ATX II, A Sodium Channel Toxin, Sensitizes Skeletal Muscle to Halothane, Caffeine, and Ryanodine

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Background: The function or expression of subtypes of the sodium ion (Na⁺) channel is altered in biopsies or cultures of skeletal muscle from many persons who are susceptible to malignant hyperthermia (MH). ATX II, a specific Na⁺ channel toxin from a sea anemone, causes delayed inactivation of the channel similar to that seen in cell cultures of MH muscle. ATX II was added to skeletal muscle to determine whether altered Na⁺ channel function could increase the sensitivity of normal skeletal muscle to agents (halothane, caffeine, ryanodine) to which MH muscle is hypersensitive.

Methods: Studies were performed of fiber bundles from the vastus lateralis muscle of persons who were deemed not MH susceptible (MH−) or MH susceptible (MH+) according to the MH diagnostic test and of strips of diaphragm muscle from rats. Preparations in a tissue bath containing Krebs solution were connected to a force transducer. ATX II was introduced 5 min before halothane, caffeine, or ryanodine.

Results: ATX II increased the magnitude of contracture to halothane in preparations from most MH−, but not MH+, human participants. After ATX II treatment, preparations from 9 of 24 MH− participants generated contractures to halothane, 3%, that were of the same magnitude as those from MH+ participants. Preparations from four of six ATX II-treated healthy participants also gave responses of the same magnitude as those of MH-susceptible participants to a graded halothane challenge (0.5–3%). The contractures to bolus doses of halothane in specimens from male participants were more than three times larger than the contractures in specimens from female participants. In rat muscle, ATX II increased the magnitude of contracture to caffeine (2 mm) and decreased the time to produce a 1-g contracture to ryanodine (1 μM).

Conclusions: ATX II, which causes delayed inactivation of the Na⁺ channel in cell cultures similar to that reported in cultures of MH+ skeletal muscle, increased the sensitivity of normal muscle to three agents to which MH+ muscle is hypersensitive. The increased sensitivity to halothane, 3%, occurred in most (79%), but not all, MH− participants, and this effect was most evident in male participants. Therefore, abnormal function of the Na⁺ channel, even if it is a secondary event in MH, may contribute to a positive contracture test result for MH. (Key words: Gender; muscle rigidity; myotonia; succinylcholine.)

MALIGNANT hyperthermia (MH) is a potentially fatal inherited hypermetabolic response to agents used during anesthesia.1–3 The primary agents that cause the MH syndrome are volatile halogenated anesthetics and succinylcholine, a depolarizing neuromuscular blocking agent. Malignant hyperthermia is a syndrome that is highly variable in presentation and can include one or all of the following signs: hyperthermia, muscle rigidity, tachycardia, lactic acidosis, and muscle breakdown (elevated creatine kinase, myoglobinuria, hyperkalemia, and so forth).1–3 Calcium regulation is altered in MH muscle,4–6 and mutations in the skeletal muscle calcium ion (Ca²⁺) release channel, or ryanodine receptor (RYR1), are associated with some cases of human MH.7

Some of the complexities in the presentation of MH may be caused by the heterogenous nature of the disorder.7–10 Not only have several mutations been identified in RYR1, that may cause various phenotypes of MH, but as many as five chromosomes have been suggested to encode proteins that potentially cause MH.10 Further-
more, certain factors can modify the expression of the syndrome, because heterozygous humans, and even swine homozygous for the porcine MH mutation, do not exhibit the syndrome at every exposure to a triggering anesthetics.

The association between muscle rigidity in response to anesthetics and MH is poorly understood. Several studies reported that approximately 50% of persons exhibiting masseter muscle rigidity during anesthesia are diagnosed as being susceptible to MH. It is unclear why the other 50% do not show positive test results for MH and other skeletal muscle abnormalities they may have; nor is it clear why in relatively few of the patients with masseter muscle rigidity full-scale MH eventually develops. However, masseter muscle rigidity can be an exaggerated response of “normal” (i.e., not MH) skeletal muscle to triggering agents. In addition, specific myotonic disorders also are associated with muscle rigidity during anesthesia, including hyperkalemic periodic paralysis (hyperPP) and paramyotonia congenita (PMC). These muscle disorders are caused by mutations in the sodium ion channel. The skeletal muscle Na⁺ channel consists of α and β subunits. The α subunit, or ion pore, can be the adult form (SkM1) or the embryonic form (SkM2). Both forms appear to be expressed in biopsies of normal human vastus lateralis muscle. There also is a third type of α subunit expressed in denervated skeletal muscle. In MH muscle from a significant number of those persons tested, the voltage-gated Na⁺ channel is altered in the relative expression of α subunits (i.e., the percentage of SkM2 is greatly reduced) and in the closing time of the channel, which is predominantly SkM1. The delayed inactivation of the fast Na⁺ currents observed in cell cultures of skeletal muscle from these persons with MH and intercostal muscle from MH swine resembles that observed in cultures from persons with hyperPP and PMC. It is not clear which of the multiple genetic defects suggested as causing MH lead to altered Na⁺ channel function or expression. Linkage studies suggested that specific mutations in SkM1 may yield a diagnosis of MH, although the exact pathophysiologic processes involved remain undefined.

ATX II is a toxin from the sea anemone that is highly selective for the Na⁺ channel. ATX II causes alterations in Na⁺ channel inactivation kinetics that serve as a model for the delayed inactivation of Na⁺ currents observed in muscle from persons with hyperPP, PMC, and MH. Although some differences between hyperPP and PMC exist at the single-channel level, the whole-cell currents observed in MH muscle cultures are similar to those in both myotonic disorders. Because muscle hypermetabolism or contractures of MH muscle are more sensitive to halothane, caffeine, or ryanodine, we wanted to determine whether a change in Na⁺ channel kinetics was sufficient to make these pathophysiologic processes hypersensitive. Muscle strips from normal and MH muscle were exposed to ATX II to determine whether functional defects in the Na⁺ channel can cause hypersensitivity to halothane, caffeine, and ryanodine.

**Methods**

*Studies in Human Skeletal Muscle*

This study was approved by the Allegheny University of the Health Sciences and University of Lille institutional review boards. Studies were conducted of muscle from persons referred for diagnosis of MH susceptibility. Muscle fascicles were isolated from biopsies of the vastus lateralis and mounted in a tissue bath (37°C) containing modified Krebs solution. Twitches were elicited electrically by supramaximal stimulation (0.2 Hz; 2-ms pulse), and the preparations were equilibrated 30–60 min before testing. Twitch kinetics were determined by computer analysis. The times to 50% and 100% of peak tension were determined from the time of muscle stimulation, and the times to 50% and 90% relaxation were determined from the peak tension.

The protocols of the North American Malignant Hyperthermia Group (NAMHG) for the bolus halothane, 3%, studies and the European Malignant Hyperthermia Group (EMHG) for the halothane dose–response studies were followed, as previously published. The contracture threshold used for a positive diagnosis by the NAMHG protocol (MH+) was a response of 0.7 g or more to halothane (3% in the gas phase; Halocarbon Laboratories, River Edge, NJ) in any one of three strips tested or a cumulative response 0.3 g or more to caffeine (2-mm concentration during a dose response; free base; Sigma Chemical Co., St. Louis, MO) in any one of three strips tested. This is the guideline suggested for genetic studies and is the most appropriate for basic science studies of MH. The suggested guideline for diagnosis (0.5 g to halothane) is more sensitive and only slightly less specific. Persons negative for MH by the NAMHG protocol (MH−) were defined as those who did not exceed

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the contracture threshold for halothane or caffeine. The contracture thresholds used for a positive diagnosis by the EMHG protocol (MHS) were 0.2 g or more to halothane (= 2% concentration during a dose response) in any one of two strips tested and a cumulative response of 0.2 g or less to caffeine (= 2 mm concentration during a dose response) in any one of two strips tested. Persons found normal according to the EMHG protocol (MHN) were defined as those who did not exceed the contracture threshold for halothane or caffeine.

The participants in the current study all were unrelated and represent a typical sample of referrals for MH diagnosis to our laboratories (located in Philadelphia, Pennsylvania, and Lille, France). They were not among the few families with sufficient members tested to determine linkage to a specific chromosome. Therefore, this sampling of 12 persons with MH most likely contains approximately 50% with mutations on chromosome 19q13.1 (possibly the ryanodine receptor) and some with mutations on different chromosomes. None of the 12 persons with MH in the study were known to have specific mutations in the ryanodine receptor (they were screened for five mutations). We have not found any of five ryanodine receptor mutations in approximately 120 of our participants whose test results were negative for MH.

After diagnostic testing, fresh muscle strips (one per condition per patient) were mounted, incubated, and equilibrated, as mentioned previously. ATX II (supplied by Dr. Lazlo Beress, Institute of Toxicology, Klinikum der Christian-Albrechts-Universität zu Kiel, Kiel, Germany) was added at a concentration of 1 μM for 5 or 60 min, as indicated, before halothane and remained in the bath during the exposure to halothane. The response of this muscle strip was compared with the average response of the muscle strips used for diagnostic testing. All studies were completed within 5 h of the biopsy procedure.

Studies in the Rat Diaphragm Preparation

This study was approved by the Allegheny University of the Health Sciences Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (200–300 g) were killed by decapitation. The entire diaphragm was removed and muscle strips were isolated, as previously described. Preparations were mounted and stimulated, as described previously, for human skeletal muscle. Preparations were pretreated with d-tubocurarine (20 μM; Sigma Chemical Co.) for 10 min to eliminate the contribution of nerve endings to the observed effects.

Curare remained in the bath throughout the study. ATX II was added at a concentration of 1 μM 5 min before caffeine or ryanodine was added and remained in the bath for the duration of the study. Caffeine was added as a dose response (0.5–2.0 mm) and ryanodine (high purity; Calbiochem, La Jolla, CA) was added as a bolus dose (1 μM). The cumulative contracture response to 2 mm caffeine and the time to reach a 1-g contracture for ryanodine were recorded.

Statistical Analysis

All values are reported as the mean ± SD. A two-tailed Student paired t test was used when comparisons were made between control and experimental preparations from the same patient. A grouped two-tailed Student t test was used when comparisons were made between groups composed of different patients. In the one case in which four groups were compared (the effects of gender on ATX II action), a one-way analysis of variance for repeated measures and subsequent Newman–Keuls test was used. All values of P ≤ 0.050 were considered significant.

Results

Effects of ATX II on Halothane, 3%, Contractures in Human Skeletal Muscle

The magnitude of halothane contracture in muscle strips during normal diagnostic conditions (i.e., without ATX II) was compared with strips tested in the presence of ATX II. ATX II significantly increased the magnitude of the contracture of MH− muscle to halothane, 3%, using the protocol of the NAMHG (fig. 1; table 1). In contrast to MH− muscle, ATX II did not significantly increase the magnitude of contracture induced by halothane (3%) in MH+ muscle (table 1). ATX II caused an increased response in 19 of the 24 MH− participants (79%) examined. Nine participants (38%) had contractures of 0.5 g or more to halothane, 3%, in the presence of ATX II, which would be considered a positive diagnosis in North American laboratories. Of the MH− specimens tested, 14 were derived from female and 10 from male participants. The three largest responses to halothane, 3%, in the presence of ATX II (1.25, 1.40, and 1.90 g) were in specimens from male participants, and the eight smallest responses (0.0–0.25 g) were in specimens from female participants. A two-way analysis of variance for repeated measures and subsequent Newman–Keuls test (P < 0.050) were used to compare the responses between male and female participants in the absence and pres-
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Fig. 1. Effects of ATX II (1 μm) on the response to halothane using the North American Malignant Hyperthermia Group (NAMHG) protocol. Preparations of vastus lateralis from a participant diagnosed as MH− by the NAMHG protocol were incubated in the absence (A) or presence (B) of ATX II for 5 min before exposure to halothane, which was administered as a bolus dose (3%) at the point indicated by the arrow. The muscle strip in A treated with halothane alone is representative of a total of four strips from the same patient with a mean ± standard deviation of 0.38 ± 0.2 g. The magnitude of the contracture response is indicated in each panel. The twitch height exceeds the maximum recordable tension in both panels to provide the maximum sensitivity for evaluating the contracture response. The increase in twitch tension caused by halothane was similar in either the absence or presence of ATX II (unpublished observations).

ence of ATX II. Although male and female participants both exhibited a significant increase in the magnitude of contractures to halothane, 3%, with ATX II (table 1), male participants had an approximately threefold greater increase in the presence of ATX II than did female participants. The difference in means between male and female participants in the response to halothane, 3%, is not significant (P = 0.557). There were no differences of significance between the number of contractures to halothane, 3%, in the presence of ATX II large enough to have been positive for MH (0.5–1.9 g) were in specimens from male participants. There was no effect of age on the response to halothane, 3%, in the presence of ATX II, because the age of those with preparations exhibiting contractures 0.5 g or more (35 ± 12 yr; n = 9) was no different (P = 0.557) from the age of those with contractures 0.5 g or less (31 ± 15 yr; n = 15).

To determine whether the time of exposure to ATX II has a significant effect on contractures to halothane, two preparations each from six MH− participants were pre-incubated with ATX II, one for 5 min and one for 60 min, before halothane exposure. The contractures to halothane, 3%, after a 5-min exposure to ATX II (0.42 ± 0.44 g; mean ± SD) were not significantly different (P = 0.781; two-tailed Student paired t test) from those after a 60-min exposure to ATX II (0.36 ± 0.27 g).

Effects of ATX II on the Response to Halothane, 0.5–3%

The EMHG halothane protocol uses a dose response to determine MH susceptibility. Therefore, in a second group of persons diagnosed by the EMHG protocol as MHS or MHN (persons with equivocal responses were excluded), we tried to determine whether ATX II also could cause a positive diagnosis in normal muscle tested by this method. The response to halothane, 2%, was significantly increased by ATX II in MHN muscle (fig. 2). Of the six MHN participants tested, preparations from four (67%) exhibited responses that would be judged as MHS; that is, they had a cumulative contracture response to halothane (≤ 2%) of 0.2 g or more. These findings were similar to those observed using the bolus dose of halothane in the NAMHG protocol. There was no greater effect of halothane in MHS muscle pretreated with ATX II (fig. 2), which corresponds with the lack of effect of ATX II on the bolus dose of halothane in MH+ muscle using the NAMHG protocol. Preparations from one of two female MHN participants and from three of four male MHN participants had positive (MHS-like) contractures to halothane in the presence of ATX II. The small sample size precludes any further analysis of gender for the EMHG test.

Effects of ATX II on Contractures to Caffeine and Ryanodine in Rat Muscle

Some MH− persons cannot be considered healthy, because they are referred for problems during anesthesia.

Table 1. Effects of ATX II (1 μm) on the Contracture Response of Human Vastus Lateralis Muscle to Halothane by the NAMHG Protocol

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>−ATX II (g)</th>
<th>+ATX II (g)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH−</td>
<td>24</td>
<td>0.15 ± 0.12</td>
<td>0.48 ± 0.47</td>
<td>0.001</td>
</tr>
<tr>
<td>Males</td>
<td>10</td>
<td>0.20 ± 0.15</td>
<td>0.77 ± 0.55</td>
<td>0.006</td>
</tr>
<tr>
<td>Females</td>
<td>14</td>
<td>0.11 ± 0.07</td>
<td>0.28 ± 0.26</td>
<td>0.026</td>
</tr>
<tr>
<td>MH+</td>
<td>6</td>
<td>2.60 ± 1.74</td>
<td>2.95 ± 2.33</td>
<td>0.776</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The response to a bolus of halothane (3%) was determined in the absence (−ATX II) or the presence (+ATX II) of the sodium channel toxin. P values ≤ 0.050 (two-tailed Student paired t test) were considered significant.

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and may have undiagnosed muscle disorders. The effects of ATX II were tested on contractures to caffeine and ryanodine in Sprague-Dawley rat diaphragm muscle, which is not known to be hypersensitive to MH-triggering agents. The use of strips of diaphragm allows multiple drugs to be tested with corresponding controls within each animal. The cumulative response to caffeine at 2 mM is used for the diagnosis of MH. ATX II significantly increased the magnitude of the cumulative contracture to 2 mM caffeine (table 2). A ryanodine contracture test has been proposed based on the greater sensitivity of MH+ muscle to this plant alkaloid toxin. The time of contracture to a 1 μM concentration has been the best method to identify MH- and MH+ persons tested with ryanodine. ATX II decreased the time to a 1-g contracture to ryanodine in the rat diaphragm by approximately one half (table 2), which is consistent with the more rapid induction of contractures by ryanodine in MH+ compared with MH- human muscle.34,55

Effects of ATX II, Halothane, Caffeine, and Ryanodine on Twitch Kinetics

In preparations from 21 MH- participants, the times to 50% peak tension (29 ± 5 ms), 100% peak tension (67 ± 13 ms), 50% relaxation (55 ± 15 ms), and 90% relaxation (115 ± 26 ms) were determined. These were not significantly different by a grouped two-tailed Student t test from preparations from 20 MH+ participants in which the times to 50% peak tension (28 ± 3 ms; P = 0.565), 100% peak tension (62 ± 8 ms; P = 0.190), 50% relaxation (49 ± 13 ms; P = 0.222), and 90% relaxation (115 ± 22 ms; P = 0.993) were also determined. Because there is no difference in twitch kinetics between MH- and MH+ participants, it was important to determine whether ATX II significantly altered the twitch characteristics of MH- muscle. The effects of ATX II (1 μM) on the twitch kinetics of human vastus lateralis muscle were evaluated in preparations from MH- participants. The times to 50% peak tension (29 ± 5 ms; P = 0.658), 100% peak tension (66 ± 13 ms; P = 0.365), 50% relaxation (56 ± 16 ms; P = 0.442), and 90% relaxation (115 ± 28; P = 0.926) for preparations from 21 MH- participants were unaltered (paired two-tailed Student t test) by ATX II after a 5-min exposure. The values for the same twitch parameters after a 60-min incubation with ATX II were also similar and not statistically different (P values 0.328-0.695; n = 6) when compared by a two-tailed Student paired t test. ATX II caused a slight (5%) decrease in twitch height (P = 0.030) after 5 min; therefore, there were no observable effects of ATX II on the twitch kinetics, in agreement with the similar twitch kinetics in MH- and MH+ muscle.

The effects of halothane, caffeine, and ryanodine on twitch kinetics were evaluated to determine whether these Ca2+-modifying drugs cause changes in the twitch not observed with ATX II. In contrast to the slight de-

Table 2. Effects of ATX II (1 μM) on the Contracture Response of the Rat Diaphragm to Caffeine and Ryanodine

<table>
<thead>
<tr>
<th>Magnitude of contracture (g)</th>
<th>n</th>
<th>ATX II</th>
<th>+ATX II</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>3</td>
<td>0.28 ± 0.21</td>
<td>0.45 ± 0.16*</td>
<td>0.038</td>
</tr>
<tr>
<td>Time to 1.0 g contracture (min)</td>
<td>3</td>
<td>20.2 ± 2.8</td>
<td>11.8 ± 1.9†</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The response to caffeine or ryanodine was determined in the absence (+ ATX II) or the presence (- ATX II) of the sodium channel toxin, ATX II. Response to caffeine (2 mM) was determined during a dose-response (0.5-2.0 mM). Time to 1.0 g contracture was used for ryanodine (1 μM). P values ≤ 0.050 (two-tailed Student paired t test) were considered significant.

* MH is associated with an increase in magnitude of contracture.
† MH is associated with a decrease in time to 1.0 g contracture.
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pressant effect of ATX II, halothane, 3% (n = 19) and 2 ms caffeine (n = 20) increased the twitch height within 5 min by approximately 57% and 83%, respectively (P < 0.001; two-tailed paired Student t test). Halothane significantly (P < 0.001) increased the time to 50% and 100% peak tension by approximately 9% and 6%, respectively. Similarly, caffeine significantly (P < 0.001) increased the time to 50% and 100% peak tension by approximately 11% and 9%, respectively. Caffeine significantly (P < 0.001) increased the time to 50% and 90% relaxation by approximately 12% and 9%, respectively. Halothane had no significant effect on these relaxation parameters. After 5 min, ryanodine 1 μM (n = 21) had no effect on the twitch kinetics yet decreased the twitch height by approximately 5% (P = 0.004). However, after 30 min, ryanodine significantly (P < 0.001) increased the time to 50% and 90% relaxation by approximately 111% and 60%, respectively. Therefore, ATX II did not have halothane, caffeine, or ryanodine-like actions, including increasing twitch height or prolonging the time to peak tension or relaxation.

Discussion

The expression and function of the Na⁺ channel are altered in MH muscle in cell cultures or in muscle from certain persons that underwent biopsy. Although linkage to SKM1 (encoded on chromosome 17) and to chromosome 3 (chromosome encoding SkM2) has been reported, it is most probable that mutations in the Na⁺ channel only account for a small percentage of MH. An important outcome of the current study is the finding that, even in the absence of mutations, a pharmacologically altered Na⁺ channel function is sufficient to induce altered contractures to halothane, caffeine, and ryanodine in some muscles. Thus, if such pathophysiologic changes were induced in muscles, a greater sensitivity to these agents might result. Therefore, the observed alterations in Na⁺ channel function or expression that might arise because of secondary responses of MH muscle to mutations in other proteins (such as the ryanodine receptor) could contribute to the development of the syndrome.

Clearly, mutations in the Na⁺ channel cause hyperPP and PMC, and these disorders are associated with rigidity in response to agents causing MH. The hypermetabolism and acidosis that are hallmarks of MH are rarely induced by volatile anesthetics in persons with these specific myotonic disorders. Rigidity is only one component of MH and it is not observed in all clinical episodes. Consequently, rigidity and hypermetabolism during anesthesia can arise independently of one another, although they often occur together in MH.

The type of altered Na⁺ channel function observed in MH muscle can contribute to a positive contracture test result for MH, as shown with the Na⁺ channel toxin ATX II. ATX II (1 μM) pretreatment of muscle alters Na⁺ currents without changing the electrically elicited twitch parameters, which corresponds with the findings of other investigators. In contrast to the lack of effect of ATX II on the rapid kinetics of the muscle twitch, the toxin can increase the magnitude of slower occurring contractures to halothane and to caffeine and can increase the rate of ryanodine contractures, all of which are characteristics of MH muscle. ATX II is known only to affect Na⁺ channels, and we did not obtain evidence for other nonspecific actions similar to the actions of halothane, caffeine, or ryanodine. ATX II has no effect on resting membrane potentials. Furthermore, BAY K 8644, which is a dihydropyridine receptor agonist, increases the separation of the halothane response between MH and normal muscle by preferentially augmenting the response of MH muscle. In contrast, ATX II decreases this separation by making normal muscle hypersensitive to halothane but by having no effect on MH muscle. All of this evidence would suggest that ATX II has a high specificity for Na⁺ channels and does not share any of the characteristics of halothane, caffeine, and ryanodine, BAY K 8644, or membrane-depolarizing agents. However, we still cannot exclude completely the possibility that ATX II may affect the function of another protein in addition to the Na⁺ channel.

The influence of ATX II on the response to halothane was varied and ranged from no effect to very marked changes, depending on the person evaluated. Of the 24 participants evaluated using the NAMHG halothane protocol, 38% would be judged as having a positive contracture test result for MH. Using the EMHG protocol, 67% of the six participants evaluated reached the threshold response for a positive halothane test result. Regarding the MH syndrome, male patients have an approximately threefold greater incidence of MH than do female patients. In our study, specimens from male participants were more likely to generate a large contracture in response to halothane in the presence of ATX II than were specimens from female participants. This is the first in vitro demonstration of a sex-related difference in response to halothane. Age is another factor that influences the expression of the MH syndrome. The

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effects of ATX II on halothane-induced contractures did not depend on age. Therefore, functional defects in the Na⁺ channel may account for some of the variability in presentation of the MH syndrome, including gender-based, but not age-based, differences.

The concept of modifiers has been invoked to explain the extreme variability in clinical presentation of the syndrome. The Na⁺ channel may be an important modifier of the syndrome; however, the current study suggests that an interplay must occur between functionally altered Na⁺ channels and an additional factor in MH muscle. An example of the type of factor that could interact with Na⁺ channels is the elevated fatty acid metabolism reported in MH muscle. Artificial elevation of intracellular fatty acids by microelectrode injection has different effects on normal and MH skeletal muscle Na⁺ channels in cell culture, possibly because fatty acids are already elevated in MH muscle. Not all persons with MH have altered Na⁺ channel expression. In a model suggested to explain the complexities of MH, a genetic defect (e.g., ryanodine receptor mutation) must trigger a second event (e.g., elevated fatty acids as an energy source to pump Ca²⁺). A third event (e.g., altered Na⁺ channel function or expression) interacting with elevated fatty acids could then modify the expression of the syndrome. This model of complex modulation could apply to other systems, such as the reported elevation in inositol 1,4,5-trisphosphate in MH muscle, or to other modifiers of Ca²⁺ regulation, such Na⁺-Ca²⁺ exchange, FK506-binding protein, triadin, and so forth.

The relatively large number of persons tested in the current study allowed us to evaluate the variability in the MH syndrome and sex-related factors that would have been impossible to interpret with a smaller sample size. The observation that ATX II did not change the contracture response of all muscles tested suggests that factors in the muscle in addition to altered Na⁺ channel function also are involved in the variability in expression of the syndrome and in the diagnostic contracture test. These factors could be genetic, hormonal, or metabolic. This may explain why some families with mutations in the Na⁺ channel exhibit positive contracture responses, whereas others that have the same mutation exhibit negative responses in the diagnostic test for MH. In addition, Na⁺ channel mutations are involved in clinical signs that could be confused with MH, such as rigidity without acidosis, as is the case with hyperPP and PMC. It is possible that subclinical myotonias or other unidentified mutations in the Na⁺ channel may also be involved in abnormal responses to anesthetics, including MH.

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