DILTIAZEM INHIBITS HALOTHANE-INDUCED CONTRACTIONS IN MALIGNANT HYPERTERMIA-SUSCEPTIBLE MUSCLES IN VITRO

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Malignant hyperthermia (MH) is a rare, but serious, complication of anaesthesia with a high mortality rate. During the syndrome, transmembranous and intracellular movements of Ca\(^{2+}\) — caused by genetically transmitted defects in calcium ion regulation — lead to pathological muscle contracture, increased energy consumption, and muscle cell death (Williams, 1976; Gronert, 1980). Dantrolene, which may block the intracellular movement of Ca\(^{2+}\) (Britt, 1984), is currently the drug of choice in the treatment of the syndrome (Nelson and Flewellen, 1983; Britt, 1984). Since muscle contracture in MH is dependent on an increase in the intracellular concentrations of unbound Ca\(^{2+}\) (Britt et al., 1982), blockade of transmembranous Ca\(^{2+}\) influx by calcium entry blockers was suggested as a possible mode of treatment (Bikhazi, Thomas and Foldes, 1979). A single observation in isolated human MH susceptible muscle showed that diltiazem was able to suppress halothane and halothane-caffeine induced muscle contracture (Iwatsuki, Koga and Amaha, 1983).

The purpose of this investigation was to assess, in a controlled study, the suppressive effect of diltiazem on halothane- and halothane-caffeine-induced contracture of MH susceptible pig muscle.

MATERIALS AND METHODS

Muscle specimens (biceps femoris) were taken from 10 MH-susceptible, inbred Poland China Pigs, and two normal control pigs (susceptibility tested at 8–10 weeks of age; halothane inhalation test) (Williams, 1976). The specimens were excised under thiopentone-oxygen-nitrous oxide anaesthesia, immediately cut into 3 x 2 x 40-mm strips and placed in a constant temperature (37 °C) organ bath containing 100 ml of modified Krebs Ringer solution (Bikhazi, Thomas and Foldes, 1979) containing calcium 1.4 mmol litre\(^{-1}\); magnesium 1.2 mmol litre\(^{-1}\); potassium 5.9 mmol litre\(^{-1}\); phosphorus 1.2 mmol litre\(^{-1}\); chloride 122.7 mmol litre\(^{-1}\); and aerated with 5% carbon dioxide in oxygen. After 60 min of equilibration the muscle was stimulated directly with supramaximal rectangular impulses (1 ms, 0.2 Hz) (Techtronics). Isometric contractions were measured by force displacement transducers (Grass FT03) and recorded continuously (HP7758). Pretension was adjusted until the

SUMMARY

The ability of diltiazem to suppress halothane and halothane-caffeine induced contractures in malignant hyperthermia (MH) susceptible pig muscle, was tested in vitro. Muscle specimens were divided into two groups and tested with a modified halothane-caffeine contracture test. One group acted as the control; the other group was pretreated with diltiazem 20 μmol litre\(^{-1}\). The control muscles developed contractures attributable to halothane and halothane-caffeine, whereas the diltiazem-treated specimens did not. Increases in muscle twitch tension as a result of halothane or halothane-caffeine exposure occurred in treated and untreated specimens, but were significantly delayed in the presence of diltiazem. Muscle exhaustion observed after halothane and halothane-caffeine exposure in the control specimens did not occur in the diltiazem treated muscles.
amplitude of muscle twitches could not be increased further (average 3 g).

After a steady state of baseline and muscle twitch tension had been established 4% halothane was added to the aeration of the organ bath for 20 min. The muscle strips were then allowed to return to a second steady state of baseline and twitch tension. The strips were again exposed to 4% halothane for 20 min and then caffeine 0.3 mmol litre\(^{-1}\) was added to the organ bath. A second muscle strip of the same specimen was treated with the same procedure except that diltiazem 20 \(\mu\)mol litre\(^{-1}\) was added to the organ bath 20 min before the administration of the halothane. Baseline shifts caused by halothane alone or by halothane-caffeine of more than 10% of control twitch tension were defined as being MH-positive. This modification of the generally accepted in vitro test (Gronert, 1979) was used to find a better relationship between muscle specimens of different strengths. The amplitude of twitch tension and the time until maximum twitch amplitude under the influence of halothane and halothane-caffeine, respectively, were determined. The differences between the control and the diltiazem-pretreated muscle strips were evaluated for statistical significance by using the Wilcoxon rank sum test and Fisher exact test. The level of significance was taken to be \(P < 0.05\).

RESULTS

Mean and standard errors of the mean of the maximum increase in muscle twitches (% of control), time to maximum twitch (min), and number of muscles which showed an increase in baseline tension caused by halothane or halothane-caffeine, respectively, are shown in table I. There were no significant differences in the percentage of maximum twitch increases between the pretreated and unpretreated muscles. However, the maximum twitch tension developed significantly later in the diltiazem-pretreated group. Increases in baseline tension which developed in the unpretreated muscle strips could be suppressed by diltiazem. After exposure to halothane, the unpretreated muscle strips developed a steady state of twitch tension 26.9±5.9% less than the control values. The diltiazem-pretreated specimens re-established a steady state of twitch tension which was equal to the control values.

Diltiazem initially decreased the control twitch tension, delayed the increase to maximum twitch tension with halothane, suppressed the elevation in baseline, and allowed re-establishment of the control twitch tension after exposure to halothane. However, in two of the preparations, diltiazem failed to suppress the halothane-caffeine-induced contractions (fig. 1).

The muscle strips taken from the two normal pigs showed no elevations in baseline during halothane or halothane-caffeine exposure with or without diltiazem pretreatment. However, the increase in time required to develop maximum twitch amplitude after diltiazem pretreatment was similar in the MH and the normal pig muscle.

DISCUSSION

The \(\text{Ca}^{2+}\)-entry blocker diltiazem suppressed halothane- and halothane-caffeine-induced contracture of MH susceptible muscle in vitro. This confirmed a single observation made in isolated human MH susceptible muscle (Iwatsuki, Koga and Amaha, 1983). \(\text{Ca}^{2+}\)-entry blockers have been shown to protect hypoxic myocardial muscle by interference with excitation-contraction coupling and suppression of an uncoupling of the oxidative phosphorylation, thereby conserving energy reserves (Watts, Koch and La Noue, 1980; Matlib et al., 1983; Hoff, 1984). The delay in the increase to maximum twitch tension and the re-establishment of control twitch tension after halothane exposure in the diltiazem-pretreated specimens would suggest similar effects in the MH-susceptible muscle. The ability to suppress contracture in MH-susceptible pig muscle induced by standard MH trigger-substances, and the well known cardiovascular effects of diltiazem and \(\text{Ca}^{2+}\)-entry blockers in general represent drug properties which should be useful in the treatment of the clinical manifestations of MH. Williams and colleagues (1985) have used diltiazem
3.4–8.9 mg kg\(^{-1}\) alone in the successful treatment of MH in five MH-susceptible pigs. Subsequent complete heart block in two pigs required adrenaline and calcium chloride to restore cardiac function.

Diltiazem may be more effective therapeutically for MH than verapamil (Zukaitis et al., 1982, 1983) in the treatment of porcine MH.

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REFERENCES


