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Desflurane Is a Trigger of Malignant Hyperthermia in Susceptible Swine

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Desflurane (difluoromethyl 1-fluoro 2,2,2-trifluoroethyl ether: CF_2-H-O-CFH-CF_3) is a potent inhalation anesthetic agent being investigated for possible clinical use. The authors examined the effects of this agent on normal swine and those from a special breeding program that were considered purebred for susceptibility to malignant hyperthermia (MH). Animals were exposed to 1 or 2 MAC or both doses of desflurane and observed for changes in end-tidal CO_2, arterial blood gases, lactate, catecholamines, core temperature, blood pressure, and heart rate. All normal swine tolerated exposure to desflurane without clinical signs of MH, but significant changes in heart rate and blood pressure were noted. In contrast, of six MH susceptible swine tested, two had unequivocal MH reactions to desflurane, defined by significant increases of end-tidal CO_2 (>50 mmHg), an increase in Pa_{CO_2} (>70 mmHg), a decrease in blood *p*H (<7.30), an increase in blood lactate concentration, and an increase in core temperature. Two other susceptible swine showed equivocal signs of MH but not until desflurane had been administered for 40–60 min. Finally, two other susceptible swine showed no signs of MH after 60 min of exposure to 2 MAC desflurane. These latter four animals all developed episodes of MH immediately after intravenous succinylcholine (2 mg/kg). The increased Pa_{CO_2}, blood lactate concentrations, and temperature, and the decrease in *p*H induced by desflurane, were successfully treated with dantrolene and supportive measures. All surviving animals were biopsied 1 to 2 weeks after the exposure to desflurane for *in vitro* contracture testing to confirm MH susceptibility. Skeletal muscle from all purebred Pietrain pigs had significantly higher sensitivities to halothane and caffeine than did the normal swine. The authors conclude that desflurane is a trigger for MH in susceptible swine. (Key words: Anesthetics, volatile: desflurane. Animal: swine; purebred Pietrain. Malignant hyperthermia: contracture test. Neuromuscular relaxants: succinylcholine.)

DESFLURANE is a new inhalation anesthetic currently under investigation for use in humans. Although animal studies have demonstrated little or no renal, hepatic, cardiovascular, or pulmonary toxicity,[1,2] the ability of desflurane to trigger malignant hyperthermia (MH) in susceptible individuals has not been studied previously. Because desflurane is structurally related to isoflurane and enflurane, which are known triggers of MH in humans[3,4] and swine[5] it is predicted to be a potential MH trigger. The relative potency of volatile anesthetics to trigger MH has been shown to vary both *in vivo*[6] and *in vitro*.[7] Thus, it is of relevance to determine if and under what circumstances desflurane would act as a trigger for an episode of MH, and whether such an episode can be treated with the usual therapeutic methods (*i.e.*, intravenous dantrolene).

This study was performed to determine if desflurane would trigger an MH episode in purebred Pietrain swine considered susceptible to MH on the basis of positive barnyard halothane challenges. We administered 1 or 2 MAC or both doses of desflurane for over 30 min to normal swine and to those susceptible to MH. Subsequently, susceptibility to MH was confirmed by an *in vivo* challenge to succinylcholine (2 mg/kg) or by *in vitro* contracture testing or by both methods. The results indicate that desflurane triggered MH in susceptible swine.

Materials and Methods

Animals

This protocol was approved by the Mayo Institutional Animal Care and Use Committee. Two groups of animals were used: 1) six Pietrain swine (weight 25.2 ± 1.6 kg; mean ± SD) obtained from a special breeding program (University of Minnesota) and considered purebred for susceptibility to MH according to positive barnyard halothane challenges[8]; and 2) five mongrel swine (weight 18.9 ± 9.9 kg) from herds without a history of MH. An intravenous catheter was inserted percutaneously into an ear vein. All animals were initially anesthetized with intravenous thiopental (5–7 mg/kg), and after tracheal intubation their lungs were mechanically ventilated with 70% N_2 and 30% O_2.

Blood Sampling

Anesthesia was maintained with incremental doses of intravenous thiopental as needed (1–3 mg/kg) while a small incision was made in the medial aspect of the thigh and a catheter inserted into a superficial branch of the femoral artery for monitoring and blood sampling. Arterial O_2 tension (Pa_{O_2}), arterial CO_2 tension (Pa_{CO_2}), and *p*H were monitored throughout the experiment by analyzing fresh arterial samples (1 ml with heparin added) with the appropriate analyzers (1304 blood gas analyzer and 482 co-oximeter, Instrumentation Laboratory, Lexington, MA). From these samples blood glucose and lac-

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tate were measured with membrane bound enzymatic analyzers (Yellow Springs Instruments, Yellow Springs, OH).

In Vivo Measurements

After insertion of monitoring catheters, a normocarbic steady state (Pa_{CO_2} 40 ± 2 mmHg) was maintained for 20 min prior to administration of desflurane (Anaquest, Madison, WI). During this period, the animals were not stimulated; if movement occurred, a small "calming" dose (1.9 ± 0.7 mg/kg) of intravenous thiopental was given. Desflurane was given through a modified DM 5000 vaporizer; end-tidal desflurane concentrations were monitored continuously with a modified and calibrated Datex analyzer (Datex Instruments, Helsinki, Finland). Steady-state inspiratory to expiratory gas concentrations were achieved, and then control swine were exposed to 1.0, 1.5, and 2.0 MAC desflurane for 20–30 min. (The MAC of desflurane in swine is approximately 9.4%.[2]) The first MH-susceptible pig was exposed to 1.0 MAC desflurane, which resulted in the slow onset of a definite episode of MH. The other five susceptible animals received only 2.0 MAC desflurane for 60 min. If a fulminant episode of MH was not triggered during that exposure, intravenous succinylcholine 2 mg/kg was administered. Once an unequivocal episode of MH occurred, as indicated by an increase in Pa_{CO_2} to greater than 70 mmHg, a decrease in blood *p*H, and increases in blood lactate, heart rate, and temperature, the animals were treated. Treatment included intravenous administration of dantrolene 3 mg/kg, hyperventilation, intravenous bicarbonate to reverse the acidosis, and other supportive measures (*e.g.*, epinephrine) as needed. Blood samples were drawn for measurement of arterial blood gases, glucose, and lactate at approximate 5-min intervals.

In Vitro Contracture Testing

All control animals and four MH-susceptible animals were maintained for 1–2 weeks after exposure to desflurane. (Two MH-susceptible animals had died after succinylcholine challenge.) The animals then were anesthetized with intramuscular ketamine, and after tracheal intubation anesthesia was maintained with 70% N_2O and 30% O_2 and intravenous thiopental. Muscle biopsies were obtained from the latissimus dorsi. Excised muscle specimens were transported to the contracture testing laboratory in oxygenated standard solution (see below for composition) at room temperature.[9] Thin muscle bundles were dissected; when possible they were >3 cm long and 2–3 mm in diameter.

The muscle bundles were mounted in experimental chambers containing gassed (95% O_2, 5% CO_2) Krebs'-Ringer's solution at 37° C. The bundles were stimulated *via* field platinum electrodes with supramaximal pulses of 1-ms duration at a frequency of 0.1 Hz.[9] Force was measured with Grass transducers (model FTO3), and the signals were recorded on an eight-channel electrostatic recorder (Astromed, West Warwick, RI). Optimal length was determined for each bundle by stretching the bundle until twitch amplitude was maximal. At least six bundles from each muscle were studied; three bundles were exposed to halothane and three to caffeine. The contracture tests were completed within a 4-h period after the biopsy.

The bathing solution contained (mM): 118.2 NaCl, 3.4 KCL, 0.8 $MgSO_4$, 2.5 KH_2PO_4, 25.0 $NaHCO_3$, 5.5 glucose (315 mOsmol/l). The *p*H of this solution was 7.4 when gassed with carbogen. Halothane was administered to the chambers *via* a continuous flow vaporizer (Tec 3, Ohmeda, West Yorkshire, England). The halothane gas concentration was monitored prior to entry into the tissue baths (Servo Gas Monitor 120, Siemens-Elema, Solna, Sweden), and the bubbling rate within each bath was controlled with Teflon® flow meters (F-1100, Gilmont and Instruments, Great Neck, NY). The actual halothane concentration within the Krebs' solution was measured by subsequent gas chromatographic analysis.[9,10] Muscle bundles were exposed to halothane concentrations of 0.5, 1.0, 2.0, and 3.0%. Caffeine (anhydrous, Sigma, St. Louis, MO) was added to the bathing solution in graded doses to produce final concentrations of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 32 mM.

The force of the twitch contractions and contractures was quantified in grams. The baseline force level from which the amplitudes of all contractures were calculated was defined as the force level just prior to the addition of the lowest concentration of any drug.[9] Contractures at any concentration of halothane exceeding 500 mg and caffeine thresholds (defined as a contracture of greater than 200 mg) at 2.0 mM or less were considered abnormal.

Data Analysis

Systemic hemodynamic and metabolic values were compared at each concentration of desflurane and at various times during administration by analysis of variance. Data from the control swine were pooled, whereas data from the swine susceptible to MH were examined individually because of the large variability in the degree and time of reaction for each animal.

Results

In Vivo Response to Desflurane

Decreases in mean arterial pressure and heart rate occurred in all animals. For example, the mean heart rates and arterial pressures for the group of animals susceptible to MH (n = 6) during the control period were 131 ± 11 beats per min and 186 ± 25 mmHg; after 5 min of des-

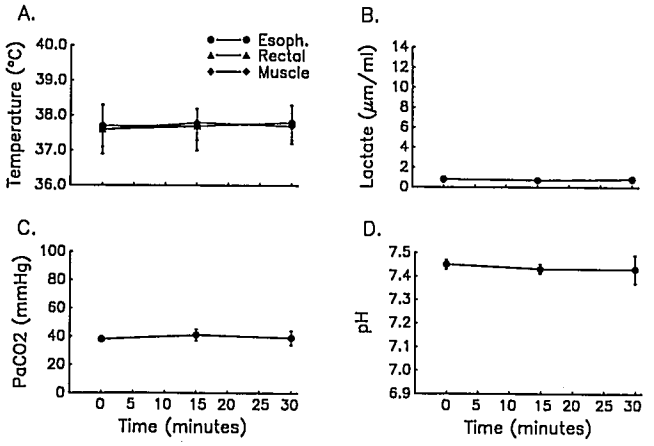

FIG. 1. The temporal relationship of changes in (*A*) temperature (rectal, esophageal and muscle), (*B*) arterial lactate, (*C*) Pa_{CO_2}, and (*D*) *p*H levels during the administration of 2 MAC desflurane in normal swine. Desflurane had little or no effect on these parameters. All data are expressed as mean values ± SD (n = 5).

flurane these parameters decreased to 71 ± 14 beats per min and 138 ± 14 mmHg, respectively. These cardiovascular parameters remained altered: after 10 min of exposure to desflurane the mean values were 76 ± 13 beats per min and 132 ± 16 mmHg, respectively. These changes were significant at a level of $P < 0.001$. The normal swine maintained steady-state values for all metabolic parameters measured (fig. 1).

The six swine susceptible to MH did not all develop signs of unequivocal MH after administration of desflurane. Two animals reacted to desflurane with fulminant MH episodes; two had equivocal episodes; and two did not respond. In the latter four swine, MH episodes were subsequently triggered with intravenous succinylcholine (2.0 mg/kg) after the desflurane exposure, and all four swine immediately showed evidence of a fulminant episode of MH. Figure 2 demonstrates the changes in Pa_{CO_2} for all six animals susceptible to MH. Figure 3 provides further metabolic data for one representative episode of MH by desflurane (purebred Pietrain 2); figure 4 shows similar data for one swine that did not react (PP 3).

Dantrolene and supportive treatment reversed the MH episodes induced by desflurane alone. This treatment also reversed the episodes in two animals in which MH was triggered with succinylcholine. However, two susceptible swine died after MH was triggered with succinylcholine despite intravenous dantrolene and supportive therapy. In addition, administration of intravenous succinylcholine caused profound hypotension in the four swine susceptible to MH in which MH was not unequivocally triggered with desflurane alone.

In Vitro Contracture Testing

Halothane concentrations measured in the Krebs' solution were within 0.25% of the chosen value. Latissimus dorsi biopsies from the surviving four susceptible animals showed positive contracture responses to both halothane and caffeine. Table 1 shows the concentration of caffeine and halothane at which fiber segments of the latissimus dorsi developed significant contractures (*i.e.*, >500 mg

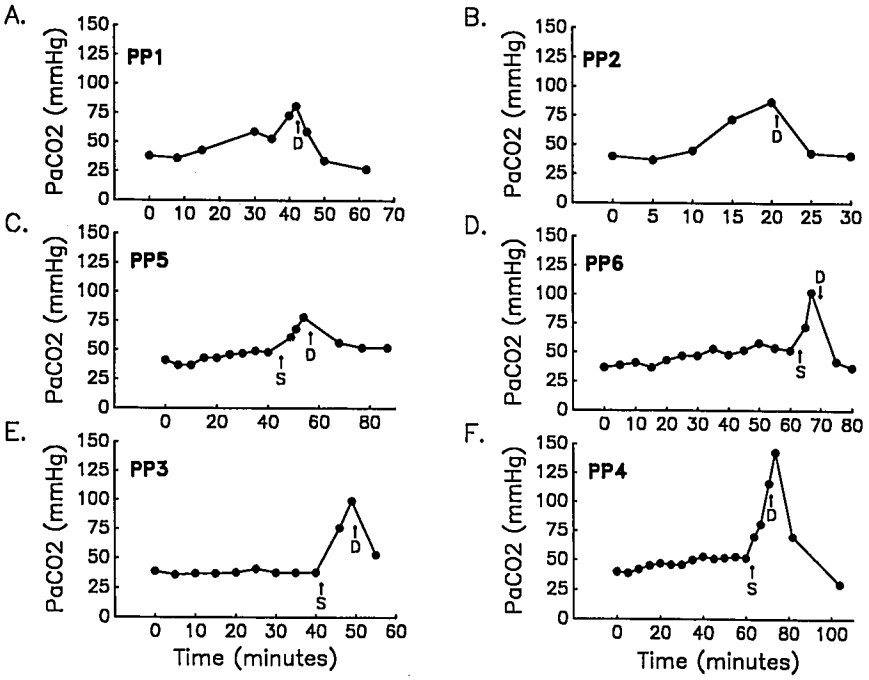

FIG. 2. Changes in arterial Pa_{CO_2} after the administration of desflurane (1 or 2 MAC), succinylcholine (2 mm/kg), and/or dantrolene (3 mg/kg) for each swine (purebred Pietrains [PP]) susceptible to MH. The arrows indicate the time at which the various agents were administered. (*A, B*) The administration of desflurane caused a substantial increase in Pa_{CO_2} in two animals (PP1 and PP2, respectively); (*C, D*) a slight increase in two others (PP5 and PP6, respectively); and (*E, F*) had little or no effect on Pa_{CO_2} in the remaining two (PP3 and PP4, respectively). Succinylcholine (2 mg/kg) caused a dramatic increase in Pa_{CO_2} in those animals to which it was administered. Dantrolene (3 mg/kg iv) initiated the return of Pa_{CO_2} to normal in all animals. Arrows indicate times of succinylcholine (S) and dantrolene (D) administration. Animal PP1 (*A*) was administered 1 MAC desflurane; all others were exposed to 2 MAC.

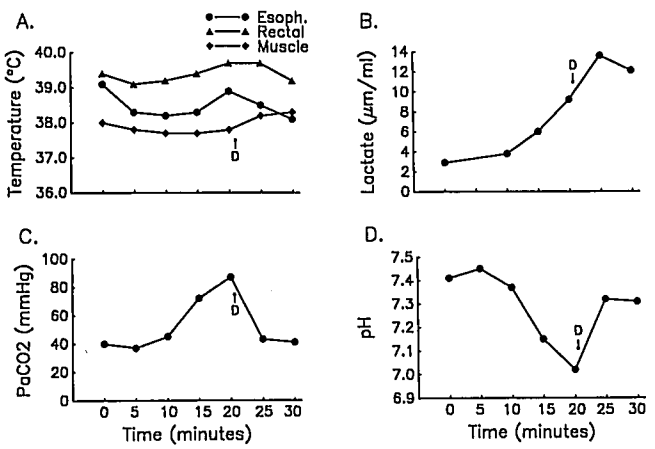

FIG. 3. Desflurane (2 MAC) triggered an episode of MH, which was treated by dantrolene and supportive therapy. Shown is the temporal relationship of changes in (*A*) temperature, (*B*) lactate, (*C*) Pa_{CO_2}, and (*D*) blood *p*H for one swine (Purebred Pietrain 2). Arrows indicate time of dantrolene (D) administration.

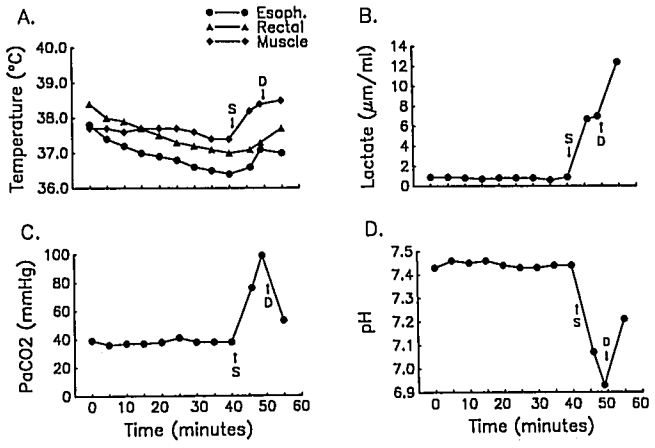

FIG. 4. Succinylcholine (2 mg/kg iv) but not desflurane (2 MAC) triggered an episode of MH in one swine susceptible to this condition (purebred Pietrain 3). The administration of dantrolene (3 mg/kg iv) and other supportive therapy was used successfully to terminate this episode, which was characterized by (*A*) elevated core, esophageal, and muscle temperatures; (*B*) elevated blood lactate levels; (*C*) elevated Pa_{CO_2}, and (*D*) acidification of the blood. *In vitro* contracture testing of muscle obtained from this animal was positive. Arrows indicate times of succinylcholine (S) and dantrolene (D) administration.

after halothane exposure and >200 mg after caffeine exposure). All normal swine had negative contracture tests.

Discussion

Desflurane triggered episodes of MH in several but not all of the swine susceptible to MH. While longer periods of desflurane exposure might have led to fulminant MH reactions in all of these purebred swine, it should be noted that all of these animals responded positively to a brief exposure of halothane (3% for <5 min) during a barnyard halothane challenge test. Furthermore, the four animals that did not exhibit unequivocal MH episodes upon or after exposure to desflurane had immediate and fulminant MH reactions to the subsequent administration of intravenous succinylcholine.

Variable responses to potent inhalation agents have been previously reported for studies *in vitro* as well as *in vivo*.[6,7] These include response differences due to varying dosages as well as differences among agents. In fact, approximately 50% of MH-susceptible humans report no occurrence of MH with previous exposures to triggering anesthetic agents.[11] Such differences between individuals or agents may be dose-related or may be due to variations in the effect of the volatile anesthetic agent on cell membrane stabilization. Other factors that affect MH triggering include core temperature[12,13] and adjuvant anesthetic agents such as opioids, barbiturates, and nondepolarizing

TABLE 1. Caffeine and Halothane Thresholds Determined by *In Vitro* Contracture Testing (Latissimus Dorsi)

Animal ID	Caffeine Threshold (mM)	Fractional Force (mM)	Halothane Threshold (%)	Contracture Test Result
PP1	1.0	1.0	0.5	Positive
PP2	1.0	2.0	3.0	Positive
PP3	1.0	1.5	0.5	Positive
PP4*				
PP5*				
PP6	0.5	1.0	0.5	Positive
N1	32.0	32.0	—	Negative
N2	6.0	6.0	—	Negative
N3	5.0	32.0	—	Negative
N4	6.0	8.0	—	Negative
N5	3.0	5.0	—	Negative

Caffeine threshold was defined as a contracture of greater than 200 mg. Fractional force was defined as the caffeine dose that induced a force amplitude of 7% or greater of the maximum induced by 32 mM caffeine. Halothane threshold was defined as a contracture of greater than 500 mg.

* Died after intravenous succinylcholine administration (2 mg/kg) as a result of fulminant episode of MH.

PP = Purebred Pietrain; N = normal.

muscle relaxants.[14,15] Hence, desflurane must be categorized with the other volatile anesthetic agents as a trigger of MH.

Incomplete penetrance and other genetic differences were believed to be minimized in our study through the use of purebred MH-positive animals obtained from a special breeding program. All swine considered susceptible to MH (*i.e.*, by breeding and by an *in vivo* halothane challenge) reacted strongly to the administration of succinylcholine and had strongly positive *in vitro* contracture tests. Prior to the administration of desflurane, body temperature was maintained in the normal range to avoid MH triggering inhibition due to hypothermia[12] or MH triggering due to hyperthermia.[13] Noninvasive methods were used in order to avoid stressing the animals and to prevent the need for adjuvant anesthetic agents. Barbiturates used for induction may have had a theoretical inhibitory influence on the onset of an MH episode; however, a 20-min steady-state period prior to the administration of desflurane was added to ensure that the thiopental concentrations were minimal at the time of desflurane exposure.

The hypotensive effect of succinylcholine in the presence of desflurane in swine susceptible to MH was dramatic and unanticipated. This response occurred immediately after intravenous administration of the drug and preceded signs of MH. All four pigs that received succinylcholine required immediate resuscitation. This effect may be species-specific, but it does not appear to relate to the purebred status of these animals. We recently have observed a similar response in mixed-breed MH-susceptible swine that were anesthetized with thiopental and N_2O.[16] Further studies are needed to elucidate the etiology of this response and its possible clinical relevance.

In summary, desflurane is a trigger for MH and is contraindicated in patients who are considered at risk for MH susceptibility. The MH responses induced by desflurane alone were successfully treated with intravenous dantrolene. Further data comparing the triggering effects of desflurane with other volatile anesthetic agents in clinically useful dosages are needed.

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