STATIC V. DYNAMIC TESTS IN THE IN VITRO DIAGNOSIS OF MALIGNANT HYPERThERMIA SUSCEPTIBILITY

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The most reliable method of establishing susceptibility to malignant hyperthermia (MH) is the in vitro pharmacological testing of skeletal muscle (Ellis et al., 1972; Moulds and Denborough, 1974; Kalow, Britt and Richter, 1977). The diagnosis is made when the tension in a specimen of muscle increases by 0.2 g or more (a “threshold”) on exposure to low concentrations of both halothane and caffeine in separate tests (European Malignant Hyperpyrexia Group, 1984; Ærpping, Ranklev and Fletcher, 1984). Although there are many different interpretations of the in vitro tests, members of the European MH-group perform them in accordance with a common procedure, and the diagnosis of susceptible (MHS), negative (MHN) or equivocal (MHE) is made according to common criteria. The diagnosis MHS requires a response at 2% or less halothane and 2 mmol litre⁻¹ or less caffeine; MHE implies a positive response to one drug only.

Most MH-investigation units use static caffeine and halothane contracture tests. In these, the length of the muscle specimen is maintained constant while caffeine or halothane is added to the muscle bath. However, in Great Britain, Denmark and Sweden, a dynamic halothane contracture test (Ellis et al., 1978) is used as well. The muscle specimen is subjected to a regular cycle of stretching and relaxation during exposure to the drug (Ellis et al., 1978; Ærpping, Ranklev and Fletcher, 1984). No description of a dynamic caffeine contracture test has been published, although it has been performed in Great Britain, Denmark and France (Ellis, Ærpping, Reiss Kozack and Möller; personal communications) as well as in our own unit. This paper describes our findings when static and dynamic tests were performed in parallel.

SUMMARY

In vitro contracture tests, in which muscle specimens are exposed to halothane or caffeine are, at present, the only generally accepted screening methods for the diagnosis of susceptibility to malignant hyperthermia (MHS). Static tests (performed with the muscle held at constant length) are used more commonly although, in addition, some MH investigation units use dynamic tests, in which the length of the specimen is varied. We have performed dynamic and static tests in parallel on muscle from 112 patients. The dynamic halothane test was more sensitive in discriminating between MHS and MH negative (MHN) individuals than the static halothane test. However, the dynamic caffeine test was less sensitive at discriminating between MHS and MHN individuals, and nothing is to be gained by including it in the investigation.

PATIENTS AND METHODS

One hundred and twelve patients were investigated for susceptibility to MH. Eighteen patients were survivors of a suspected MH-reaction and 92 were relatives of known or suspected MH-probands. One patient had known central core disease, and one was the father of two teenage boys who had died suddenly without explanation (Ranklev, Fletcher and Krantz, 1985).

For control purposes, biopsies were obtained from 12 healthy subjects undergoing minor surgery on the leg. All muscle biopsies (including the controls) were taken from the vastus medialis muscle during extradural analgesia with mepivacaine, or during thiopentone, fentanyl and nitrous oxide in oxygen anaesthesia.

For the in vitro test, pieces of muscle (15–25 mm long and 2–3 mm in diameter) were suspended in
a tissue bath, and perfused with Krebs solution at 37 °C, bubbled with preheated 5% carbon dioxide in oxygen. Viability of the specimen was demonstrated by supramaximal stimulation at 0.2 Hz. Specimens were exposed to increasing concentrations of either halothane or caffeine, and the muscle tension measured by a displacement transducer and recorded on paper (European Malignant Hyperpyrexia Group, 1984; Ørding, Ranklev and Fletcher, 1984).

In the dynamic test the muscle was allowed to rest at zero or low tension for 3 min and was then stretched at a rate of 4 mm min⁻¹ for 1.5 min to a tension of approximately 2 g, left for 1 min and then returned (at the same rate) to its initial length. The whole cycle took 7 min (Ellis et al., 1978; Ørding, Ranklev and Fletcher, 1984). After three such cycles 0.5, 1, 2 and 3% halothane or caffeine 0.5, 1, 1.5, 2, 3 and 4 mmol litre⁻¹ were added for one cycle each. In the static test the muscle was stretched to approximately 2 g and left for 20–30 min before halothane or caffeine was added in the same concentrations as in the dynamic test. Each concentration was maintained until a new plateau had been achieved or 3 min had elapsed.

The criteria for susceptible, equivocal or negative results were as agreed by the European Malignant Hyperpyrexia Group (1984) except that, in addition, the results of the dynamic caffeine test were taken into account. The test giving the greatest increase in tension was used to make the diagnosis. The tests were performed in randomized order. Student's t test was used for statistical comparison within each group.

RESULTS

No control specimen, in either this study or a previous one (Ørding, Ranklev and Fletcher, 1984), demonstrated a contracture when exposed to 2% or less halothane or 2 mmol litre⁻¹ or less caffeine.

Twenty-five patients were diagnosed as MHS, 20 MHE and 67 MHN. In both the MHS-group (P = 0.008) and the MHE-group (P = 0.002), thresholds with halothane were obtained at lower concentrations in the dynamic test than in the static test. Five MHS-patients and all but two MHE had normal static halothane tests, but abnormal dynamic tests. Five patients in the MHE-group, but none in the MHS-group had normal dynamic halothane tests (fig. 1).

In the caffeine test, thresholds were obtained at significantly lower concentrations in the dynamic than in the static test in both the MHE (P = 0.004) and MHN-groups (P = 0.006). However, no significant difference was seen between the static and dynamic caffeine tests in the MHS group (P = 0.5) (fig. 2).
DISCUSSION

In the establishment of MH susceptibility, the only procedure which has stood the test of time is in vitro pharmacological testing of muscle. Nevertheless, the method has not been completely satisfactory because of the dearth of acceptable control data, that is results from vastus muscle obtained in good condition from healthy individuals anaesthetized in the same way as patients undergoing investigation for MH susceptibility. Thus criteria have, to some extent, been defined arbitrarily.

It is gratifying that results from normal control muscle have so far upheld the criteria established by the European MH-group (1984). However, more controls are necessary for greater diagnostic safety and for the further clarification of the status of the MHE group, most of whom would have been classified as MH susceptible before the formation of the European MH Group.

The present results demonstrate that the dynamic halothane test is more sensitive in discriminating between MHS and MHN patients than the static halothane test; if the static halothane test alone were used, many MH susceptible patients could be misdiagnosed, apparently having only a pathological caffeine test. Regardless of which criteria for MHS are chosen, it is true to say that the dynamic halothane test is the more sensitive, since the overwhelming majority of points in figure 1 are on or above the line of identity. With caffeine, thresholds were obtained at significantly lower concentrations in the dynamic than in the static test in the MHN-group, whilst there was no difference in the MHS-group. The dynamic caffeine test is, thus, of less value in discriminating between MHS and MHN patients than the static caffeine test.

The results from the MHE-group are more difficult to interpret. Only four MHE-patients had a pathological contracture with caffeine (and only in the dynamic test), but not with halothane. It is impossible to ascertain their true status from the history and pedigree. The other 14 MHE-patients had normal responses to caffeine in both tests. Because of their pedigree, at least two of these must be susceptible. Both had a pathological contracture to the dynamic, but not the static halothane tests, and would have been diagnosed as MHN without the use of the dynamic test.

At present the European consensus of opinion is that both the halothane and the caffeine tests are necessary to diagnose MH susceptibility, although this opinion may be revised when more control data become available. Both static and dynamic halothane tests are considered necessary, as our results confirm. However, nothing would appear to be gained by including a dynamic caffeine test in the procedure.

REFERENCES


