of dantrolene and quinacrine, a phospholipase A₂ inhibitor (1 and 10 μM, respectively; unpublished observations), suggesting

a specific action of halothane or SCh. Further evidence for specificity, as demonstrated in the present study, is the ability to single out a unique human population—the MH⁻/MMR⁺ patients. Previous studies using PLA₂ antagonists suggest that the synergism observed with SCh and halothane is mediated by some effect on PLA₂ activity, either directly or through increased Ca²⁺ availability to the enzyme.¹⁰

MH and MMR appear to be related, as about 60% of those referred for MH diagnosis and having a history of MMR are found to be MH⁺ by the halothane contracture test. There appears to be no difference between the response of MH⁺/MMR⁺ and MH⁺/MMR⁻ patients to halothane or SCh, alone or in combination. If the pathophysiology of MH and MMR is the same, then why are about 40% of MMR referrals not diagnosed as MH positive by the muscle biopsy test? A link between MH and MMR was observed by using the combination of SCh and halothane to induce contractures. Preparations from MH⁺/MMR⁺, MH⁺/MMR⁻, and MH⁻/MMR⁺ individuals responded to halothane in the presence of SCh with contractures significantly greater than did those from MH⁻/MMR⁻ patients. Thus, MH⁻/MMR⁺ individuals form a group with a unique pattern of responses to the two contracture tests of halothane alone (no contracture) and halothane in the presence of SCh (contracture). A similar approach using humans¹⁹ or the pig model²⁰ has defined three MH phenotypes with different responses to halothane and caffeine alone and to caffeine in the presence of halothane. Perhaps the MH⁻/MMR⁺ group in the present study is similar to the intermediate group of Kalow *et al.*¹⁹ or the phenotype K reported by Nelson *et al.*²⁰

The contracture to halothane with SCh present is most likely mediated through a different mechanism than the contracture to SCh in the presence of halothane, as *d*-tubocurarine (50 mM) antagonizes only the contractures to SCh (manuscript in preparation). This is in agreement with the *in vivo* observation that curare can antagonize SCh-induced, but not halothane-induced, MH episodes.^{17,21} We believe that a third, separate mechanism is also required for contracture induction by either of these agents. This mechanism is likely PLA activity.¹⁰

Halothane-induced contractures are known to correlate with MH susceptibility.⁵⁻⁷ The present study suggests that contractures induced by halothane in the presence of SCh correlate with MH and MMR. In contrast, contractures induced by SCh in the presence of halothane do not appear to correlate with masseter muscle rigidity susceptibility.

We do not propose the use of SCh in combination with halothane in patient diagnosis at this time. A

normal control patient population in which muscle biopsies are incidental to other surgical procedures or in which muscles are biopsied for purposes other than the diagnosis of MH⁵ must be tested before the diagnostic value of the halothane-SCh interaction can be determined. The interaction between halothane and SCh does have special interest, since, unlike the halothane-caffeine combination that is used only as a laboratory means of MH diagnosis, SCh and halothane are frequently used in combination clinically.

The *in vitro* model used in the present study may prove useful in mechanistic investigations of MH and MMR. The relationship between MH and MMR appears more than coincidental. Preexposure to SCh results in contractures to halothane that are similar for preparations from MH+ patients and patients with a history of MMR. These results suggest that, in addition to MH+ and MH-, a third population (MH-/MMR+) can be identified by the halothane-contraction test.

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