MICROCALORIMETRIC STUDIES IN MALIGNANT HYPERPYREXIA SUSCEPTIBLE INDIVIDUALS

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The only generally accepted method of diagnosing susceptibility to malignant hyperpyrexia (MHS) is in vitro testing of muscle (Kalow, Britt and Richter, 1977; Ellis et al., 1978). However, since this is a time-consuming technique which requires an open muscle biopsy, a simpler method of diagnosis would be of value.

Although the primary manifestation of malignant hyperthermia (MH) is an increase in heat production in muscle, platelets also contain contractile elements and have been the focus of attention. Indeed, abnormalities of platelet metabolism and aggregation have been reported (Solomons, Tan and Aldrete, 1978; Zsigmond, Penner and Kothary, 1978) in association with MH.

We have measured in vitro the heat produced by exposing small specimens of muscle to halothane and by exposing platelets to halothane and caffeine.

PATIENTS AND METHODS

Muscle preparation

A muscle biopsy was taken from 26 individuals being investigated for susceptibility to MH, and from seven normal controls during other operations. MH-suspected individuals were identified as MH susceptible (MHS), or MH negative (MHN) after the in vitro exposure of the muscle to halothane and to caffeine (Ørding, Ranklev and Fletcher, 1984). All muscle biopsies from MH-suspected individuals were from the vastus medialis and included the motor point. The biopsies from the control patients were taken from the rectus abdominis, gluteus maximus or gastrocnemius. Anaesthesia for the biopsies was either femoral nerve blockade or thiopentone-fentanyl-nitrous oxide in oxygen.

After excision the muscle was stored and transported at 4 °C in Krebs–Ringer phosphate (KRP) buffer solution containing glucose 8.25 mmol litre⁻¹ and insulin 0.1 iu ml⁻¹. The fibres were dissected to about 1 mm diameter (about 50 mg weight) and placed in the calorimeter within 30 min of excision.

Platelet suspensions

Venous blood samples were collected, from 14 individuals being investigated for MH sensitivity, in 10-ml Vacutainer tubes containing 25 U.S.P. units of sodium heparin (Kabi, Sweden) and 5% Dextran 80 solution 2 ml (Pharmacia, Sweden). Platelet rich plasma (PRP) suspensions were prepared as described by Levin (1971). The concentration of platelets in the cell suspensions was about 150 x 10⁹ litre⁻¹. Duplicate platelet and leucocyte counts were made by phase contrast microscopy. No erythrocytes were observed in the cell suspensions used.

Calorimetric measurements

The studies on muscle were performed in a perfusion calorimeter (Wadsö, 1974) in which the
fluid was maintained at 37 °C by a heat exchanger to ensure minimal heat loss from the system. Heat production was measured as the change in voltage produced in a thermopile placed round the calorimeter vessel. After 30 min the perfusion fluid was changed to one equilibrated with 4% halothane.

The studies on platelets were performed in microcalorimeters of the thermopile heat conduction type (Spink and Wadso, 1976). The samples were enclosed in 1.0-ml stainless steel ampoules. Measurements were made under static conditions at 37 °C as described by Monti and Wadso (1977). Two samples were measured simultaneously. Corrections were made for the heat production of leucocytes in the PRP suspensions (2% of the total heat production (Bandmann, Monti and Wadso, 1975)) and for the final pH to pH 7.4 (Monti and Wadso, 1977). The coefficient of variation for the method has previously been determined to be 4% (Monti and Wadso, 1977).

In the caffeine–platelet study, caffeine diluted in KRP-buffer was added to PRP to a final concentration of 4 mmol litre$^{-1}$. The control ampoule contained PRP and KRP buffer solution. Samples from nine patients (five MHS and four MHN) were tested. In the halothane–platelet study, platelets were suspended in autologous plasma and the reactant ampoule was equilibrated with 3% halothane for 10 min before measurements were begun. Samples were obtained from one MHS and two MHN patients. In addition, two equivocal patients (MHE (European MH group, 1984)) were tested.

Statistics

Differences were tested by the Mann–Whitney test for unpaired data.

RESULTS

Effect of halothane on heat production in muscle

Figure 1 shows a typical muscle calorimetry tracing. Before the addition of halothane there was a continuous decrease in heat production, possibly reflecting a decrease in the availability of substrate in the cells. The addition of halothane was, in all instances, followed by an increase in the rate of heat production; on many occasions the increase was enough to reverse the trend.

Heat production was measured as the area under the “hump” in the tracing (the shaded area in

FIG. 1. Microcalorimetry tracing showing effect of halothane on muscle metabolism.

There were no significant differences between MHS ($n = 12$), MHN ($n = 14$) and normal controls ($n = 7$).

Effect of caffeine on heat production by platelets

Heat production was determined with and without the addition of caffeine to a final concentration of 4 mmol litre$^{-1}$. In all instances heat production decreased in the suspensions containing caffeine. There were no significant differences between MH-positive and MH-negative patients.

Effect of halothane on heat production by platelets

In four patients (one MHS, two MHN and one MHE) halothane caused an increase in heat production; in one equivocal patient heat production decreased.

DISCUSSION

Measurement of heat production in cells by calorimetry gives information about total cellular metabolic activity. The present method of muscle fibre preparation has been used extensively by other investigators (Lundholm et al., 1975) who
were unable to demonstrate morphological or functional alterations in the specimens (Bylund et al., 1976). Muscle for this test was excised and tested at the same time as the muscle used for the in vitro halothane and caffeine tests, during which satisfactory viability was demonstrated by repeated electrical stimulation. In the model described above, halothane increased the metabolism of muscle tissue, but there was no significant difference between MHS and MHN individuals. The muscle specimen was not stretched during the test. In the in vitro test for MH sensitivity, a halothane contracture cannot usually be demonstrated at zero tension. It may, therefore, be necessary to stretch the muscle to 1–2 g tension in order to observe a difference between MHS and MHN individuals.

Halothane had a variable effect on heat production by platelets, increasing it in four patients and decreasing it in one. Although the MHS patient showed the greatest increase in heat production, the differences were too small to be of any diagnostic value. More importantly, halothane has a tendency to make platelets aggregate. Because aggregation per se causes an increase in heat production by platelets (Ross, Fletcher and Jamieson, 1973) we do not believe that this method can have any diagnostic value.

Caffeine, on the other hand, decreased heat production in both MHS and MHN platelets, although the effect of caffeine on the intact organism is to increase heat production (Acheson et al., 1980). Other xanthine derivatives have been shown to have the same effect on platelets (Monti and colleagues, in preparation).

We conclude that the microcalorimetric methods described above cannot be used to distinguish between tissues from MHS and MHN individuals.

REFERENCES