'Picture Frame' Fibres in a Carrier of the Trait for Malignant Hyperpyrexia

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SUMMARY

A member of a family which was known to be susceptible to malignant hyperpyrexia, who was identified as a carrier by the presence of an elevated serum creatine-phosphokinase, has been investigated further. Muscle was examined biochemically, and the study included the sarcoplasmic ATPase-activity, actinomycin, Mg\textsuperscript{2+} ATPase activity, ATP, phosphocreatine and glucose-6-phosphate. In addition, the calcium uptake by the sarcoplasmic reticulum was studied.

The histochemical analysis of the muscle revealed the presence of a new fibre type characterised by a dense rim of ATPase activity, which gives the impression of a 'picture-frame'.

Ultra microscopic study revealed changes in the mitochondria and areas of myofibrillar disruption with swelling of the sarcoplasmic reticulum.


Various diseases of muscle in carriers of the trait for malignant hyperpyrexia have been described.1,4 The patient described in this article was identified as a carrier by an estimation of the serum creatine phosphokinase activity. He is a symptom-free, 19-year-old member of a family which is known to be susceptible.

Electron microscopy of a muscle biopsy specimen was performed and his carrier state was also confirmed by histochemistry and the muscle response in vitro to halothane exposure. Sarcoplasmic reticulum and biochemical studies were also performed.

METHOD

Muscle was removed from the left quadriceps femoris under local anaesthesia, and care was taken not to infiltrate the muscle. Appropriate specimens were removed for histological, histochemical, electron microscopic, muscle tension and biochemical studies. The specimen for histo-

logical and histochemical examinations was removed according to standard techniques, and 10-μm-sections were cut on a cryostat. The sections were processed in the usual way for NAD diaphorase, phosphorylase, ATPase at pH 4.3, 4.6, 9.4 and, after prior treatment with EDTA at pH 4.5. The muscle was also stained with haematoxylin and eosin (H and E), PAS, and a modified trichrome.

The muscle for electron microscopy was maintained at resting length, buffered with cacodylate and fixed in 4% osmium. Thin sections were cut, stained and viewed with a Siemens Eimskop electron microscope.

Sarcoplasmic reticulum (SR) and actomyosin were prepared by a standard ultracentrifugal method.5 SR ATPase activity and calcium uptake and actomyosin Mg\textsuperscript{2+}-activated ATPase activity were measured as previously described.6 ATP, phosphocreatine and glucose-6-phosphate were measured enzymically by the method of Lamprecht and Stein.7 Alkali-soluble protein in the specimen for biopsy was rendered soluble by the method of Lilienthal et al.8, and determined by the biuret method.

All the solutions which were used were prepared in glass-double distilled water, and chemicals were of analytical grade, where possible. Counting of Ca was carried out with a Packard Tri-carb liquid scintillation counter and Instagel scintillation fluid. CaCl\textsubscript{2} was obtained from the Radiochemical Centre, UK.

The halothane contracture test was carried out on a separate strip of muscle, in a muscle-bath, at 37°C, by a method similar to that of Ellis et al.9 Tension was recorded with a Statham U3C force transducer on a Beckman Dynograph. Halothane (Fluothane; ICr Ltd, Johannesburg) was introduced to the Ringer solution (pH 7.3) in the muscle bath via a calibrated Fluotec MkII vapouriser at a level of 3.2%.

RESULTS

The histology of the muscle as revealed by H and E and the modified trichrome stain, was normal. PAS staining revealed a normal glycogen distribution. Histochemical examination for phosphorylase was normal and a number of the smaller fibres were noted to be type 2. The NAD-diaphorase reaction was normal. The ATPase studies at pH 4.3, 4.6, 9.4 and 4.5 with prior treatment with EDTA, were abnormal in that a rim of excessive ATPase activity (Figs 1, 2, 3) was noted in many type 2 fibres, and particularly at the lower pH range. The characteristics of these fibres identified them as Type 2c, according to the classification of Brooke and Kaiser.10 In addition to the picture frame impression of this increased activity,
there were blob-like collections of increased activity in the subsarcolemma and within the fibres (Fig. 4).

Electron microscopic study revealed areas of mitochondrial accumulation, many of which were swollen, and contained only remnants of cristae (Fig. 5). There were also widespread areas where swelling of the sarcoplasmic reticulum and disruption of the normal myofibrillar pattern were noted.

The halothane contracture test carried out on a fresh viable muscle strip, was positive (Fig. 6). The increase in tension caused by halothane was evident after 5 minutes' exposure to the anaesthetic and it continued to increase for 20 minutes after first exposure. Halothane was then discontinued, whereupon the tension slowly fell to almost the initial value.

Table I shows the ATPase activity and calcium uptake by the SR, and the Mg²⁺-activated ATPase activity of actomyosin. Both the ATPase activity and the rate of calcium uptake by the SR were in the normal range. The most significant finding is the (approximate) one-half decrease in the total calcium uptake by the SR. In the patient reported here, the actomyosin ATPase activity was 40% greater than the mean activity of the same enzyme observed in 3 other carriers of the trait for malignant hyperpyrexia. However, the activity was just 9% more than the upper limit of the range of actomyosin ATPase activity in clinically normal muscle (range:  

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**Fig. 1.** ATPase preparation at pH 4.3 showing intense activity at the periphery of fibres with subsarcolemmal accumulations (x 80).

**Fig. 2.** ATPase preparation at pH 4.5 with prior treatment with EDTA, showing the intense peripheral activity of the involved fibre population, with occasional areas of intense central activity and subsarcolemmal activity (x 80).

**Fig. 3.** ATPase preparation at pH 9.4 showing peripheral activity with central areas of intense ATPase in many involved fibres (x 80).

**Fig. 4.** ATPase preparation at pH 4.3 demonstrating areas of subsarcolemmal and mid-fibre excessive ATPase activity (x 320).
FIG. 5. Fine structure, demonstrating a collection of mitochondria, several of which are swollen, and show only remnants of cristae (x 10 000).

Fig. 6. The rise in tension in the muscle strip in response to halothane exposure. The arrow indicates the point of halothane withdrawal, and the subsequent fill interim is plotted.

0.218 - 0.272 μmol Pi per mg protein per minute). In this case the levels of glucose-6-phosphate and ATP in the muscle were normal while there was a small decrease in the level of phosphocreatine (10.5 μmol/g compared with normal range of 13.1 - 18.3 μmol/g). The alkali-soluble protein was also in the normal range.

DISCUSSION

This patient, a carrier of the trait for malignant hyperpyrexia which was originally diagnosed by his elevated serum creatine phosphokinase activity, also showed evidence of sensitivity to halothane as determined by muscle strip exposure.

Work on the SR in malignant hyperthermia has been extensive, and although the SR has been implicated as the probable site of the primary biochemical defect in the malignant hyperthermia syndrome, we recently showed that halothane, the causative agent of MH in most cases, had no inhibitory effect on the calcium-accumulating ability of the isolated SR from susceptible individuals, at anaesthetic concentrations. However, the rate of SR Ca++-dependent ATPase activity, rate of calcium uptake and total calcium uptake were decreased in the muscle from the susceptible individuals. The present case

The normal rates of ATPase activity and calcium uptake by the SR, while total uptake of calcium was halved. The ‘basal’ or calcium-independent ATPase activity of the SR was 0.556 μmol Pi/mg protein/min, an entirely normal value.

The normal rates of ATPase activity and calcium uptake by the SR in this case of malignant hyperthermia show that the syndrome may exist without any coexisting abnormality in the function of the SR, and suggest further that the SR is not primarily involved in the triggering of the syndrome by halothane. Sreter found that the rate of SR ATPase activity and calcium uptake decreased after experimental denervation of rat muscle, while the total calcium uptake rose sharply in the initial period of denervation and eventually fell below normal. The rate of calcium uptake by the SR is probably a better measure of the integrity of the physiological functioning of the SR, since the functional role of the SR depends largely on the speed with which it removes calcium ions from the sarcoplasm in the phase of relaxation.

Since the rate of calcium uptake varies according to the muscle fibre type, we suggest that fibre type counts be made and correlated with this sarcoplasmic function. Slow fibres have a lower calcium-uptake than fast fibres.

TABLE I. BIOCHEMICAL CHARACTERISTICS OF THE MUSCLE OF A PATIENT WITH MALIGNANT HYPERTHERMIA

<table>
<thead>
<tr>
<th>Sarcoplasmic reticulum</th>
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<tbody>
<tr>
<td>Ca++-dependent ATPase activity (μmol Pi/mg protein/ min, 37°C)</td>
<td>0.877</td>
</tr>
<tr>
<td>'Basal' ATPase</td>
<td>0.556</td>
</tr>
<tr>
<td>Calcium uptake Rate (μmol/mg protein/min, 37°C)</td>
<td>0.561</td>
</tr>
<tr>
<td>Total (μmol/mg protein, 37°C)</td>
<td>0.961</td>
</tr>
<tr>
<td>Mg++-activated actomyosin ATPase activity (μmol Pi/ mg protein/min, 30°C)</td>
<td>0.296</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>10.5</td>
</tr>
<tr>
<td>ATP</td>
<td>5.9</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>0.5</td>
</tr>
<tr>
<td>Alkali-soluble protein (mg protein/g muscle)</td>
<td>198.0</td>
</tr>
</tbody>
</table>
We believe that an alteration in fibre population accounts largely for the low uptake in patients with central core disease, as found by Isaacs et al.\textsuperscript{11}

The most interesting feature of this case relates to the presence of excessive patchy and peripheral activity of ATPase in the small type 2 fibres. This provides yet another histopathological state which must be added to the list of pathologies of muscle which occur in carriers of the trait for malignant hyperthermia. This peripheral concentration of ATPase activity has not been described before. The biochemistry of increased activity of this muscle conforms to the histochemical appearance.

This abnormal appearance was confined to ATPase activity and was not seen in the muscle sections processed for phosphorylase and NAD diaphorase, and all the histochemical processes were carried out on the same muscle at the same time, which excluded the possibility of 'pie' artifact.\textsuperscript{12}

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REFERENCES


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