Diltiazem and nifedipine reduce the *in vitro* contracture response to halothane in malignant hyperthermia-susceptible muscle

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The effects of diltiazem (1 μM) and nifedipine (1 μM) were examined separately on the in vitro *halothane tests for malignant hyperthermia (MH) susceptibility. Eighteen patients with MH susceptibility were diagnosed as MH-susceptible (MHS) according to the protocol of the European MH Group. In addition, halothane tests were carried out in the presence of either diltiazem (ten patients) or nifedipine (eight patients). These two calcium channel blockers significantly reduced the halothane contracture. Furthermore, in five of the ten MHS patients tested in the presence of diltiazem as well as in five of the eight MHS patients tested in the presence of nifedipine the halothane contracture test could be classified as negative. It is concluded that the presence of clinical concentrations of either diltiazem or nifedipine in the muscle bath affects the* in vitro *discrimination for MH susceptibility to halothane.*

Les effets du diltiazem (1 μM) et de la nifédipine (1 μM) ont été étudiés séparément sur le test halothane in vitro *de dépistage de l'hyperthermie maligne (HM). Des lambeaux musculaires provenant de dix huit patients sensibles à l'HM ont été testés suivant le protocole de l'« European MH Group ». Le test à l'halothane a été réalisé seul, puis en présence de diltiazem (dix patients) ou de nifédipine (huit patients). Le prétraitement des lambeaux musculaires au diltiazem classe cinq des dix sujets susceptibles comme négatif à l'halothane. De même cinq des huit lambeaux musculaires prétraités à la nifédipine classe ces sujets susceptibles comme négatif au test à l'halothane. Il semble donc que la présence de concentrations cliniques de diltiazem ou de nifédipine dans le liquide de perfusion affecte les résultats du test à l'halothane.*

Key words
ANAESTHETICS, VOLATILE: halothane;
HYPERTHERMIA: malignant;
PHARMACOLOGY: calcium channel blockers, diltiazem, nifedipine.

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The most widely accepted test for susceptibility to malignant hyperthermia (MH) in humans is the *in vitro* halothane-caffeine contracture test. The test determines abnormal sensitivity of a muscle specimen to caffeine or halothane added to the bathing solution.[1–4] Various drugs such as procaine,[5] dantrolene[6] or propanolol[7] have been described to interfere with the *in vitro* halothane and caffeine contracture tests in humans. The effects of calcium entry blockers such as diltiazem have been examined poorly and, to our knowledge, those of nifedipine have never been tested on MH-susceptible human muscle. Because calcium entry blockers have gained widespread use in the treatment of patients with cardiovascular diseases,[8] this could be troublesome, for some of these patients may undergo elective muscle biopsy for MH diagnosis.

In the present study, we have examined the effects of diltiazem and nifedipine at a concentration of 1 μM on halothane-induced contractures of susceptible human muscle.

Methods

Eighteen patients presenting for a diagnostic muscle biopsy as part of an investigation for MH participated in the study. All were MH-susceptible according to the protocol of the European MH Group.[4] None was taking drugs which might influence skeletal muscle contractility. The study protocol was approved by the Medical Ethics Committee and informed consent was obtained from the patients for removing extra muscle.

Human muscle biopsies

The biopsies were obtained from the vastus medialis muscle under femoral nerve block anaesthesia with lidocaine. The biopsy consisted of one large muscle lump from which several muscle strips (approximately 15 mm long and 2 mm diameter) were carefully dissected. One end of the muscle strip was pinned to the silicone bottom of the tissue bath which was perfused continuously (4–5 $ml \cdot min^{-1}$) with Krebs-Ringer (KR) solution at 37° C. The KR solution was of the following composition (mM): NaCl 118.1, KCl 3.4, $CaCl_2$ 2.5, $MgSO_4$ 0.8, KH_2PO_4 1.2, $NaHCO_2$ 25; glucose 11.1. The pH was 7.35 ± 0.05 and the solution was bubbled with carbogen (95 per cent O_2, 5 per cent CO_2). The other end of the strip was attached by a thin silk thread to a force transducer (Bioscience dynamometer UFI and Biological amplifier 120). The preparations were stimulated directly using silver electrodes with rectangular current pulses of 2 ms duration and twice the threshold intensity, delivered at a frequency of 0.2 Hz by a stimulator CEA-DAM model GP1-GE2198. If a muscle strip did not contract and relax in response to this stimulation, it was discarded and another strip was dissected from the biopsy. The preparation was stretched until the amplitude of muscle twitches could not be increased further and allowed to stabilize during 15 min of isometric relaxation. Baseline and twitch tension were continuously recorded at low speed on a kompensograph C1013 Siemens.

CAFFEINE CONTRACTURE TEST

Pure caffeine dissolved in KR solution was added stepwise (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, 16 and 32 mM final bath concentration) to a muscle strip which was exposed to each concentration for 3 min.

HALOTHANE CONTRACTURE TEST

Halothane was delivered to the carbogen flow using a calibrated vaporizer (Fluotec Mark III) and each concentration (0.5, 1.0, 1.5, 2 and 3.0 vol%) was maintained for three minutes.

IN VITRO DIAGNOSIS OF MH SUSCEPTIBILITY

All patients were investigated according to the European MH protocol.[4] The criteria for MH susceptibility (MHS) are an increase in resting tension of at least 0.2 g at 2 mM caffeine or less and at 2 vol% of halothane or less. Halothane and caffeine effects were always tested on separate strips.

EXPERIMENTAL PROTOCOL

All experiments were conducted with halothane. The caffeine test was only performed to establish the diagnosis of MH susceptibility. In additional muscle strips obtained from the same biopsies, diltiazem (1 μM) or nifedipine (1 μM) were added to the KR solution ten minutes before the administration of halothane according to the same procedure as described above. The choice of the drug tested was randomized but not the sequence of tests because it was first necessary to secure enough viable tissue for the diagnostic tests. Nifedipine solution was protected from exposure to light during the experiments. The 18 MHS patients were divided in two groups. Ten patients for the diltiazem (Group one) and eight patients for the nifedipine test (Group two). The experimental protocol was performed only once on one muscle strip dissected from the same biopsy (i.e. the same patient). Hence, three muscle strips from the same biopsy were used: two for diagnosis and one for the experimental protocol. The increase in resting tension was determined. Results are expressed as mean ± SEM. Differences between the means of the groups for maximum contracture response to each concentration of halothane were analyzed using Student's t test for independent samples at a level of significance of $P < 0.05$.

Results

All the patients developed a caffeine contracture of 0.2 g or more (0.35 ± 0.05 g) with 2 mM caffeine. Futhermore, halothane alone induced contracture from the lowest concentration of 0.5 per cent and these contractures exceeded the threshold of 0.2 g for the 18 patients at two per cent (0.69 ± 0.12 g). According to the protocol supported by the European MH Group, these patients were thus classified susceptible to malignant hyperthermia (MHS). This halothane-induced contracture was not significantly different in the two groups of unpretreated muscle strips (Figure 1). At two per cent halothane, the contracture was 0.63 ± 0.16 g in the diltiazem group, and 0.77 ± 0.2 g in the nifedipine group. As indicated in Figure 1, a ten-minute preincubation with 1 μM diltiazem reduced the halothane contracture ($P < 0.05$). This was observed in the ten MHS patients and in five out of the ten patients the halothane contracture was completely blocked. Hence, in these five patients, contracture tests of muscle in the presence of diltiazem could be classified as negative to halothane.

The halothane-induced contracture in muscle strips from the second group was also significantly reduced after ten-minute pretreatment with nifedipine (Figure 1) and was demonstrated in eight out of eight muscle preparations. In four preparations, the halothane contracture was completely blocked and in another preparation the contracture was less than 0.2 g. Thus, in five of the eight patients, the contracture test of muscle in the presence of nifedipine could be classified as negative to halothane. The distribution of patients on each side of the contracture

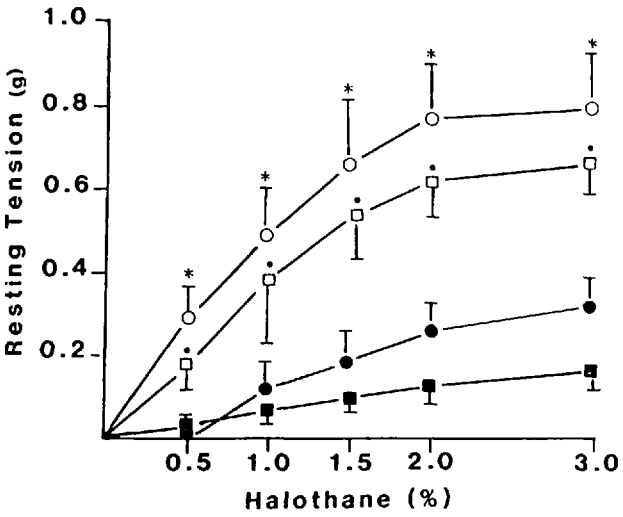

FIGURE 1 Effects of diltiazem or nifedipine on the response to halothane in diagnostic muscle biopsies from MHS patients. Four plots are shown: (□) muscle strips without diltiazem (Group one) (n = 10); (■) muscle strips with diltiazem from the same biopsies of Group one (n = 10); (○) muscle strips without nifedipine (Group two) (n = 8); (●) muscle strips with nifedipine from the same biopsies of the second group (n = 8); mean ± SEM. Statistically significant differences between the unpretreated and pretreated groups at all concentrations ($P < 0.05$) *Compared with ● $P < 0.05$. •Compared with ■ $P < 0.05$.

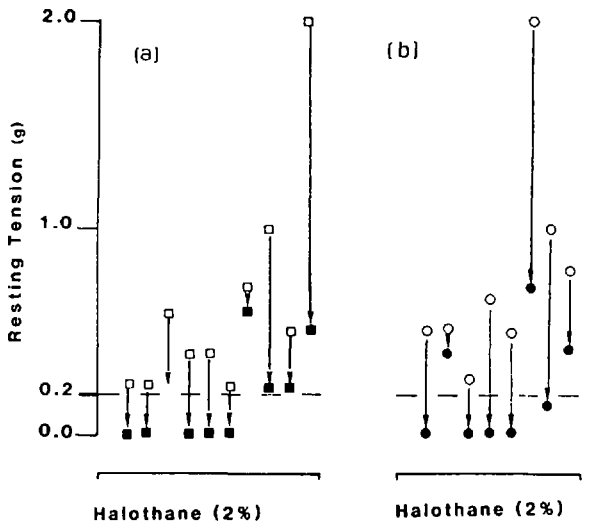

FIGURE 2 (a) and (b) Distribution of patients on both sides of the contracture threshold (0.2 g) for two per cent halothane concentration in the absence of diltiazem (□) (a) or nifedipine (○) (b) and in the presence of 1 μM diltiazem (■) (a) or nifedipine (●) (b). Horizontal dotted line: contracture threshold at two per cent halothane.

threshold for a concentration of halothane of two per cent with and without diltiazem or nifedipine is shown in Figure 2 a and b.

Discussion

The present study demonstrated that both diltiazem and nifedipine at the concentration of 1 μM were able to affect the diagnosis of MH susceptibility based on the *in vitro* muscle contractures in response to halothane. The effects of diltiazem on halothane-induced contracture have already been investigated in skeletal muscle from MH susceptible pigs but very high concentrations were required to inhibit contracture responses: 20 μM[9] and 80 μM[10] respectively. Our results also confirmed a single observation made on isolated human MH susceptible muscle where 7 μM diltiazem prevented the halothane-induced contracture.[11] It is of interest to note that the concentration of diltiazem used in the present study was more comparable to the clinical range. Plasma concentrations of diltiazem close to 1 μM are easily attainable since diltiazem is known to have a large first-pass metabolism in the liver that would result in a considerable inter-individual variation in plasma concentration.[12,13] Hence diltiazem in therapeutic concentration affects the *in vitro* halothane test for discrimination of MH susceptibility by inducing false negative results in some samples from MHS patients.

Diltiazem and nifedipine were tested at the same concentration and similar effects with both drugs were observed. These data do not confirm a previous report by Gallant and Goeth who found that diltiazem and nifedipine produced contradictory effects when used at the same concentration on mammalian skeletal muscle.[14] Although a concentration of 1 μM is outside the therapeutic range, plasma nifedipine concentrations may be close to 1 μM in some patients.[15] Furthermore, it has been commonly used *in vitro* in muscle obtained from various animal species.[16] Hence the action of nifedipine on halothane-induced contracture observed in this study cannot be considered as non-specific and clinically irrelevant for the MHS patients. However, it is not known whether plasma concentrations of nifedipine and diltiazem are a good guide to their muscle concentrations.

Similar reduction or suppression of halothane-induced contracture has been observed when another calcium entry blocker, verapamil, was added to the muscle bath or when extracellular Ca^{++} was removed,[17] thus providing evidence that halothane-induced contracture may depend on an extracellular source of Ca^{++}. Nevertheless, the influence of diltiazem or nifedipine on halothane-induced contracture in MHS patients is sufficiently notable for it to be taken into account in the interpretation of contracture tests.

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