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Comparison of the in vitro caffeine-halothane contracture test with the Ca-induced Ca release rate test in patients suspected of having malignant hyperthermia susceptibility

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Abstract

Purpose. We compared the results of the in vitro caffeine-halothane contracture test (CHCT) according to the protocols of the North American Malignant Hyperthermia Group (NAMHG) and the European Malignant Hyperthermia Group (EMHG) with the Ca-induced Ca release (CICR) rate test in the same patients with suspected malignant hyper thermia (MH).

Methods. Five normal controls and 16 patients suspected of having MH susceptibility were studied. Muscle biopsies were usually obtained from the musculus vastus lateralis. Diagnostic cutoff points and procedures for CHCT protocols were as described in the original and renewal versions of NAMHG and EMHGs. The CICR rate test was performed according to the protocol reported by Endo et al.

Results. All five normal controls and two patients with abortive MH, two with postoperative hyperthermia, and three with high serum creatine kinase levels were normal in the three tests. Three patients with MH reactions and one patient with a history of masseter spasm were classified as MH positive according to NAMHG criteria and MH susceptible and MH equivocal according to EMHG criteria. There were five cases with discordant results between the CHCT and CICR rate tests.

Conclusion. We propose that muscle biopsy for diagnosis of MH susceptibility should combine the CHCT with the CICR rate test, which may identify the defective site of Ca release channels.

Key words: In vitro caffeine-halothane contracture test (in vitro CHCT), Ca-induced Ca release rate test (CICR), Malignant hyperthermia (MH), Malignant hyperthermia susceptibility (MHS)

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Introduction

Malignant hyperthermia (MH) is a pharmacogenetic skeletal muscle disease with heterogeneous inheritance characterized by abnormalities in intracellular calcium metabolism secondary to a variety of genetic mutations in the skeletal muscle excitation-contraction coupling [1]. It is well known that MH crisis is caused by enhanced Ca release from intracellular stores [2]. The increased Ca release produces an ionic Ca overload in the sarcoplasm of the muscle cell, which induces muscle contracture as well as accelerated glycolytic and aerobic metabolism [3]. One of the defective sites in patients susceptible to MH is the Ca release channel of the sarcoplasmic reticulum (SR) in skeletal muscle cells [4]. However, several other triggering mechanisms of MH reactions have been proposed, such as a mutation of the α_1-subunit of the dihydropyridine-sensitive L-type voltage-dependent Ca-channel receptor [5], and the α_1-subunit of the sodium channel in the transverse tubules [6]. MH susceptibility (MHS) in humans has been believed to be independent of sex, age, or race. Over 10 cases of MH crisis have occurred during a few million operations with anesthetic each year in Japan. In Japan, MH reactions during operation were more frequent in children than in adults and were approximately three times more frequent in males than in females [7]. Diagnostic muscle biopsy from patients suspected of having MHS has been performed with the Ca-induced Ca release (CICR) rate test using a chemical skinned fiber originated by Endo et al. [8–10]. Quite a few cases have been diagnosed by the in vitro caffeine-halothane contracture test (CHCT) in Japan. However, there is some confusion about MHS diagnosed by the different tests, and there have been no studies to investigate whether the results obtained by the CHCT correspond to those obtained by the CICR rate test for the diagnosis of MHS. We, therefore, compared the results obtained by

the CHCT with those obtained by the CICR rate test on the same muscle sample from 16 patients suspected of having MHS and 5 normal controls.

Materials and methods

This study was the collaborative work of two facilities. Patients suspected of having MHS were referred to one of the two facilities from local hospitals. Muscle specimens were obtained from 16 patients suspected of having MHS and 5 normal controls, for example, by amputation of the upper leg or benign tumor around the knee during various kinds of local, regional, or general anesthesia while undergoing muscle biopsy or surgery. The study protocol was approved by the Ethical Committee of Shiga University of Medical Science and the institutional committees of the hospitals treating the patients. Written informed consent for muscle biopsy was obtained from each patient. Muscle fascicles were biopsied from the vastus lateralis (17 patients), rectus femoris (2 patients), gluteus maximus (1 patient), or erector spinalis (1 patient) muscles. The specimens for the CHCT were transported in 1.5 h from 11 neighboring hospitals to the laboratory at the Shiga University of Medical Science at room temperature in Krebs-Ringer's solution aerated with 5% CO_2 – 95% O_2. Specimens for the CICR rate test were transported in the cooled relaxing solution to the laboratory at the Department of Anesthesiology and Critical Care Medicine at Hiroshima University within 24 h by commercial shipping services.

In vitro caffeine-halothane contracture test

Muscle bundles were dissected from muscle specimens and suspended in two parallel tissue baths of Krebs-Ringer's solution and bubbled with 5% CO_2 – 95% O_2 at 37°C. The first bundle was tested within 2 h and the last bundles within 5 h. The approximate dimensions of the muscle bundles were 15–17 mm in length with a thickness of 2–3 mm and a weight ranging from 40 to 100 mg. One end of the bundle was fixed on a sample board, and the other end was attached by a 3–0 silk suture to a force displacement transducer (NEC, Japan) with a peak tension capacify of 10 g. Each bundle was stimulated with a supramaximal electric current in 2-ms pulses at 0.2 Hz, through platinum wire electrodes and a nerve stimulator (NEC, Tokyo, Japan). The viability of the muscle specimens was confirmed by the response of a single twitch contraction to the electric current. Muscle bundles from each patient were exposed to caffeine alone and halothane alone according to the European MH Group (EMHG) protocol [11,12] and the North American MH Group (NAMHG) protocol [13]. Because the quantity of muscle specimens had to be limited in order to avoid the sequelae of muscle weakness in patients, the caffeine alone and the halothane alone test in both protocols were each performed using one muscle bundle (Table 1). All of the experiments were completed within 5 h. The diagnostic cutoff points were adopted from the original standard of each group as reported [11–13].

Ca-induced Ca release rate test

The transported muscle specimen was dissected into a small bundle 8–12 mm in length, 1–2 mm in width, and 1 mm in thickness. Then it was chemically skinned with the relaxing solution containing saponin at a concentration of 50 μg·ml^{-1} for 30 min at 20–25°C. A single skinned fiber or a few skinned fibers (1.5–2.5 mm in length and 75–100 μm in width) were isolated and were stretched in a microbath (190 μl). One end of the skinned fiber was connected to a strain gauge transducer (AE801, Serso Nor, Horten, Norway). The other end was tied to a tungsten hook with a micro-manipulator at 20°C. The isometric tension was measured using an amplifier (DSA601B, NMB, Tokyo, Japan) and the time-tension curve triggered by 25 mM caffeine was recorded on a personal computer (PC9801UX, NEC) through the A/D converter board. The CICR rate was measured as follows. First, the skinned fiber was loaded with a fixed amount of Ca into SR in the presence of 3.5 mM MgATP. Then it was exposed to various concentrations of Ca (free, 0.3, 1.0, 3.0, and 10 μM) at various times (t) from 0.1 to 6 min, which triggered CICR without ATP to prevent Ca uptake. Finally, the remaining amount of Ca in the SR was assayed by 25 mM caffeine, which thoroughly released the entire amount of stored Ca in the SR in the presence of ATP and consequently increased tension. The higher the Ca concentration ex-

Table 1. Experimental conditions of in vitro CHCT

Test	NAHMG	EMHG
Caffeine	0.5, 1, 2, 4, 8, 32 mM[a] maximal contracture plateau	0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 mM[a] maximal contracture plateau
Halothane	3.0% for 10 min	0.5, 1.0, 2.0, 3.0 %[a] for 3 min

[a] Incremental dose

posure, the lower the rise in tension. The area under the time-tension curve was calculated on a personal computer and corresponded to the Ca content in the SR. A control procedure without the triggering CICR step was performed. The control time-tension curve and the area (S_o) were obtained. Next, the test procedure was performed, including the triggering CICR step at one Ca concentration in t minutes. In the same way, the area (S_t) was taken. The Ca release rate was calculated with S_o, S_t, and t by the formula: rate of Ca release (min^{-1}) = $-[\log e\ (S_t/S_o)]/t$. These procedures were repeated at each Ca concentration, and each Ca release rate was calculated[14]. The measurements were finished within 48 h after muscle biopsy and were performed five times per subject. The average CICR rates at various Ca concentrations were calculated. The resulting curve patterns of mean CICR rate at various Ca concentrations were classified into accelerated and unaccelerated groups based on the data from the CICR rate test performed in the Laboratory of Anesthesiology and Critical Care Medicine at the University of Hiroshima. If the CICR rate at each Ca concentration exceeded or was equal to the mean value plus two standard deviations from the normal control data of 45 subjects who were not MHS, the specimen was classified as accelerated. If the CICR rate of the subject was in the 95% confidence area around the mean of a normal distribution curve, the subject was classified as unaccelerated.

Results

The diagnostic classification results using both the CHCT protocols and the CICR rate test, depending on the individual criteria, are shown in Table 2. The results of the CICR rate at each Ca concentration are shown in Table 3. The five normal controls showed normal values in the three tests. Therefore, complete accordance between the CHCT and CICR rate test was obtained in all normal controls. Cases 1 and 2 were diagnosed as having MHS according to the EMHG criteria and as being MH positive according to the NAMHG criteria. Both patients were classified as accelerated according to the CICR rate test. Cases 3 and 4 had a slightly different diagnosis, i.e., MH equivocal with abnormal results from the caffeine test (MHEc) and MH equivocal with abnormal results from the halothane test (MHEh) (see Table 1) according to the EMHG protocol, MH positive according to the NAMHG protocol, and accelerated according to the CICR rate test. MHEc and MHEh may be regarded as MH susceptible from a clinical point of view. Therefore, complete accordance between the results of the CHCT and CICR rate criteria was obtained in the four patients whose MH crisis had been triggered during general anesthesia with halogenated anesthetics and/or succinylcholine (SCC). Cases 9, 10, 14, 15, and 16 were classified as MHS or MHEh according to EMHG criteria and MH positive according to NAMHG criteria, but were classified as unaccelerated according to the CICR test criteria. Discordance between the CHCT and CICR rate test was found in these five cases. Because there is no clinical difference between MHS and MH equivocal (MHE) according to the EMHG definition, these five cases were placed in the same category of MH susceptibility, whether EMHG or NAMHG criteria were used. There was complete accordance between the results of two CHCTs determined by the original version of both MH group protocols in all cases, except for case 16, in whom the EMHG protocol could not be performed because of the time limit. Using a new version of the standard for NAMHG (MH positive: >0.5 g at 3% halothane) [15], cases 10, 14, and 15 were classified as MH negative by NAMHG and MHS or MHEh by EMHG. Therefore, there was still discordance between the CHCT obtained with NAMHG criteria and the CICR rate test in case 9, and there also was discordance between the results of the two CHCTs in cases 10, 14, and 15.

Discussion

CHCT using biopsied muscle fascicles with caffeine alone and halothane alone is considered the most accurate diagnostic test for susceptibility to MH at present [15]. The details of the CHCT protocol for EMHG and NAMHG, such as the dimensions of muscle bundles, conditions of electrical stimuli, and concentrations of test reagents, were standardized. However, there are still some differences in the exposure time of the specimens to the test reagents and in the sequence of the concentrations of halothane and caffeine. The EMHG protocol uses a threshold concentration of caffeine and halothane that produces a 0.2 g contracture. On the other hand, the NAMHG protocol uses a cutoff point of contracture in grams obtained by exposure to a concentration of 2 mM caffeine or 3% halothane as specified in the original version of the protocol. Nevertheless, diagnosis of susceptibility to MH should belong to the same category, whether the EMHG or the NAMHG protocol is used. There is an inherent category difference between the two CHCTs. MHE in the EMHG protocol is not included in the NAMHG protocol. It is reasonable that MH positive in NAMHG may include MHS and MHE in the EMHG protocol from a clinical standpoint. Even if such a definition is accepted, a false negative result must be avoided. Studies subjecting the same specimens to the two CHCT protocols have reported a few cases misclassified into the category of susceptibility to MH in pigs [16] and humans [17]. This study showed

Table 2. Results of CHCT and CICR rate test

Case No.	Age (yr)	Sex	Biopsy due to	NAMHG (g)		EMHG (threshold)		Classification		
				2 mM caffeine	3% Halothare	Caffeine (mM)	Halothare (%)	NAMHG	EMHG	CICR
1	37	M	History of fulminant MH crisis	1.4	1.4	0.5	0.5	MH +	MHS	Accl
2	31	M	Fulminant MH reactions	1.4	4.2	1	1	MH +	MHS	Accl
3	20	M	Fulminant MH reactions	0.3	0.9	2	3	MH +	MHEc	Accl
4	20	F	History of MMR	0	0.3	>4	0.5	MH +	MHEh	Accl
5	23	M	Postoperative fulminant MH	0	−0.2	>4	>3	MH −	MHN	Unac
6	12	F	Postoperative fulminant MH	0	0	3	>3	MH −	MHN	Unac
7	47	M	Abortive MH	0	0	3	>3	MH −	MHN	Unac
8	30	F	History of abortive MH	−0.2	−0.1	>4	>3	MH −	MHN	Unac
9	28	M	Preoperative high CKemia (185 IU·l^{-1})	0.4	1.2	2	1	MH +	MHS	Unac
10	52	M	Preoperative high CKemia (720 IU·l^{-1})	0	0.3	3	2	MH +	MHEh	Unac
11	59	F	Preoperative high CKemia (328 IU·l^{-1})	0	0	4	>3	MH −	MHN	Unac
12	21	F	Preoperative high CKemia (6192 IU·l^{-1})	−0.5	0	3	3	MH −	MHN	Unac
13	58	M	Preoperative high CKemia (466 IU·l^{-1})	0	−0.2	>3	>3	MH −	MHN	Unac
14	41	M	Rhabdomyolysis	0	0.2	3	2	MH +	MHEh	Unac
15	19	M	Neuroleptic malignant syndrome	0.2	0.3	2	2	MH +	MHS	Unac
16	69	F	Mother of case No. 1	0.3	0.2	—	—	MH +	—	Unac
17	47	F	Normal control	0	−0.1	>4	>3	MH −	MHN	Unac
18	31	F	Normal control	0.1	0	>3	>3	MH −	MHN	Unac
19	52	F	Normal control	0	−0.1	4	>3	MH −	MHN	Unac
20	76	M	Normal control	0.1	0	>4	>3	MH −	MHN	Unac
21	68	M	Normal control	−0.1	0	>4	>3	MH −	MHN	Unac

NAMHG, North American Malignant Hyperthermia Group; EMHG, European Malignant Hyperthermia Group; MHS, malignant hyperthermia susceptible; MHN, MH nonsusceptible; MHEh, MH equivocal with abnormal results from halothane test; MHEc, MH equivocal with abnormal results from caffeine test; MMR, masseter muscle rigidity; Accl, accelerated; Unac, Unaccelerated. MH positive is defined by NAMHG cutoff point if $a >= 0.2$ g contracture after exposure to 3% halothane or $a >= 0.2$ g contracture after exposure to 2 mM caffeine is demonstrated [13]. EMHG protocol classifies patients into three groups according to the following criteria [11, 12]: MHS: caffeine threshold (0.2 g) at caffeine concentration of 2 mM or less, and halothane threshold of 2% halothane or less. MHN: caffeine threshold at caffeine concentration of 2 mM or more without halothane threshold at 2% halothane or less. MHE: all other results

Table 3. Mean rate of Ca release at free, 0.3, 1.0, 3.0, and 10 μM Ca^{2+} and classification of CICR rate in 21 cases

No.	Age (yr)	Sex	Classification	*n*	Rate of Ca release (min^{-1})				
					Ca^{2+} free[a]	0.3 μM Ca^{2+}	1.0 μM Ca^{2+}	Ca^{2+} 3.0 μM	Ca^{2+} 10 μM
1	37	M	Accl	6	0.098 ± 0.035	0.148 ± 0.094	0.515 ± 0.308	2.381 ± 1.247	4.063 ± 1.220
2	31	M	Accl	5	0.128 ± 0.040	0.239 ± 0.043	1.102 ± 0.375	5.638 ± 2.590	10.71 ± 5.910
3	20	M	Accl	4	0.113 ± 0.049	0.107 ± 0.062	0.180 ± 0.070	1.145 ± 0.405	3.796 ± 0.574
4	20	F	Accl	4	0.105 ± 0.038	0.095 ± 0.021	0.181 ± 0.029	1.030 ± 0.168	4.464 ± 0.168
5	23	M	Unac	4	0.042 ± 0.012	0.048 ± 0.015	0.067 ± 0.004	0.272 ± 0.034	0.954 ± 0.185
6	12	F	Unac	4	0.057 ± 0.023	0.056 ± 0.022	0.091 ± 0.028	0.445 ± 0.196	1.345 ± 0.613
7	47	M	Unac	4	0.083 ± 0.042	0.082 ± 0.040	0.097 ± 0.025	0.554 ± 0.190	2.013 ± 1.330
8	30	F	Unac[b]	4	0.131 ± 0.049	0.077 ± 0.024	0.108 ± 0.056	0.589 ± 0.289	2.021 ± 1.310
9	28	M	Unac	4	0.068 ± 0.022	0.034 ± 0.008	0.092 ± 0.035	0.372 ± 0.128	1.141 ± 0.289
10	52	M	Unac	4	0.073 ± 0.017	0.048 ± 0.004	0.073 ± 0.018	0.409 ± 0.175	1.485 ± 0.258
11	59	F	Unac	4	0.055 ± 0.010	0.059 ± 0.031	0.071 ± 0.037	0.404 ± 0.183	1.719 ± 0.396
12	21	F	Unac	4	0.047 ± 0.012	0.042 ± 0.005	0.055 ± 0.012	0.309 ± 0.100	1.205 ± 0.321
13	58	M	Unac	4	0.058 ± 0.027	0.062 ± 0.012	0.079 ± 0.019	0.317 ± 0.086	2.633 ± 0.735
14	41	M	Unac	4	0.083 ± 0.019	0.051 ± 0.013	0.078 ± 0.022	0.303 ± 0.120	1.207 ± 0.640
15	19	M	Unac	4	0.068 ± 0.018	0.050 ± 0.009	0.077 ± 0.012	0.397 ± 0.142	1.000 ± 0.261
16	69	F	Unac	4	0.063 ± 0.013	0.045 ± 0.010	0.071 ± 0.014	0.345 ± 0.067	0.960 ± 0.183
17	47	F	Unac	4	0.055 ± 0.018	0.043 ± 0.007	0.065 ± 0.009	0.271 ± 0.056	1.631 ± 0.317
18	31	F	Unac	2	0.045	0.050	0.069	0.323	1.260
19	52	F	Unac	4	0.068 ± 0.013	0.052 ± 0.014	0.082 ± 0.027	0.365 ± 0.045	1.206 ± 0.155
20	76	M	Unac	3	0.064 ± 0.020	0.052 ± 0.009	0.070 ± 0.008	0.291 ± 0.099	1.077 ± 0.259
21	68	M	Unac	4	0.073 ± 0.015	0.049 ± 0.016	0.081 ± 0.020	0.450 ± 0.189	1.333 ± 0.380
			Mean+2 × SD of 45 normal subjects		0.119	0.090	0.139	0.673	3.043

Accl, accelerated; Unac, unaccelerated. All values of cases are expressed as means ± SD except for No.18, for which only the mean is given

[a] Rate of Ca release at Ca^{2+} free means rate of Ca leakage from SR, not CICR rate

[b] The rate only at Ca^{2+} free (Ca leakage rate from SR) was over mean + 2 × SD of normal subjects, and the rates at other Ca^{2+} concentrations were within normal limits in case No. 8. Therefore, we classified this case into the unaccelerated group

no misclassification between the two original versions of the CHCT carried out on 15 patients (case 16 was excluded) and 5 normal controls. From clinical decisions, we obtained 100% accordance between the results using the original protocols of the EMHG and the NAMHG. This result demonstrates that the two original CHCTs appear to be accurate tests for detecting MHS. After the definition of the specified threshold of muscle tension was changed in the new version of the NAMHG protocol, the sensitivity and specificity of the protocol appeared to reach an acceptable level [18]. However, when the new version of the cutoff point (>0.5 g at 3% halothane, >0.3 g at 2 mM caffeine in the NAMHG protocol) was applied to our results, the accordance rate between the two CHCTs decreased to 83%.

Several types of skinned-fiber tests for diagnosis of MH have been reported [19, 20]. This is the test to determine the sensitivity of skinned fibers for contracture obtained by caffeine or halothane according to the same principle used to measure the threshold of contracture in CHCT. One comparative study showed that the caffeine skinned fiber tension test showed a strongly positive relationship with CHCT, and the authors concluded that this test could be used as a complement test because of a decrease in the required sample size and a longer possible time period from biopsy to test, but not that the test could be used as a substitute for CHCT [19]. Using 50% glycerol-EGTA pretreated skinned fibers, Adnet et al. showed a significantly higher sensitivity to caffeine in type I fibers (slow-twitch, slow oxidative) than in type II fibers (fast-twitch, fast glycolytic) from MHS and MH nonsusceptible patients [21]. The difference in Ca sensitivity of the contractile system between skeletal muscle fiber types in humans was found in EGTA-treated skinned fiber in which SR function was destroyed. The type I fiber was reported to have a lower threshold for causing tension than the type II fiber [22]. The CICR rate test proposed by Endo et al. using saponin-treated skinned fibers which kept SR function measures the relative rate of release of Ca from SR after stepwise exposure to triggering ionic Ca concentrations. This test does not depend on the Ca sensitivity of the contractile system and uptake of Ca into the SR, but it does depend on the Ca releasing function in the SR. Therefore, the CICR rate has been considered not to differ between fiber types, and thus the test may be an alternative diagnostic method for MHS that identifies the functional acceleration of the Ca-induced Ca release mechanism in the SR. This test

may detect a defect in the Ca release channel of the SR. However, there is no direct evidence of accordance between the genotype of the ryanodine receptor and the phenotype of the CICR rate test result. There have been several reports on accelerated Ca-induced Ca release reaction using fragmented SR separated from MHS swine [23–25]. Using this chemically skinned fiber with saponin, Ohta and coworkers [14] demonstrated that SR from the MHS swine showed an accelerated rate of CICR, but SR from a normal pig did not. The human CICR rate test using a saponin-skinned fiber demonstrated that approximately 80% of fulminant MH cases based on body temperature criteria in Japan were classified as accelerated according to the CICR rate test [7]. Our cases, except for the five normal controls, were suspected of being MHS because of their medical history and/or laboratory data. Four cases (cases 1, 2, 3, and 4) in which MH reactions occurred during anesthesia were classified into the same category of MHS as determined by two CHCTs and the CICR rate test. Therefore, none of the three diagnostic tests resulted in false positive and/or false negative findings, because five normal controls and four MH cases were categorized as normal and MHS. Postoperative hyperthermia cases (case 5 and 6) were determined to be normal by the two CHCTs and the CICR rate test. Cases with preoperative high serum creatine kinase levels (high CKemia), however, were placed in the heterogeneous classification. Different pathophysiologic mechanisms may cause the elevation of CK levels at rest. MH is one of the diseases in which high CKemia occurs at rest. However, some cases of MH showed normal CK levels at rest [26]. Most cases of high CKemia have myopathy. The pathology of skeletal muscle in case 9 revealed that the biceps muscle contained fibers of various sizes, predominantly type II fibers. Even when a new version of the NAMHG criteria was used, this case was still determined to be a discordant case that was categorized as MHS according to the two CHCTs and as unaccelerated according to the CICR rate test. The other two cases of high CKemia showed discordance between EMHG and NAMHG according to the new criteria. In case 10, hypothyroidism was identified by the increased serum T3 level, and after supplementary treatment with thyroid hormone, the T3 level became normal. Knee surgery was performed uneventfully under spinal anesthesia. The CK level became normal after the surgery. Two cases of abortive MH based on temperature criteria were determined to be normal by the three diagnostic tests. These patients may have had a different entity that causes MH-like reactions. Probably a special test, such as the 1% halothane plus caffeine test [27] or the 1% halothane plus SCC test [28], would reveal the entity causing the defect.

Abnormal contracture of muscle fascicles exposed to 3% halothane is caused, not only by extraordinary sensitivity of the SR to a triggering ionized Ca for the release of Ca, but also by a high level of inositol 1, 4, 5-trisphosphate in the excitation-contraction coupling sites [29]. Hypersensitivity of contractile protein to ionized Ca may also increase the contractility of actomyosin interaction [30]. Any phenomenon that induces an increase of ionic Ca concentration in cytoplasm may cause an in vitro abnormal contracture of the muscle bundle [16]. All cases with an accelerated CICR rate showed abnormal results in the CHCT. It was found that halothane and caffeine increased the CICR rate even in fascicles from normal controls as well as those from MHS patients [9]. Muscle bundles that are particularly sensitive to the CICR mechanism have a left-shifted dose-response curve of the Ca release rate on a series of triggering Ca concentrations without the presence of caffeine or halothane. It is reasonable to assume that muscle fascicles with abnormalities in te Ca-induced Ca release mechanism in the SR have a lower threshold of contraction to caffeine and/or halothane.

The MH-positive results obtained by CHCT in patients with mutations in the Ca release channel of the SR are indistinguishable from the results obtained in patients predisposed by mutation of the dihydropyridine receptor [5] or sodium-channel receptors in the transverse tubules [6]. After treatment with saponin, the sarcolemma and transverse tubules of the muscle bundle presumably are not intact in the excitation-contraction coupling mechanism. It is not clear whether or not muscle fascicles with a defect in the sarcolemma, transverse tubules, or other organelles retain an enhanced CICR mechanism. The CICR rate test using a saponin-treated skinned fiber may detect only the abnormalities in the SR. MHS may include heterogeneous disorders affecting the regulation of the ionic Ca concentration in cytoplasm, resulting not only in defects in the Ca release channel of the SR but also in defects in organelles regulating Ca metabolism. A positive response to the CHCT has been reported in presumed MH-negative subjects [31], as well as in patients with various neuromuscular disorders [32] and neuroleptic malignant syndrome [33]. Any genetic or acquired disorder in cytoplasmic Ca ion control may result in an in vitro abnormal contracture of the muscle bundle. Therefore, we accept that there is some discordance between an abnormal contracture result in the CHCT and normal (unaccelerated) CICR rate in patients suspected of having MHS.

In conclusion, this comparative study using three MHS diagnostic tests on the same specimens suggests that the entity of the positive result is not homogeneous, but heterogeneous. The CICR rate test using a chemically skinned fiber with saponin can identify the abnor-

mal CICR mechanism in the SR. Therefore, it may be acceptable that there are discordances between the results obtained by the CHCT and those obtained by the CICR rate test on the same muscle fascicles from patients suspected of having MHS. The CHCT is an accurate and useful screening test for MHS caused by various abnormal functions of cell organelles in skeletal muscle. The CICR rate test may specify an abnormal Ca release mechanism in the SR and may exclude other defects causing MH. Therefore, it is important that, in muscle biopsy for the diagnosis of MHS, the CHCT be combined with specifying tests, such as the ryanodine-contracture test [34] and/or the CICR rate test.

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