IDENTIFICATION OF SUSCEPTIBILITY TO MALIGNANT HYPERPYREXIA IN SWINE

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SUMMARY

In vitro muscle contracture responses in swine susceptible to malignant hyperpyrexia (MH) were similar to those found in muscle from humans susceptible to this anaesthetic complication, confirming the suitability of the pig as an animal model for studying MH. The results suggest that there are different degrees of susceptibility to MH. Whichever drug was used, there was some overlap in the contracture responses between susceptible animals and controls, suggesting that the most accurate way of identifying susceptibility to MH is to use a variety of chemical agents, the best of which seem to be halothane, caffeine, suxamethonium and potassium chloride. Thymol, which is used as a preservative in commercial preparations of halothane, potentiates halothane contractures, but it is not known if this is significant clinically.

Individuals who are susceptible to malignant hyperpyrexia (MH) have an underlying disease of muscle. The most precise means of identifying susceptibility to MH is pharmacological testing in vitro on muscle from the individual in question.

Several different pharmacological tests are used. One examines the contracture response to caffeine (Kalow et al., 1970), another to halothane (Ellis et al., 1972), and a third studies the contracture response to a variety of agents including caffeine, halothane, suxamethonium and potassium chloride (Moulds and Denborough, 1974).

In the present study an attempt has been made to define the most reliable pharmacological tests for identifying susceptibility to MH, using susceptible swine as an animal model.

MATERIALS AND METHODS

Pure Landrace, pure Large White or crossbred Landrace-Large White swine were screened for susceptibility to MH using halothane anaesthesia. Anaesthesia was induced with 3% halothane in oxygen via a face mask, and maintained with 1-1.5% halothane. Rectal temperature was monitored continuously using a thermistor. MH was diagnosed by a rapid increase in temperature, stiffness of the legs, abnormal respiration and blotchy cyanosis of the skin.

As soon as the diagnosis was made, the halothane was discontinued and the animal was cooled by sponging with water or alcohol. Tracheal intubation and artificial ventilation with pure oxygen was carried out and procaine hydrochloride 3-5 mg kg⁻¹ and sodium bicarbonate were given i.v. if necessary.

All the control swine failed to develop abnormal symptoms or signs or an increase in temperature after halothane anaesthesia for 1 h followed by suxamethonium 2 mg kg⁻¹ i.v. Nineteen of 326 swine were susceptible, of which 10 died during the challenge. One pig which died developed MH only after being given halothane and suxamethonium. The remainder developed the syndrome with halothane alone. The nine survivors consisted of five Landrace, three crossbred Landrace-Large White and one Large White.

The mean maximum body temperature during the challenge in the 19 susceptible swine was 40.2 ± 1.2 (SD) °C and the mean time to peak temperature from the beginning of halothane administration was 27.3 ± 14.3 min. The mean increase in temperature was 2.0 ± 1.0 °C.

Muscle biopsy and pharmacological testing

Muscle biopsy was performed in nine swine susceptible to MH and 16 control swine. Each pig was premedicated with ketamine hydrochloride 4-5 mg kg⁻¹ i.m. and diazepam 0.5-1 mg kg⁻¹ i.m. Tracheal intubation was performed after anaesthesia was induced with thiopentone 3-4 mg kg⁻¹. Anaesthesia was maintained with nitrous oxide in oxygen, supplemented with thiopentone.
Biopsy specimens (4 cm long, 1 cm wide and 0.5 cm thick) were removed from the gracilis muscle after it had been clamped, and placed immediately in a muscle buffer at 37 °C bubbled with 5% carbon dioxide in oxygen (carbogen). The methods of preparing the muscle strips for pharmacological testing and for measurement of tension have been described previously (Moulds and Denborough, 1974). The maximum ischaemic time from excision of the muscle to the end of the experiment was 5 h. The initial resting tension for each preparation was 1 g. Before commencing the contracture experiments a single electric stimulus of 30 V was used to confirm the suitability of the muscle preparation. The stock solutions of the drugs used were caffeine 100 mmol litre⁻¹ in muscle buffer, KCl 4 mol litre⁻¹ in water, 5% suxamethonium chloride in water and thymol 50 mmol litre⁻¹ in ethanol. Halothane was administered by passing carbogen through a calibrated Dragewick vaporizer.

Dose–response curves for each drug were obtained at 37 °C. For suxamethonium, potassium chloride and thymol, single bolus injections were used. For caffeine, successive doses were added as soon as the maximal contracture plateau induced by the previous dose of caffeine had been reached. Subsequently a dose was selected for each drug which appeared to give the maximal separation between susceptible and control swine, and the efficacy of the different drugs in identifying susceptibility to MH was compared.

Statistical analysis

Rigorous statistical analysis of the data was difficult, as there were large differences in the number of muscle preparations taken from individual pigs. One-way analysis of variance, of the type used for random effects and unequal sample size, was performed on the results from MH-susceptible pigs where the data allowed. The muscle preparations used for any particular drug test in the control group came from a large number of swine and there were few preparations from any individual pig. For this reason, the data were unsuitable for such an analysis, since the within-pig variance could not be estimated accurately. The variances for the tests in the control group result mainly from between-pig variability.

P values were obtained using Student's t test for unequal population variances, there being a greater variability in the MH-susceptible swine than in the control swine.

The methods used were those described by Snedecor and Cochran (1967).

RESULTS

The results of the analyses of variance are summarized in table I. Although variance ratios (F ratio) were not significant at either the 1 or 2.5%, levels, they strongly suggest that heterogeneity exists in the MH-susceptible pig group, in that between-pig variability seemed larger than within-pig variability. Thus it was decided to estimate the components of variance and use these estimates to calculate the standard errors. In a single tension measurement the results show that, for the different drugs, between 77 and 88% results from within-pig differences and only 12–23% is associated with between-pig differences.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of muscle preparations</th>
<th>Between animals Mean square</th>
<th>Between animals d.f.*</th>
<th>Within animals Mean square</th>
<th>Within animals d.f.</th>
<th>Calculated F ratio</th>
<th>Tabulated F ratio (2.5% point)</th>
<th>Components of variance of a single measurement Between animals</th>
<th>Variance estimate of grand mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% Halothane</td>
<td>58</td>
<td>0.45</td>
<td>7</td>
<td>0.22</td>
<td>50</td>
<td>2.0</td>
<td>2.6</td>
<td>0.032</td>
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<tr>
<td>Caffeine</td>
<td>47</td>
<td>0.32</td>
<td>8</td>
<td>0.13</td>
<td>38</td>
<td>2.5</td>
<td>2.6</td>
<td>0.038</td>
<td>0.127</td>
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<td>2 mmol litre⁻¹</td>
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<tr>
<td>KCl</td>
<td>34</td>
<td>1.40</td>
<td>8</td>
<td>0.92</td>
<td>25</td>
<td>1.5</td>
<td>2.8</td>
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<tr>
<td>Suxamethonium</td>
<td>46</td>
<td>0.08</td>
<td>8</td>
<td>0.04</td>
<td>37</td>
<td>1.9</td>
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<td>Thymol</td>
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<td>3</td>
<td>0.14</td>
<td>26</td>
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<td>3.7</td>
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<td>100 µmol litre⁻¹</td>
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* Between-animal d.f. + 1 = number of animals.
Dose-response curves for caffeine, suxamethonium, potassium chloride and thymol are shown in figures 1-4.

Each of the drugs produced greater contractures in muscle from swine susceptible to MH than in controls. The mean concentration of caffeine required to increase the tension of muscle by 1 g was $4.6 \pm (SEM) 0.7$ mmol litre$^{-1}$ in the swine susceptible to MH and $9.5 \pm 0.7$ mmol litre$^{-1}$ in the controls ($P<0.001$).

Halothane induced contracture in muscle from susceptible swine at concentrations of 0.5%, 1% and 3%, but induced little or no contracture in the control muscle (fig. 5).

The optimal concentration of each drug for separating susceptible from normal swine was decided by inspection of the dose–response curves. The concentrations selected were caffeine 2 mmol litre$^{-1}$, suxamethonium 1 mmol litre$^{-1}$, KCl 80 mmol litre$^{-1}$, thymol 100 µmol litre$^{-1}$ and 3% halothane. Responses to these concentrations of the different drugs in swine susceptible to MH were compared with the responses in controls; the standard errors in the susceptible swine data being calculated from the components of variance as in Snedecor and Cochran (1967). The results are shown in table II and figure 6. The mean muscle contraction was significantly greater ($P<0.001$) in the susceptible swine than in the
controls with each of the drugs (table II), but there was a wide scatter of results in susceptible swine with each of the drugs, and also some overlap in the results with the controls in each case (fig. 6).

In a single tension measurement on muscle from a randomly chosen MH-susceptible pig, the results show that between 77% and 88% of the variance for different drugs resulted from within-pig differences and only 12–23% was associated with between-pig differences. The within-pig differences reflect experimental factors which include a difference in the size of the muscle preparation, the temperature of the muscle bath and the ischaemic time from excision of the muscle to the end of the experiment.

Even when the maximum possible care is taken in the preparation and in vitro testing of muscle samples from both humans and swine, there is overlap in the amount of contracture induced by each of the agents currently used in identifying susceptibility to MH. This implies that the most accurate means of identifying susceptibility to MH is not to use a single predictive test, but to expose the muscle in vitro to a variety of chemical agents, the most efficient of which, at present, seem to be caffeine 2 mmol litre⁻¹, 3% halothane, suxamethonium 1 mmol litre⁻¹ and KCl 80 mmol litre⁻¹.

Since thymol induces contracture of skeletal muscle (Ebashi, 1965) and because thymol is used as a preservative in commercial preparations of halothane, the effects of thymol on muscle were examined also in the present investigation. This seemed important as thymol appears to have a mode of action which is similar to but much more powerful than caffeine (Ogawa, 1970). With a low concentration of thymol (100 μmol litre⁻¹) the mean muscle contracture was greater in susceptible than in control swine, but there was more overlap with thymol than with the other drugs used. It is possible that a lower concentration of thymol (such as 75 μmol litre⁻¹) might identify susceptible muscle more precisely (fig. 4).

Although thymol is a potent inducer of skeletal muscle contracture, the contractures induced by
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Fig. 6. Contractions induced by caffeine 2 mmol litre\(^{-1}\), 3\% halothane, suxamethonium 1 mmol litre\(^{-1}\), thymol 100 \(\mu\)mol litre\(^{-1}\) and KCl 80 mmol litre\(^{-1}\) in MH-susceptible swine (MHS) and in controls. Each symbol represents the contraction induced in a single muscle preparation.

Fig. 7. Potentiation of thymol contracture by halothane in muscle from a pig susceptible to MH. The change in tension is shown on the vertical axis with time in minutes on the horizontal axis. Thymol 50 \(\mu\)mol litre\(^{-1}\) induced a contracture of 0.45 g in the presence of 3\% halothane, but had no effect on its own.

halothane in muscle from swine susceptible to MH did not appear to be related to the presence of thymol as preservative. Special halothane preparations free from thymol also induced contracture in MH muscle. Halothane does potentiate the muscle contraction induced in susceptible swine muscle by thymol (fig. 7), and thymol potentiates halothane and potassium contractures (Okumura, Crocker and Denborough, 1978—unpublished observations), but it is not known if the thymol concentration in blood during halothane anaesthesia is sufficient to potentiate the halothane muscle contracture in an individual who is susceptible to MH.

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REFERENCES


**IDENTIFICATIONS DE LA SUSCEPTIBILITE A L'HYPERPYREXIE MALIGNE CHEZ LES PORCS**

**RESUME**

Les réactions à la contracture du muscle *in vitro* ont été similaires, chez les porcs susceptibles à l'hyperpyrexie maligne (MH), à celles observées sur les muscles humains susceptibles à cette complication anesthésique, ce qui confirme que le porc convient comme animal type pour étudier la MH. Les résultats laissent penser qu'il y a différents degrés de susceptibility à la MH. Quel que soit le médicament que l'on ait utilisé, il y a eu certains chevauchements dans les réactions à la contracture entre les animaux susceptibles et les animaux témoins, ce qui laisse penser que la méthode la plus précise d'identifier la susceptibility à la MH est d'utiliser une variété d'agents chimiques, parmi lesquels les meilleurs semblent être l'halothane, la caféine, le suxaméthonium et le chlorure de potassium. Le thymol que l'on utilise comme agent de préservation dans les préparations commerciales d'halothane, peut entraîner les contractures par l'halothane, mais on ne sait pas si cela a une importance du point de vue clinique.

**IDENTIFICAZIONI DEI PORCELLINI SUSCETTIBILI ALLA HIPERPYREXIA MALIGNA**

**RISUMEN**

Las respuestas de contractura muscular *in vitro* fueron semejantes en los cerdos susceptibles a la hiperpirexia maligna (MH) a las producidas en el músculo humano susceptible a esta complicación anestésica, confirmandose la conveniencia del cerdo como animal modelo para estudiar la MH. Los resultados sugieren que hay diferentes grados de susceptibleidad a la MH. Con cualquiera de las drogas empleadas, se produjo cierta superposición de respuestas de contractura entre los animales susceptibles y los controles, lo que sugiere que la forma más precisa de identificar la susceptibleidad a la MH es el empleo de una variedad de agentes químicos, los mejores de los cuales parecen ser el halotano, cafeína, suxametonio y cloruro potásico. El timol, que se emplea como preservativo en preparaciones comerciales de halotano, da potencia a las contracturas de halotano, pero no se sabe si esto tiene significado clínico.