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Porcine Malignant Hyperthermia: Critical Temperatures for In Vivo *and* In Vitro *Responses*

*Thomas E. Nelson, Ph.D.**

Malignant hyperthermia (MH) can be triggered in swine either by stress or by certain anesthetic agents. In humans, MH commonly occurs in patients previously exposed uneventfully to triggering anesthetics. This variability in expressivity of the MH syndrome is a combination of unknown genetic and environmental factors. A hypothesis was tested that a fall in rectal temperature following general anesthesia can prevent the MH syndrome in susceptible patients. Nine littermate Pietrain pigs with MH were exposed to halothane after their rectal temperatures were stabilized at 35°, 36°, and 37° C during thiopental/nitrous oxide anesthesia. The *in vivo* MH metabolic, cardiopulmonary, and contracture responses were attenuated at the lower rectal temperatures. The effect of varying temperatures on biopsies of skeletal muscle from these animals showed a marked decrease in contracture response to halothane when the muscle was cooled to 25° C. Studies on the Ca^{2+} uptake process and on Ca^{2+} channel-Ca^{2+} release properties of isolated sarcoplasmic reticulum (SR) membranes showed that increasing incubation temperatures from 25° to 38° C increased the Ca^{2+} uptake rate by the SR Ca^{2+} pump and also increased the probability of Ca^{2+}-induced Ca^{2+} opening of a Ca^{2+} channel and the release of stored Ca^{2+}. This study indicates that temperature can have a marked effect on the expressivity of the MH defect at the whole animal, isolated tissue, and fragmented membrane levels of organization. Since many surgical patients' temperatures decrease after induction and anesthesia, this may explain one environmental factor that determines the incidence, rate, and magnitude of the MH syndrome. (Key words: Hyperthermia, malignant. Muscle: sarcoplasmic reticulum. Temperature.)

MALIGNANT HYPERTHERMIA (MH) is a life-threatening hypermetabolic syndrome triggered by combinations of genetic and environmental factors. The fact that many known MH-susceptible (MHS) individuals have received MH-triggering anesthetic agents without developing MH[1–3] is perplexing and as yet unexplained. Environmental factors include exposure to inhaled, potent anesthetics and depolarizing skeletal muscle relaxants. Other environmental factors such as stress[4,5] have been postulated as factors that may affect predisposition to the development of MH in a susceptible individual during surgical anesthesia.

In the current study, a hypothesis was tested that mild hypothermia makes it more difficult to trigger MH and decreases symptom severity. The effects of different temperatures were tested on 1) halothane-induced MH in pigs, 2) halothane-induced contracture in biopsies of skeletal muscle, and on 3) Ca^{2+} uptake and Ca^{2+} release functions of sarcoplasmic reticulum membranes isolated from MH skeletal muscle.

* Professor of Anesthesiology.

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Address reprint requests to Dr. Nelson: Department of Anesthesiology, The University of Texas Health Science Center at Houston, Houston, Texas.

Methods

Nine littermate, MH-susceptible, purebred Pietrain pigs were used for the *in vivo* halothane challenge and for the *in vitro* skeletal muscle contracture experiments. Both parents of this litter are MH-susceptible and 100% of the offspring are MH-susceptible, making pre-screening for MH unnecessary. Another litter of MH-susceptible Pietrain pigs from the same parents as the litter used for the *in vivo* study was used to obtain skeletal muscle for the isolation and study of sarcoplasmic reticulum membrane function. The protocols were approved by our institutional Animal Welfare Committee.

In Vivo Study

An MH anesthetic challenge protocol using each pig was performed at rectal temperatures of 35°, 36°, and 37° C in three separate experimental periods defined as experiments I, II, and III. The sequence defining the temperature established during each exposure to halothane was randomly assigned. Each halothane challenge protocol proceeded as follows. Induction of anesthesia was produced by thiopental (25 mg/kg) intravenously administered through an indwelling ear vein catheter. After induction of anesthesia, the trachea of each animal was intubated, and the lungs were mechanically ventilated with a 70% N_2O/30% O_2 mixture. During the control period, a catheter was inserted into the saphenous artery, and rectal temperature was adjusted to the desired level by appropriate application of heat or cooling. Rectal temperature was maintained at the desired level by wrapping the pig in a blanket through which temperature-regulated water was circulated.

Although the method of rectal temperature regulation was crude, a satisfactory "clamping" of rectal temperature was achieved during the initial period of halothane administration to allow testing of the hypothesis. If rectal temperature began to increase above the experimental set-point after halothane administration, all efforts to control temperature were discontinued. The first step was to stop circulation of heated water through the blankets and then to completely uncover the animal and expose

the body to ambient air temperature of 20° C. If, on the other hand, rectal temperature began to decrease below the experimental set-point, then efforts were made to maintain rectal temperature by simply increasing temperature of the water circulating through the warming blankets. As necessary, additional thiopental was administered for maintenance of an anesthetic state during the control period.

After rectal temperature was stabilized to the predetermined level (±0.2° C), halothane (2% inspired) was initiated and continued either for 1 h or until an unequivocal MH syndrome developed. Unequivocal MH was diagnosed when three of the following symptoms were observed: tachycardia, increasing rectal temperature, skeletal muscle rigidity, or increasing end-tidal CO_2 production. At this point, halothane was discontinued, and if the syndrome developed to life-threatening stages, MH was treated with cooling and with dantrolene sodium (1–2 mg/kg iv). At 5-min intervals throughout the experiment, arterial blood was obtained for lactate, blood gas, and *p*H measurements. Heart rate, blood pressure, rectal temperature, minute volume, end-tidal CO_2, and respiratory rate also were measured. Each animal had MH symptoms initiating at varying times after halothane administration. For this reason, peak values or values at times when changes were maximal were selected for statistical comparisons in some cases, and in others, values were averaged at regular time intervals after halothane initiation. Each animal was allowed to recover for 26 days after experiment I and 11 days after experiment II. Each animal had an uneventful recovery from experiments I and II, and except for one animal saved for breeding, all were killed by KCl injection at the end of experiment III. During the control period of the third anesthetic, biopsies of the gracilis muscle were done for *in vitro* contracture studies.

In Vitro Studies

Fascicles of gracilis muscle measuring approximately 2 mm in diameter and 3 cm in length were dissected *in situ*, tied to wooden applicator sticks, and cut from the body and placed in Krebs-Ringer solution equilibrated with carbogen (95% O_2/5% CO_2) at room temperature. Each fascicle was removed from the applicator stick and mounted vertically in a glass muscle contraction chamber. Each muscle chamber contained 100 cc of Krebs-Ringer solution equilibrated with carbogen at either 25°, 33°, 35°, or 37° C. After increasing stimulating voltage (20–25 V; 0.4–0.8 A) and muscle length for maximum twitch tension, two fascicles were exposed to 3% halothane in carbogen bubbled through the Krebs-Ringer solution. The peak isometric contracture tension response to 3% halothane was measured at each temperature studied.

Heavy and light sarcoplasmic reticulum (SR) membrane vesicles were isolated by differential centrifugation from homogenized biopsies of longissimus dorsi and gracilis skeletal muscle by methods described previously.[6] The rate of oxalate-facilitated Ca^{2+} transport was measured in light SR at different temperatures as follows. Dual wavelength (absorbance, 650–700 nm) spectrophotometry and the Ca^{2+} indicator dye, arsenazo III, were used to measure changes in Ca^{2+} concentration outside of the SR. A cuvette containing 1 ml volume of the following components was used for Ca^{2+} uptake: SR, 0.05 mg/ml; KCl, 150 mM; histidine, 10 mM (*p*H, 6.8); K-oxalate, 5 mM; NaN_3, 5 mM; arsenazo III, 30 μM; and MgATP, 1 mM. The Ca^{2+} uptake was initiated by the addition of Ca^{2+} (75 μM) to the cuvette. Cuvette temperature was maintained by a circulating water bath. Uptake of Ca^{2+} was measured in the presence and absence of ruthenium red (200 nmol/l) to block ruthenium red-sensitive Ca^{2+} release pathways. The threshold for Ca^{2+}-induced Ca^{2+} release was measured by methods detailed previously.[7] Briefly, the same cuvette contents as described above for the Ca^{2+} uptake experiments were used except that K-oxalate was omitted, the SR concentration was 1.0 mg/ml, and Ca^{2+} was added in 2-nmol increments until the Ca^{2+} removed by the SR was partly released by Ca^{2+} channel-opening events.[7] The total amount of added Ca^{2+} that was taken up by the SR before the occurrence of Ca^{2+} release was recorded as the threshold for Ca^{2+} release. The Ca^{2+} uptake and Ca^{2+} release were measured at 20°, 25°, 30°, 33°, 35°, and 37° C. Statistical procedures included analysis of variance for general effects and Neuman-Keuls test for mean comparisons.

Results

In Vivo

At the time halothane was initiated, and for 20 min thereafter, average rectal temperature was within 0.2° C of the experimental temperature set-point for each of the pig groups (fig. 1). During this same 20-min period, the maximum deviation for a single animal was +0.70° C for the 35° C group, +0.45° C for the 36° C group, and +0.4° C for the 37° C group of pigs. Each of these animals subsequently had decreases in rectal temperature closer to the experimental temperature set-point. Before therapeutic intervention, the time from halothane administration at which mean rectal temperature had increased by 0.5° C was 40 min for the 37° C group and 55 min for the 36° C group. At 60 min and with no therapeutic intervention, the average rectal temperature for the 35° C group had increased by 0.21° C.

The halothane-induced increase in arterial blood lactate concentration in MHS pigs was not the same at dif-

ferent rectal temperatures. Lactate concentrations increased from five to nine times control concentrations within 30 min after halothane administration and when rectal temperature was 37° C. When rectal temperature was 36° C compared to 37° C, increases in arterial lactate concentration were smaller ($P < 0.05$) and slower in onset (fig. 2). In two of the nine animals challenged with halothane at rectal temperatures of 36° C, only small, insignificant increases in lactate occurred after 50 min. When rectal temperature was nearer to 35° C, lactate concentration was lower ($P < 0.05$) than when rectal temperature was 37° or 36° C (fig. 2). In four of nine animals with rectal temperatures at 35° C, halothane anesthesia produced no significant change in arterial lactate concentration. Peak lactate concentration during halothane anesthesia was determined for each animal, and mean values were determined for each rectal temperature. At rectal temperatures of 37° C, mean peak lactate concentration was greater by 20.9 mmol/l ± 18.5 SD and 49.9 mmol/l ± 35.5 SD than for mean peak values at rectal temperatures of 36° C and 35° C, respectively. Regression analysis of mean peak lactate concentration *versus* rectal temperature for this study showed that for each 1° C increase in rectal temperature between 35° C and 37° C, there is a corresponding increase in peak arterial blood lactate of 22 mmol/l.

Following administration of halothane, average CO_2 production increased in the MH pigs at each of the three rectal temperatures (fig. 3). The onset of increased CO_2 production occurred 15 min after initiating halothane administration, but rate of CO_2 production was statistically significantly greater ($P < 0.05$) in pigs with rectal temperatures at 37° C than in those with rectal temperatures at 35° and 36° C. Before any animal was treated with dantrolene, rate of increase in CO_2 production was 1.00 ± 0.06 cc·(min^{-1} kg^{-1})·min^{-1} at 37° C *versus* 0.35 ± 0.02 and 0.42 ± 0.02 cc·(min^{-1} kg^{-1})·min^{-1} at 35° and 36° C, respectively. The rate of CO_2 production at 37° C is statistically significantly different ($P < 0.05$) from those at 35° and 36° C, and the later values did not differ statistically.

Clinical consequences of exposure of these MHS pigs at varying rectal temperatures in relation to diagnosis of the syndrome were as follows. At rectal temperatures of 35° C, two of the nine pigs developed MH symptoms severe enough to become life-threatening, *i.e.*, tachyarrhythmias and a hypermetabolic state that would not be expected to spontaneously revert to normality. Consequently, each pig was treated with dantrolene (1–2 mg/kg iv) after 60 min of halothane anesthesia. Clinical value extremes for each of these two animals (mean ± SD for the nine animals given in parentheses) were as follows: rectal temperature increase, 2.1° and 1.0° C (0.5 ± 0.68° C); base deficit, −11.1 and −4.6 mEq/l (−2.8 ± 4.1 mEq/

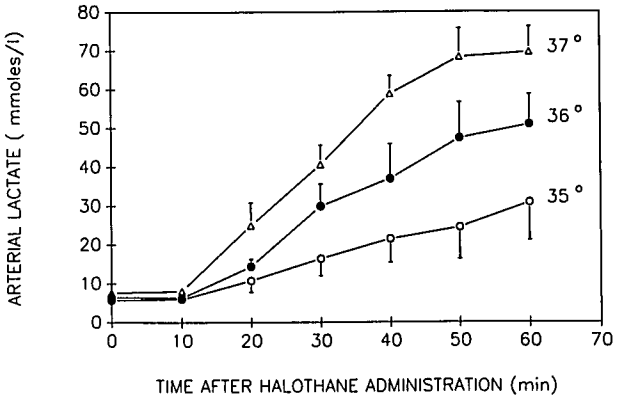

FIG. 2. Effect of initial rectal temperature on blood lactate levels during the MH syndrome in pigs. Each graph represents the average ± SD values for nine MH pigs, randomly assigned to each of the three initial rectal temperatures.

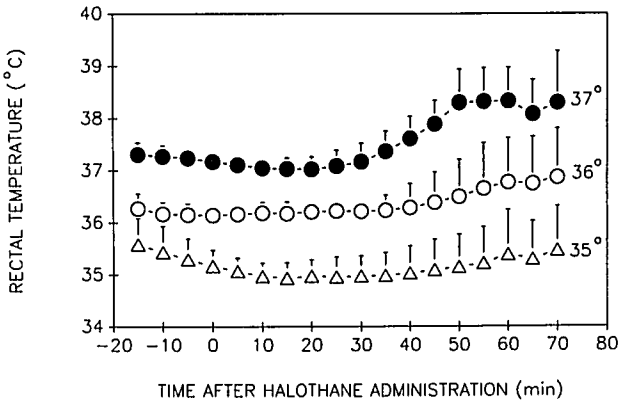

FIG. 1. Effect of initial rectal temperature on the change in rectal temperature during the MH syndrome in pigs. Each graph is composed of the average ± SD. Values for nine MH pigs, each randomly assigned to each different temperature.

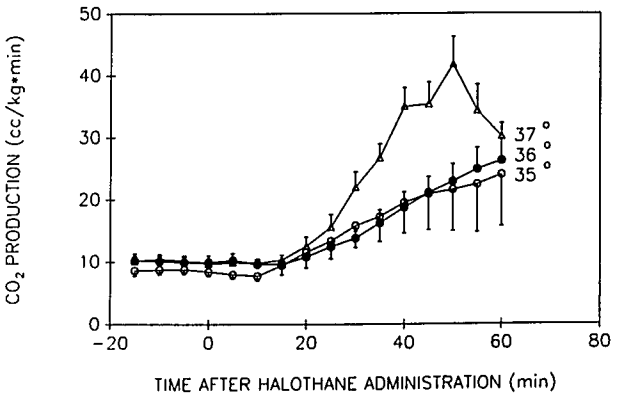

FIG. 3. Effect of initial rectal temperature on carbon dioxide production during the MH syndrome in pigs. Each graph represents the average (±SD) of nine animals, each exposed to halothane at three different initial rectal temperatures.

l); heart rate, 230 and 205 bpm (125 ± 55 bpm); and arterial blood lactate, 24.4 and 21.4 mmol/l (7.4 ± 8.6 mmol/l). Only one of these animals exhibited skeletal muscle rigidity. At rectal temperatures of 36° C, five of nine pigs required dantrolene treatment and seven of nine developed skeletal muscle rigidity. Mean ± SD of extreme values for the nine pigs at rectal temperatures of 36° C were as follows: rectal temperature increase, 1.3 ± 0.48° C; base deficit, −7.0 ± 2.4 mEq/l; heart rate, 159 ± 30 bpm; and arterial blood lactate, 9.9 ± 3.8 mmol/l. Seven of the nine pigs exposed to halothane at rectal temperatures of 37° C required treatment with dantrolene, and all nine developed skeletal muscle rigidity. Mean ± SD of extreme values for this group at 37° C rectal temperature were as follows: rectal temperature increase, 1.8 ± 0.5° C; base deficit, −12.1 ± 3.9 mEq/l; heart rate, 198 ± 17 bpm; and arterial lactate 21.3 ± 8.5 mmol/l.

In Vitro

The average contracture amplitude induced by halothane at 25° C was 26% of the contractures occurring at 33°–37° C ($P < 0.05$) (fig. 4). There was no statistically significant difference among the average contractures to halothane at 33°, 35°, and 37° C. The temperature dependence of *in vitro* halothane contracture of MH muscle is different from that for the MH syndrome *in vivo* in that skeletal muscle was not highly activated by halothane at a rectal temperature of 35° C, whereas at 33° C *in vitro*, halothane produced contractures that did not differ significantly from those produced at 37° C. However, the relationship between amplitude of *in vitro* contractures and clinical severity of an MH episode is unknown.

In light fractions of SR membrane isolated from the MH skeletal muscle, rate of ATP-dependent Ca^{2+} uptake increased ($P < 0.05$) with temperatures from 25° to 38° C (fig. 5). A Ca^{2+} channel blocker (ruthenium red, 0.5 mM) did not alter the rate of Ca^{2+} uptake in these light SR fractions and it did not change the effect of increasing temperature to increase rate of Ca^{2+} uptake. In the heavy SR fractions, the amount of Ca^{2+} that could be actively loaded into the SR vesicle until Ca^{2+} release occurred was altered by temperature (fig. 5). The threshold Ca^{2+} load (nmol of Ca^{2+}/mg of SR) for Ca^{2+}-induced Ca^{2+} release was not statistically significantly different between 25° *versus* 30° C. Increasing temperature from 30° to 35° C and to 38° C caused a decrease of the Ca^{2+} threshold (fig. 5), and this was statistically significantly different for 30° and 35° C *versus* 38° C ($P < 0.05$).

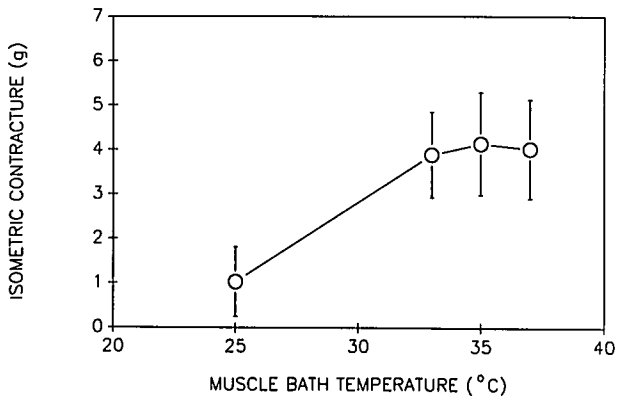

FIG. 4. Effect of incubation temperature on the halothane-induced isometric contracture response of biopsied MH pig skeletal muscle. Values are averages ± SD of 18 observations, *i.e.*, two fascicles from each of nine pigs.

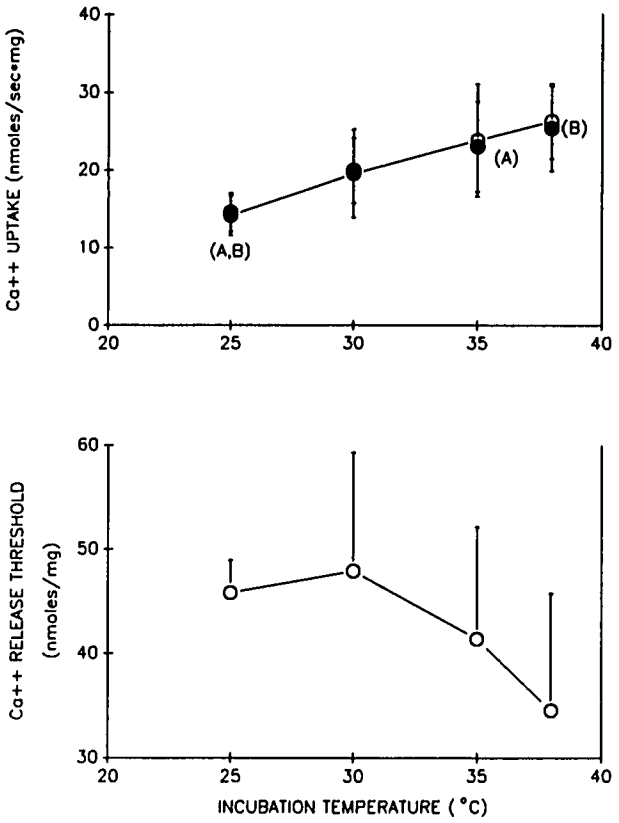

FIG. 5. Effect of incubation temperature on rate of Ca^{2+} uptake (upper panel) and on threshold for Ca^{2+}-induced Ca^{2+} release in sarcoplasmic reticulum vesicles isolated from MH pig muscle. Ca^{2+} uptake in the absence (*A*) and in the presence (*B*) of 200 nmoles/l of ruthenium red, a blocker of Ca^{2+} release pathways. Values are the average ± SD of two determinations for each of nine animals, *i.e.*, 18 observations.

Discussion

The magnitude of effects produced by halothane that are manifested as MH syndrome in intact animals, contracture response of biopsies of muscle fascicles, and loss in Ca^{2+} regulatory function by isolated SR membranes is markedly altered by temperatures below the pig's normal

rectal temperature. The average normal rectal temperature in awake, resting pigs is 39° C; thus, the experimental set-point for rectal temperatures selected for this study (*i.e.*, 37°, 36°, and 35° C) are 2°, 3°, and 4° C below normal pig rectal temperature. In humans, this would correspond to rectal temperatures of 35°, 34°, and 33° C.

It has been suggested that thiopental may provide protection against MH,[8] but studies confirming this suggestion are lacking. In this study, the average total thiopental dose for the three temperature groups was 31.8, 31.0, and 33.1 mg/kg for the 35°, 36°, and 37° C groups, respectively, but these differences are not sufficient to explain the results obtained. It could be argued that the metabolism of thiopental is reduced at lower rectal temperatures and therefore more remained to protect those animals at lower rectal temperatures. Our study cannot rule out this possibility.

Another possible explanation for the temperature effects in this study is the Q_{10} effect, *i.e.*, a two- to threefold increase in reaction rate per 10° C temperature increase. During the MH syndrome in pigs with a rectal temperature experimental set-point of 37° C, average peak CO_2 production increased from 9.7 to 41.7 cc/kg^{-1} · min^{-1}, while average rectal temperature increased from 37.2° to 38.3° C. This change in CO_2 production of 32 cc/kg^{-1} · min^{-1} with a corresponding change of 1.1° C in rectal temperature produces a Q_{10} greater than expected values (32/1.1; Q_{10} = 29). Values reported for temperature effects on oxygen consumption in anesthetized patients are in the range of 6% per 1° C.[9] The change in peak CO_2 production per 1 °C is three times the value before MH trigger and represents a 300% increase for a 1° C rise in rectal temperature. No difference in CO_2 production was observed for responses at 35° *versus* 36° C rectal temperatures, a finding that also argues against a simple Q_{10} effect.

Malignant hyperthermia is thought to be initiated by the anesthetic agents' effects to produce sustained increases in myoplasmic Ca^{2+}. Using a Ca^{2+} ion-specific microelectrode technique, myoplasmic Ca^{2+} was observed to increase during porcine MH, and treatment with dantrolene reversed the abnormal increase of myoplasmic Ca^{2+}.[10] Using fura-2 to measure intracellular Ca^{2+}, lower concentrations of caffeine were shown to release myoplasmic Ca^{2+} and induce contracture in biopsies of MH pig skeletal muscle fascicles.[11] The maximal halothane-induced contractures of MH skeletal muscle in this study were observed at 33° to 37° C, whereas contractures at 25° C were reduced by 73% from those occurring at 33° to 37° C. We cannot explain exactly why the maximum *in vitro* halothane contractures occurred at 33° to 37° C while the rectal temperature effect on the MH syndrome was more proportional across this temperature range. We reported the temperature dependence of *in vitro* halothane contractures in MHS pig muscle,[12] and this was confirmed in Denborough's laboratory.[13]

Some factors that bear on this apparent discrepancy are presented. 1) In anesthetized pigs, the skeletal muscle temperature may be as much as 2° C lower than rectal temperature* and what we measured rectally may differ from the actual skeletal muscle temperature at which MH-related events occur. 2) Homeostasis in a cut skeletal muscle fascicle is probably much different from the *in vivo* environment, and this may alter the temperature dependence of the process. 3) The relationships between core rectal temperature and skeletal muscle temperature in the anesthetized state are dependent on several variables (*i.e.*, muscle blood flow and insulation from heat loss or gain to the environment) that make a prediction of one from the other very difficult. It is my contention that simply demonstrating a temperature dependence of mechanisms that may be related to development of the MH syndrome does not prove cause and effect but definitely attracts interesting speculation. For the MH syndrome, most of the variables measured in the intact animal (*i.e.*, V_{CO_2}, temperature, heart rate, and blood lactate) are probably secondary to the hypermetabolic state of the skeletal muscle cell.

Results from studies of the isolated SR membranes may provide some clues for how temperature may be influencing myoplasmic Ca^{2+} concentration in MH muscle. The fact that increasing temperature increased the rate of active Ca^{2+} uptake by SR from MH muscle suggests that temperature alteration of this mechanism does not produce the increase in myoplasmic Ca^{2+}, but in fact, higher temperature effect on Ca^{2+} uptake would reduce the Ca^{2+} level and tend to protect against MH. The calculated Q_{10} effect (*i.e.*, two- to threefold increase in reaction rate per 10° C increase in temperature) for Ca^{2+} uptake was 2.0 over temperatures ranging from 25° to 38° C, a value in the range for Q_{10} effects. The threshold for Ca^{2+}-induced Ca^{2+} release decreased as temperature increased from 30° to 38° C, and this can be interpreted as an effect of increasing temperature to increase Ca^{2+} release from the SR. The Ca^{2+}-induced Ca^{2+} release mechanism probably reflects Ca^{2+} channel-opening events, and one possible effect of increasing temperature is to increase probability of the Ca^{2+} channel open state time. The net effect of this would be to increase Ca^{2+} release from the SR. There is evidence that this Ca^{2+} channel is defective in MH pig skeletal muscle and contributes to initiation of the MH syndrome.[6,14,15] If, by decreasing rectal temperature, the probability for open state time of the abnormal MH Ca^{2+} channel is reduced,

* Unpublished observations.

then this could explain how lower rectal temperature could protect against MH. We previously reported that levels of volatile anesthetics below 1 MAC increased the rate of Ca^{2+} uptake and decreased the threshold for Ca^{2+}-induced Ca^{2+} release.[7] If Ca^{2+}-induced Ca^{2+} release is a mechanism by which halothane produces MH, then it could be expected that lower rectal temperatures would protect against this halothane effect.

In summary, we measured the effects of different temperatures on the MH syndrome in intact animals, on *in vitro* contracture of biopsies of muscle fascicles, and on Ca^{2+} regulating functions by isolated SR membranes. Lowering rectal temperature, muscle bath temperature, or the media temperature in which SR membrane Ca^{2+} uptake and release properties are measured each has an effect to reduce the probability for halothane to produce abnormal MH-related responses. This study supports the thesis that some human patients may not develop MH, even when triggering anesthetic agents are administered, because a critically low temperature has inhibited or reduced the effect of halothane. Temperature studies on SR membranes from MH pig muscle suggest that the theory for increased myoplasmic Ca^{2+} during MH is valid and that as the temperature of a Ca^{2+} release channel is decreased, the probability of that channel opening abnormally is decreased.

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