ADENYLATE KINASE AND MALIGNANT HYPERPYREXIA

L. A. MARJANEN AND M. A. DENBOROUGH

SUMMARY

The isoenzyme pattern and activity of adenylate kinase was studied in muscle and red blood cells from individuals and swine shown to be susceptible to malignant hyperpyrexia. No abnormality in adenylate kinase isoenzyme pattern or activity was found.

Malignant hyperpyrexia (MH) occurs in individuals and swine with an underlying disease of skeletal muscle (Denborough, 1980). The enzyme adenylate kinase (AK), a phosphotransferase, catalyses the reversible reaction: 2 ADP ⇌ ATP + AMP, occurs in many tissues and plays an important role in regulating the concentration of cellular adenine nucleotide (Noda, 1973). During an episode of MH muscle adenosine triphosphate (ATP) is depleted (Harrison et al., 1969). However, although a deficiency of skeletal muscle adenylate kinase was reported in one family susceptible to MH (Schmitt, Schmidt and Ritter, 1974), in another investigation no abnormality was found in skeletal muscle adenylate kinase in MH (Cerri et al., 1981).

As a result of the disparity in these findings investigations were undertaken on adenylate kinase in both red blood cells and skeletal muscle from man and swine susceptible to MH. The findings were compared with those from normal individuals and non-susceptible swine.

METHODS

Susceptibility to MH

In all instances susceptibility was assessed on muscle biopsy samples by pharmacological methods which have been described previously (Moulds and Denborough, 1974; Okumura, Crocker and Denborough, 1979).

Measurement of adenylate kinase

Preparation of samples. Adenylate kinase was extracted from muscle biopsy samples in four volumes of Tris-HCl 5 mmol litre⁻¹ with Na-EDTA 0.5 mmol litre⁻¹ pH 8.0 unit as described by Cerri and colleagues (1981).

Red cell lysates were prepared by mixing one volume of packed cells with two volumes of water (Fildes and Harris, 1966).

Electrophoresis. Starch gel electrophoresis of muscle and red blood cell AK was carried out by the method described by Smith (1968), at 4°C using a Tris-citric acid buffer, pH 7.0 unit (Schmitt, Schmidt and Ritter, 1974), and a staining solution in 1% agar (Fildes and Harris, 1966).

AK activity. AK activity was assayed spectrophotometrically by coupled enzyme assay systems (Fildes and Harris, 1966). Both forward (ADP utilization) and backward (ADP formation) reaction systems were used.

Protein concentration was measured by the method of Lowry and co-workers (1951).

AK inhibitors. Isoenzymes of AK can be distinguished by the use of inhibitors of AK activity (Khoo and Russell, 1972; Russell et al., 1974), and with this in mind the effect of the sulphhydryl reacting reagents silver nitrate, M-ethylmaleimide (NEM) and p-hydroxymercuribenzoate (p-OH-MB) on the AK enzyme patterns and AK activity were assessed.

RESULTS

Starch gel electrophoresis

Muscle. In human muscle (and red blood cells) three distinct types of electrophoretic pattern are recognized and are referred to as AK 1, AK 2-1 and AK 2 (Fildes and Harris, 1966). About 90% of individuals in the English population have the AK 1 phenotype and about 10% have the AK 2-1 phenotype. AK 2 is very uncommon.

In the present study, the electrophoretic pattern

© The Macmillan Press Ltd 1982
of muscle AK was investigated in 10 individuals who were susceptible to MH and in 12 who were not. Each of these showed the characteristic AK\(^1\) pattern (fig. 1A). The AK isoenzyme pattern was studied also in muscle from 15 pigs susceptible to MH and in four controls. A pattern similar to human muscle AK\(^1\) was found in each animal (fig. 1B). However, each component in porcine AK moved faster than the component in human AK. Also, in porcine AK, the minor, faster moving components were not always clearly seen. There were no differences in the isoenzyme pattern between susceptible and control pig muscle.

**Red blood cells.** The red blood cell AK phenotype in two patients who were susceptible to MH and in two controls was AK\(^1\) (fig. 1A). In addition, 20 blood samples from random blood donor controls were studied. One AK\(^2-1\) phenotype was found (fig. 1A). The others were all AK\(^1\). The AK pattern in human red blood cells was the same as in human muscle.

In porcine red blood cells the AK pattern was the same in two pigs which were susceptible to MH as in two pigs which were not. The AK pattern in porcine red blood cells was the same as in porcine muscle (fig. 1B).

**AK activity studies**

The activities which were measured spectrophotometrically in muscle are shown in table I. Greater AK activity was found in porcine samples than in human preparations, but no statistically significant differences in AK activity were found between MH-susceptible humans or swine and controls (Student's \(t\) test).

**Effect of specific inhibitors**

Specific inhibitors of AK invariably abolished the
TABLE I

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Specific activity†* (AOD min⁻¹/mg protein)</th>
<th>No samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control human</td>
<td>10.2 ± 4.7</td>
<td>11</td>
</tr>
<tr>
<td>MH-susceptible human</td>
<td>8.9 ± 3.7</td>
<td>12</td>
</tr>
<tr>
<td>Control porcine</td>
<td>26.4 ± 2.3</td>
<td>6</td>
</tr>
<tr>
<td>MH-susceptible porcine</td>
<td>23.6 ± 4.1</td>
<td>8</td>
</tr>
</tbody>
</table>

AK electrophoretic patterns and AK activity in all the samples tested, regardless of whether they were from humans or swine, and whether or not they were susceptible to MH.

DISCUSSION

The demonstration of an abnormality in AK activity in malignant hyperpyrexia would be not only of theoretical interest but could also have considerable practical significance. The use of a simple enzyme test to screen for susceptibility to MH would be a considerable advance in the prevention of the anaesthetic complication. At present, the only definitive way to identify susceptibility to MH is to carry out a complicated in vitro muscle test under stringent conditions (Denborough, 1980).

The observation that adenylate kinase was deficient in a family susceptible to MH (Schmitt, Schmidt and Ritter, 1974) prompted the present investigation in which adenylate kinase activity has been examined in humans and swine which were susceptible to MH and in controls. In the present study, no deficiency in the activity or isoenzyme pattern of adenylate kinase in either skeletal muscle or red blood cells was found in MH-susceptible humans or swine. Although no statistically significant difference was observed in the present study between control and MH muscle, low AK activities were occasionally found in both groups. In these instances only the major band of the electrophoretic pattern of AK was visible, and larger amounts of samples were required to show the presence of the minor, fast-moving components.

REFERENCES


ACKNOWLEDGEMENT

The help given by Dr Max Blake with starch gel electrophoresis is gratefully acknowledged.

ADENYLATE KINASE ET HYPERTHERMIE MALIGNE

RESUME

Le profil des isoenzymes et l'activité de l'adénylate kinase ont été étudiés dans le muscle et les hématoles venant de patients et de porcs dont on avait prouvé qu'ils étaient prédisposés à l'hypertthermie maligne. Il n'a pas été trouvé d'anomalies du profil des isoenzymes ou de l'activité de l'adénylate kinase.
ZUSAMMENFASSUNG
Das Isoenzym-Muster und die Aktivität der Adenylat-Kinase wurden im Muskel und den roten Blutkörperchen von Menschen und Schweinen, die sich empfindlich für die maligne Hyperthermie gezeigt hatten, untersucht. Es wurde kein abnormales Enzymmuster oder abnormale Enzymaktivität gefunden.

SUMARIO
Se estudió la estructura y la actividad de la enzima kinasa de adenilato en los músculos y en los glóbulos rojos de la sangre de individuos y de cerdos que mostraron susceptibilidad a la hiperpirexia maligna. No se encontró anormalidad alguna en la estructura o actividad de dicha enzima.