4-Chloro-m-cresol is a Trigger of Malignant Hyperthermia in Susceptible Swine

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Background: 4-Chloro-*m*-cresol (4-CmC) induces marked contractures in skeletal muscle specimens from individuals susceptible to malignant hyperthermia (MHS). In contrast, 4-CmC induces only small contractures in specimens from normal (MHN) patients. 4-CmC is a preservative within a large number of commercially available drug-preparations (*e.g.*, insulin, heparin, succinylcholine), and it has been suggested that 4-CmC might trigger malignant hyperthermia. This study was designed to investigate the effects of 4-CmC *in vivo* and *in vitro* in the same animals.

Methods: After approval of the animal care committee, six Pietrain MHS and six control (MHN) swine were anesthetized with azaperone 4 mg/kg intramuscularly and metomidate 10 mg/kg intraperitoneally. After endotracheal intubation, lungs were mechanically ventilated (inspired oxygen fraction 0.3) and anesthesia was maintained with etomidate $2.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and fentanyl $50 \ \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Animals were surgically prepared with arterial and central venous catheters for measurement of hemodynamic parameters and to obtain blood samples. Before exposure to 4-CmC *in vivo*, muscle specimens were excised for *in vitro* contracture tests with 4-CmC in concentrations of 75 and 200 μM. Subsequently, pigs were exposed to cumulative administration of 3, 6, 12, 24, and 48 mg/kg

4-CmC intravenously. If an unequivocal episode of malignant hyperthermia occurred, as indicated by venous carbon dioxide concentration ≥ 70 mmHg, pH ≤ 7.25, and an increase of temperature ≥ 2°C, the animals were treated with dantrolene, 3.5 mg/kg.

Results: All MHS swine developed malignant hyperthermia after administration of 4-CmC in doses of 12 or 24 mg/kg. Venous carbon dioxide concentration significantly increased and pH significantly decreased. Temperature increased in all MHS animals more than 2°C. Blood lactate concentrations and creatine kinase levels were significantly elevated. All MHS swine were treated successfully with dantrolene. In contrast, no MHN swine developed signs of malignant hyperthermia. After receiving 4-CmC in a concentration of 48 mg/kg, however, all MHN animals died by ventricular fibrillation. The *in vitro* experiments showed that both concentrations of 4-CmC produced significantly greater contractures in MHS than in MHN specimens.

Conclusions: 4-CmC is *in vivo* a trigger of malignant hyperthermia in swine. However, the 4-CmC doses required for induction of malignant hyperthermia were between 12 and 24 mg/kg, which is about 150-fold higher than the 4-CmC concentrations within clinically used preparations. (Key words: Caffeine; dantrolene; halothane; *in vitro* contracture test.)

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MALIGNANT hyperthermia (MH) is an uncommon inherited disorder of skeletal muscle, characterized by a hypermetabolic response to all commonly used inhalational anesthetics and depolarizing muscle relaxants.[1] The clinical syndrome includes muscle rigidity, hypercapnia, tachycardia, and myoglobinuria as result of increased CO_2 production, oxygen consumption, and muscle-membrane breakdown. In humans and animals susceptible to MH (MHS) it is generally accepted that an increase in the level of myoplasmic free Ca^{2+} is the cause of the syndrome.[2] Various hypotheses have been proposed to account for the increase of intracellular Ca^{2+} levels, *e.g.*, a defect in the Ca^{2+} release channel of the sarcoplasmic reticulum (ryanodine receptor),[3,4] an abnormality in the excitation–contraction coupling mechanisms,[5] and alterations in the inositol phosphate metabolism of skeletal muscles.[6-8] The pharmacology of MH in humans is complex, and it has been suggested that either physiologic or pharmacologic mediators may cause or modulate MH syndrome.[5] A variety of different

drugs has been proposed to trigger MH. This assessment is underlined by the fact that a large number of substances can activate the ryanodine receptor (*e.g.*, volatile anesthetics, caffeine, cresol).[9] Thus, it has been speculated that stimulation with these drugs of the abnormal ryanodine receptor in MHS individuals can induce an excessive increase of intracellular Ca^{2+} levels and subsequently trigger MH.

In 1986 it was shown that a commercial preparation of succinylcholine containing 4-chloro-*m*-cresol (4-CmC) as preservative induced contractures in skeletal muscle specimens from MHS swine, whereas pure succinylcholine produced no contracture.[10] Thus, the authors used 4-CmC as test drug and demonstrated *in vitro* contractures in MHS muscles but not in specimens from MH normal (MHN) animals. These results were confirmed by *in vitro* contracture studies in skeletal muscle from MHS patients.[11,12] These observations appear to be explained by results of experiments in which 4-CmC was found to be a specific activator of ryanodine receptor mediated Ca^{2+} release in skeletal muscle cells.[13] Recent studies showed that 4-CmC affinity of $[^{3}H]$-ryanodine binding to MHS vesicles was two-fold greater than that in normal tissue.[14] Furthermore, 4-CmC increased the open probability of the isolated Ca^{2+} release channel in the same concentration range effective in $[^{3}H]$ryanodine binding and Ca^{2+} flux measurements.[15]

Regarding the results of *in vitro* contracture and biochemical studies with 4-CmC and its wide distribution as a preservative in a large number of preparations (*e.g.*, insulin, heparin), it has been suggested that 4-CmC might be a trigger agent for MH. The purpose of the current study was therefore to investigate for the first time the effects of 4-CmC *in vivo* and *in vitro* in the same MHS and MHN swine.

Materials and Methods

Following approval by the animal care committee, six MHS Pietrain (weight, 28.4 ± 2.9 kg) and six MHN Hampshire (27.6 ± 3.1 kg) swine from a special breeding program at the Federal Breeding Center in Mölln, Germany, were investigated. The genotype of MH susceptibility was determined by DNA analyses from ear tissue of the pigs used in this study. Susceptibility to MH also was verified using the *in vitro* contracture test (IVCT) method.[16]

In Vivo *Experiments*

Each animal was fasted overnight with free access to water. All animals were premedicated with azaperone 4 mg/kg intramuscularly (Stresnil, Janssen, Neuss, Germany) and metomidate 10 mg/kg intraperitoneally (Hypnodil, Janssen, Neuss, Germany). Subsequently an intravenous catheter was inserted percutaneously into an ear vein. The animals were initially anesthetized with intravenous etomidate 0.3 mg/kg (Hypnomidate, Janssen, Boulogne, France) and the trachea intubated without administration of a muscle relaxant. After endotracheal intubation, lungs were mechanically ventilated (inspired oxygen fraction 0.3) and anesthesia was maintained with etomidate 2.5 mg · kg^{-1} · h^{-1} intravenously, fentanyl 50 μg · kg^{-1} · h^{-1} intravenously, and nitrous oxide (66%) in oxygen. Animals were surgically prepared with arterial and central venous catheters for measurement of hemodynamic parameters and to obtain blood samples. A cannula was inserted into the femoral artery for pressure monitoring and blood sampling. A multilumen central venous catheter was placed into the right internal jugular vein for blood sampling (lumen 1), administration of the test drug (lumen 2), and anesthetics (lumen 3). A second cannula was inserted in a femoral vein for additional drug (dantrolene) or fluid infusion (5–10 ml · kg^{-1} · h^{-1} intravenously of Ringer's solution. Before MH was triggered *in vivo*, muscle specimens were excised from hind limb for subsequent IVCTs. During all surgical procedures, the animals were maintained normothermic at 37.0 ± 0.2°C using both radiant heat applied to the body and warming blankets.

Blood gases (arterial oxygen saturation [Sa_{O_2}], venous carbon dioxide pressure [P_{CO_2}], and pH) and blood lactate were monitored by analyzing fresh arterial and venous samples using a blood gas analyzer (ABL625, Radiometer, Copenhagen, Denmark). Mechanical ventilation was adjusted to maintain P_{CO_2} at 40 ± 2 mmHg. The rate and volumes of the inspired gases remained constant for the rest of the study. Once ventilation was adjusted to provide a stable, normal P_{CO_2} (minimum of 30 min), resting values for all variables were obtained.

After this control period the *in vivo* experiments were started by cumulative administration of 4-CmC. Administration of 4-CmC started with a dosage of 3 mg/kg intravenously, followed by increasing doses of 6, 12, 24, and 48 mg/kg intravenously, each over 20 min. Once an unequivocal episode of MH occurred, as indicated by $P_{CO_2} \geq 70$ mmHg, pH ≤ 7.25, and an increase of temperature of ≥ 2°C, administration of further doses of

4-CmC was stopped. and the animals were treated with dantrolene 3.5 mg/kg intravenously.

During the experiments hemodynamic variables (heart rate, mean arterial pressure, and central venous pressure), end-tidal carbon dioxide concentration (ET_{CO_2}), rectal temperature (in degrees Celsius), blood gas concentrations (Sa_{O_2}, P_{CO_2}, and pH) and lactate levels were measured every 5 min. Creatine kinase (CK) in serum was measured each 10 min. After all experiments were completed, the pigs were killed using magnesium chloride solution (10%).

In Vitro Experiments

Muscle bundles were excised carefully and stored in Krebs-Ringer solution equilibrated with carbogen (95% oxygen:5% carbon dioxide). The muscle bundles then were dissected into 6–10 strips (length: 15–25 mm; width: 2–3 mm; weight: 120–250 mg). Only viable muscle samples (twitch response to supramaximal stimulation ≥ 10 mN) were used for the IVCTs according to the European MH Group (EMHG) protocol.[16] Each muscle specimen was secured with silk sutures to a fixed point and connected with a force-displacement transducer (Lectromed, Welwyn Garden City, UK). The specimens were suspended in a 20-ml tissue bath perfused with Krebs-Ringer solution bubbled with carbogen continuously; temperature was kept constant at 37°C; pH was 7.4.

The muscles were stimulated electrically with square waves to achieve a supramaximal response by the HSE Stimulator Type 215/I (Hugo Sachs Elektronik, March, Germany) with a duration of 1 ms and a frequency of 0.2 Hz. Contracture curves were displayed on a Linseis L2200 II (Selb, Germany) and recorded with a computer-based data-evaluation program (MusCo, RS BioMedTech, Sinzing, Germany). The resting lengths of the specimens were measured before testing, and the initial baseline tension prior to testing was achieved by stretching the samples slowly to 150 ± 10% of the resting length.

Swine were first classified by IVCT according to the procedure of the EMHG.[16] In each animal a minimum of two samples was tested with each drug. The IVCT gave halothane and caffeine thresholds as follows: MHS = muscle contractures ≥ 2 mN at a caffeine concentration of 2.0 mM or less and a halothane threshold concentration of 0.44 mM or less; MHN = muscle contractures ≥ 2 mN at a caffeine concentration of 3.0 mM or more and a halothane threshold concentration greater than 0.44 mM.

After investigation of MH susceptibility, viable muscle specimens were subjected to in vitro contracture tests with 4-CmC. After stable baseline tension for at least 10 min was achieved, 4-CmC was administered in concentrations of 75 and 200 μM as bolus to the tissue bath. The in vitro effects of 4-CmC were observed for at least 45 min and included measurement of contracture development and muscle-twitch responses.

The following chemicals were used: 4-CmC (Aldrich-Chemie, Steinheim, Germany; purity > 99%), dantrolene (Procter and Gamble, Weiterstadt, Germany), caffeine (Sigma, Deisenhofen, Germany), and halothane (Hoechst, Frankfurt, Germany). Solutions were prepared fresh before each investigation in carboxygenated Krebs-Ringer solution at 37°C and administered directly to the tissue bath.

Statistical Analysis

Statistical evaluation of our data was performed using a computer-based statistical program (SPSS, Chicago, IL). Unless otherwise indicated, the data from the in vivo experiments are indicated as means ± SD. The data obtained during the in vivo experiments were compared with two-way analysis of variance followed by Mann-Whitney and Student t tests.

The in vitro data are presented as medians and ranges. The effects of 4-CmC on contracture development were assessed with repeated-measures analysis of variance. If appropriate, subsequent comparisons were performed using Scheffé's post hoc method. Results were considered significant if P values were less than 0.05.

Results

All MHS swine developed typical signs of MH during administration of the 12- or 24-mg/kg dose of 4-CmC. MH syndrome could be treated successfully with dantrolene in all susceptible animals. In contrast, none of the MHN swine developed signs of MH during the experiments. Therefore, the highest 4-CmC concentration of 48 mg/kg was administered. Five of six MHN animals died directly after this dose because of ventricular fibrillation.

Heart rate increased after administration of 4-CmC in all MHS swine. Following 12 mg/kg 4-CmC, heart rate increased significantly from 80 ± 7 beats/min to 111 ± 19 beats/min, and to 135 ± 22 beats/min after 24 mg/kg 4-CmC. In contrast, heart rate in control swine remains stable between 61 ± 5 and 86 ± 9 beats/min. Baseline values for mean arterial pressure were comparable in both groups. In the MHS group a slight decrease from 76 ± 5 mmHg to 65 ± 7 mmHg was observed, whereas
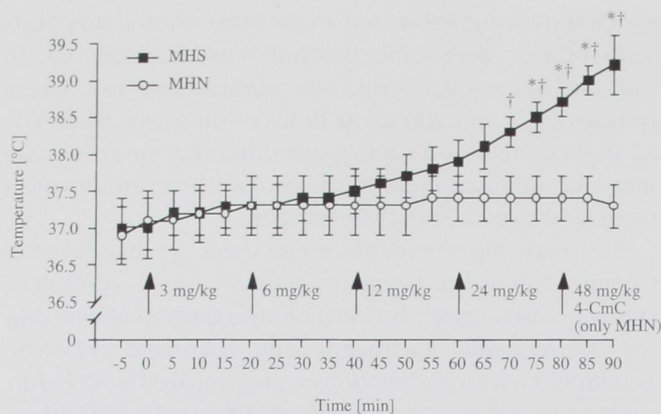
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Fig. 1. Effects of cumulative intravenous administration of 4-chloro-*m*-cresol (4-CmC) on rectal temperature of malignant hyperthermia susceptible (MHS) swine (*filled squares*; n = 6) and normal (MHN) swine (*open circles*; n = 6). Data are mean ± SD. *P < 0.05 *versus* MHN, †P < 0.05 *versus* baseline at 0 min.

in the MHN group mean arterial pressure showed no changes following administration of the test drug. 4-CmC induced a significant increase in the body temperature of the MHS animals of more than 2°C (fig. 1), but in none of the control swine.

Following 12 mg/kg 4-CmC, P_{CO_2} in the MHS group increased from normal baseline values significantly up to 67.6 ± 6.6 mmHg (fig. 2). After 24 mg/kg 4-CmC, P_{CO_2} increased to a maximum of 86.0 ± 8.2 mmHg. In the MHN group no increase of venous P_{CO_2} was registered. These venous P_{CO_2} changes were reflected in (ET_{CO_2}) measurements with a maximum of 79.5 ± 8.6 mmHg in the MHS group and normal values in the MHN group. Sa_{O_2} decreased following cumulative administration of
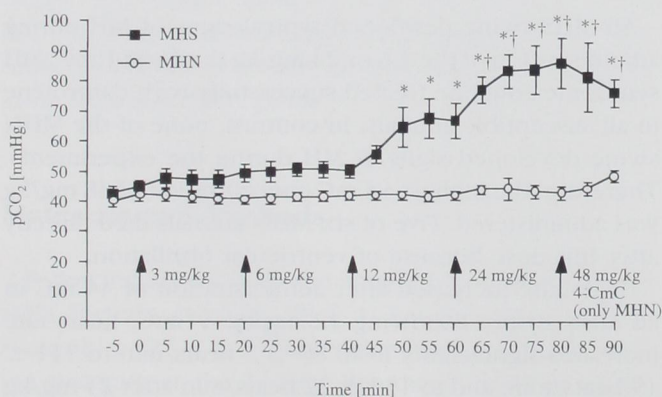

Fig. 2. Effects of cumulative intravenous administration of 4-chloro-*m*-cresol (4-CmC) on venous carbondioxide concentration (P_{CO_2}) of malignant hyperthermia susceptible (MHS) swine (*filled squares*; n = 6) and normal (MHN) swine (*open circles*; n = 6). Data are means ± SD. *P < 0.05 *versus* MHN, †P < 0.05 *versus* baseline at 0 min.
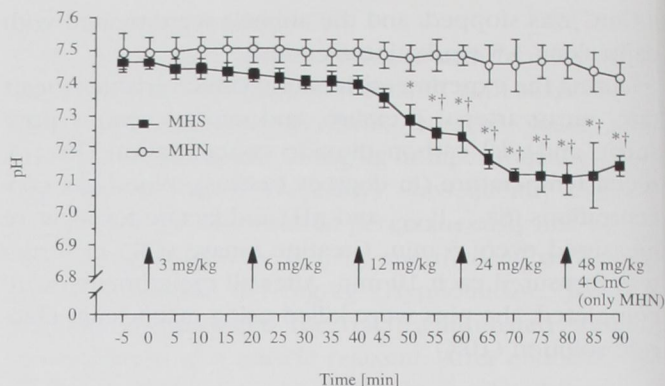

Fig. 3. Effects of cumulative intravenous administration of 4-chloro-*m*-cresol (4-CmC) on pH of malignant hyperthermia susceptible (MHS) swine (*filled squares*; n = 6) and normal (MHN) swine (*open circles*; n = 6). Data are mean ± SD. *P < 0.05 *versus* MHN, †P < 0.05 *versus* baseline at 0 min.

4-CmC from 100% to a minimum of 83.6 ± 3.3% in the MHS swine. In the controls Sa_{O_2} was stable throughout the experiments.

Administration of 12 mg/kg 4-CmC induced a significant decrease of pH to 7.23 ± 0.05 in the MHS group, and the highest concentration of 4-CmC used in this protocol led to a decrease to 7.1 ± 0.05 (fig. 3). In the MHN animals only a slight decrease of pH from 7.48 ± 0.06 to 7.43 ± 0.03 was measured. All MHS animals showed a significant enhanced venous lactate concentration after receiving 4-CmC of 5.2 ± 0.6 μM (12 mg/kg) and 7.4 ± 0.8 μM (24 mg/kg), whereas none of the MHN swine developed changes in venous lactate concentration during the experiments. Mean CK in rest were significantly elevated in the MHS group to 1286 ± 378 U/l compared with the values in the control group of 402 ± 56 U/l. Throughout the investigation CK values increased in the MHS group to a maximum of 2,525 ± 450 U/l. The CK concentration in the MHN group remained unchanged.

Once an unequivocal MH episode occurred the animals were treated with dantrolene. Administration of dantrolene reversed the syndrome induced by 4-CmC in all cases. Heart rate decreased to 100.0 ± 17.5 beats/min and mean arterial pressure to 75.3 ± 3.4 mmHg. Temperature decreased to 37.8 ± 0.5°C. Venous P_{CO_2} was 54.3 ± 4.3 mmHg, and ET_{CO_2} concentration decreased to 52.0 ± 3.1 mmHg. Sa_{O_2} normalized under dantrolene treatment and reached values of 98.2 ± 0.5%. The pH increased to 7.28 ± 0.02, lactate levels decreased to 4.6 ± 0.8 mM, and CK values decreased to 1,108 ± 398 U/l.

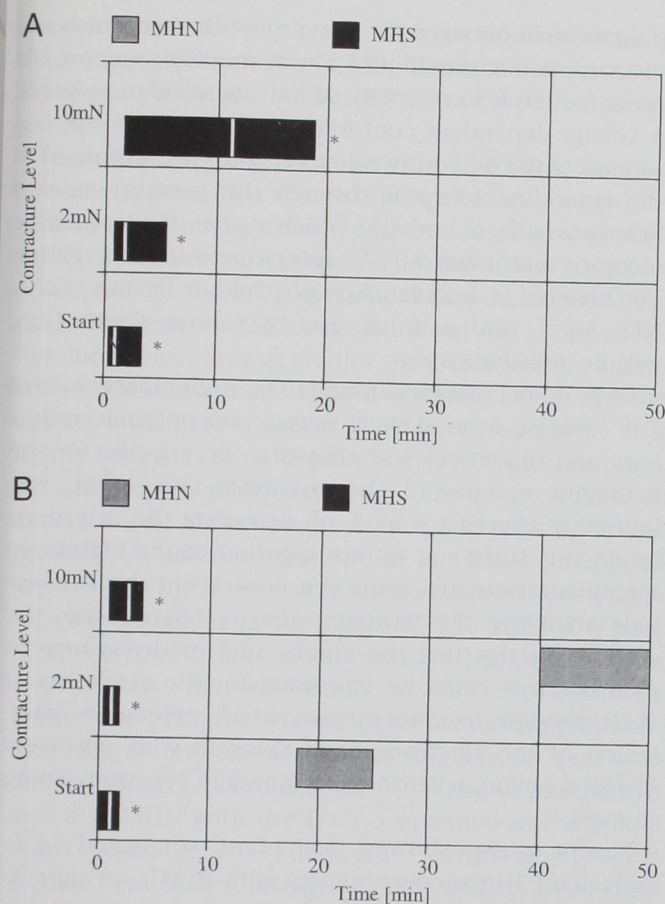Skeletal muscle specimens from all MHS swine devel-

Fig. 4. Contracture development following bolus administration in concentrations of 75 μM (*A*) and 200 μM (*B*) 4-chloro-*m*-cresol (4-CmC) in skeletal muscle preparations of malignant hyperthermia susceptible (MHS; n = 12) and normal (MHN; n = 12) swine. Contracture course is characterized by the attainment of three different levels in minutes: start of contracture development, contractures of 2 mN, and contractures of 10 mN. Data are medians and ranges. *$P < 0.05$ *versus* MHN.

oped abnormal contracture responses to halothane and caffeine, whereas none of the control specimens produced abnormal contractures according to the EMHG protocol verifying the MH status. Administration of 4-CmC produced contractures in all MHS specimens. In contrast, after administration of 75 μM 4-CmC none of the MHN specimens developed contractures, and 200 μM 4-CmC produced contractures in only 3 of 12 MHN specimens. The results of the different concentrations of 4-CmC at the distinct contracture levels are shown in figures 4A and B. Contractures following 75 μM 4-CmC started after 0.5 min, reached a contracture level of 2 mN after 1.6 min, and reached the contracture level of 10 mN after 12.0 min (fig. 4A). The contracture maximum of 20 mN was reached after 27.9 min (fig. 5). In the MHN

preparations no contractures were observed. After administration of 200 μM 4-CmC the different contracture levels in the MHS group were reached after 0.5 min (start), 0.9 min (2 mN), and 1.5 min (10 mN) as shown in figure 4B. The maximum of contracture development of 25 mN was achieved after 7.1 min (fig. 5). In the MHN specimens only small contractures were observed in response to 200 μM 4-CmC. Contracture start and level of 2 mN were only reached in 3 of 12 specimens, and none of the specimens developed contractures of $\geq$ 10 mN. There was no overlap in the range of times between both groups.

Discussion

Our results demonstrate that 4-CmC in very large doses is a trigger of MH in MHS swine. Further, dantrolene administration treated cresol-induced MH syndrome successfully. In contrast, none of the MHN swine developed signs of MH. Under *in vitro* conditions 4-CmC produces marked contractures in skeletal muscles from MHS but only small contractures in control specimens. Based on these findings, it is suggested that 4-CmC is a trigger of MH and can be used as a specific test agent for diagnosis of MH susceptibility.

Derivatives of cresol are commonly used in pharmaceutical drug preparations such as insulin, hormones, and heparin as preservatives. Regarding the results from biochemical, pharmacologic, and previous contracture
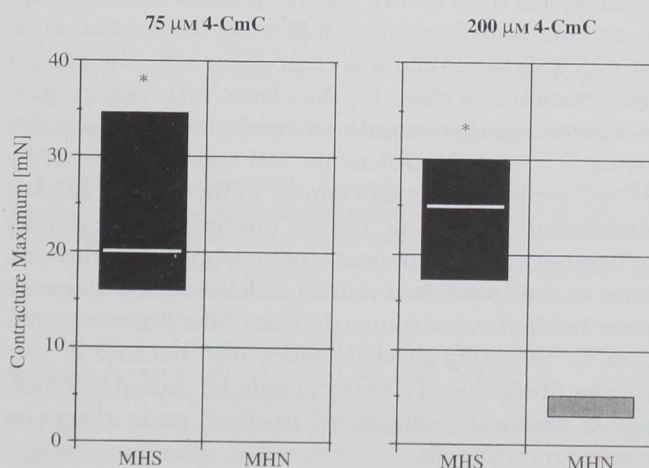

Fig. 5. Contracture maximum following bolus administration in concentrations of 75 μM (*left*) and 200 μM (*right*) 4-chloro-*m*-cresol (4-CmC) in skeletal muscle preparations of malignant hyperthermia susceptible (MHS; n = 12) and normal swine (MHN; n = 12). Data are medians and ranges. *$P < 0.05$ *versus* MHN.

studies it has been suggested that cresol derivatives could trigger MH. Furthermore, a case of MH syndrome after administration of an insulin preparation containing cresol in a patient with ketoacidotic diabetic decompensation was presented recently.[17] After treatment with the insulin, this patient became restless and complained of increasing myalgia. His temperature rose immediately from 37.6°C up to 41.4°C. He lost consciousness, and respiration was insufficient. The patient required endotracheal intubation and mechanical ventilation. Because of the similarity between this clinical picture and MH, dantrolene was administered and the patient was cooled. Under these conditions the patient's body temperature decreased to 37.5°C within 75 min. Minute ventilation was reduced continuously 3 h after the temperature peak of 41.7°C. CK values in blood reached a maximum of 12,848 U/l on the 5th day and came back to normal limits within 14 days. Three months later this patient was diagnosed as MHS by the IVCT. During this clinical course the patient did not receive any of the "classic" MH triggers such as volatile anesthetics and succinylcholine. Therefore, it has been suggested that the cresol within the insulin preparation induced this syndrome. It has been discussed that this could also be a case of human stress syndrome. However, the patient had received catecholamines for therapy of hemodynamic instability after the MH episode without any MH-type signs and had no signs of stress symptoms prior or after this clinical episode.

In these *in vivo* experiments, all MHS swine developed typical signs of MH, *e.g.*, tachycardia, hypercapnia, fever, and muscle rigidity, following administration of 4-CmC. This syndrome was comparable with the clinical presentation described for the classic MH triggers such as volatile anesthetics and succinylcholine in susceptible swine.[18,19] With dantrolene the MH episodes induced by 4-CmC were treated successfully. In the control animals administration of 4-CmC did not produce muscle rigidity or hemodynamic, pulmonary, or metabolic disturbances; none of the established criteria for clinical MH diagnosis were fulfilled. After administration of the highest 4-CmC dose of 48 mg/kg all MHN swine died because of ventricular fibrillation. This effect might be caused by blockade of cardiac K^+ channels[20] or direct toxic effects on cardiac muscle cells.

It is widely believed that MH susceptibility is caused by abnormal calcium metabolism within the skeletal muscle fiber.[2] The site of this defect presumably is a calcium-release channel of the skeletal muscle sarcoplasmic reticulum, *e.g.*, the ryanodine receptor, which is the foot-

plate protein between the dihydropyridine receptor and the sarcplasmic reticulum.[5] The ryanodine receptor is a protein complex consisting of four identical monomers. A voltage-dependent conformational change in the α_{1S}-subunit of the dihydropyridine receptor is transmitted to the ryanodine receptor through the protein segment linking motifs II and III, which opens the ryanodine receptor and induces Ca^{2+} release into the myoplasm. The channel is modulated by physiologic ligands (Ca^{2+}, ATP, Mg^{2+}, and so forth) and by various agents (*e.g.*, volatile anesthetics).[5]

4-Chloro-*m*-cresol was found to be a potent activator of Ca^{2+} release from skeletal muscle sarcoplasmic reticulum, and this effect was shown to be specific for the ryanodine receptor.[13] The possibility that 4-CmC was indirectly affecting $[Ca^{2+}]_i$ by damaging the cell membrane was ruled out by investigation of the effects on membrane potential using the fluorescent dye bis-oxonol. Analyzing the structure–activity relationship, the authors suggest that the chloro- and methyl-groups in chlorocresols could be important for the activation of the ryanodine receptor-mediated Ca^{2+} release by interaction of the electronegative chloride with positively charged amino acids in the ryanodine receptor gating domain.

Recent studies showed that 4-CmC stimulated Ca^{2+}-activated [^{3}H]ryanodine binding with an EC_{50} of approximately 100 μM which is about 10 times lower than that of caffeine.[14] In the same concentration range, 4-CmC directly activated the isolated Ca^{2+} release channel reconstituted into planar lipid bilayers from the luminal and from the cytoplasmic site. This effect was caused by an increase in the number and duration of open events. Further experiments showed that 4-CmC affinity of [^{3}H]ryanodine binding to MHS vesicles from porcine sarcoplasmic reticulum was twofold higher than that in normal tissue.[15]

These results can explain the effects of 4-CmC *in vivo* as well as the effects on force development in skeletal muscle preparations. Under *in vitro* conditions 4-CmC induced marked contractures in MHS but only small contractures in MHN skeletal muscles.[10–12] 4-CmC induced caffeine-like contractures in the muscle specimens; however, in contrast to caffeine, which induces contractures in millimolar concentrations, the threshold concentration for 4-CmC was between 25 and 75 μM compared with control fibers. In MHN specimens 4-CmC concentrations $\geq$ 100 μM were needed for contracture induction. Regarding these results it has been concluded that an IVCT with bolus administration of 4-CmC in a

concentration of 75 μM might be an improvement in diagnosing susceptibility to MH. Therefore, in the latest European protocol a 4-CmC IVCT was included as optional test method.[16]

Regarding the results of the 4-CmC IVCT in porcine specimens from the current study and from contracture testing using human muscle preparations, a greater variability in contracture response in the latter has been observed.[11,12] It can be speculated that these results are caused by genetic differences in human and porcine MH. Porcine genetic linkage studies show that a single amino acid mutation, arginine 615 to cysteine, in the skeletal muscle ryanodine receptor gene on chromosome 6 is tightly linked to the MH phenotype.[21] The corresponding mutation in the human ryanodine receptor gene is localized on chromosome 19q13.1–2 region.[22,23] At present, 17 different single-point mutations have been identified in the human skeletal-muscle ryanodine receptor gene in families with disposition to MH.# The incidences of the various mutations have been reported as 2–10% each. A combination of different mutations within one pedigree has not been demonstrated. However, about 50% of susceptible families do not have linkage of MH to chromosome 19,[24] and in one patient tested as MHN with the IVCT a mutation in the ryanodine receptor gene was detected.[25] Furthermore, recent studies have shown linkage to DNA markers from chromosomes 1, 3, 5, 7, and 17 and the MHS phenotype.[26] To estimate the influence of certain mutations on IVCT results, further studies have to determine the genetic effects on the variability of contracture-test responses with 4-CmC as has already been done with halothane and caffeine.[27]

The 4-CmC concentrations used for the *in vivo* experiments were approximately 150-fold higher than the concentrations within clinically used preparations. Therefore, it has to be discussed whether administration of 4-CmC is dangerous in MHS patients. In contrast to genetically susceptible swine, MH is a heterogeneous disorder in humans. Whereas susceptible swines respond relatively uniformly to MH triggers, the clinical picture of MH in humans is variable. Some patients respond with fulminant crisis to first administration and also to low concentrations of triggering substances; other studies report no occurrence of MH with previous exposures to known triggers.[28] Furthermore, some pa-

tients present only minor symptoms of MH after receiving large doses of anesthetics. Other factors that affect MH triggering include core temperature[29] and adjuvant anesthetic agents such as opioids, barbiturates, and nondepolarizing muscle relaxants.[1] Therefore, it can be hypothesized that MH in humans might be induced by lower 4-CmC concentrations as used in the presented study. Furthermore, administration of cresols can induce cardiac dysrhythmias and cerebral convulsions by K^+ channel block.[30] Regarding the specific risks of administration of cresols, the number of preparations containing cresol derivatives has decreased during recent years.

The presented findings indicate that 4-chloro-*m*-cresol is a trigger of MH *in vivo*, and in contrast to the classic MH triggers cresols could induce MH apart from anesthetic procedures. Whether this is relevant to the effects of this drug in humans remains unclear. *In vitro* contracture testing with bolus application of 4-CmC could improve evaluation of MH susceptibility. The variability of contracture-test responses in porcine and human muscle preparations might be explained by genetic differences. Further studies should be designed to investigate the effects of certain genetic mutations on contracture-test results and to define thresholds for a precise discrimination between MHS and normal individuals.

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