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Malignant Hyperthermia of Poland China Swine:

Studies of a Myogenic Etiology

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Compared with muscle from normal swine, muscle slices from a strain of malignant-hyperthermia-susceptible (MHS) swine *in vitro* showed greater depletion of ATP and greater production of lactate, and halothane increased the ATP depletion and lactate production in MHS muscle slices. No difference between the calcium uptake capacities of specimens of sarcoplasmic reticulum isolated from normal and from MHS swine was detected. Halothane increased the amounts of Ca^{++} bound by sarcoplasmic reticulum from both control and MHS muscle. The data suggest an underlying myopathy in MHS muscle, the nature of which appears to be rapid utilization of muscle ATP and loss of glycolytic control mechanisms.

BRITT AND KALOW[1,2] recently reviewed the characteristics of malignant hyperthermia in man. The discovery of a similar, if not identical, syndrome in certain breeds of swine[3-6] has provided an appropriate animal model for further investigations. The establishment of a colony of malignant-hyperthermia-susceptible (MHS) swine in our laboratory has permitted us to study this inherited condition further.

There is notable evidence that skeletal muscle is the target tissue in anesthetic-induced malignant hyperthermia. Halothane has a direct effect upon the depletion of ATP in muscle from susceptible swine *in vitro*.[4] Extreme muscular rigidity is frequently an early clinical sign, although in man it does not always occur.[1,2,4,6] When succinylcholine is administered to susceptible swine, there is immediate coarse fasciculation with hypertonicity of most, if not all, skeletal muscles, followed by hyperthermia.[5,6] A regional response characterized by coarse fasciculation and rigidity in a single limb occurs when succinylcholine is injected into the femoral artery of that limb.[6,¶] Administration of tubocurarine does not inhibit muscular rigidity, and delayed onset of rigidity has been observed in a limb in which the blood supply was restricted by a tourniquet.[7] These observations suggest a defect distal to the myoneural junction. The question arises, where in the muscle cell does the defect occur?

Contraction and relaxation of twitch-type skeletal muscle fibers probably depend upon the functional characteristics of four of its major structural elements[8]: 1) the surface membrane and transverse tubules which conduct action potentials necessary to bring the total fiber into contraction; 2) the longitudinal array of the sarcoplasmic reticulum synaptically related to the transverse system, which regulates myoplasmic flux of ionic calcium; 3) muscle mitochondria, which in addition to supplying energy as ATP may provide secondary regulation of myoplasmic ionic calcium flux; 4) the myofibrillar array with its contractile proteins, including myosin and actin, which are activated by adequate quantities of Ca^{++}, Mg^{++}, and ATP.

Kalow *et al.*[9] suggested that abnormal metabolism in the skeletal muscle of MHS human beings causes the distribution of intracellular calcium to be affected by drugs. Study of biopsied muscle strips from two patients who recovered after developing muscular rigidity during malignant hyperthermia revealed an in-

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¶ L. W. Hall, personal communication.

TABLE 1. Depletion of Porcine Muscle ATP during Exposure to Carbogen and to Carbogen with 4 Per Cent Halothane *in Vitro**

	Fresh-frozen	O_2/CO_2 KRB 30 min	O_2/CO_2 + 4 Per Cent Halothane KRB 30 min
μM ATP per gm muscle			
Control swine (n = 12)	3.19*	1.87	1.75
	0.17†	0.12	0.09
MHS swine (n = 12)	3.14	1.02	0.59
	0.16	0.10	0.06
Percentage ATP depleted			
Control swine		37.50	41.50
MHS swine		67.50	81.20

* *Conditions of Assay*: Two separate muscle biopsies were taken from each pig and sliced into three pieces, each weighing approximately 350 mg. Two slices were quick-frozen in liquid N_2, two incubated 30 min in Krebs-Ringer bicarbonate (KRB) through which 95 per cent O_2/5 per cent CO_2 was bubbled at 2 l/min, and two pieces incubated 30 min in KRB through which 4 per cent halothane in 95 per cent O_2/5 per cent CO_2 was passed. Following incubation, samples were frozen in liquid N_2. Percentage ATP depletion was calculated by:

$$\frac{\text{ATP concentration in fresh-frozen muscle} - \text{ATP concentration after incubation}}{\text{ATP concentration in fresh-frozen muscle}} \times 100$$

* Mean.
† SE.

creased sensitivity to caffeine-induced contraction which was potientiated by halothane. In addition, these authors observed that halothane inhibited calcium accumulation by the isolated sacroplasmic reticulum. Berman *et al.*[10] also postulated that halothane caused liberation of calcium ions from binding sites in the muscle, resulting in activation of myosin ATPase and glycogenolysis.

Methods and Materials

The care of control and MHS swine used for this study has been described.[6] Potentially susceptible swine were selected from a strain of swine in which the occurrence of malignant hyperthermia had been recognized. Biopsies of the longissimus dorsi muscle were obtained during thiamyal sodium–nitrous oxide–oxygen anesthesia. The depletion of muscle ATP *in vitro* was studied by the technique of Harrison *et al.*,[4] ATP being determined enzymatically[11] after extraction by trichloroacetic acid. Samples of the incubation medium were taken at various times during incubation for the determination of lactic acid production. Lactate was determined enzymatically.[12] The sarcoplasmic reticulum (SR) was isolated by the procedure of Martonosi and Feretos.[13] The SR fragments were extracted for an hour with 0.6 M KCl–5 mM histidine (*p*H 7.3) to remove contaminating actomyosin,[14] and the final SR fragments were obtained by centifugation at 48,000 × g for an hour. The medium for studies of calcium uptake contained the following: 0.1 M KCl–5 mM histidine (*p*H 7.3), 5 mM ATP, 5 mM $MgCl_2$, 5 mM oxalate, 2 mM $^{45}CaCl_2$, and SR, 0.02 mg protein/ml. The reaction was started by addition of SR to the medium. After incubation for 5 minutes at 25 C, the SR was removed by filtration through 0.45-μ millipore filters. Samples removed from the incubation medium before and after filtration were plated on planchettes, and radioactivity was counted by a gas-flow proportional counter (Nuclear, Chicago). Calcium binding was calculated from the difference between the radioactivity before and after filtration. Four per cent halothane vapor in carbogen (95 per cent O_2 and 5 per cent CO_2) 2 l/min, was passed over the SR fragments for 5 minutes at 25 C.

Muscle samples for ATP depletion studies *in vitro* were obtained during screening for susceptible swine, or before and during experi-

TABLE 2. Porcine Muscle Lactate Production during Exposure to Carbogen and to Carbogen with 4 Per Cent Halothane *in Vitro*

	Time of Incubation (Min)					
	1	2	4	8	16	30
MHS swine (n = 3) Carbogen	4.50*	7.40	12.30	18.10	27.20	35.20
	0.27†	0.55	2.55	3.23	6.43	8.19
4 Per Cent halothane	6.20	8.50	14.60	19.60	27.70	40.20
	0.65	1.34	1.27	2.00	0.75	0.90
Control swine (n = 3) Carbogen	4.50	5.10	7.50	8.80	13.70	17.00
	0.75	0.40	1.32	1.65	2.42	3.59
4 Per cent Halothane	4.00	4.90	6.60	9.10	12.10	16.50
	0.40	0.87	0.89	2.06	2.20	2.95

See table I for incubation procedure.
* Mean values expressed as μM lactate/gm muscle.
† SE.

ments[6] designed to determine responses to halothane inhalation *in vivo*. All muscle samples for ATP depletion and SR calcium accumulation experiments were obtained from swine which had had no previous exposure to anesthetics known to trigger malignant hyperthermia. Muscle for the SR calcium accumulation experiments was obtained from three control and three MHS swine, and two of the latter were subsequently challenged with halothane, with resulting acute fatal malignant hyperthermia. The third MHS pig has not been challenged, but susceptibility would seem to be confirmed by a 75 per cent decrease of muscle ATP during exposure to halothane *in vitro*. The three control swine similarly challenged with halothane did not develop malignant hyperthermia and recovered uneventfully.

Results

Depletion of Muscle ATP and Lactate Production in Vitro

Harrison *et al.*[4] initially reported the abnormally high depletion of ATP in MHS porcine muscle during exposure to carbogen *in vitro*. Addition of halothane vapor increased the ATP depletion. By this method they were able to identify susceptible Landrace swine. Results of similar studies of Poland China swine from our laboratory are summarized in table 1. There was no significant difference between the depletion of ATP in control swine muscle *in vitro* incubated in the absence and in the presence of halothane. MHS muscle incubated in Krebs–Ringer's solution and exposed to carbogen had an average depletion

TABLE 3. Results of Calcium-binding Studies in Isolated Porcine Sarcoplasmic Reticulum

	Sarcoplasmic Reticulum Concentration (mg SR Protein/gm Muscle)	Binding Capacity (μM Ca/mg)	Binding Capacity of Halothane-treated Sarcoplasmic Reticulum (μM Ca/mg)
Control swine (n = 3)	1.59*	11.84	15.60
	0.46†	2.84	4.00
MHS swine (n = 3)	1.51	13.26	14.40
	0.22	2.93	2.52

* Mean.
† SE.

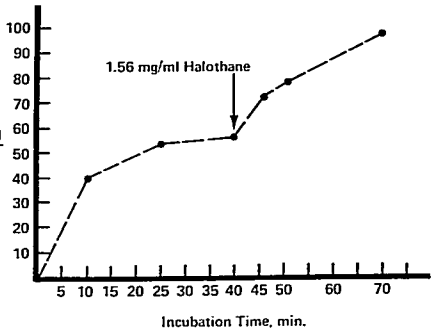

FIG. 1. Effect of halothane on calcium binding in isolated MHS porcine sarcoplasmic reticulum in the absence of oxalate. Conditions for assay are described in the text, except that oxalate was omitted. Uptake of Ca^{++} was measured for 40 minutes until uptake was constant. Addition of halothane increased Ca^{++} uptake.

of 67.5 per cent of the ATP content, or 30 per cent more ATP depletion than control muscle treated identically. Four per cent halothane in the gas phase induced a further 13.7 per cent increase in the depletion of ATP in MHS muscle. No differences between the ATP levels of fresh-frozen MHS and control muscles were detected.

The values for lactate production are listed in table 2. The presence of 4 per cent halothane in the gas phase produced a 14 per cent increase in lactate from MHS muscle, incubated for 30 minutes, but was without effect upon lactate from control muscle. In the absence of halothane, MHS muscle produced twice as much lactate (t test, $P < 0.05$) as did normal muscle.

Calcium Uptake by Isolated Sarcoplasmic Reticulum

No difference between the calcium uptake capacities of SR isolated from control and from MHS swine was found (table 3). When isolated SR was treated with 4 per cent halothane in 95 per cent O_2/5 per cent CO_2, both control and MHS SR had increased calcium uptake. The halothane-treated SR from control swine had a greater increase (average 32 per cent) than that from MHS swine (average 9 per cent). This data plotted in figure 1 show that, in the absence of oxalate and following steady-state conditions of calcium uptake and flux, addition of halothane increases calcium uptake by the SR.

Discussion

Elevated serum CPK levels,[6] abnormally high depletion of ATP *in vitro*, and marked lactate production from MHS muscle in the absence of anesthetic suggest a pre-existing myopathy which predisposes to stress. Such a hypothesis is supported by a 20 per cent mortality from acute stress of MHS swine in our colony. These deaths were triggered by fighting, escape from confinement, or moderate exertion, and were characterized by muscle rigidity, hyperthermia, and early onset of rigor mortis. Acute-stress deaths and high serum CPK levels have also been reported in MHS Pietrain swine.[15] Elevated serum CPK levels have been observed in a patient surviving malignant hyperthermia[16] and among relatives of a family in which three deaths due to malignant hyperthermia occurred.[17] We have challenged with halothane 17 swine, each having considerable depletion of muscle ATP *in vitro*, and each has developed acute, fatal malignant hyperthermia. ATP depletion *in vitro* and lactate production are high in MHS muscle in the absence of halothane, suggesting a pre-existing myopathy which, as regards ATP depletion and lactate production, is enhanced by halothane.

There are at least three major sources of ATP production in muscle tissue: creatine phosphate, mitochondrial oxidative phosphorylation, and glycolysis. During malignant hyperthermia there are decreases in both muscle glycogen[7] and ATP[4,7] and an increase of

blood lactate,[4, 6, 7] despite high blood P_{O_2} levels.[6, 7] These findings indicate either localized muscle anaerobiosis[7] or impaired oxidative phosphorylation. Although halothane can uncouple mitochondrial oxidative phosphorylation,[18-20] such an effect does not explain the abnormal depletion of MHS muscle ATP *in vitro* in the absence of halothane. Studies of muscle *in vivo*[4, 7] and *in vitro*[4] indicate rapid utilization of readily available ATP (via creatine phosphate), with stimulation of glycolysis due to loss of the negative modulator ATP and production of stimulator ADP and/or AMP. Impaired oxidative phosphorylation or inability to meet the rate of ATP utilization would ultimately deplete ATP.

The observations of Kalow *et al.*[9] that in MHS muscle halothane potentiates an enhanced sensitivity to caffeine-induced contracture and also inhibits calcium accumulation by isolated sarcoplasmic reticulum suggest that muscle contraction occurs prior to ATP depletion. We have not observed halothane inhibition of calcium uptake by sarcoplasmic reticulum isolated from MHS swine. A species difference, or the fact that the human tissue was obtained from patients who had recovered from malignant hyperthermia, whereas the MHS swine muscle was from animals not previously exposed to halothane, might explain this discrepancy.

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