

Low Molecular Weight Proteins in Human Malignant Hyperthermic Muscle

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Malignant hyperthermia (MH) is a pharmacogenetic disorder provoked by volatile anesthetics and depolarizing muscle relaxants. The preoperative diagnosis of MH is difficult because it requires a large muscle biopsy and a laboratory dedicated to such diagnostic studies. The authors performed electrophoresis of six muscles taken from MH patients or their relatives to determine whether the protein composition is different from normal muscle. MH muscle was found to contain large amounts of two low molecular weight proteins (15,000 daltons and 13,500 daltons) that are not present in normal muscle. Although it has not been determined that these differences are specific for MH, this finding eventually might be of assistance in diagnosing MH. (Key words: Hyperthermia; malignant. Muscle, skeletal. Proteins.)

ELECTROPHORESIS of human muscle recently has been used to demonstrate alterations in the protein constituents of diseased muscle.^{1,2} We have performed electrophoresis of muscle taken from patients who previously experienced malignant hyperthermia (MH) and from members of a family with a strong history of MH to determine any underlying difference in protein composition from normal human muscle.

MH is a metabolic syndrome triggered by volatile anesthetics and depolarizing muscle relaxants and characterized by elevated temperature, muscle rigidity, and metabolic acidosis.³ Clinical and pharmacologic evidence indicates that the skeletal muscles of MH-susceptible patients are abnormal.³ A single biochemical defect of

skeletal muscle has not yet been detected to explain the abnormal pharmacologic response of the MH patients. There have been conflicting reports of abnormalities of Ca^{2+} transport in skeletal muscle fractions.⁴⁻⁷ Elevations of myophosphorylase A and adenylcyclase also have been found.^{8,9} Thus far, none of these abnormalities have been reported consistently from one group of investigators to another. In this communication we demonstrate the marked increase of two low molecular weight proteins in the soluble fraction of vastus lateralis muscle taken from subjects who either have MH or are members of an MH family.

Methods

All specimens were obtained with the informed consent of the subjects and approval of the Human Subject Committee. MH muscle was taken from six subjects (M_1 , M_2 , M_3 , M_4 , M_5 , M_6). M_1 represents muscle from a 19-year-old man who developed elevated temperature and serum creatine kinase activity (CPK) after enflurane anesthesia accompanied by succinylcholine and pancuronium. Excised vastus lateralis muscle was examined for contracture and twitch characteristics and found to be abnormal.¹⁰ M_2 , M_3 , and M_4 represent muscle from relatives of a known MH family. M_2 was taken from a 14-year-old boy with an elevated resting CPK level, M_3 from his mother whose second cousin died of MH, and M_4 from a 22-year-old daughter who had a positive caffeine contracture test. M_5 was taken from a 23-year-old male who developed fever, rigidity, and metabolic acidosis during anesthesia. He had a markedly elevated CPK level ($>8,000$ U) postoperatively. Halothane contracture test was positive for MH. Muscle histology was consistent with an earlier episode of rhabdomyolysis. M_6 was taken from a 13-year-old boy with King-Denborough syndrome manifested by scoliosis, cryptorchidism, high arched palate, and an MH episode 5 years before biopsy. During a halothane anesthetic with succinylcholine, he developed marked rigidity, metabolic acidosis, and a PaCO_2 of 130 mmHg, despite vigorous ventilation. Postoperatively, he had an elevated CPK (3,100 U). His muscle histology was normal.

Normal vastus lateralis muscle was obtained from six control patients, (17 to 43 years of age) undergoing

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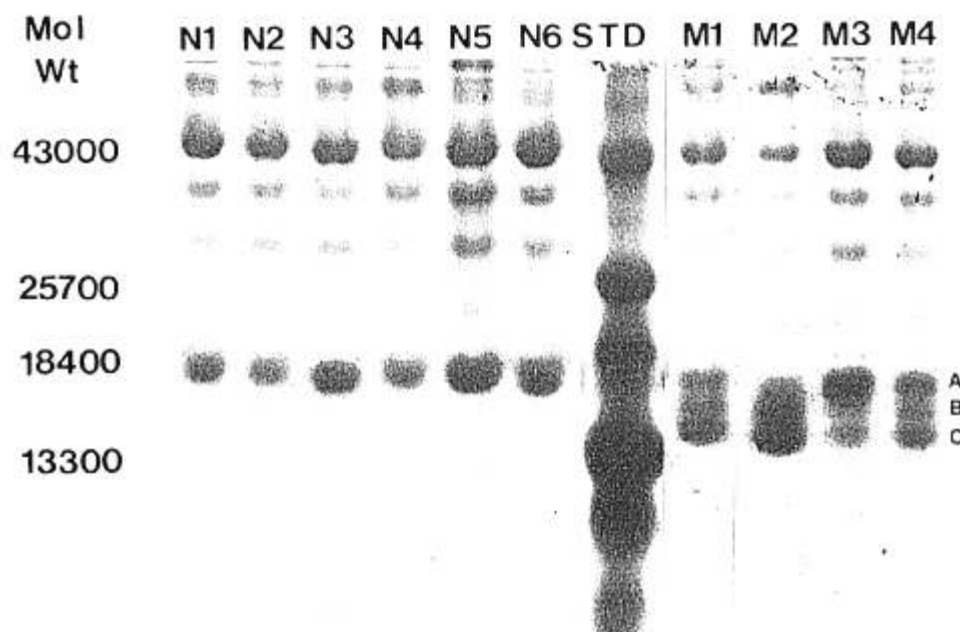


FIG. 1. Fifteen per cent SDS-polyacrylamide gel electrophoresis of soluble proteins from human vastus lateralis: N₁-N₆, normal muscle; STD, standard known molecular weight proteins; and, M₁-M₄, MH (M₁) and MH related (M₂-M₄) patients. A represents a protein found in all muscles examined and is labeled to serve as an internal reference. Labels B and C represent proteins of 15,000 d and 13,500 d irrespectively.

orthopedic surgery for knee injuries. None had a family history of muscle disease or of an abnormal response to anesthesia. Muscle weighing approximately 400 mg was homogenized immediately, centrifuged, and frozen or frozen and stored before fractionation. All muscle and muscle fractions were stored at -65°C . The muscle was weighed, minced with scissors, and suspended in 5 volumes of 10 mM KCl and 250 mM sucrose at pH 7.3, 4°C . The suspension was homogenized with a Tekmar "tissumizer" at 80% maximal speed for 30 s and filtered through two layers of cheesecloth. The filtrate was diluted 1:1 with the above buffer and centrifuged at a force of $49,000 \times g$ for 35 min; supernatant and pellet were recovered from this centrifugation. Electrophoresis was performed on the supernatant. Protein concentration was determined by the Coomassie binding technique.¹¹

SODIUM DODECYL SULFATE (SDS) POLYACRYLAMIDE ELECTROPHORESIS

Samples were diluted 1:1 with 2% SDS and 2% β mercaptoethanol and heated for 3 min at 90°C . The supernatant was treated as above and 12.5 μg of protein applied to 15% polyacrylamide gels with a 3.5% stacking gel. This system provided an accurate assessment of low molecular weight proteins.^{2,11} Electrophoresis was performed at pH 7.0 in 0.1 M phosphate buffer on 0.5 mm slab gels at 5 v/cm. Proteins of known molecular weight (ovalbumin 43,000 dalton (d), α -chymotrypsinogen

25,700 d, lysozyme/cytochrome C13,300 d, and bovine trypsin inhibitor 6,200 d) were electrophoresed on the same gel. Gels were stained with Coomassie blue and destained with 10% acetic acid, 10% isopropanol. Molecular weights of unknown proteins were determined by comparison with the migration of the standard proteins. Optical density scans of the stained gels were performed on a Gelman ACD 15[®] densitometer.

Acrylamide, N,N,methylene bisacrylamide, N,N,N,N,tetramethylethylenediamine, and Coomassie blue all were purchased from Bio-Rad Laboratories, Richmond, California.

Results

Electrophoretic patterns of muscle supernatant proteins from six normal, one MH, and three MH-related patients are shown in figure 1. Two extra densities are present near the front of the gel in the samples from the MH and MH-related patients. The extra densities, corresponding to labels B and C in figure 1, represent proteins of approximately 15,000 d (B) and 13,500 d (C) molecular weight. The density labeled A represents a protein found in all samples and is labeled to serve as an internal reference. The estimated molecular weight is based on a comparison of electrophoretic patterns with known molecular weight proteins.

Figure 2 demonstrates the electrophoretic pattern in two additional MH patients, M₅ and M₆. Both have definitive MH episodes 6 months and 5 years before muscle biopsy was obtained, respectively. Both lanes M₅ and M₆ show the presence of the extra low molecular

¹¹ Bethesda Research Laboratories: A procedure for gel electrophoresis of low molecular weight proteins. *Biologie* 1: 6, 1981.

weight densities (B and C) that are observed in patients M_1 through M_4 .

The optical density scan of the electrophoretic gels of a normal sample, an MH-related sample, and an MH sample are shown in figure 3. The abscissa represents the distance the protein constituents have migrated, and the ordinate indicates the relative optical density of the sample. The area under each peak represents the relative amount of each protein constituent. Distinct differences in peaks are apparent in the low molecular weight region (right side of figure). In the scan from the MH patient's muscle two peaks (B and C) are evident that

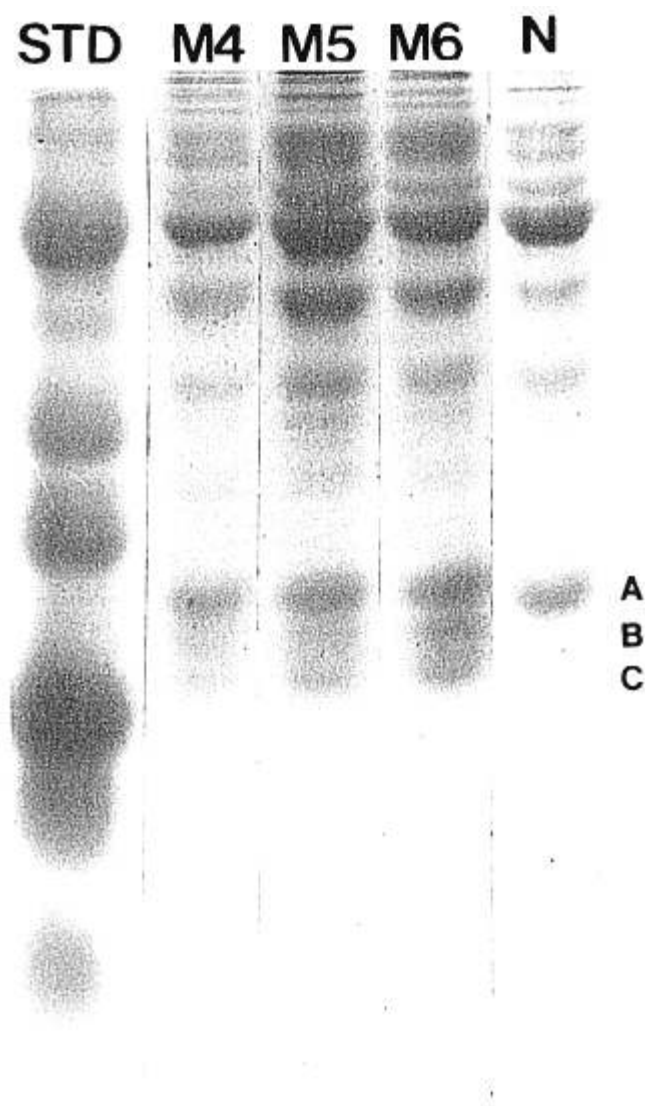


FIG. 2. Fifteen per cent SDS-polyacrylamide gel electrophoresis of soluble proteins from human vastus lateralis: STD, standard known molecular weight proteins M4, MH-related patient (fig. 1); M5, MH patient; M6, MH patient with King-Denborough syndrome; N, normal muscle.

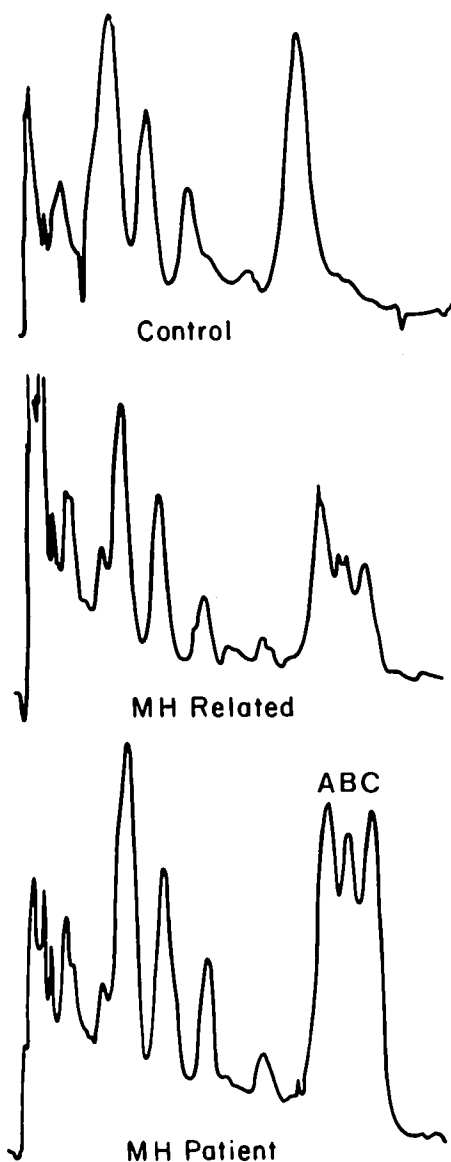


FIG. 3. Representative optical density scan of 15% SDS-polyacrylamide gels. Gels scanned at 595 nm. The abscissa represents the distance migrated along the gel, and the ordinate represents the relative optical density.

do not appear in the pattern from normal muscle. Peaks B and C are also present but to a somewhat lesser extent in the pattern from the MH-related patient. The optical density patterns in the remainder of the gel (left half) are quite similar for all three samples; they are, however, not exactly the same.

Discussion

We have observed the presence of two low molecular weight proteins in the supernatant fraction of homoge-

nized human MH vastus lateralis muscle that were not seen in normal human vastus lateralis muscle. These two proteins have molecular weights of 15,000 d and 13,500 d, respectively. The proteins are soluble, based on their separation into the supernatant phase by a high-speed centrifugation. We have not ruled out the possibility that proteins B and C are peptide fragments from proteolysis of larger soluble, myofibrillar, or membrane-bound proteins rather than native proteins. This alternative, if proven, would suggest that the population of proteolytic enzymes in MH muscle is more active than normal muscle.

The measurement of low molecular weight proteins in MH muscle might be useful as a diagnostic tool for distinguishing MH from normal patients. Until a greater number of MH and normal patients have been screened, it is premature at this time to use this distinction in protein composition as a diagnostic criterion for MH. It is also uncertain whether these findings are unique to muscle from MH patients as opposed to muscle from patients with other muscle diseases.

We have confined our electrophoretic comparison between normal and MH muscle to vastus lateralis muscle because the other muscle types that we have examined had different electrophoretic patterns and because vastus lateralis was the muscle most often biopsied for diagnostic purposes. The question also arises as to whether the extra proteins in MH muscle are involved in the pathophysiologic mechanism of MH. As yet, we have no information regarding this. The molecular weights of the extra proteins are similar to that of several well-known Ca^{2+} binding proteins such as parvalbumin, calmodulin, and troponin C. The Ca^{2+} binding characteristics of the MH proteins are unknown at this time but are being examined.

In conclusion, we have observed two low molecular weight proteins of 15,500 d and 13,500 d that are found

in large amounts in MH vastus lateralis muscle but not in normal vastus lateralis muscle.

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