British Journal of Anaesthesia 1993; 70: 76–79

IN VITRO EFFECT OF EPHEDRINE, ADRENALINE, NORADRENALINE AND ISOPRENALINE ON HALOTHANE-INDUCED CONTRACTURES IN SKELETAL MUSCLE FROM PATIENTS POTENTIALLY SUSCEPTIBLE TO MALIGNANT HYPERTHERMIA

A. URWYLER, K. CENSIER, M. D. SEEBERGER, J. M. ROTHENBÜHLER, M. A. KAUFMANN AND J. DREWE

SUMMARY

We have measured the effects of ephedrine, adrenaline, noradrenaline and isoprenaline on halothane-induced contractures in muscle biopsies from patients potentially susceptible to malignant hyperthermia (MH). At concentrations of 4–24 mmol litre^{-1}, ephedrine induced in vitro *contractures in halothane 0.44 mmol litre^{-1}-prechallenged muscle, whilst adrenaline, noradrenaline and isoprenaline had no effect. There was a shift of the ephedrine concentration–response curve to the left and an increased maximum muscle contracture in the MH susceptible group compared with the MH negative group ($P < 0.001$). We conclude that ephedrine increased halothane-induced muscle contractures* in vitro *either by an unknown pharmacological mechanism or by an adrenergic stimulation which was different from those of the other investigated adrenoceptor agonists.* (Br. J. Anaesth. 1993; **70**: 76–79)

KEY WORDS

Hyperthermia: malignant. Muscle: halothane-induced contracture. Sympathetic nervous system, pharmacology: adrenaline, ephedrine, isoprenaline.

Malignant hyperthermia (MH) is characterized clinically by a life-threatening hypermetabolic reaction that is attributed to an underlying muscle disease. Skeletal muscle from MH susceptible (MHS) individuals demonstrates abnormal *in vitro* contractures to halothane, in contrast with muscle bundles from MH negative (MHN) subjects [1, 2]. *In vitro* muscle contractures are characterized by an increase in baseline tension and are related to an increased myoplasmic calcium concentration $[Ca^{2+}]_i$ [3]. There is controversy as to whether or not the sympathetic system is involved primarily or as a secondary response to MH [4], and less is known about the effects of adrenergic agents on muscle metabolism in humans with an acute MH episode. Therefore, we have investigated the effects of different adrenoceptor agonists on halothane-induced muscle contractures *in vitro* in patients potentially susceptible to MH. Some of these results have been published previously in abstract form [5].

PATIENTS AND METHODS

We studied 42 potentially MHS individuals after approval by the University of Basel's Hospital Ethics Committee. All patients gave informed written consent. Biopsies were taken from the vastus medialis muscle under regional anaesthesia with a femoral nerve block using 1.5% mepivacaine 5–7.5 mg kg^{-1}. The muscle tissue was maintained in Krebs–Ringer solution oxygenated with 5% carbon dioxide in oxygen (carbogen) until the experiments were performed. Muscle bundles, approximately 2.5 cm long and 2–3 mm in diameter, were mounted in a test bath containing Krebs–Ringer solution. Temperature was maintained at 37 °C and the bath was bubbled continuously with carbogen. Supramaximal electrical stimulation at 0.2 Hz was used to demonstrate the viability of the muscle bundle. MH susceptibility was evaluated with two halothane and two caffeine tests on four different muscle bundles, and MH diagnosis was made according to a standardized procedure [2, 6].

Surplus muscle bundles were challenged for 10 min with halothane 0.44 mmol litre^{-1} in the test bath, corresponding to 2% in the gas phase, followed by incremental doses of adrenaline ($n = 3$), noradrenaline ($n = 6$), isoprenaline ($n = 3$) or ephedrine ($n = 42$). Twelve bundles were pretreated with incremental doses of ephedrine 4–24 mmol litre^{-1} before the halothane 0.44 mmol litre^{-1} challenge. All drugs were dissolved in Krebs–Ringer solution (pH 7.4) before addition to the test bath. In preliminary experiments, concentrations between 1 nmol litre^{-1} and 1 mmol litre^{-1} of all adrenergic agents studied

A. URWYLER, M.D., K. CENSIER, M. D. SEEBERGER, M.D., M. A. KAUFMANN, M.D., J. DREWE, M.D. (Department of Anaesthesia and Research); J. M. ROTHENBÜHLER, M.D. (Department of Surgery); University of Basel, Kantonsspital, CH-4031 Basel, Switzerland.
Accepted for Publication: July 7, 1992.

did not initiate any muscle contractures [unpublished results], and thus each adrenoceptor agonist was evaluated at greater concentrations: 4, 8, 12, 16 and 24 mmol litre^{-1}. Drug concentrations were maintained for at least 3 min or until a steady state in baseline tension was achieved. Muscle contractures were recorded for each concentration of the various agents. The baseline value just before addition of the first dose of an adrenergic agonist was used as the zero level.

Drugs were obtained from the following commercial sources: halothane from Halocarbon Laboratories, Hackensack, NJ, U.S.A.; pure caffeine from Merck, Darmstadt, Germany; (−)-adrenaline hydrochloride (HCl), (−)-noradrenaline HCl, (−)-isoprenaline HCl, (−)-ephedrine HCl from Sigma Chemical Co., St Louis, MO, U.S.A.

Data analysis

Ephedrine concentration–response curves were calculated by non-linear regression analysis according to the sigmoidal maximum effect model (Hill equation) (SAS, Release 6.03, U.S.A.). Data are presented as mean (SEM) and 95% confidence intervals (95% CI). Statistical analyses were performed using multiple one-sided or two-sided Student's t tests with Bonferroni correction as appropriate. $P < 0.05$ was considered to represent a significant difference between the groups.

RESULTS

Sixteen individuals were classified as MHN, while 26 patients were considered to be clinically MHS (fig. 1). The clinically MHS group consisted of 17 individuals who had abnormal contracture tests to halothane and caffeine challenges and a second group of nine MH equivocal patients (MHEh) with abnormal halothane but normal caffeine contracture tests.

In muscle bundles from 12 patients (MHN: $n = 5$; MHS: $n = 7$), administration of ephedrine before halothane did not produce any contractures. However, when halothane was added to the test bath after pretreatment with ephedrine, all muscle bundles demonstrated contractures (12.6 (3.6) mN (95% CI 2.6–22.6 mN) in the MHN group compared with 43.9 (10.3) mN (95% CI 18.6–69.1 mN) in the MHS group).

After the halothane 0.44 mmol litre^{-1} challenge, there were no muscle contractures in bundles of MHS individuals after addition of adrenaline, noradrenaline or isoprenaline to the test bath, whilst ephedrine induced dose-dependent muscle contractures in all these muscle bundles (table I, fig. 2).

There was a significant shift of the ephedrine concentration–response curve to the left for the MHS group compared with the MHEh and MHN groups (table II, fig. 3). Eight patients had to be excluded for the calculations of EC_{50} and the maximum effect (Emax), because there were no muscle contractures in response to ephedrine ($n = 4$ in the MHN group) or because Emax was not achieved in the range of the investigated concentra-

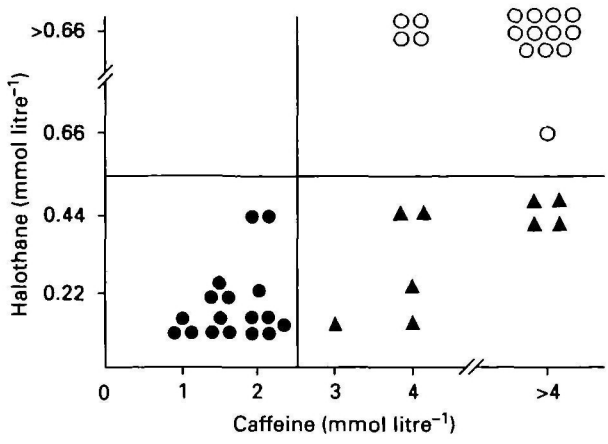

FIG. 1. Classification of 42 patients investigated with *in vitro* muscle contracture tests according to the procedure of the European Malignant Hyperpyrexia Group [2]. For the diagnosis of MH susceptibility the threshold concentrations of halothane and caffeine were used which initiated an *in vitro* muscle contracture ⩾ 2 mN. MHN (○) ($n = 16$) = MH negative; MHS (●) ($n = 17$) = MH susceptible (abnormal halothane and abnormal caffeine contracture tests); MHEh (▲) ($n = 9$) = MH equivocal (abnormal halothane but normal caffeine contracture tests).

TABLE I. *In vitro contractures (⩾ 2 mN) initiated by various adrenoceptor agonists in 29 muscle bundles from MHS subjects. Each adrenoceptor agonist was evaluated at increasing concentrations (4, 8, 12, 16 and 24 mmol litre^{-1}) in the presence of halothane 0.44 mmol litre^{-1}*

	Number of contractures	Number tested
(−)-Ephedrine	17	17
(−)-Adrenaline	0	3
(−)-Noradrenaline	0	6
(−)-Isoprenaline	0	3
(−)-Ephedrine after (−)-adrenaline	3	3
(−)-Ephedrine after (−)-noradrenaline	6	6
(−)-Ephedrine after (−)-isoprenaline	3	3

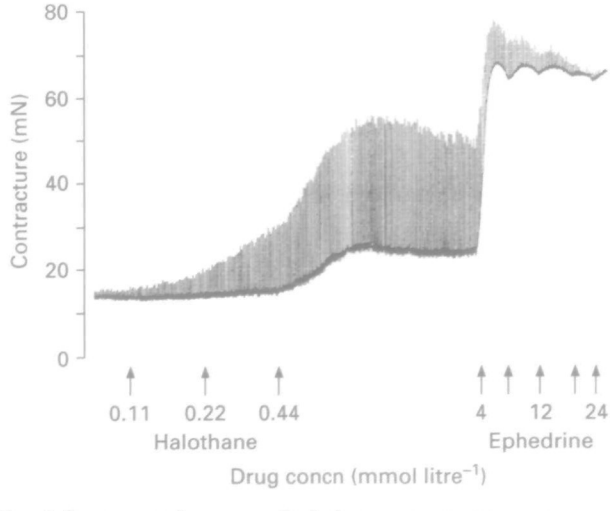

FIG. 2. Representative curve of halothane and ephedrine effects on the *in vitro* muscle contracture of an MHS individual. Contractions were elicited using supramaximal electrical stimulation (0.2 Hz) to prove the viability of the muscle bundle. Halothane and ephedrine were added to the test bath in increasing concentrations as indicated (labels 8 and 16 (mmol litre^{-1}) omitted for ephedrine, for clarity.)

TABLE II. In vitro *effect of ephedrine on halothane-induced muscle contractures (mean (SEM) [95% confidence intervals]). Significant changes (multiple one-sided t test with Bonferroni correction): *P < 0.05 (compared with MHN group); †P < 0.05 (compared with MHEh group); ***P < 0.001 (compared with MHN group)*

	n	Emax (mN)	EC_{50} (mmol litre^{-1})
MHN	12	17 (3.2) [10–24]	11.8 (1.1) [9.3–14]
MHEh	7	20 (3.9) [11–30]	9.4 (1.9) [4.8–14]
MHS	15	29 (4)* [20–38]	4.6 (1)†*** [2.5–6.8]

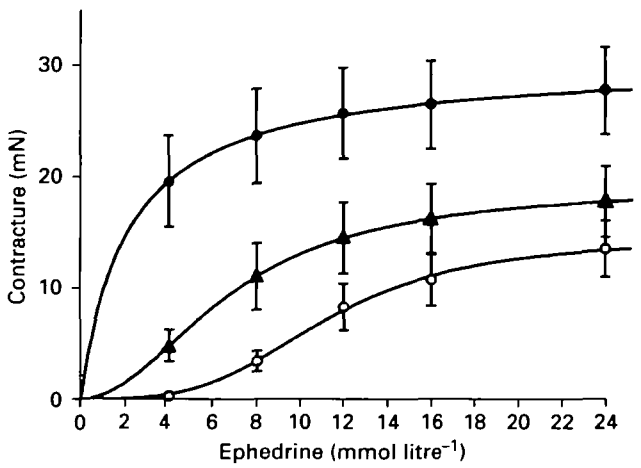

FIG. 3. Mean (SEM) *in vitro* effect of ephedrine on halothane-induced contractures in muscle bundles from patients potentially susceptible to malignant hyperthermia. MHS (●) (n = 17) = MH susceptible (abnormal halothane and caffeine contracture); MHEh (▲) (n = 9) = MH equivocal (abnormal halothane but normal caffeine contracture); MHN (○) (n = 16) = MH negative.

tions (n = 1 in the MHN group, n = 2 in the MHEh group and n = 1 in the MHS group).

DISCUSSION

In this study, ephedrine 4–24 mmol litre^{-1} increased halothane-induced *in vitro* muscle contractures, whilst adrenaline, noradrenaline and isoprenaline had no effect. There was a statistically significant shift of the ephedrine concentration–response curve to the left in the MHS group compared with the MHN group. These data suggest the possibility that ephedrine increased muscle metabolism *in vitro*. However, the exact mechanism of the *in vitro* effect of ephedrine on skeletal muscle is speculative.

In general, ephedrine is considered to have direct and indirect sympathomimetic effects, but the exact pharmacological mechanism(s) is not known [7]. Because inositol 1,4,5-triphosphate (IP_3) has been found to be increased in muscle tissue from MHS pigs and MHS humans [8, 9], one could speculate that ephedrine might increase intracellular IP_3 concentrations and thereby increase halothane-induced muscle contractures. Alternatively, or in addition to IP_3, it may be that ephedrine effects are mediated indirectly by cyclic AMP, comparable to the effects of theophylline or caffeine on skeletal muscle *in vitro* [10]. Recent work has shown the possible involvement of a mutation of the ryanodine receptor gene as the primary defect in MHS individuals [11, 12]. Therefore, it may be interesting to determine if ephedrine interacts directly or indirectly with the ryanodine receptor.

If ephedrine initiates muscle contractures by an adrenergic effect, this mechanism must differ from classical adrenergic stimulation because adrenaline, noradrenaline and isoprenaline did not have similar effects. Little is known about the adrenergic effects on halothane-induced *in vitro* muscle contractures. To our knowledge, only noradrenaline in porcine skeletal muscle [13] and salbutamol, a $beta_2$-adrenoceptor agonist, in human skeletal muscle have been investigated [14]. Both adrenoceptor agonists did not potentiate halothane-induced muscle contractures. Our study provides additional evidence that the sympathomimetic system does not initiate MH primarily [15, 16], as we did not find any contractures in muscle bundles from MHS individuals after challenges with adrenaline or noradrenaline.

The clinical relevance of *in vitro* muscle investigations, as performed in the present study, is unclear, as we used ephedrine in concentrations of 4–24 mmol litre^{-1}, 1000–2000-fold greater than clinically relevant plasma concentrations. However, these large concentrations of ephedrine *in vitro* could reflect the requirement that effective tissue concentrations can be achieved by diffusion, in contrast to the situation *in vivo* when ephedrine is brought into muscle tissue by capillary circulation. Clinically, ephedrine increases energy expenditure [17, 18] and has been proposed as a thermogenic drug for the treatment of obesity [19] and cyclical hypothermia [20] or for the improvement of cold tolerance [21]. Leg oxygen consumption increased 60% after ephedrine administration and it was speculated that 50% of the increase in oxygen consumption induced by ephedrine may take place in skeletal muscle [17]. In anaesthetics, ephedrine is used for vasopressor therapy in hypotension as a result of regional or general anaesthesia [21]. To our knowledge, there are no clinical data suggesting ephedrine as a MH trigger. However, these data suggest the use of alternative adrenergic drugs if cardiovascular instability is associated with a hypermetabolic state in MHS individuals.

REFERENCES

1. Ellis FR, Harriman DGF, Keaney NP, Kyei-Mensah K, Tyrrell JH. Halothane-induced muscle contracture as a cause of hyperpyrexia. *British Journal of Anaesthesia* 1971; **43**: 721–722.
2. European Malignant Hyperpyrexia Group. A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. *British Journal of Anaesthesia* 1984; **56**: 1267–1269.
3. 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. *Pflügers Archiv* 1988; **411**: 648–653.
4. Gronert GA, Mott J, Lee J. Aetiology of malignant hyperthermia. *British Journal of Anaesthesia* 1988; **60**: 253–267.
5. Urwyler A, Censier K, Rothenbühler JM. *In vitro* effect of ephedrine in malignant hyperthermia susceptible muscle. *Journal of the Neurological Sciences* 1990; **98** (Suppl.): 520.
6. Urwyler A, Funk B, Censier K, Drewe J. Effect of halothane equilibration kinetics on *in vitro* muscle contractures for

malignant hyperthermia screening. *Acta Anaesthesiologica Scandinavica* 1992; **36**: 115–118.
7. Hoffman BB, Lefkowitz RJ. Catecholamines and sympathomimetic drugs. In: Gilman A, Rall TW, Nies AS, Taylor P, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. New York: Pergamon Press, 1990; 187–220.
8. Foster PS, Gesini E, Claudianos C, Hopkinson KC, Denborough MA. Inositol 1,4,5-triphosphate phosphatase deficiency and malignant hyperpyrexia in swine. *Lancet* 1989; **2**: 124–127.
9. Scholz J, Troll U, Schulte am Esch J, Hartung E, Patten M, Sandig P, Schmitz W. Inositol-1,4,5-triphosphate and malignant hyperthermia. *Lancet* 1991; **337**: 1361.
10. Flewellen EH, Nelson TE. Is theophylline, aminophylline, or caffeine (methylxanthines) contraindicated in malignant hyperthermia susceptible patients? *Anesthesia and Analgesia* 1983; **62**: 115–118.
11. MacLennon DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt BA, Worton RG. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* (*London*) 1990; **343**: 559–561.
12. McCarthy TV, Healy JMS, Heffron JA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K. Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. *Nature* (*London*) 1990; **343**: 562–564.
13. Sim ATR, White MD, Denborough MA. Effects of adenylate cyclase activators on porcine skeletal muscle in malignant hyperpyrexia. *British Journal of Anaesthesia* 1987; **59**: 1557–1562.
14. Bendixen D, Ording H. Influence of salbutamol on the in vitro muscle response to caffeine and halothane in malignant hyperthermia. *Acta Anaesthesiologica Scandinavica* 1990; **34**: 658–661.
15. Gronert GA, White DA. Failure of norepinephrine to initiate porcine malignant hyperthermia. *Pflügers Archiv* 1988; **411**: 226–228.
16. Haeggendal J, Joensen L, Carlsten J. The role of sympathetic activity in initiating malignant hyperthermia. *Acta Anaesthesiologica Scandinavica* 1990; **34**: 677–683.
17. Astrup A, Buelow J, Madasen J, Christensen NJ. Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *American Journal of Physiology* 1985; **248**: E507–E515.
18. Vallerand AL, Jacobs I, Kavanagh MF. Mechanism of enhanced cold tolerance by an ephedrine–caffeine mixture in humans. *Journal of Applied Physiology* 1989; **67**: 438–444.
19. Dulloo AG, Miller DS. Prevention of genetic fa/fa obesity with an ephedrine–methylxanthines thermogenic mixture. *American Journal of Physiology* 1987; **252**: R507–R513.
20. Flynn MD, Sandeman DD, Mawson DM, Shore AC, Tooke JE. Cyclical hypothermia: successful treatment with ephedrine. *Journal of the Royal Society of Medicine* 1991; **84**: 752.
21. Ramanthan S, Grant GJ. Vasopressor therapy for hypotension due to epidural anesthesia for Caesarean section. *Acta Anaesthesiologica Scandinavica* 1988; **32**: 559–565.