

Effect of Bay K 8644 on the Magnitude of Isoflurane and Halothane Contracture of Skeletal Muscle from Patients Susceptible to Malignant Hyperthermia

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Isoflurane has a lesser ability than halothane to induce contracture in malignant hyperthermia (MH) muscle *in vitro*. This does not necessarily imply that isoflurane is not as potent an MH trigger as halothane *in vivo*. A hypothesis was tested that *in vitro* treatment with Bay K 8644, an activator of both the dihydropyridine receptors as well as the sodium channels of the T-tubules, potentiates isoflurane-induced MH-susceptible skeletal muscle contracture. In addition to the usual halothane-caffeine test, other muscle bundles were exposed to 10 μM Bay K 8644-halothane and equipotent anesthetic concentrations (expressed in multiple minimum alveolar concentration [MAC]) of isoflurane either alone or combined with Bay K 8644. In 14 MH-susceptible muscle bundles, the mean maximum contracture induced by 2 MAC isoflurane was 0.20 ± 0.22 g (mean \pm SD), and this value was significantly less than that obtained with 2 MAC halothane (0.68 ± 0.40 g). Bay K 8644 did not induce muscle contracture on its own but consistently enhanced both the 0.5 MAC isoflurane and halothane to the same maximal isometric tension (1.09 ± 0.35 g and 1.11 ± 0.37 g, respectively). Such an effect was not observed in the MH-nonsusceptible group. Under the conditions of this *in vitro* study, 0.5 MAC isoflurane appears to be as potent as halothane in inducing muscle contracture in skeletal muscle bundles from individuals susceptible to MH. (Key words: Anesthetics, volatile; halothane; isoflurane. Hyperthermia: malignant. Pharmacology, calcium agonist: Bay K 8644.)

AN UNDERLYING GENETIC DEFECT predisposes certain individuals to the possibility that a malignant hyperthermia (MH) episode could be induced by triggering anesthetic agents under certain conditions such as patient's temperature.¹ Patients susceptible to MH are usually identified on the basis of results of the *in vitro* halothane-caffeine test.^{2,3} Compared to isoflurane, halothane is more

potent with respect to inducing contracture in MH muscle *in vitro*^{4,5} and to triggering MH in pigs *in vivo*.⁶ However, these results contrast with many clinical reports of fulminant MH crisis induced by isoflurane.⁷⁻¹³

Bay K 8644, a calcium (Ca^{2+}) agonist that acts both on the dihydropyridine receptors and sodium (Na^+) channels located in the T-tubules,¹⁴⁻¹⁶ has been reported to potentiate strongly the *in vitro* positive response to halothane in MH-susceptible (MHS) muscle bundles.¹⁷ It is possible that Bay K 8644 also influences the isoflurane-induced contracture *in vitro* and thus could mimic some condition that may cause isoflurane to be as potent an MH trigger as is halothane. We therefore investigated the effects of Bay K 8644 on the *in vitro* response to increasing concentrations of isoflurane in muscle bundles from MHS and MH-nonsusceptible (MHN) patients. Furthermore, these effects were compared with those obtained with halothane under the same experimental conditions.

Materials and Methods

DIAGNOSIS OF MALIGNANT HYPERTHERMIA SUSCEPTIBILITY

Thirty patients presenting for a diagnostic muscle biopsy as part of an investigation for MH participated in the study, which was approved by the Lille University Studies Ethics Committee. Informed consent was obtained from the patients for removing extra muscle. The biopsies were taken from the vastus lateralis muscle. Dimensions of the muscle bundles isolated for mounting in the tissue bath were 15–20 mm (length) by 2–3 mm (diameter). The preparation and stimulation of the muscle bundles, experimental apparatus, and methods of delivery of halothane have been described elsewhere.¹⁷ Halothane concentration was measured in the bath by gas-liquid chromatography as described below. Caffeine free base was dissolved in Krebs-Ringer solution. All patients were investigated according to the protocol supported by the European Malignant Hyperpyrexia Group.²

MATERIALS

Isoflurane was mixed with carbogen by means of a calibrated vaporizer (Isotec®). The anesthetic concentration in the gas phase was monitored with an infrared calibrated

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analyzer (Normac[®], Datex, Finland). The anesthetic concentrations used were 0.75, 1.5, 3.0, and 4.5 vol% isoflurane. These concentrations are roughly equivalent to 0.5, 1, 2, and 3 MAC multiples of halothane in humans at 37° C.^{18,19} The anesthetic concentrations obtained in the Krebs-Ringer solution were measured by gas-liquid chromatography to determine the amount of anesthetic present in the test solutions. A Varian 1400[®] gas chromatograph equipped with a flame ionization detector and a Porapak Q[®] 3.17-mm by 150-cm column was used for determination of anesthetic concentrations.²⁰ A 60-ml flask containing 100 μl of the solution equilibrated 15 min with the anesthetic was maintained at 60° C (above the boiling point of each anesthetic) for 20 min before injection of 1 ml gas into the apparatus, previously calibrated with known concentrations of each anesthetic (head space technique).

The anesthetic concentrations measured in the experimental solution (n = 6) after 3 min of continuous bubbling at equivalent multiples of MAC were as follows: 0.5 MAC: 0.09 ± 0.01 mM for halothane, 0.22 ± 0.01 mM for isoflurane; 1 MAC: 0.20 ± 0.02 mM for halothane, 0.44 ± 0.02 mM for isoflurane; 2 MAC: 0.49 ± 0.03 mM for halothane, 0.91 ± 0.03 mM for isoflurane; and 3 MAC: 0.72 ± 0.05 mM for halothane, 1.15 ± 0.04 mM for isoflurane. Racemic (±) Bay K 8644 powder (Bayer, Pharma) containing 50% of relative proportions of the enantiomers was dissolved in dimethyl sulfoxide; the choice of the concentration has been described previously.¹⁷ Because Bay K 8644 was dissolved in 0.1% dimethyl sulfoxide, a preliminary test in this solution was necessary. Dimethyl sulfoxide in Krebs-Ringer solution did not show any effect on baseline tension with or without isoflurane in both MHN and MHS preparations (n = 4 for each group).

EXPERIMENTAL PROCEDURE

Additional muscle bundles obtained from the same biopsies (*i.e.*, separate muscle bundles not previously exposed to either halothane or caffeine) were exposed to: 1) increasing concentrations of isoflurane or 2) 10 μM Bay K 8644 for 10 min before and during the administration of either halothane or isoflurane. Each protocol was performed on a separate muscle bundle from the same patient. The sequence of the tests was not randomized because it was first necessary to secure enough viable tissue for the diagnostic tests. Thus, the experimental procedures were performed on tissue remaining after the diagnostic tests.

STATISTICAL ANALYSIS

Comparisons between groups of MHS and MHN patients, between anesthetics with or without Bay K 8644, and at equivalent MAC multiples were made by repeated measures analysis of variance using a computer program (SAS system). For multiple comparisons among groups where indicated by results of analysis of variance, Duncan's multiple range test was used. Values of *P* < 0.05 or less were regarded as significant. Results are expressed as mean ± standard deviation of the mean (SD).

Results

IN VITRO DISCRIMINATION OF MALIGNANT HYPERTHERMIA SUSCEPTIBILITY

Muscle bundles from 14 patients developed a caffeine contracture of 0.31 ± 0.11 g at 2 mM caffeine and a contracture of 0.68 ± 0.40 g at 2 vol% (2 MAC) halothane.

TABLE 1. Contracture Amplitudes of 56 Muscle Bundles from 14 Patients Susceptible to Malignant Hyperthermia in Response to Either 2 MAC Halothane or Isoflurane and Either 0.5 MAC Halothane or Isoflurane in the Presence of Bay K 8644

MHS Patient	2 MAC Halothane (g)	2 MAC Isoflurane (g)	10 μM Bay K 8644 + 0.5 MAC Halothane (g)	10 μM Bay K 8644 + 0.5 MAC Isoflurane (g)
1	0.5	0.0	1.1	1.4
2	0.3	0.0	1.0	1.5
3	0.6	0.2	1.1	0.7
4	0.8	0.0	1.4	1.3
5	1.1	0.4	0.8	1.4
6	0.3	0.0	1.1	0.4
7	0.2	0.3	0.5	1.3
8	0.3	0.0	1.1	1.0
9	1.5	0.7	2.0	1.6
10	1.1	0.5	1.1	0.9
11	0.6	0.2	0.8	0.6
12	1.2	0.3	1.2	1.0
13	0.5	0.0	0.8	1.0
14	0.7	0.3	1.6	1.2
Mean ± SD	0.68 ± 0.40	0.20 ± 0.22	1.11 ± 0.37	1.09 ± 0.35

MHS = malignant hyperthermia-susceptible.

According to the protocol supported by the European Malignant Hyperpyrexia Group, these patients were classified as MHS. The most positive response for 2 vol% halothane for each MHS patient is provided in table 1. Sixteen patients did not develop any significant contracture at the above concentrations and were classified as MHN.

ISOFLURANE CONTRACTURE

Thirty muscle bundles (one strip for each patient) were tested with increasing concentrations of isoflurane. Six of the 14 muscle bundles from the MHS patients did not develop any contracture in response to isoflurane even at the highest concentration of 3 MAC (table 1). Figure 1A illustrates the absence of contracture in one of the 6 muscle bundles. The maximum contracture recorded in the 8 other MHS muscles was obtained at 2 MAC isoflurane.

The muscle responses (mean \pm SD) of the MHS and MHN groups to increasing concentrations of isoflurane and equianesthetic concentrations of halothane are expressed graphically in figures 2A and 2B. In the 14 MHS muscle bundles, the mean maximum contracture induced by 2 MAC isoflurane was 0.20 ± 0.22 g, and this value was significantly less than that obtained with 2 MAC halothane (table 1). The differences in muscle contractures between the two agents were significant at 0.5, 1, 2, and 3 equivalent MAC multiples (fig. 2A). No effect of isoflurane was observed on baseline tension in MHN muscle from the 16 MHN patients (fig. 2B).

BAY K 8644-ISOFLURANE CONTRACTURE

A typical example of the difference in the contracture response to isoflurane in two MHS muscle bundles from the same biopsy specimen with and without Bay K 8644

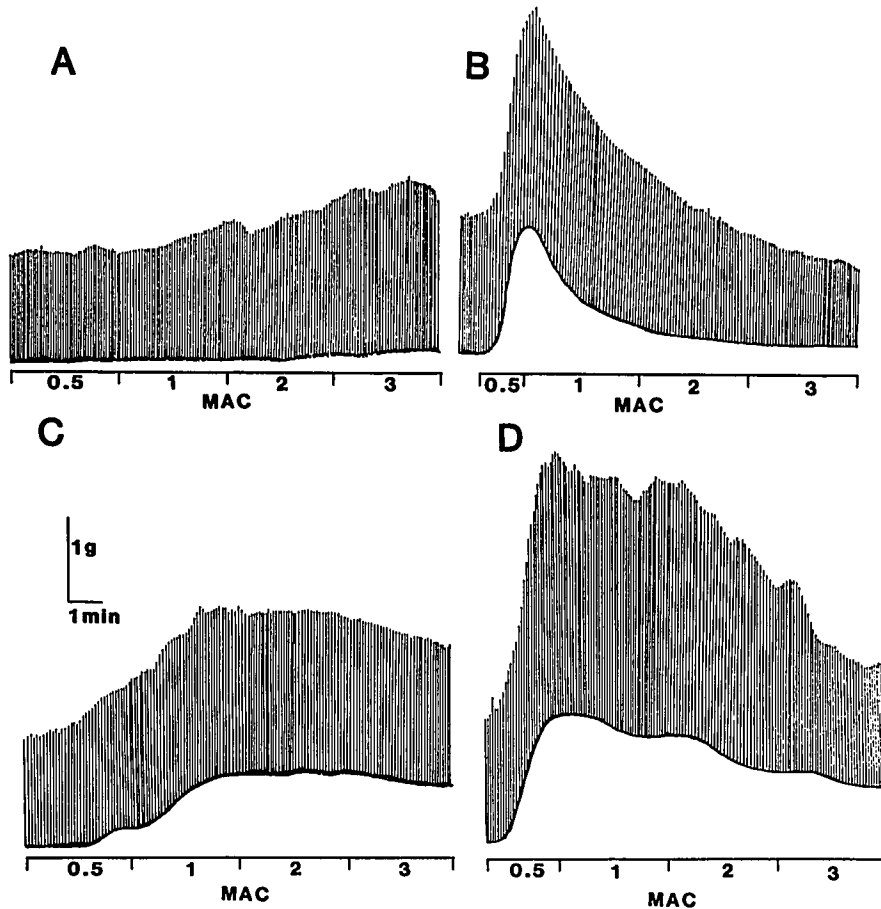


FIG. 1. Typical *in vitro* responses of malignant hyperthermia-susceptible muscle to increasing concentrations of isoflurane (A), isoflurane in the presence of Bay K 8644 (B) halothane (C), and halothane in the presence of Bay K 8644 (D). Each concentration of isoflurane or halothane was applied for 3 min, or, in case of contracture, until a plateau was achieved. Muscle bundles from patient 4 exhibited no contracture at 2 MAC isoflurane and a 0.8-g contracture at 2 MAC halothane. Preincubation with Bay K 8644 enhanced the response to both the isoflurane and equianesthetic concentration of halothane. Muscle bundles from the same biopsy exhibited thus a 1.2-g contracture to 0.5 MAC isoflurane and a 1.4-g contracture to 0.5 MAC halothane.

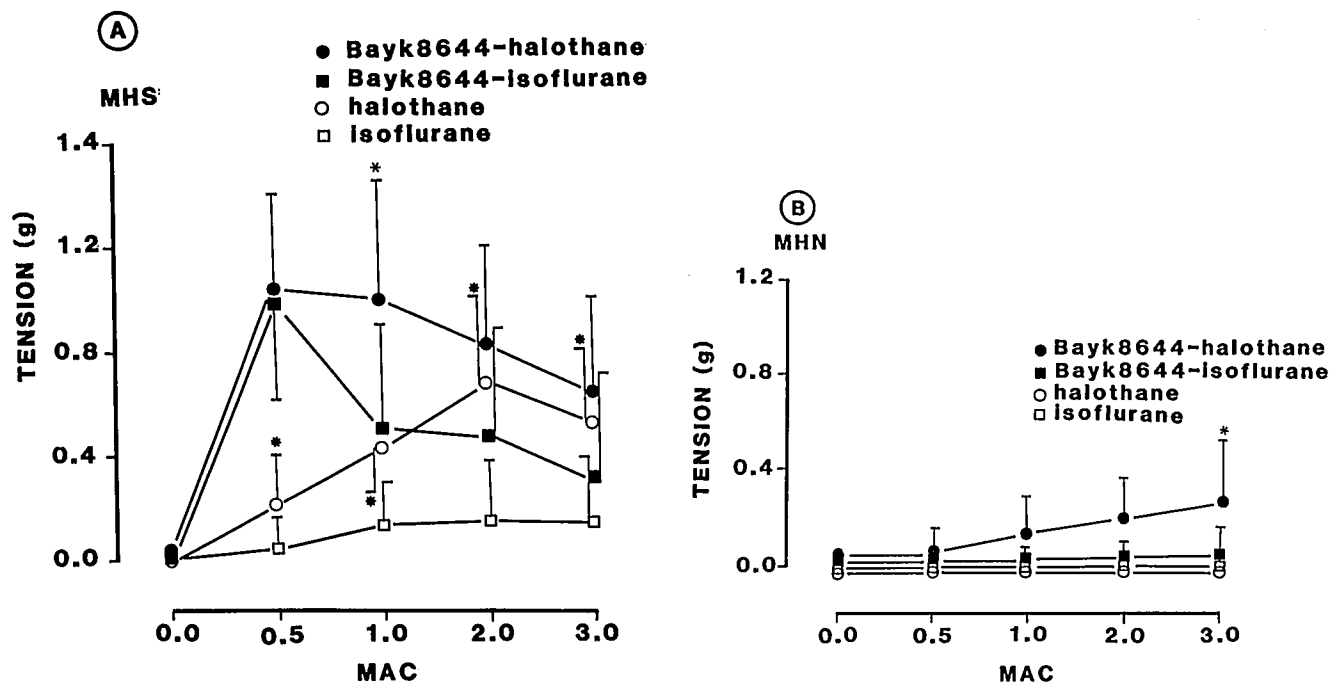


FIG. 2. Cumulative dose-response relationships to isoflurane and halothane for contracture responses in muscle bundles from 14 malignant hyperthermia-susceptible (MHS) patients (A) and 16 malignant hyperthermia-nonsusceptible (MHN) patients (B). Mean \pm SD values were plotted as a function of anesthetic concentration (MAC) during cumulative exposure in the absence (open symbols) or in the presence (closed symbols) of the Ca^{2+} agonist Bay K 8644. A: Starburst indicates $P < 0.05$, statistically significant differences between halothane versus isoflurane contracture within group of MHS patients. Asterisk indicates $P < 0.05$, statistically significant differences between Bay K 8644-halothane versus Bay K 8644-isoflurane contractures within group of MHS patients. B: Asterisk indicates $P < 0.05$, statistically significant difference between Bay K 8644-halothane contracture versus Bay K 8644-isoflurane within group of MHN patients.

is shown in figures 1A and 1B. In the MHS group, preincubation for 10 min with Bay K 8644 considerably enhanced the isoflurane contracture (fig. 2A). This contracture was significant at 0.5, 1, and 2 MAC isoflurane concentrations when compared with the effects of isoflurane alone. The effect was particularly marked with the lowest concentration of isoflurane. The muscle bundles developed a contracture of 1.09 ± 0.33 g as soon as 0.5 MAC isoflurane was added to the carbogen flow (table 1). This was observed in 14 muscle bundles from the 14 MHS patients. No significant difference between isoflurane and halothane thus was found at this equivalent MAC multiple in the presence of Bay K 8644 (fig. 2A). Comparison of Bay K 8644-0.5 MAC isoflurane-induced contracture between the six muscles bundles of the six MHS patients that did not develop contracture with isoflurane alone and the eight other MHS muscle bundles that had a contracture with isoflurane alone showed no significant difference: the mean \pm SD value was 1.10 ± 0.40 g for these six MHS patients and 1.08 ± 0.34 g for the eight patients' bundles that reacted with isoflurane alone.

As the isoflurane concentration was increased, the addition of Bay K 8644 was always associated with a greater

tension than that in the absence of Bay K 8644; however, the developed tension was less than that at 0.5 MAC. In the MHN group, the addition of Bay K 8644 in the Krebs-Ringer solution was not associated with a dose-dependent increase in tension induced by isoflurane (fig. 2B). Significant differences were observed between groups of MHS and MHN muscle bundles for Bay K 8644-isoflurane contracture at 0.5, 1, 2, and 3 MAC isoflurane concentrations.

BAY K 8644-HALOTHANE CONTRACTURE

A typical example of the difference in contracture response of MHS muscles from the same patient with and without Bay K 8644 is illustrated in figures 1C and 1D. As previously observed with another group of MHS patients,¹⁷ the 14 muscle bundles from the 14 MHS patients developed a contracture of 1.11 ± 0.37 g as soon as 0.5 MAC halothane was added to the carbogen flow (fig. 2A), and this contracture was significantly higher from 0.5 to 1.0 MAC halothane within MHS groups of muscle bundles tested with halothane alone (fig. 2A). In the MHN group, preincubation for 10 min with Bay K 8644 did not significantly enhance the effects of halothane except for the highest concentration of 3 MAC halothane (fig 2B).

Discussion

The main finding of the present study is that the magnitude of contracture response produced by incremental concentrations of isoflurane on muscle bundles from MHS patients is markedly enhanced by Bay K 8644. Thus, it was found that the maximal isometric tension did not differ quantitatively from that observed with the equianesthetic concentration of 0.5 MAC halothane under the same experimental conditions. Consequently, even though homeostasis in a cut skeletal muscle bundle is probably different from that *in vivo*, the lowest concentration of isoflurane appears to have the same ability to induce contracture in MHS muscle bundles under this experimental condition.

Previous *in vitro* studies reported that halothane has the greatest ability to potentiate the caffeine-induced contracture both in animal⁴ and MHS patients.⁵ It is assumed that contracture elicited by caffeine reflects facilitated opening of the Ca²⁺-release channels of the terminal cisternae of the sarcoplasmic reticulum (SR).²¹ This channel opening leads to the release of stored Ca²⁺, similar to the pathophysiologic defect believed to be present in MH.^{22,23} However, mechanisms whereby isoflurane and halothane induce contractures in MH muscle remain unknown. To date, there has been no direct evidence to implicate an abnormal Ca²⁺-release mechanism in the *in vitro* isoflurane- or halothane-induced contracture. Indeed, the hypothesis of an increased sensitivity of SR Ca²⁺ release mechanism refers to studies having no relationship to anesthesia since caffeine or Ca²⁺ was used instead of anesthetic agents.²²⁻²⁴ In normal skeletal muscle, isoflurane has similar intracellular mechanisms of action as halothane,^{25,26} and equianesthetic concentrations of isoflurane and halothane induce the same amount of Ca²⁺ release from SR.²⁵ This seems to indicate that the difference in MH muscle contracture response observed in our study between isoflurane and halothane *in vitro* does not directly implicate a difference in SR Ca²⁺ release responses to these agents. The lower potency of isoflurane with respect to inducing contracture in MH muscle *in vitro* contrasts with clinical reports of fulminant MH crisis *in vivo*.⁷⁻¹³ Many of the *in vivo* conditions are not reproduced *in vitro*. It is thus possible that drugs showing marked response *in vivo* may exhibit only small effects *in vitro*.

In the current study, Bay K 8644 potentiated the lowest concentration of both isoflurane and halothane, which then resulted in the same maximum contracture. It has been demonstrated that the dihydropyridine receptors, which also mediate the entrance of a very small current of extracellular Ca²⁺ into the muscle fiber,²⁷ act as the voltage sensors involved in the process of charge movement and in the modulation of the Ca²⁺ release channel of the SR.²⁸⁻³⁰ Bay K 8644 acts on the dihydropyridine

receptor of the T-system¹⁴ and activates also the fast Na⁺ current,^{15,16} which may contribute to a Na⁺-induced Ca²⁺ release mechanism previously described in skeletal muscle.³¹ Even though the current study does not provide any insight into the intracellular mechanisms of the excitation-contraction coupling, it can be hypothesized that the presence of Bay K 8644 in the medium may alter the capacity of dihydropyridine receptors to regulate the Ca²⁺ release channel, thereby allowing isoflurane to be as potent a trigger of MH muscle contracture as halothane. Furthermore, the ability of Bay K 8644 to activate the functional Ca²⁺ channels of the T-system may supply more Ca²⁺ for isoflurane contracture. Finally, the activation of the Na⁺ channels, which have been found to be functionally abnormal in MH muscle,³² may contribute to an increase in myoplasmic Ca²⁺ concentration.

Environmental factors such as temperature can have a marked effect on the expressivity of the MH defect.¹ Other factors, such as stress, have been postulated to be conditions that may affect predisposition to a MH reaction in a susceptible patient,³³ but studies confirming this suggestion are lacking. In the current study, the activation of the dihydropyridine receptors and Na⁺ channels is proposed as one of the environmental conditions that leads to isoflurane to be as potent a trigger as halothane. It is likely that these two types of protein structures are subject to regulation by mechanisms other than the voltage dependence.^{34,35}

In summary, the data presented in the current study suggest that activation of dihydropyridine receptors and Na⁺ channels of the T-system by means of Bay K 8644 potentiates the isoflurane-induced contracture of MH muscle *in vitro*. Therefore, this *in vitro* pharmacologic association may be representative of one of the *in vivo* conditions whereby isoflurane is as potent an MH trigger as halothane. Because to date there has been no evidence to implicate directly an abnormal Ca²⁺ release response to anesthetic agents,³⁶⁻³⁸ the role of T-system receptors and/or channels in the development of isoflurane and halothane contracture should be evaluated.

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