

Xenon does not induce contracture in human malignant hyperthermia muscle[†]

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Xenon has many characteristics of an ideal anaesthetic agent. It is not known whether xenon is a safe alternative to the potent inhalational anaesthetics in patients susceptible to malignant hyperthermia (MH). We investigated the effect of xenon, halothane and caffeine on muscle specimens of 31 individuals, referred to the MH Unit of the University of Ulm, and performed genetic epidemiology. Thirteen individuals were classified as MH susceptible and 18 as MH negative. Xenon 70% did not cause an increase in baseline tension of any MH-susceptible muscle specimen in contrast to halothane and caffeine. The evoked twitch response increased transiently in MH-susceptible and normal specimens indicating a mechanism independent of MH susceptibility. These results suggest that xenon, in concentrations up to 70% may be a safe anaesthetic for MH-susceptible patients.

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Malignant hyperthermia (MH) is an autosomal dominant, pharmacogenetic disorder of skeletal muscle. First recognized in 1960,¹ MH is triggered by volatile anaesthetics and depolarizing muscle relaxants leading to a life-threatening hypermetabolic state² with a clinical age-dependent incidence of ranging from 1 in 3000 to 1 in 100 000 anaesthetics.^{3–5}

The main causative gene (RYR1) encodes the high molecular weight ligand-gated calcium channel, the ryanodine receptor type 1^{6,7} located in the sarcoplasmic reticulum (SR). Over 20 mutations have been reported in the RYR1 gene on chromosome 19q12-13.2, accounting for approximately 20% of all cases. By linkage, more than 50% of families are linked to RYR1^{8,9} indicating that many mutations are unidentified. Two mutations in the gene on chromosome 1q encoding the α_1 -subunit of the skeletal muscle dihydropyridine receptor (DHPR) have also been described, each in a single pedigree (for review see¹⁰). In the muscle, volatile anaesthetics cause an increase in myoplasmic calcium concentration¹¹ leading to muscle contracture and hypermetabolism. These effects are utilized in the in-vitro contracture test (IVCT)¹², the diagnostic gold standard.¹³

Since 1939 the inert gases have been known to exert anaesthetic properties at hyperbaric pressure.¹⁴ In addition, xenon produces anaesthesia at atmospheric pressure.¹⁵

Xenon was first used for anaesthesia in man in 1951.¹⁶ It causes no significant increases in plasma catecholamine concentrations or adverse effects on myocardial function.¹⁷ With a minimum alveolar concentration (MAC) of 71%, it is more potent than nitrous oxide (MAC 105%) and its very low blood/gas solubility coefficient (0.14 vs 0.47 of N₂O) leads to rapid induction and recovery from anaesthesia. However, its high cost has prevented its use in routine clinical practice. The development of recycling devices, closed circuit anaesthesia systems, new preparations, such as lipid-bound i.v. solutions, and the decrease in cost of xenon indicate that this may change.¹⁸ One possible use is as an alternative to potent inhalational anaesthetics in MH-susceptible patients. To test the potential of xenon to trigger MH, we investigated the effect of xenon on muscle specimens of MH-susceptible patients and normal individuals in the IVCT.

Methods

With approval by the Ethics Committee of the University of Ulm, 31 patients referred to the MH Unit of the University of Ulm for assessment of MH susceptibility, consented to

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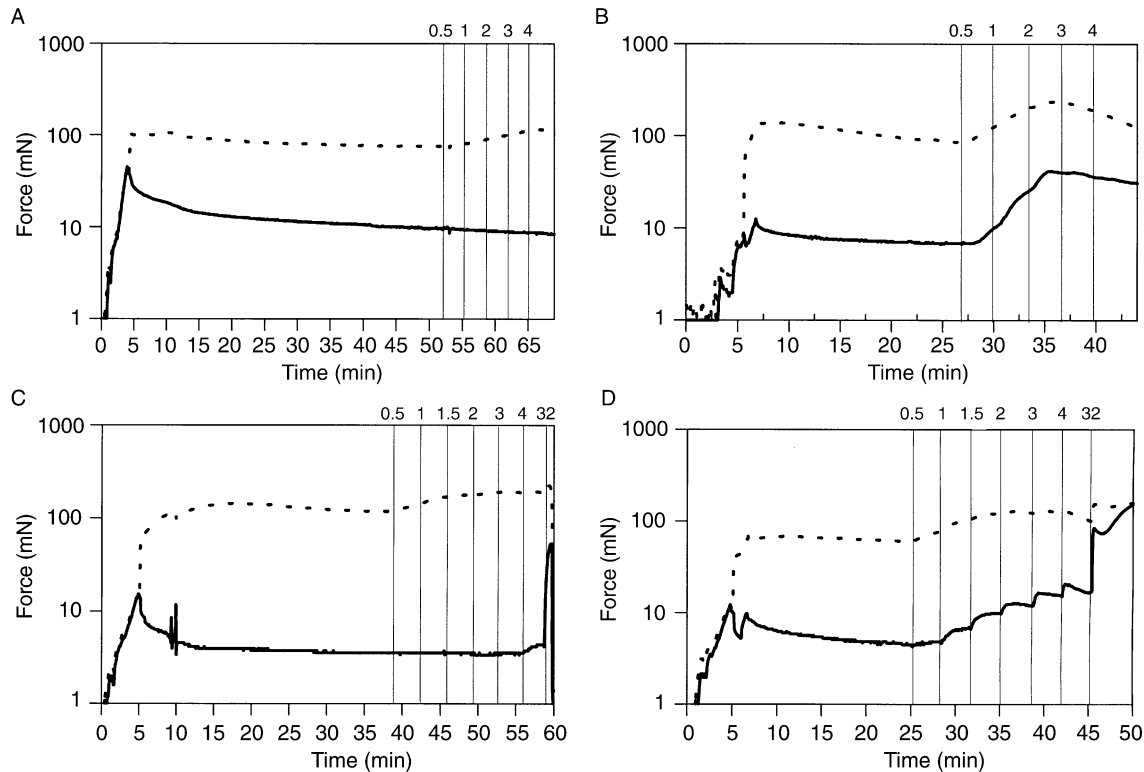


Fig 1 IVCT trace of a MHN (A) and MHS (B) muscle specimen during exposure to halothane (0.5–4.0%). MHS specimens showed a marked increase in baseline tension and twitch force. The vertical lines indicate the application of the halothane concentration specified on top of the graph (in %). IVCT trace of a MHN (C) and MHS (D) muscle specimen during exposure to caffeine (0.5–32 mM) MHS specimens showed a marked increase in baseline tension and twitch force. The vertical lines indicate the application of the caffeine concentration specified on top of the graph (in mM).

participate in this study. The patients belonged to 28 non-related families. No patient had a clinically evident, neuromuscular disease.

In vitro contracture test

Muscle bundles were excised from the quadriceps muscle under regional anaesthesia (3 in 1-block: 1% prilocaine plus 0.5% bupivacaine). The IVCT was performed on viable muscle fascicles (twitch response to supramaximal electrical stimulation ≥ 10 mN) according to the European MH Group protocol.¹² Muscle strips were stretched to 150% of their initial length and electrically stimulated (frequency 0.2 Hz, square pulse, 1 ms duration). After equilibration, administration of halothane (cumulative: 0.5, 1, 2, 3, and 4% v/v equivalent to muscle bath concentrations of 0.11, 0.22, 0.44, 0.66 and 0.88 mM) or caffeine (cumulative: 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 32 mM) was started and the baseline tension prior to drug application and at each drug concentration was measured continuously using a force transducer (Fa. RS Biomed Tech, Sinzing, Germany). The halothane concentration was increased at 3-min intervals and monitored by infrared spectroscopy (Iris, Draeger, Luebeck, Germany). Additionally, the concentrations of halothane in the muscle bath were checked by gas chromatography. Caffeine was added cumulatively for each concentration (3 min) from a freshly prepared and warmed stock solution

(100 mM at 37°C). The concentration of caffeine in the bath solution was determined by UV-spectrophotometry. MH status was assigned according to the European MH group protocol: MHS (MH susceptible) muscle showed abnormal contractures of ≥ 2 mN in at least one muscle specimen exposed to halothane ≤ 2 mM and one exposed to caffeine ≤ 2 mM, MHE (MH equivocal) only to halothane (MHEh) or caffeine (MHEc) and MHN (normal) muscle strips showed no abnormal response.

Xenon test

A xenon test was performed with one or two viable and supernumerary muscle strips of each individual tested by the standard IVCT. Xenon was obtained from Messer-Griesheim (Duisburg, Germany) in a composition of 70% xenon, 25% oxygen and 5% carbon dioxide which, by its buffering capacities, plays an important role in stabilizing the pH of the bath solution. The preparation, stretching and stimulation of the muscle bundles were done as described for the diagnostic IVCT. After equilibration of the muscle bundle (a baseline tension decrease of < 2 mN over 10 min) the gassing mixture was switched from carboxygen (95% oxygen, 5% carbon dioxide) to the xenon mixture for 10 min. Baseline tension and evoked twitch force were recorded continuously.

Genetic testing

All families were tested for the following mutations on the RYR1 gene by restriction enzyme analysis or single strand conformation analysis (SSCA) on genomic DNA according to the methods of Brandt and co-workers:¹⁹ Cys35Arg, Arg163Cys, Gly248Arg, Gly341Arg, Ile403Met, Tyr522Ser, Arg533His, Arg552Trp, Arg614Cys, Arg614Leu, Arg2163Cys, Arg2163His, Arg2163Pro, Val2168Met, Thr2206Met, Thr2206Arg, Gly2434Arg, Arg2435His, Arg2435Leu, Arg2454His, Arg2454Cys, Arg2458Cys and Arg2458His. Additionally, all families were screened for the presence of the Arg1068His and Arg1086Cys mutations in the gene encoding the α_1 -subunit of the L-type voltage-dependent calcium channel (dihydropyridine receptor, DHPR). Each patient from a family with a known mutation was individually tested for the presence of this mutation. Genomic DNA was isolated from blood preserved in ethylenediaminetetraacetic acid.

Statistics

The increase in evoked twitch force is presented as the percentage change in comparison to the force before application of the test agents (predrug vs postdrug value). A contracture was determined as the change in baseline tension from the lowest value reached during the test. Inter-group comparisons were performed with the Student's *t*-test for unpaired samples. A *P* value <0.05 was considered to indicate statistical significance. All analyses were performed with StatView 4.5™ (Abacus Concepts, Berkeley, CA, USA).

Results

IVCT

Typical IVCT traces for halothane and caffeine are plotted in Figure 1. During exposure to either substance, the MHS strips initially show a more pronounced evoked twitch force increase compared to MHN, but reach a plateau and then decrease due to the development of contractures. The evoked twitch force in MHN specimens, in the absence of contractures, increases with increasing drug concentration (Figure 2). The medians and interquartile ranges of the contractures in the 13 MHS and 18 MHN individuals are presented in Table 1.

Xenon test

Following application of 70% xenon, no increase in baseline tension, i.e. contracture, was observed in muscle specimens of MHS patients or MHN individuals (Table 1). The evoked twitch force, however, showed a slight, but transient increase compared to the predrug force which was more pronounced in muscle specimens of MHS patients than of MHN individuals: the median of the maximal increase was

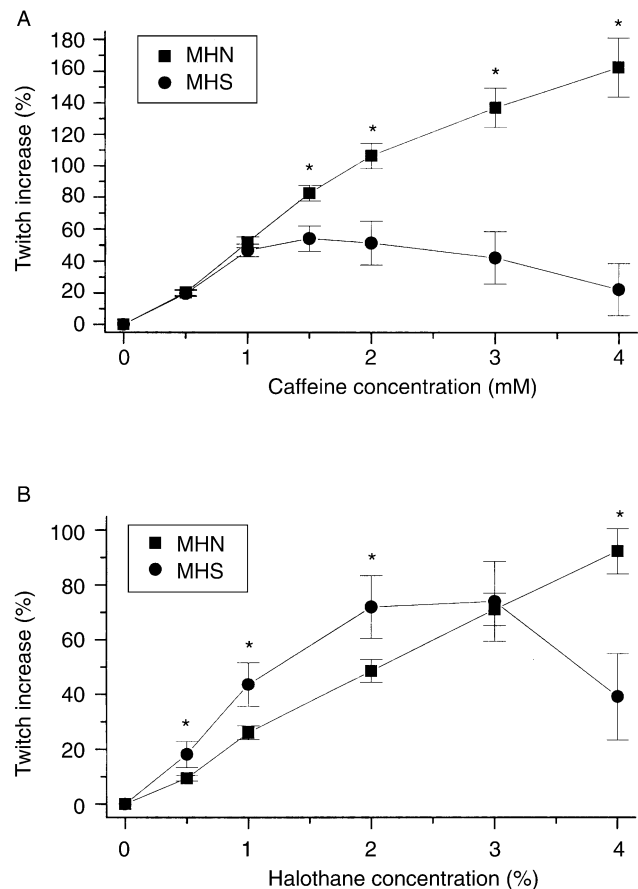


Fig 2 Dose-response curves of the twitch force due to exposure to caffeine (A) and halothane (B). Twitch force increase is given in percent relative to the predrug value. Error bars indicate SEM. Values that are significantly different are marked by an asterisk (*).

56.7% and 33.5%, respectively (Table 1). To demonstrate the viability of MHS specimens used in the xenon tests, 3% halothane was administered after discontinuation of xenon and was found to cause a contracture in all cases (Fig. 3C).

Genetic testing

Screening of the 28 families for all known RYR1 and two DHPR mutations (α_1 -subunit) revealed the Gly2434Arg mutation in three MHS patients, belonging to two unrelated families, and the Arg614Cys mutation in two unrelated patients. All of these patients had a positive IVCT result (MHS). No other mutation could be detected.

Discussion

Contractures in the IVCT are induced by increases of myoplasmic calcium and therefore represent a simple *in vitro* model for testing the potential of anaesthetic agents to trigger an MH episode. Therefore, if xenon also causes a pathological increase in myoplasmic calcium, contractures in the IVCT should be expected. However, in our investigations, we found no contractures in either muscle strips of

Table 1 Baseline contracture and twitch force increase of the muscle bundles after exposure to halothane, caffeine or xenon. Values are given as median. 25th and 75th centiles are noted in brackets. *P*-values of the unpaired *t*-test MHS vs MHN are indicated

MH status	Contracture (mN)			Twitch force increase (%)		
	Halothane (2%)	Caffeine (2 mM)	Xenon (70%)	Halothane (2%)	Caffeine (2 mM)	Xenon (70%)
MHN (<i>n</i> =18)	-0.8 (-1.1 / 0)	-1.0 (-2.0 / 0.4)	-0.6 (-0.9 / -0.1)	43.6 (35.9 / 65.4)	104.5 (80.1 / 125.2)	33.5 (24.5 / 45.6)
MHS (<i>n</i> =13)	6.3 (3.7 / 15.3)	6.2 (2.5 / 11.7)	-0.9 (-1.5 / -0.2)	63.6 (46.6 / 98.2)	60.1 (30.15 / 71.3)	56.7 (41.0 / 62.4)
<i>P</i> -value	0.003	0.007	0.25	0.026	0.005	0.015

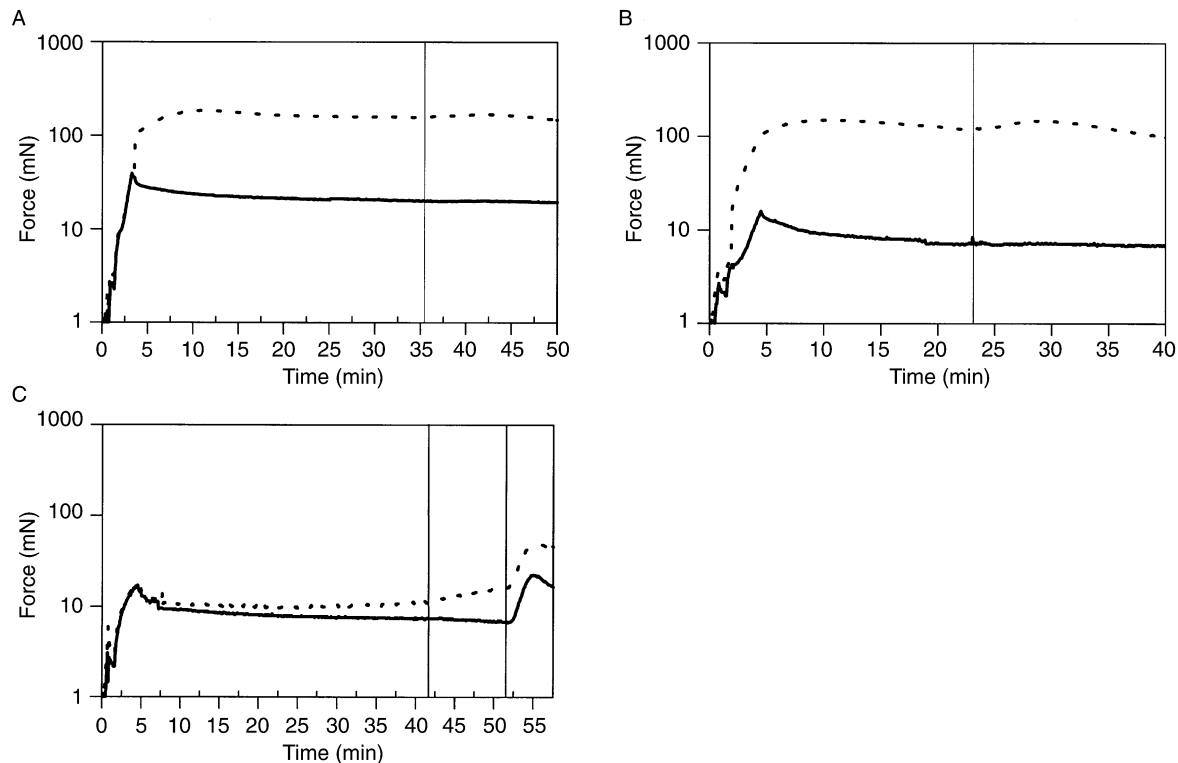


Fig 3 IVCT trace of a MHN (A) and MHS (B) muscle specimen during exposure to xenon (70%). No increase in baseline tension, but a slight increase in twitch force is notable. The vertical line indicates the start of the application of 70% xenon. IVCT trace of a MHS (C) muscle specimen during successive exposure to xenon (70%) and subsequently to halothane (3%). A marked increase in baseline tension is visible only after halothane exposure. The first vertical line indicates the start of the application of 70% xenon, the second indicates discontinuation of xenon and simultaneous start of 3% halothane exposure.

MH susceptible patients or in normal individuals despite proof of contracture responsiveness of the tested MHS bundles.

Four of the 11 tested MHS families (36%) carried one of the two most common RYR1 mutations (Arg614Cys or Gly2434Arg). This frequency of mutations in the investigated patients corresponds to those previously reported.¹⁹ The samples tested may therefore be considered representative of the MH population.

The application of all three test agents increased the evoked twitch force in the IVCT, a phenomenon presumably due to slight, but controllable elevation of sarcoplasmic calcium release.²⁰ Remarkably, this effect is consistently

more pronounced in MHS specimens than in MHN, but the increase induced by xenon in its maximal clinically applied dose is significantly smaller than that observed with either halothane or caffeine. The evoked twitch force increase on application of xenon indicates the substance readily penetrates the muscle strip and lack of penetration is unlikely to be the reason for lack of contracture in response to xenon. Recently, xenon was shown to inhibit plasma membrane calcium-ATPase (PMCA) leading to increased resting $[Ca^{2+}]_i$, enhanced peak $[Ca^{2+}]_i$, and delayed reuptake following stimulation in brain tissue.²¹ As an increase in intracellular calcium in skeletal muscle could lead to an increase in muscle force and as PMCA is also present in

skeletal muscle, this mechanism, independent of RYR1, could be responsible for the observed evoked twitch force increase in accordance with earlier findings of passive non-ryanodine receptor-mediated calcium release in MH.²²

In vivo effects of xenon on MH susceptible patients have not yet been tested. In an animal model of MH, the purebred Landrace, Pietrain and Poland China pigs,²³ Froeba and co-workers²⁴ have demonstrated the complete absence of any haemodynamic, gas-exchange or metabolic response indicative of MH during 2 h of xenon administration. In contrast, in all animals the subsequent administration of halothane initiated fatal MH episodes. Considering the results of the *in vivo* experiments in the pig model and the *in vitro* results in humans, it is reasonable to conclude that xenon (up to 70%) will not trigger MH during anaesthesia in man.

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References

- Denborough M, Lovell R. Anaesthetic deaths in a family. *Lancet* 1960; **2**: 45
- Gronert GA, Antognini JF. Malignant hyperthermia. In: Miller RD, ed. *Anesthesia*, 5th edn. New York: Churchill Livingstone, 1999
- Ryan JF. Malignant hyperthermia. In: Cote CJ, Ryan JF, Todres ID, Goudsouzian NG, eds. *A practice of anesthesia for infants and children*, 2nd edn. Philadelphia: Saunders, 1993: 417–428
- Kalow W, Britt BA, Chan FY. Epidemiology and inheritance of malignant hyperthermia. *Int Anesthesiol Clin* 1979; **17**: 119–39
- Ørding H. Incidence of malignant hyperthermia in Denmark. *Anesth Analg* 1985; **64**: 700–4
- MacLennan DH, Duff C, Zorzato F, et al. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* 1990; **343**: 559–61
- McCarthy TV, Healy JM, Heffron JJ, et al. Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. *Nature* 1990; **343**: 562–4
- Deufel T. Malignant hyperthermia is genetically heterogeneous. Problems in search for molecular genetic markers. *Fortschr Med* 1992; **110**: 49
- Ball SP, Dorkins HR, Ellis FR, et al. Genetic linkage analysis of chromosome 19 markers in malignant hyperthermia. *Br J Anaesth* 1993; **70**: 70–5
- Jurkat-Rott K, McCarthy T, Lehmann-Horn F. Genetics and pathogenesis of malignant hyperthermia. *Muscle Nerve* 2000; **23**: 4–17
- Iaizzo PA, Klein W, Lehmann-Horn F. Fura-2 detected myoplasmic calcium and its correlation with contracture force in skeletal muscle from normal and malignant hyperthermia susceptible pigs. *Pflug Arch* 1988; **411**: 648–53
- The European Malignant Hyperpyrexia Group. A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. *Br J Anaesth* 1984; **56**: 1267–9
- Larach MG. Should we use muscle biopsy to diagnose malignant hyperthermia susceptibility? *Anesthesiology* 1993; **79**: 1–4
- Behnke AR, Yarbrough OD. Respiratory resistance, oil-water solubility, and mental effects of argon, compared with helium and nitrogen. *Am J Physiol* 1939; **126**: 409–15
- Lawrence JH, Loomis WF, Tobias CA, Turpin FA. Preliminary observations on the narcotic effect of xenon with a review of values for solubility of gases in water and oil. *J Physiol* 1946; **105**: 197–205
- Cullen SC, Gross EG. The anesthetic properties of xenon in animals and human beings, with additional observations on krypton. *Science* 1951; **113**: 580–2
- Marx T, Froeba G, Wagner D, Baeder S, Georgieff M. Effects on haemodynamics and catecholamine release of xenon anaesthesia compared with total i.v. anaesthesia in the pig. *Br J Anaesth* 1997; **78**: 326–7
- Marx T, Froeba G, Wagner D, Baeder S, Georgieff M. Retrieval and purification of xenon in anaesthesia. *Acta Anaesthesiol Scand* 1996; **40**: 217
- Brandt A, Schleithoff L, Jurkat-Rott K, Klingler W, Baur C, Lehmann-Horn F. Screening of the ryanodine receptor gene in 105 malignant hyperthermia families: novel mutations and concordance with the *in vitro* contracture test. *Human Mol Genet* 1999; **8**: 2055–62
- Gallant EM, Mickelson JR, Roggow BD, Donaldson SK, Louis CF, Rempel WE. Halothane-sensitivity gene and muscle contractile properties in malignant hyperthermia. *Am J Physiol* 1989; **257**: C781–6.
- Horn JL, Janicki PK, Franks JJ. Lack of effect of flurothyl, a non-anesthetic fluorinated ether, on rat brain synaptic plasma membrane calcium-ATPase. *Life Sci* 1998; **64**: 179–83
- Louis CF, Zulkernan K, Roghair T, Mickelson JR. The effects of volatile anesthetics on calcium regulation by malignant hyperthermia-susceptible sarcoplasmic reticulum. *Anesthesiology* 1992; **77**: 114–25
- Lucke JN, Hall GM, Lister D. Porcine malignant hyperthermia. I: Metabolic and physiological changes. *Br J Anaesth* 1976; **48**: 297–304
- Froeba G, Marx T, Pazhur JR, et al. Xenon does not trigger malignant hyperthermia in susceptible swine. *Anesthesiology* 1999; **91**: 1047–52
- Gallant EM, Godt RE, Gronert GA. Mechanical properties of normal and malignant hyperthermia susceptible porcine muscle: effects of halothane and other drugs. *J Pharmacol Exp Ther* 1980; **213**: 91–6
- Gronert GA, Heffron JJ, Taylor SR. Skeletal muscle sarcoplasmic reticulum in porcine malignant hyperthermia. *Eur J Pharmacol* 1979; **58**: 179–87
- Iaizzo PA, Lehmann-Horn F, Taylor SR, Gallant EM. Malignant hyperthermia: effects of halothane on the surface membrane. *Muscle Nerve* 1989; **12**: 178–83