PAPERS AND ORIGINALS

Biochemical Basis of Malignant Hyperpyrexia

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Summary

Pharmacologically-induced muscle contracture in vitro has been used as a model to study the biochemical basis of malignant hyperpyrexia. In 15 susceptible subjects halothane, succinylcholine, and potassium chloride all produced an abnormal muscle contracture, and the caffeine-induced contracture was greater than normal. The contractures were reproducible only in the presence of extracellular calcium ions. The fact that such dissimilar pharmacological stimuli all induced contracture in the affected muscle suggests that the essential abnormality in the muscle cell in malignant hyperpyrexia is an impaired binding of calcium ions to the membranes of the sarcoplasmic reticulum and the sarcolemma. Exposure of these membranes to halothane, succinylcholine, and other anaesthetic agents then leads to a rapid and abnormally large release of calcium into the myoplasm, which in turn gives rise to all the clinical features of the syndrome.

Introduction

Malignant hyperpyrexia is a rare but often fatal complication of general anaesthesia. First described by Denborough and Lovell (1960), it has since been found to occur in patients who suffer from one of a number of myopathies (King *et al.*, 1972), which are usually dominantly inherited (Denborough *et al.*, 1962; Britt *et al.*, 1969) and often subclinical (Isaacs and Barlow, 1973). The main manifestations of the syndrome are a rapid rise in body temperature, generalized muscular rigidity, and a severe metabolic acidosis (Britt and Kalow, 1970). These could all be explained by a raised calcium ion concentration in the myoplasm (Kalow *et al.*, 1970; Denborough *et al.*, 1973).

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Caffeine has long been known to cause contracture of frog skeletal muscle (Axelsson and Thesleff, 1958), principally by causing a release of calcium ions into the myoplasm from the sarcoplasmic reticulum (Frank, 1962; Weber, 1968), and it has been suggested that a caffeine-produced contracture might be used as a model for studying malignant hyperpyrexia (Kalow *et al.*, 1970; Strobel and Bianchi 1971). Halothane potentiates the caffeine-produced contracture in frog (Strobel and Bianchi, 1971) and normal human skeletal muscle (Moulds and Denborough, 1972), and procaine inhibits both the caffeine-produced contracture of frog muscle (Feinstein and Paimre, 1969) and the halothane-potentiated caffeine contracture of normal human muscle (Moulds and Denborough, 1972). There have been fewer studies of the effects of these drugs on muscle from patients susceptible to malignant hyperpyrexia, but halothane produces a spontaneous contracture in such muscle (Ellis *et al.*, 1972; Moulds and Denborough, 1972) which is partially reversed by procaine (Moulds and Denborough, 1972). Also muscle from patients susceptible to malignant hyperpyrexia gives an enhanced caffeine contracture (Kalow *et al.*, 1970).

We have examined in detail the "pathopharmacology" of muscle from patients susceptible to malignant hyperpyrexia to gain a clearer understanding of the biochemical basis of this syndrome and to try to develop a definitive test to identify individuals who are at risk of developing malignant hyperpyrexia.

Subjects and Methods

A total of 20 subjects were studied from six different families. Four were unrelated male survivors of malignant hyperpyrexia who had a dominantly-inherited subclinical myopathy. The other 16 were relatives of patients who had suffered from malignant hyperpyrexia. Eleven of them were considered to be affected by the myopathy because they had raised serum creatine phosphokinase levels, and five whose serum creatine phosphokinase levels were normal were thought to be unaffected by the myopathy and were included as controls. Informed consent was obtained in each case.

Tissue for biopsy was obtained under local anaesthesia from motor points in the vastus lateralis muscle. The specimens were made into preparations about 1·5 cm long and 3 mm

wide and isometric tension was measured as described previously (Moulds and Denborough, 1972). Resting tension was 1 g, and the temperature of the bath in which they were kept was maintained at 37°C. The bath was filled with a solution as previously described (Moulds and Denborough, 1972) and it could be rapidly emptied and refilled with fresh solution. Drugs were added to the bath by disposable syringe. Halothane was added either by bubbling carbogen sequentially through two flasks—each containing 500 ml of a solution of 18 mmol of halothane per litre of water—before it was bubbled into the bath, or by injecting into the bath a solution of halothane 18 mmol halothane/l. dissolved in bath solution. The succinylcholine was obtained from standard ampoules (Anectine 50 mg/ml) and the procaine was made up as a 10% solution of procaine hydrochloride in 0·1% sodium metabisulphite.

Up to 15 preparations could be made from each sample of muscle. These did not usually deteriorate when stored in the bath solution at 37°C and bubbled with carbogen. Hence, despite some variation in response, there were ample preparations to test all the drug combinations on each biopsy specimen.

Results

The muscle from the 11 relatives affected by the myopathy responded to the various drugs qualitatively in the same way as the muscle from four survivors of malignant hyperpyrexia. We shall refer to the muscle from all these subjects as malignant hyperpyrexia muscle. The muscle from the five relatives unaffected by the myopathy and acting as controls responded to the drugs qualitatively in the same way as a large series of normal rectus abdominis muscle which had been studied earlier. We shall refer to the muscle from the five relatives as normal muscle.

Typical contractures produced by the various drugs in malignant hyperpyrexia muscle are compared with those produced in normal muscle. No result is given unless it was present in all the patients, with only a few exceptions due to technical reasons.

TEMPERATURE

The experiments were performed at 37°C but the preparations were made up at room temperature. When the muscle which was at room temperature was lowered into the bath at 37°C a contracture was provoked in malignant hyperpyrexia muscle but not in normal muscle (figs. 1 and 5).

HALOTHANE

Halothane produced a large contracture in all the samples of malignant hyperpyrexia muscle either when it was bubbled into the bath solution (fig. 1) or when it was injected into the bath to give a final concentration of about 0·75 mM (fig. 6). In normal muscle halothane gave no more than a small, slower contracture, and usually none at all. After halothane was removed rapid relaxation occurred, but there were at least one, and usually two, rebound contractures which gradually became smaller and slower (fig. 1). Re-exposure to halothane usually produced a contracture similar to but often smaller than that produced by the first exposure.

CAFFEINE

Small doses of caffeine (4 mmol/l.), which in normal muscle produced only a small, slow contracture, produced a much larger and more rapid contracture in malignant hyperpyrexia muscle (fig. 2). This was reversible on removal of the caffeine, and a similar though often smaller contracture occurred on re-exposure to caffeine. Large doses of caffeine (16 mmol/l.) produced a more rapid contracture in the malignant hyperpyrexia muscle than in the normal muscle but the height of the contracture was not usually any greater.

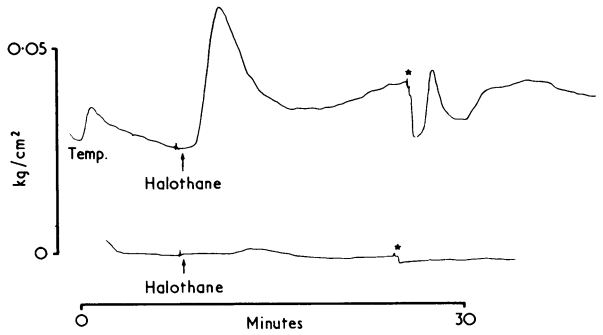

FIG. 1—Response of malignant hyperpyrexia muscle (upper tracing) and normal muscle (lower tracing) to temperature change and then exposure to halothane vapour. Also shown is effect of removal of halothane by changing bath (indicated by star in each tracing).

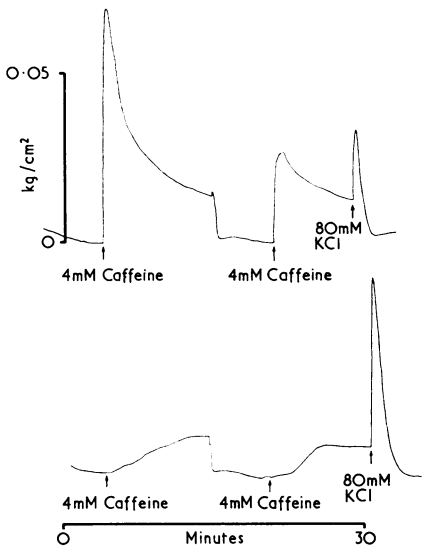

FIG. 2—Response of malignant hyperpyrexia muscle (upper tracing) and normal muscle (lower tracing) to 4 mmol caffeine/l. Also shown are effects of removal of caffeine after about 15 minutes, re-exposure to 4 mmol caffeine/l., and finally addition of 80 mmol KCl/l. in presence of 4 mmol caffeine/l.

SUCCINYLCHOLINE

The response of malignant hyperpyrexia muscle to a large dose (10 mg) of succinylcholine was a large, long, and sustained contracture, whereas there was no contracture in normal muscle (fig. 3). Removal of the succinylcholine produced rapid relaxation then a rebound contracture similar to that which occurred after stopping halothane. Re-exposure to succinylcholine produced a contracture similar to that on first exposure (fig. 3).

POTASSIUM CHLORIDE

In single frog muscle fibres activation of the contractile process begins when the sarcolemmal membrance potential falls

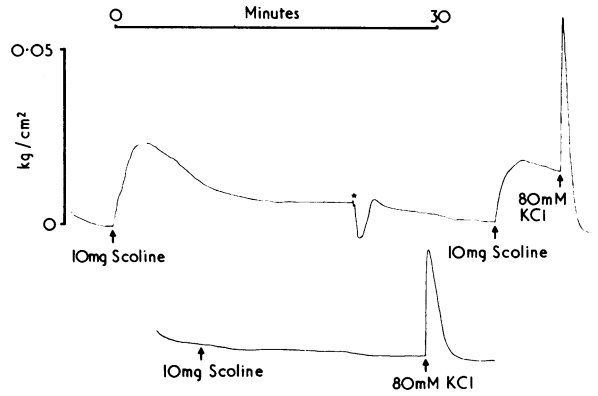

FIG. 3—Response to 10 mg succinylcholine (Scoline) of malignant hyperpyrexia muscle (upper tracing) and normal muscle (lower tracing). Malignant hyperpyrexia muscle has had bath changed and succinylcholine removed (indicated by star) and has then been re-exposed to succinylcholine. Also shown in each case is effect of the addition of 80 mmol KCl/l. in presence of succinylcholine.

from its resting value of about −90 mV to −54 mV (Hodgkin and Horowicz, 1960; Littgau and Oetliker, 1968). This can be artificially produced by increasing the potassium ion concentration in the extracellular fluid to 25 mmol/l., and activation is complete if the external potassium concentration is raised to about 60 mmol/l. (Luttgau and Oetliker, 1968). In the present investigation the effect of depolarization of the sarcolemmal membrane was studied by adding potassium chloride to the bath, and a dose of 80 mmol KCl/l. was used to ensure complete activation of the contractile process.

By itself 80 mmol KCl/l. produced a large contracture in malignant hyperpyrexia muscle (fig. 4). In normal muscle it usually gave no discernible contracture when added alone but gave a large contracture when potentiated by either caffeine (fig. 2) or succinylcholine (fig. 3). The presence of 80 mmol KCl/l. also partially inhibited a subsequent caffeine-induced contracture in normal muscle but in malignant hyperpyrexia muscle this inhibitory effect was less (fig. 4).

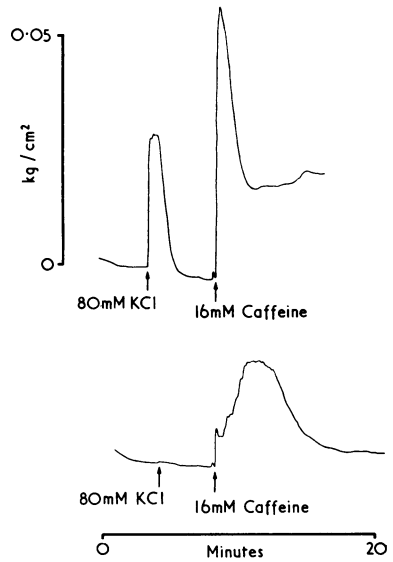

FIG. 4—Response to 80 mmol KCl/l. and then to 16 mmol caffeine/l. of malignantpyrexia muscle (upper tracing) and normal muscle (lower tracing).

Because the enhanced contracture produced in malignant hyperpyrexia muscle by 80 mmol KCl/l. might have been due to a change in the threshold for activation of the contractile response we studied this in three samples of malignant hyperpyrexia muscle by adding small incremental doses of KCl. Contracture started in two samples at between 20 and 30 mmol KCl/l. and between 30 and 50 mmol KCl/l. in the third. These are normal levels and they suggest that the threshold linking membrane deplorization to contractile response is normal in malignant hyperpyrexia muscle.

PROCAINE

Procaine added either before or after exposure of malignant hyperpyrexia muscle to halothane consistently inhibited contracture (fig. 5). It also inhibited the abnormal contracture produced in malignant hyperpyrexia muscle by succinycholine.

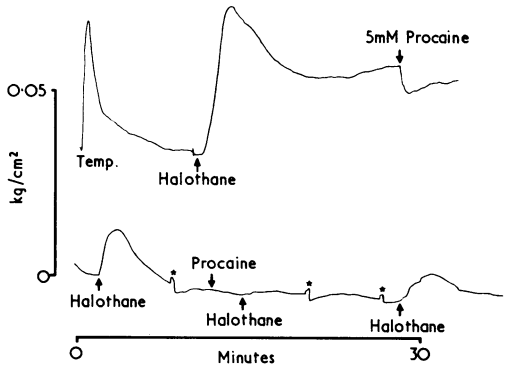

FIG. 5—Response of malignant hyperpyrexia muscle to procaine added after halothane-induced contracture (upper tracing) and before halothane (lower tracing). In lower tracing muscle has been exposed to halothane by injection three times, with second halothane exposure being preceded by administration of 5 mmol procaine/l. Halothane was removed by changing bath (indicated by star).

EXTRACELLULAR CALCIUM

To study the role of extracellular calcium ions in the generation of the halothane contracture muscle was sequentially exposed to halothane either in a normal bath solution or in one without any calcium ions. Malignant hyperpyrexia muscle gave a contracture when first exposed to halothane in the absence of extracellular calcium ions, but was then unable to give a further contracture on re-exposure to halothane. When calcium ions in their normal concentration was readded to the bath solution, the muscle again gave a contracture on exposure to halothane (fig. 6).

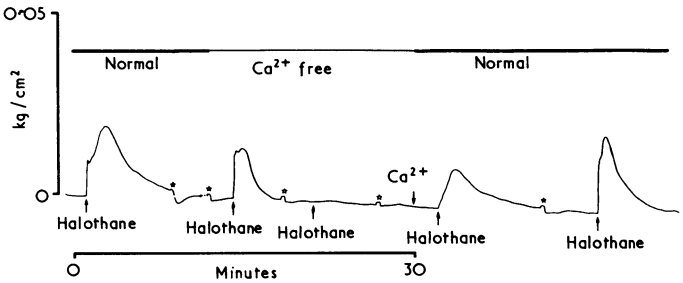

FIG. 6—Response of malignant hyperpyrexia muscle to five sequential exposures to halothane (hal.) by injection, showing disappearance of contracture in absence of extracellular calcium ions and its restoration by readdition of calcium ions. Halothane was removed by changing bath to either normal bath (closed star) or calcium-free bath (open star). Calcium-free period is shown by interrupted line at top of fig. Calcium was readded to bath to make normal bath concentration of 2.5 mmol Ca^{2+}/l.

Similar experiments showed that, if the initial contracture in a calcium-free bath was produced by 4 mmol caffeine/l. rather than by halothane, the muscle then failed to give a contracture when exposed to caffeine or halothane until calcium was readded to the bath solution.

Discussion

One of the purposes of the investigation was to gain more information about the basic defect in muscle which leads to the development of malignant hyperpyrexia on exposure to various anaesthetic drugs, particularly succinylcholine and halothone. It confirmed that malignant hyperpyrexia muscle gives a large contracture on exposure to halothane. Unlike some investigators (Kalow *et al.*, 1970) we found this to be a consistent response which occurred in all four survivors of malignant hyperpyrexia and in all 11 affected relatives of patients who had suffered from malignant hyperpyrexia. Our investigation also confirmed that malignant hyperpyrexia muscle is more sensitive to caffeine than normal muscle. Furthermore, it showed that the most consistent and striking feature of malignant hyperpyrexia muscle is its ability to give an increased contracture when exposed to a wide variety of stimuli, including the seemingly unrelated chemical stimuli of halothane, caffeine, succinylcholine, potassium chloride, and the physical stimulus of a temperature change. Such an indiscriminate enhancement of the contractile response must therefore involve one of the basic regulatory mechanisms of muscle contraction.

The strength of a muscle contraction is a function of the concentration of free calcium ions in the myoplasm (Weber and Murray, 1973). Thus the enhanced contracture in malignant hyperpyrexia muscle could be produced by any one of, or a combination of, three mechanisms: (1) a decreased reuptake of calcium ions from the myoplasm by the sarcoplasmic reticulum; (2) an increased entry of calcium ions into the myoplasm from the extracellular fluid; or (3) an increased release of calcium ions from the calcium-storing membranes in the muscle cell, principally the sarcolemma and the sarcoplasmic reticulum.

The first of these is probably not the most important, because of the rapidity with which the contractures occur and also because relaxation occurs so quickly after the contracture produced by the various drugs, especially potassium chloride. The second also cannot be solely responsible for the enhancement because the contractures still occur in the absense of extracellular calcium ions. But all the results could be explained by the third mechanism. The relative contributions of sarcolemmal and sarcoplasmic reticular calcium release and whether "trigger calcium" participates in the generation of a normal muscle contraction are not yet known, and so it is possible that abnormalities in both the sarcoplasmic reticulum and the sarcolemma may be involved in the massive release of calcium which occurs in malignant hyperpyrexia.

The experiments showing the importance of extracellular calcium ions in maintaining halothane contracture suggest that there must be a tight coupling between the release of calcium ions stored in the intracellular membranes and their replacement by extracellular calcium ions which presumably must cross the sarcolemmal membrane to enter the cell. In malignant hyperpyrexia a defect in the sarcolemmal membrane might therefore allow calcium ions in the extracellular fluid to replace more easily the calcium ions which have been released from the intracellular membranes.

Our investigations provide strong evidence to support the suggestion that the clinical syndrome of malignant hyperpyrexia is produced by a raised concentration of calcium ions in the myoplasm. They also suggest that these calcium ions have been abnormally released because of an inherited defect in the calcium-storing membranes of the muscle cell which makes them more sensitive to a wide variety of physicochemical stimuli.

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