PORCINE MALIGNANT HYPERTHERMIA: CAFFEINE CONTRACTURE OF SINGLE SKINNED MUSCLE FIBERS

MAKOTO ARAKI¹, AKIO TAKAGI¹, TAKEHISA FUJITA¹ and TOSHIMITSU MATSUBARA²

¹National Center for Nervous, Mental and Muscular Disorders, Kodaira, Tokyo 187, and ²Department of Veterinary Medicine, University of Azabu, Sagamihara, Kanagawa 229, Japan

ABSTRACT

Using skinned muscle fibers, the response to caffeine was compared between malignant hyperthermia susceptible (MHS) and control swines. The caffeine concentration for the threshold and half maximum contracture was markedly lower in MHS swines than in controls. These results agree with those in human malignant hyperthermia (MH). Dantrolene did not exhibit any effect on the dose-response relationship of caffeine contracture in the skinned fiber from MHS swines. The muscle fiber diameter was significantly increased in every fiber type of MHS swines. The pathogenesis of porcine MH and effect of dantrolene were discussed.

Malignant hyperthermia (MH) is one of the serious complications of general anesthesia, characterized by hyperthermia, metabolic acidosis, circulatory failure and muscle contracture (2, 4). Malignant hyperthermia susceptible (MHS) swine is a suitable animal model for studying a similar, if not identical, syndrome in humans (15). In the present study, skinned fibers were prepared from muscles of MHS swines, and response to caffeine was analyzed *in vitro*. Since human and porcine MH has been successfully treated with dantrolene (10, 11), the effect of this agent on the caffeine contracture was also examined.

MATERIALS AND METHODS

From two MHS Berkshire and two control Yorkshire swines, the gracilis muscle was biopsied under anesthesia with sodium pentobarbital. MHS swines were identified by a challenge with 6% halothane (19). By this procedure, MHS swines presented hyperthermia, contracture of extremities and abdominal muscles, and elevation of serum creatine kinase activity. Excised muscle specimens were placed in the relaxing solution (G-5) on ice (Table 1) (24). Single muscle fibers were prepared and mechanically

skinned (14). The experiment was performed at $22 \pm 2^\circ\text{C}$ within 3 days after biopsy.

The dose-response relationship of caffeine contracture was analyzed and compared between MHS and control muscles. For this, the contracture was induced by caffeine in various concentrations after the sarcoplasmic reticulum (SR) of skinned fibers was loaded with Ca (Fig. 1). To load the SR with Ca, the skinned fiber was incubated for 2 min in the solution at pCa 6.5 (Table 1). Peak tension of caffeine contracture at a given concentration was compared to the maximum contracture, which was induced by 40 mM caffeine, and the relative tension was calculated. When the effect of dantrolene was examined, the Ca-loaded fiber was washed twice with G-0.1 containing 25 μM dantrolene for 30 sec, and then exposed to caffeine and dantrolene. The muscle type of single fiber was determined by activation pattern with Ca or strontium ion as previously reported (22). All the fibers examined except two were type 2 and the other two fibers were of intermediate type (20).

The response of the contractile system to Ca was analyzed on fibers treated with Brij 58 to abolish the function of SR. The fiber was incubated for 30 min in G-2 containing 0.5% (w/v) Brij. pCa-tension relationship was analyzed (22)

Fig. 1 The schema for quantification of caffeine contracture. A, C: control runs. B: test run. After the SR of the fiber was loaded with Ca by incubating it in solution at pCa 6.5 for 2 min, caffeine contracture was induced in G-0.1 solution. In B, contracture was first induced by X mM caffeine. The peak tension of this contracture was compared to the mean peak tension of contracture in A and C, and relative tension was calculated.

Table 1 *Constituents of Solutions*

	Relaxing solution			Ca solution
	G-5	G-2	G-0.1	pCa 6.5
ATP-Na ₂	4	4	4	4
Tris	20	20	20	20
Maleate	20	20	20	20
MgSO ₄	4	4	4	4
EGTA	5	2	0.1	10
CaSO ₄	0	0	0	1.30
KMS*	101	105	108	92

Concentrations are expressed in mM. *Potassium methansulfonate

and compared between MHS and control muscle fibers. Ratio of peak tension of contracture by 40 mM caffeine to P_0 (maximum contracting force of the fiber) was adopted as a Ca uptake index. For a rough estimation of Ca uptake activity of the SR, Ca uptake index was calculated and compared between MHS and control muscle fibers.

For histological and histochemical examinations, muscles were frozen in isopentane chilled with liquid nitrogen, and frozen sections (10 μ m thick) were obtained. Sections were stained with hematoxylin and eosin (H-E), modified Gomori trichrome, and a battery of histochemical

methods including routine ATPase (alkaline preincubation), ATPase with preincubation at pH 4.6 and 4.2 (5), and NADH-tetrazolium reductase (NADH-TR). Type 1, 2A, 2B and 2C muscle fibers were classified according to the criteria of Brooke and Kaiser (3), and the diameter of each muscle type was measured with an MDP-AM03 semiautomatic image analyzer (Kontron, Munich, F.R.G.). For electron microscopical examinations, parts of the biopsied specimen were fixed in cacodylate-buffered 2% glutaraldehyde solution for 2 h. After rinsing in the same buffer, they were postfixed in the lanthanum-OsO₄-collidine solution (Revel and Karnovsky, 1972) for 4 h, dehydrated in ascending series of ethanol and embedded in Epon-resin. The data were analyzed by *t*-test of Cochran-Cox.

Solutions

Constituents of various solutions are listed in Table 1. Caffeine (0.25–40 mM) and dantrolene (25 μ M) were dissolved in the G-0.1 solution. Free Ca ion concentration was calculated on the assumption that the apparent binding constant of Ca-EGTA is $5 \times 10^5 \text{ M}^{-1}$ in the present buffer system (17). The pH and the ionic strength of the solution were adjusted to 6.80 and 0.20 M, respectively. All the chemicals were of special reagent grade.

RESULTS

The dose-response relationship of caffeine contracture was compared between MHS and control muscle fibers. The relative tension of contracture was significantly larger in MHS than in controls in caffeine concentrations higher than 1 mM (Fig. 2). The concentration of caffeine, which caused 10% or more of the maximum contracture, was taken as the threshold. The threshold of caffeine for contracture was 1.10 ± 0.66 mM (mean $\pm$ SD) for MHS swines and 5.17 ± 2.74 mM for controls ($P < 0.001$; Table 2). Caffeine concentration at half maximum contracture was 1.09 ± 0.70 mM for MHS swines and 6.58 ± 2.84 mM for controls ($P < 0.001$; Table 2). The difference was similarly significant when results were compared among individual MHS and control swines. The results demonstrated that skinned fibers from MHS swines produced a larger contracture by caffeine in concentrations lower than in controls.

The effect of dantrolene on caffeine contracture was studied in MHS muscle fibers. Dantrolene had no effect on the dose-response relationship (Fig. 3). Similarly, dantrolene had no effect on caffeine concentration at the threshold and at half maximum contracture (Table 2).

In both MHS and controls, the contractile system started to contract at pCa 6. Maximum contracting force was obtained at pCa 5.5 or pCa 5.0. There was no difference in pCa-tension relationship between MHS swines and controls. The Ca uptake index was 0.75 ± 0.15 (n=10) for MHS swines and 0.79 ± 0.11 (n=9) for controls. There was no significant difference between them.

In morphological studies, the only abnormal finding was the difference in muscle fiber diam-

Fig. 2 The dose-response relationship of caffeine contracture was compared between MHS swines and controls. Values were larger in MHS (closed circles) than in controls (open circles) in caffeine concentrations above 1 mM. The difference was significant as given below. 1.0 mM ($P < 0.01$); 2.0 mM ($P < 0.001$); 4.0 mM ($P < 0.001$); 6.0 mM ($P < 0.004$); 8.0 mM ($P < 0.003$); 10.0 mM ($P < 0.006$)

Fig. 3 The effect of dantrolene was analyzed for the caffeine contracture of MHS muscle fibers. Dantrolene (25 μ M) had no effect on caffeine dose-response relationship.

Table 2 Caffeine Concentration at the Threshold and at Half Maximum Contracture

Caffeine concentration at the threshold of contracture		
MHS	1.10 ± 0.66 mM	(n=10)
MHS+Dantrolene	0.92 ± 0.58 mM	(n=5)
Control	5.17 ± 2.74 mM	(n=9)*
Caffeine concentration at half maximum contracture		
MHS	1.09 ± 0.70 mM	(n=10)
MHS+Dantrolene	1.01 ± 1.12 mM	(n=5)
Control	6.58 ± 2.84 mM	(n=9)*

Values represent mean $\pm$ SD (numbers of experiments). The value* was lower in MHS swines than in controls ($P < 0.001$). Addition of 25 μ M dantrolene had no effect on the threshold and on half maximum contracture.

eter between MHS and control swines. Variability of muscle fiber diameter was larger in MHS swines than in controls, and diameter distribution of type 1, type 2A and type 2B fibers was shifted to the right in MHS swines (Fig. 4). Mean diameter was 62.5 ± 20.6 μ m for MHS swines (n=856) and 53.8 ± 17.0 μ m for controls (n=917) ($P < 0.001$). The enlargement of fiber diameter

Fig. 4 The distribution of muscle fiber diameter. In MHS muscles, larger fibers were increased in every muscle fiber type.

Table 3 Mean Fiber Diameter and Fiber Types

Muscle fiber type	1	2A	2B
Diameter (μm)			
MHS	61.6 $\pm$ 17.9	71.2 $\pm$ 23.0	62.2 $\pm$ 21.1
Control	42.6 $\pm$ 15.3	54.6 $\pm$ 15.2	56.3 $\pm$ 16.6
Percentage of fiber (%)			
MHS	15.4	7.1	77.5
Control	15.3	8.5	76.2

The diameter was significantly larger in MHS swine than in controls in the every muscle fiber type ($P<0.005$).

was also observed when type 1, type 2A or type 2B fiber was compared separately ($P<0.005$; Table 3). No type 2C fiber was detected in both MHS swines and controls. The electron microscopy did not disclose any alteration of cytoarchitecture (micrographs not shown).

DISCUSSION

MHS swines were first described by Hall *et al.* in 1966 (9). In the swine industry, porcine stress

syndrome or pale, soft and exudative pork has provoked much concern; the porcine MH seems closely related in its pathogenesis to these syndromes (15). Muscle bundles from MHS swines gave abnormal responses *in vitro* to halothane or caffeine as in human MH (1). Since halothane or caffeine mainly interferes with the function of SR, the fragmented SR has been studied in porcine MH. However, no consistent abnormality was found in the Ca uptake activity (8). Recently, Ca release mechanism was also evaluated using the fragmented SR (12). In the SR of MHS swines, Ca-induced Ca release mechanism was enhanced as in the case of human MH (16, 18).

We have analyzed caffeine contracture of the skinned fiber *in vitro*. In patients who had recovered from episodes of MH, contracture was induced with lower concentration of caffeine than in controls (21). In the present study, analysis of caffeine contracture was extended to porcine MH. In skinned fibers from MHS swines, caffeine concentrations for the threshold and for half maximum contracture were much lower than in controls. There was no difference in pCa-tension relationship between MHS swines and controls. In the present study, activity of Ca uptake was compared only in skinned fibers incubated at pCa 6.5 for 2 min. Under this condition, the Ca uptake index was the same between MHS swines and controls; we have not yet examined whether Ca-loading capacity of the SR differs in solutions at other pCa between MHS and controls. In our observations, the mechanism of Ca release from the SR was concluded to be abnormal in porcine MH. Since caffeine releases Ca from the SR by activating Ca-induced Ca release (6), it is suggested that this mechanism is abnormal in porcine MH, as was already shown in human MH by Endo *et al.* (7).

In histological studies of the porcine MH muscle, the only abnormal finding was an increase of hypertrophic muscle fibers. Previous studies had not disclosed any specific abnormality in the ultrastructure of muscle cells (23). Concerning the pathogenesis of porcine MH, we have currently no established theory. The abnormal release of Ca from the SR might be a primary genetic disorder of the membrane specific to the syndrome. On the other hand, it might be an epiphenomenon accompanying a degenerative process of the skeletal muscle. In human MH syndrome, diseases underlying MH episodes were heterogeneous (21). This fact might support the latter hypothesis.

Dantrolene had been effective in controlling porcine MH (10). It was also effective in certain,

but not all, human MH syndrome (11). Anderson and Jones demonstrated that halothane-induced contracture of MHS muscles *in vitro* was suppressed by dantrolene (1). Concerning the effect of dantrolene on the fragmented SR, results are still inconclusive (16, 18). In the present study using the skinned muscle fibers, dantrolene did not influence the dose-response relationship of caffeine contracture in MHS swines. We did not examine the effect of dantrolene on skinned fibers of control swines. Dantrolene is a blocker of the excitation-contraction coupling and inhibits release of Ca from the SR (13). It was reported that the drug directly influenced the SR and decreased the amount of Ca release from the SR (18). Our present observations are in apparent contrast with previous reports, in that dantrolene did not influence caffeine contracture. This does not necessarily exclude the possibility that dantrolene has a direct influence upon the Ca-induced Ca release *in vivo*. In the skinned fiber preparation, a factor or factors which make the SR susceptible to dantrolene may be lost. The mode and site of action of dantrolene remain to be elucidated.

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