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In-Vitro and *In-Vivo* Effects of the Local Anaesthetic Dibucaine on Malignant Hyperthermia – Susceptible and Normal Swine

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Abstract

Background: Ten case reports have described clinical signs and symptoms of malignant hyperthermia (MH) that developed following local anaesthetics. In this study, the in-vitro effects of dibucaine, an amide local anaesthetic, were compared in skeletal-muscle samples from MH-susceptible (MHS) and normal (MHN) swine. In addition, we investigated the in-vivo MH trigger potency of dibucaine in MHS swine.

Methods: Trigger-free general anaesthesia was induced in six MHS and six MHN swine. Muscle biopsies were obtained. In the in-vivo experiment, cumulative doses of dibucaine (1, 2, 4, 8, 16, and 32 mg/kg) were administered i.v. In the in-vitro contracture test (IVCT), dibucaine was added cumulatively to muscle specimens from eight MHS and eight MHN muscles (bath concentrations: 0.1, 0.2, 0.4, 0.6, and 1.0 mmol/l).

Results: In the in-vivo test, no signs or symptoms of MH were observed. In-vitro contractures developed in all muscle specimens, but they developed significantly faster and were more intense in MHS than in MHN muscles.

Conclusion: Dibucaine is not a MH trigger in swine. The IVCT with dibucaine demonstrated overlaps between the diagnostic groups, so that the possibility of 100 % accurate discrimination between MHS and MHN individuals by this means does not seem likely.

Introduction

Malignant hyperthermia (MH) is an uncommon inherited pharmacogenetic syndrome of skeletal-muscle cells in humans and various animal species. The basis is a genetic disorder of intracellular calcium homeostasis caused by a defect in the ryanodine receptor type 1 (RyR1). It is usually triggered by halogenated volatile anaesthetics and depolarizing muscle relaxants, leading to a hypermetabolic state. The clinical symptoms vary in severity from mild to potentially lethal forms, including tachycardia, hypercapnia, hypoxemia, muscle rigidity, hyperthermia, and rhabdomyolysis.

Despite the fact that regional anaesthesia is generally considered to be safe in MH-susceptible (MHS) patients, nine case reports describing clinical signs and symptoms of putative MH following spinal or epidural anaesthesia have been published [1-9]. A further case of MH caused by intravenous (i.v.) lidocaine, which had been administered as treatment for ventricular arrhythmias, was reported [10].

These case reports suggest that local anaesthetics (LA) might also be triggers of MH. It is thus imperative that the safety of regional anaesthesia in patients susceptible to MH undergoes further investigation.

Dibucaine, a commonly used amide LA, has been shown to enhance the rate of Ca^{2+} release from skeletal-muscle sarcoplasmic reticulum (SR) [11] and to stimulate ryanodine binding in skeletal-muscle preparations [12].

Therefore, the aim of this study was on the one part to compare the in-vitro effects of dibucaine in skeletal muscles from MHS and normal (MHN) swine, and on the other to investigate the in-vivo MH trigger potency of dibucaine in MHS swine.

Material and Methods

Following approval by the animal-care committee (University Hospital Hamburg-Eppendorf, Hamburg, Germany), 6 MHS Pietrain and 6 MHN German landrace male and female swine, aged 3–4 months, from a special breeding program at the Federal Breeding Center in Mölln, Germany, were investigated. The MH genotype of the swine was determined by DNA analysis of ear tissue to check for the presence of the *C1843T* point mutation on chromosome 6, indicating MH susceptibility [13,14].

Anaesthesia was induced in all swine by administration of 10 mg/kg ketamine and 0.5 mg/kg midazolam intramuscularly. After insertion of an i.v. line into an ear vein, anaesthesia was deepened by administration of 10 µg/kg fentanyl and 2 mg/kg propofol i.v. The trachea was intubated without administration of a muscle relaxant. The swine were mechanically ventilated, and anaesthesia was maintained by continuous i.v. administration of propofol and fentanyl and inhalation of 70% nitrous oxide in 30% oxygen. Monitoring included electrocardiography (ECG), pulse oximetry, and rectal temperature measurement. Radiant heat application and warming blankets were used to maintain a stable body temperature. A catheter was inserted into the femoral artery for blood pressure monitoring and obtaining blood samples. A multilumen central-venous catheter was surgically placed in the right internal jugular vein for blood sampling, application of the

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test drug, administration of anaesthetics, and infusion of crystalloid fluid (5–10 ml/kg/h Ringer's solution). Prior to starting the in-vivo experiments, muscle specimens were excised from the hind limb for the i.v. contracture test (IVCT) investigations.

In-vitro Experiments

In contrast to the *in-vivo* test, muscle specimens from 8 MHS Pietrain and 8 MHN German landrace swine were used for this experiment. The seventh and eighth specimens were taken from pigs that were scheduled to subsequently undergo *in-vivo* tests with a different substance [15]. All *in-vivo* investigations were performed within 5 h after the muscle biopsy. Muscle bundles were excised carefully and dissected into single strips (length, 15–25 mm; width, 2 – 3 mm). The method and test set-up of the IVCT were in accordance with the European MH Group protocol [14]. One viable muscle specimen from each of the 8 MHS and 8 MHN swine with a twitch response to supramaximal stimulation of 10 millinewtons (mN) or more was used for the IVCT, with cumulative administration of dibucaine at bath concentrations of 0.1, 0.2, 0.4, 0.6 and 1.0 mM every 5 min. After a stable baseline tension was reached for at least 10 min, dibucaine was added directly to the tissue bath every 5 min in order to obtain bath concentrations of 0.1, 0.2, 0.4, 0.6, and 1.0 mM. The in-vitro effects of dibucaine on contracture development and twitch response in the muscle specimens were continuously observed for 60 min.

In-vivo Experiments

The in-vivo experiments were performed on six MHS and six MHN swine. Prior to administration of the test substance, a steady state of all measured variables was achieved for at least 30 min. A bolus of 0.5 mg/kg dibucaine was given i.v. followed by increasing dibucaine doses of 1, 2, 4, 8, 16, and 32 mg/kg every 20 min. The clinical development of MH was defined by the presence of two of the following three conditions: p_aCO_2 greater than 70 mmHg, pH less than 7.20, and increase in body temperature greater than 2.0°C. In the event that a MH crisis occurred, further administration of dibucaine was to be stopped. During the experiments, hemodynamic variables (heart rate [HR], mean arterial pressure [MAP], central-venous pressure [CVP]), end-tidal carbon dioxide concentration, rectal temperature (°C), blood-gas parameters (arterial oxygen saturation, p_aCO_2, pH), and lactate concentrations were measured every 5 min. After all experiments were completed, the pigs were euthanised using magnesium chloride solution (10%).

Statistical analysis

Statistical evaluation was performed using a computer-based program (SPSS Inc., Chicago, USA). All data are presented as mean and standard deviation. Inter-group differences of dibucaine on contracture development *in-vitro* were analyzed using ANOVA. The data obtained during the in-vivo experiments were compared using two-way ANOVA for repeated measures followed by Scheffe's test. Results were considered significant if *P* values were less than 0.05.

Results

The *in-vitro* dibucaine experiments produced contractures in a concentration-dependent manner in all muscle specimens. However, contractures developed significantly more intense in MHS than in MHN muscles (Figure 1). Contractures in the MHS group started after administration of 0.1 mmol/l dibucaine with 1.5 ± 0.9 mN and reached a maximum at 1.0 mmol/l with 14.0 ± 3.6 mN. In contrast, MHN specimens developed contractures beginning at a dibucaine concentration of 0.4 mmol/l with 0.6 ± 0.3 mN, and the contractures at 1.0 mmol/l dibucaine with 5.5 ± 0.8 mN were significantly smaller than in the MHS group.

The initial muscle twitch amplitudes, with 40.9 ± 38.0 mN in the MHS group, were not significantly lower compared to the MHN group, with 71.4 ± 45.0 mN (Figure 2). Both study groups demonstrated a decline in twitch amplitudes throughout the experiment, which can be explained by the negative inotropy of dibucaine. The inter-group analysis showed a significant difference in the MHS muscles after 0.1 and 0.2 mmol/l dibucaine.

In-vivo Experiments

Neither MHS nor MHN swine developed significant changes in p_aCO_2, pH, or body temperature after administration of dibucaine. Hence, no MH crisis was seen in any of the animals. The clinical course of p_aCO_2, pH, body temperature, MAP, HR, and the number of live animals during the investigation are summarised in Fig. 3. After a median dose of 3.5 mg/kg dibucaine, the mean p_aCO_2 was 33.3 mmHg (30.9 - 38.4 mmHg) in the MHS and 37.0 mmHg (32.2 - 54.3 mmHg) in the MHN swine. The mean pH at this time point of the investigation was 7.43 (7.41 - 7.46) in the MHS and 7.35 (7.28 - 7.43) in the MHN animals. The mean body temperature was 37.7 °C (36.3 - 38.2 °C) in the MHS swine and 38.3 °C (37.3 - 38.8 °C) in the MHN swine. Higher doses of dibucaine did not induce changes in these parameters, but did cause terminal ventricular fibrillation. All animals died at the latest after administration of a cumulative dose of 63.5 mg/kg dibucaine.

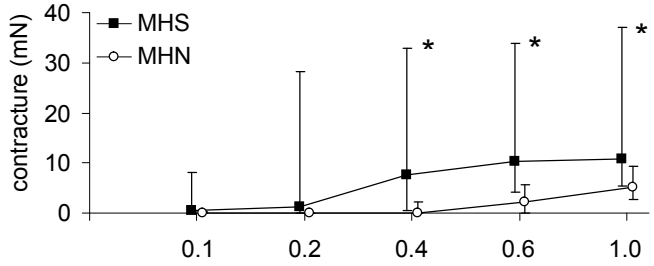

Figure 1: Contracture development following cumulative administration of dibucaine in concentrations of 0.1, 0.2, 0.4, 0.6, and 1.0 mM in skeletal muscle specimen of malignant hyperthermia-susceptible (MHS, n=8) and normal (MHN, n=8) swine. The value "0" refers to the last contracture before the application of 0.1 mM dibucaine. Dots and squares, respectively, indicate medians; error bars illustrate ranges. Intergroup difference: *P <0.05.

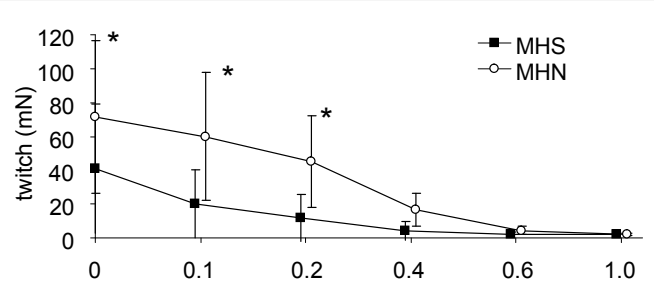

Figure 2: Muscle twitch response (mN) in an in-vitro contracture test with 0.1, 0.2, 0.4, 0.6, and 1.0 mM dibucaine in skeletal muscle specimens of malignant hyperthermia- susceptible (MHS; n=8) and normal (MHN; n=8) swine. The value "0" refers to the last twitch before the application of 0.1 mM dibucaine. Dots and squares, respectively, indicate means; error bars illustrate standard deviation. Intergroup difference: *$P < 0.05$.

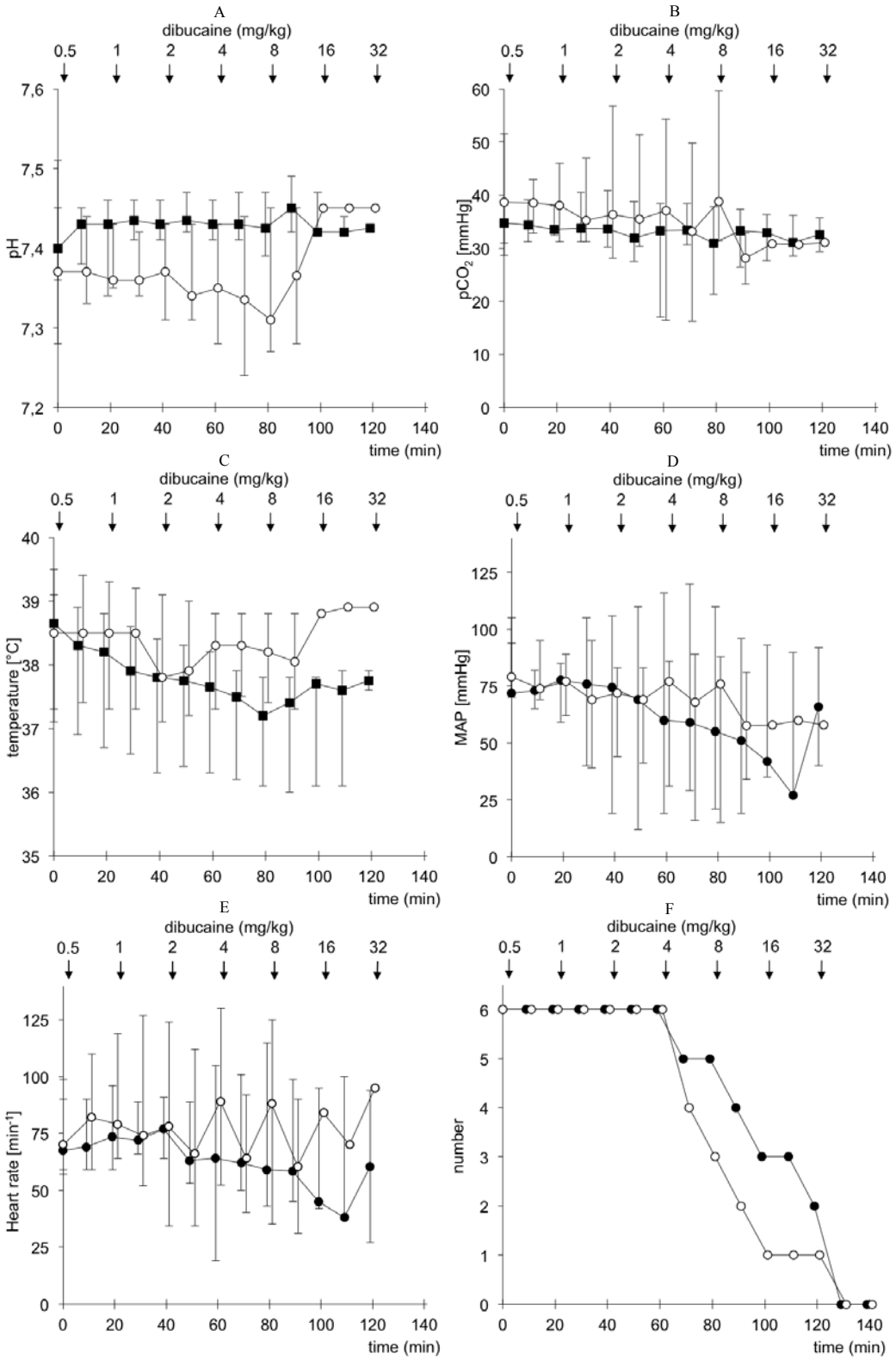

Figure 3: Course of pH (a) p_aCO_2 (b), rectal body temperature (c), mean arterial pressure (MAP) (d), heart rate (e), and number of animals alive (f) during cumulative administration of dibucaine in concentrations (0.5 -1 - 2 - 4 - 8 - 16 - 32 mg/kg) in 6 MH susceptible (MHS, squares) and 6 MH normal (MHN, dots) swine. The value "0" refers to the steady-state condition before the administration of 0.1 mM dibucaine. Dots and squares, respectively, indicate medians; error bars illustrate ranges. Intergroup difference: $*P < 0.05$

Discussion

Systemic administration of dibucaine, even in highly toxic doses, did not induce MH in anaesthetised MHS swine in-vivo. Both the clinical signs and symptoms as well as the laboratory parameters were compatible with the findings in dibucaine intoxication, for which there was sufficient pharmacological evidence. Therefore, the use of this amide LA in MHS patients would appear to be safe. It might even be speculated that this finding could be extrapolated to apply to all amide LAs.

The question arises as to how the case reports of suspected MH following administration of LAs should be interpreted, when taken the results of this study into account. In general, it can be ascertained that the differential diagnosis of MH is extensive, in particular when it involves abortive or mild forms with highly variable clinical presentations. Some of the possible pathologies include malignant neuroleptic syndrome, serotonin syndrome, amphetamine intoxication, sepsis, thyrotoxic crisis, heat stroke, pheochromocytoma, and rhabdomyolysis from other causes. In addition, a successful response to dantrolene therapy is often misinterpreted as indicating proof of MH. The following cases demonstrate that the clinical diagnosis of MH is only rarely nonambiguous, and that in these cases an IVCT was not or could not be performed to confirm the diagnosis.

Sheu et al. from Taiwan reported clinical symptoms that were compatible with a fulminant MH crisis in a patient who had undergone spinal anaesthesia with 12 mg tetracaine [1]. The differential diagnosis of septic shock could not be ruled out in this case. An IVCT could not be performed, as this test is not available in Taiwan.

Motegi et al. reported an apparent case of a mild MH reaction following epidural anaesthesia with 2% procaine in a patient with previous clinically suspected MH after general anaesthesia [2]. The symptoms could, however, also have been due to a reperfusion syndrome: 10 min after deflation of a tight pneumatic tourniquet (500 mmHg for 60 min), the upper extremities became rigid, body temperature increased from 36.8 to 37.1°C, and the HR increased from 94 to 126/min. The ECG showed a bigeminal rhythm. After i.v. administration of 0.9 mg/kg dantrolene (which would actually be a subtherapeutic dose for MH), 40 mEq sodium bicarbonate, and 50 mg phenytoin, the clinical symptoms and laboratory parameters had normalised. A post-symptomatic IVCT was not performed.

In another case, an extremely high dose of i.v. lidocaine - that was given for the treatment of a ventricular arrhythmia - was presumed to cause a MH crisis [10]. In this patient, who had a history of chronic renal failure, it would appear more likely that lidocaine intoxication was the cause of both general and focal seizures followed by drug-induced rhabdomyolysis: "... a convulsion occurred ... generalised muscular twitching was observed, but the patient remained conscious and alert ... he gradually became drowsy. Muscular twitching developed in the facial muscles, the eyes were deviated upward, and both the upper and lower extremities were flaccid ... cardiopulmonary arrest occurred ... successful resuscitation ... body temperature rose to over 41°C ... CPK (1,393 U/l) and serum myoglobin (2,489 ng/ml) were elevated". The patient died from cerebral causes, so that in this case as well no IVCT could be performed.

In a 25-year-old female in whom MH was assumed [7], premedication with 15 mg diazepam and 0.5 mg atropine was administered intramuscularly. I.v. Infusion of dextran 70,000 was started; 30 min after epidural injection of 300 mg lidocaine and 50 mg bupivacaine with adrenaline, the following signs and symptoms occurred: cyanosis, hyperthermia with warm, dry skin, muscle rigidity, hyperventilation, and tachycardia. Hypotension and hyperkalaemia were not seen, and no rhabdomyolysis or creatine kinase (CK) elevation was reported. The differential diagnosis in this case includes a pyogenic reaction to dextran or a central anticholinergic syndrome. The subsequent symptomatic therapy – without dantrolene – was successful.

Another putative MH reaction was reported [9] in a 65-year-old male: epidural analgesia was begun by administering a bolus of 300 mg lidocaine followed by a continuous epidural lidocaine infusion. General anaesthesia was then induced using thiopental, succinylcholine, and topical lidocaine. No volatile anaesthetics were used. The operation lasted 5 hours. During the procedure, 600 ml stored whole blood was transfused. Fifteen minutes after admission to the recovery room, the patient developed shivering and metabolic acidosis and the temperature rose to 40°C. Mild CK elevation (267 U/L) was reported. No other symptoms typical of MH were reported. Therapy consisted of administration of 15 mg triflupromazine, sodium bicarbonate, physical cooling, and induction of trigger-free general anaesthesia to terminate the shivering. This therapy was successful, although the patient showed persistent subfebrile temperatures for three subsequent days. If this was indeed a case of abortive MH, the most probable trigger was succinylcholine. A further differential-diagnostic possibility, however, could be a pyogenic reaction to the whole-blood transfusion.

Katz and Krich described a patient with a possible clinical MH reaction in whom spinal anaesthesia was performed [6]: "Spinal puncture was performed with a great deal of difficulty and a free return of cerebrospinal fluid was not obtained. Tetracaine 10 mg was instilled. "Spotty" anaesthesia to the level of the second lumbar dermatome was obtained which was not adequate for the operation and repeated local infiltrations of procaine 0.5 per cent were required." Six hours after the spinal anaesthetic, the patient's temperature rose from 95° to 101°F. This was the only sign that was compatible with a possible MH episode, and it responded to symptomatic therapy. It seems highly unlikely that this was actually a MH event.

Unfortunately, in none of the reported cases of presumed MH after LA administration were post-symptomatic investigations undertaken.

Excessive elevation of the myoplasmic Ca^{2+} concentration due to an increased opening probability of the RyR1 leads to the hypermetabolic state of skeletal muscle that is typical of MH. However, numerous substances influence the opening capacity of the RyR1. It has been demonstrated that many LAs interact with the RyR1, so that these agents can modulate RyR1-mediated Ca^{2+} release [12].

In past studies, the effects of LAs on [3H] ryanodine binding to microsomes obtained from skeletal muscle were measured. Since only the open conformational state of the RyR binds ryanodine, [3H] ryanodine binding can be used as an index of Ca^{2+} release channel activity in the sarcoplasmic reticulum (SR). In these studies, LAs showed both inhibitory and stimulatory properties, which were in part dependent on their concentration and on the particular assay conditions (ionic strength, pH, ATP).

As early as 1980, it was shown by Schon that dibucaine increases myoplasmic Ca^{2+} concentrations in skinned skeletal muscle. Dibucaine alters ryanodine binding in a biphasic manner: stimulation at low concentrations (>100/μM) and inhibition at higher concentrations [11]. In our experiments, we opted to use increasing cumulative doses of dibucaine to allow for potential concentration-related factors,

Bupivacaine in a concentration of 5 mM enhanced and 10 mM inhibited [3H] ryanodine binding to skeletal- muscle microsomes [16]. Stimulative effects of lidocaine and prilocaine on [3H] ryanodine binding have also been reported [11].

The ester LAs tetracaine and procaine decrease [3H]ryanodine binding to skeletal muscle RyR and decrease the opening probability of the RyR1 [15-18]. Furthermore, tetracaine suppresses SR calcium release in enzymatic isolated skeletal-muscle fibers [18]. In addition, a nonspecific action of various LAs on lipid bilayers to increase the Ca^{2+} permeability of SR membranes is highly likely.

The varied interactions at the RyR1 and the lipid bilayers are probably responsible for the variable myotoxicity of the LAs, but presumably do not involve a causal relation with MH for any of these substances. Additionally, in-vitro studies have shown that low pH and low ATP concentrations are associated with a decreased opening probability of the RyR1. These are exactly the conditions, however - metabolic acidosis and anaerobic metabolism due to ATP depletion - that develop in the myoplasm during a MH episode. It could thus be hypothesised that the LA-related RyR1 opening possibility decreases during a MH crisis.

MHS and MHN skeletal muscles showed an in-vitro increase followed by a marked decrease in muscle twitch, indicating muscle fatigue, which is explained by the negative inotropy of dibucaine. MHS muscle demonstrated contracture development at significantly lower dibucaine concentrations compared with MHN specimens. However, with all dibucaine dosages used, overlapping of individual values between both diagnostic groups was observed, so that 100% accurate differentiation between MHS und MHN specimens was not possible. It thus appears that further IVCT studies with dibucaine using larger numbers of animals may not be useful in facilitating the diagnosis of MH susceptibility.

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