A SCREENING TEST FOR THE MALIGNANT HYPERPYREXIA PHENOTYPE USING SUXAMETHONIUM-INDUCED CONTRACTURE OF MUSCLE TREATED WITH CAFFEINE, AND ITS INHIBITION BY DANTROLENE

P. J. HALSALL AND F. R. ELLIS

SUMMARY

Suxamethonium induced a contracture in caffeine pretreated human muscle in vitro. The contracture was significantly greater \( (P < 0.001) \) with MHS muscle compared with muscle from normal subjects. This reaction is now used as an additional screening test for the MHS phenotype. The contracture was prevented by pretreatment with dantrolene.

The diagnosis of susceptibility to malignant hyperpyrexia (MHS) depends on the demonstration of halothane-induced contracture of a muscle biopsy specimen at 37 °C in vitro (Ellis et al., 1978). Although the diagnosis of MHS is made in our laboratory from the results of the halothane contracture test, the caffeine and caffeine/halothane sensitivities of the muscle are determined routinely in a manner similar to that described by Kalow, Britt and Richter (1977), with the modification that all investigations are made at 37 °C. Moulds and Denborough (1974) used potassium chloride and suxamethonium, in addition to halothane and caffeine, to induce contracture of MHS muscle. We have rarely obtained significant contractures of MHS muscle with suxamethonium alone. However, Moulds (personal communication) described a method combining caffeine and suxamethonium which induces a contracture in normal muscle. It was decided to study this combination as a possible additional method of screening MHS patients.

The effect of dantrolene on the contracture produced by this drug combination was studied in view of its potential importance in the treatment of the patient developing MH and the associated muscle contracture.

METHODS

Twenty consecutive patients being screened for MH were studied. All were apparently healthy, aged 12–45 yr. The muscle biopsy was taken across the motor point in the left vastus medialis muscle under general anaesthesia (Cain and Ellis, 1977). The biopsy specimens were placed in fresh oxygenated Krebs solution and transported to the laboratory at room temperature. Fresh muscle fibres measuring 20 mm × 2 mm × 1 mm were dissected from the gross biopsy specimens and placed in a 2-ml tissue bath and perfused with fresh oxygenated Krebs solution at 37 °C. Moulds and Denborough (1974) used potassium chloride and suxamethonium, in addition to halothane and caffeine, to induce contracture of MHS muscle. We have rarely obtained significant contractures of MHS muscle with suxamethonium alone. However, Moulds (personal communication) described a method combining caffeine and suxamethonium which induces a contracture in normal muscle. It was decided to study this combination as a possible additional method of screening MHS patients.

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Statistical evaluation of the results was made using Student's t test for unpaired means and differences between the means were assessed using two-tailed probability distribution tables.

RESULTS

Of the 20 patients studied, 10 were found to be MHS and 10 were normal (MHN) according to the results of the halothane challenge test.

TABLE I. Mean changes in tension (g) ± SEM developed by muscle samples after administration of suxamethonium 6.29 mmol litre⁻¹ with tissue bath perfused with Krebs solution containing caffeine 4 mmol litre⁻¹

<table>
<thead>
<tr>
<th>Tension (g)</th>
<th>Significance between MHS and MHN groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHS</td>
</tr>
<tr>
<td>1st exposure</td>
<td>4.8 ± 0.82</td>
</tr>
<tr>
<td>2nd exposure</td>
<td>5.72 ± 0.84</td>
</tr>
</tbody>
</table>

From table I it can be seen that the muscle contractions induced by the suxamethonium/caffeine combination in a perfused tissue bath were significantly greater in the MHS group compared with the MHN group. Typical tracings produced by caffeine and suxamethonium are shown in figures 1A (MHS) and

![Fig. 1. A](image1)

From these tracings it may be seen that the response produced by MHS muscle occurred rapidly, whereas that produced by MHN muscle occurred slowly, if at all. There was no significant difference between the weights of muscle samples in the two groups (P > 0.9).

The results of the effect of dantrolene on muscle contracture induced by caffeine/suxamethonium are shown in table II. Dantrolene 0.04 mmol litre⁻¹ was effective in preventing the induced contractures in most muscle samples from both MHS and MHN patients. A statistical evaluation of these findings is

![Fig. 2. A](image2)

FIG. 2. A: Trace showing the effect of suxamethonium 6.29 mmol litre⁻¹ on normal caffeinated muscle. B: Trace showing the effect of dantrolene 0.04 mmol litre⁻¹ on the contracture produced by normal muscle with suxamethonium 6.29 mmol litre⁻¹ and caffeine 4 mmol litre⁻¹.

2A (MHN). From these tracings it may be seen that the response produced by MHS muscle occurred rapidly, whereas that produced by MHN muscle occurred slowly, if at all. There was no significant difference between the weights of muscle samples in the two groups (P > 0.9).

The results of the effect of dantrolene on muscle contracture induced by caffeine/suxamethonium are shown in table II. Dantrolene 0.04 mmol litre⁻¹ was effective in preventing the induced contractures in most muscle samples from both MHS and MHN patients. A statistical evaluation of these findings is

![Fig. 2. B](image3)

**TABLE II. Mean changes in tension (g) developed by muscle samples after administration of suxamethonium 6.29 mmol litre⁻¹ with perfusion of Krebs solution containing dantrolene and caffeine**

<table>
<thead>
<tr>
<th>Tension (g)</th>
<th>MHS</th>
<th>MHN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st exposure</td>
<td>0.16 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>2nd exposure</td>
<td>0.48 ± 0.2</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

**TABLE III. Tests of significance between tables I and II for MHS and MHN groups**

<table>
<thead>
<tr>
<th></th>
<th>MHS comparison</th>
<th>MHN comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td>1st exposure</td>
<td>5.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2nd exposure</td>
<td>5.59</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
shown in table III. Figures 1b (MHS) and 2b (MHN) show the typical response to caffeine/suxamethonium after pretreatment with dantrolene. In the three muscle samples most sensitive to the combination of caffeine and suxamethonium, dantrolene 0.04 mmol litre\(^{-1}\) was insufficient to prevent a contracture occurring, although the contracture height was reduced. In two of these samples the concentration of dantrolene was increased to 0.08 mmol litre. This greater concentration further reduced the contracture height, but did not prevent its occurrence. The difference between the weights of muscle samples in the MHS and MHN treated and untreated groups was not statistically significant (\(P > 0.4\)).

**DISCUSSION**

Normal muscle responds to caffeine by producing a gradual increase in tension as a result of both the release of Ca\(^{2+}\) from storage sites in the sarcoplasmic reticulum (SR) and by preventing re-accumulation of Ca\(^{2+}\) by the SR which allows relaxation (Bianchi, 1963; Herz and Weber, 1965). Suxamethonium alone never produces any change in tension in normal muscle in vitro. In our experience, only in two patients from one family has suxamethonium alone ever produced a contracture of MHS muscle in vitro. Thus, the contracture induced by the caffeine/suxamethonium combination must be a synergistic action. Since suxamethonium is a large, charged molecule which does not cross a charged cell membrane, it is reasonable to suppose that it is acting at the cell membrane rather than in the cell itself. It may be that small increases in movement of “trigger” Ca\(^{2+}\) across the cell membrane with suxamethonium (the first part of excitation-contraction coupling), have an explosive effect on the intracellular Ca\(^{2+}\) translocations in the sarcoplasmic reticulum already stimulated by caffeine (the second part of excitation-contraction coupling). Hence the immediate dramatic increase in tension seen after the drug combination (fig. 1).

The prevention by dantrolene of the caffeine/suxamethonium contracture suggests that a least two of these drugs act at the same site in the excitation-contraction coupling system. Dantrolene may uncouple this system by its effects on calcium mobilization either at the plasma membrane site or in the sarcoplasmic reticulum (Ellis and Bryant, 1972; Nelson, 1978).

The synergistic interaction of caffeine and suxamethonium distinguishes between MHS and MHN muscle. Unlike the halothane contracture test, the caffeine/suxamethonium test produces a graded response and is a useful additional test when investigating a patient’s susceptibility to MH. The result of the halothane contracture test still remains the main diagnostic criterion.

It is hoped to use the new test to help to distinguish between the possibly various MHS phenotypes suggested by Kalow, Britt and Richter (1977) and Ellis and others (1978).

**REFERENCES**


**ESSAI DE DEPISTAGE DU PHENOTYPE D’HYPERPYREXIE MALIGNE A L’AIDE DE LA CONTRACTURE DU MUSCLE TRAITE A LA CAFEINE, PROVOQUEE PAR LE SUXAMETHONIUM DE MEME QUE SON INHIBITION PAR LE DANTROLENE**

**RESUME**

Le suxaméthonium a provoqué *in vitro* la contracture d’un muscle humain prétraité à la caféine. La contracture a été beaucoup plus forte (*P < 0,001*) avec les muscles réagissant à l’hyperpyrexie maligne qu’avec les muscles des sujets normaux. On se sert maintenant de cette réaction comme essai de dépistage supplémentaire pour le phénotype d’hyperpyrexie maligne. La contracture a été empêchée par un prétraitement au dantrolène.