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Malignant Hyperthermia Phenotype

Hypotension Induced by Succinylcholine in Susceptible Swine

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Background: **Succinylcholine causes immediate and severe arterial hypotension in swine with the malignant hyperthermia phenotype. The underlying mechanisms are unknown.**

Methods: **Malignant hyperthermia–susceptible (MHS; n = 10) and normal swine (n = 5) were anesthetized with thiopental. The following were monitored: electrocardiogram; arterial blood pressure; pulmonary artery, central venous, and left and right ventricular pressure; cardiac output; end-tidal carbon dioxide; core temperature; peripheral-blood flows; and arterial blood gases. After a control period, 2 mg/kg succinylcholine was given intravenously. Three MHS animals received 1 mg/kg vecuronium and two MHS animals received 2.5 mg/kg dantrolene intravenously. The effects of succinylcholine on left and right ventricular pressure and contractility were analyzed in isolated hearts. The effects of 0.06 mM succinylcholine on isometric tension development were recorded in isolated femoral artery rings.**

Results: **Succinylcholine caused an early, severe decrease in blood pressure, cardiac output, left ventricular pressure, and left ventricular contractility in MHS swine but not in normal swine; no significant differences were found in heart rate, right ventricular parameters, systemic vascular resistance, and preload (pulmonary diastolic pressure, central venous pressure). The succinylcholine-induced hypotension and associated effects were not prevented by dantrolene. However, pretreatment with high-dose vecuronium prevented not only the cardiovascular depression, but also MH. In addition, no phenotypic differences of succinylcholine on contractility or left ventricular pressure were observed in the isolated working hearts. Similary, succinylcholine did not cause a significantly different relaxation in rings in either phenotype.**

Conclusion: **Succinylcholine-induced hypotension occurred before muscle hypermetabolism in MHS swine. Succinylcholine had no differential physiologic effects on either the isolated heart or on isolated arteries. This hypotension could not be prevented by dantrolene but was prevented by pretreatment with high-dose vecuronium. Thus, an indirect mechanism such as the release of a cardiac depressant from skeletal muscle may have caused this hypotensive response. (Key words: Anesthetics; depolarizing neuromuscular blocking agents; nondepolarizing neuromuscular blocking agents.)**

MALIGNANT hyperthermia (MH) is a genetic disease characterized by muscle hypermetabolism, and its pathophysiologic basis is considered to be defects in the calcium regulation metabolism within skeletal muscle.[1] In humans, this disorder has a heterogenetic origin. In the swine model, a single point mutation in the *RYR1* gene is responsible for this phenotype.[2]

Succinylcholine, a depolarizing muscle relaxant, is a potent trigger of MH.[1,3] Administration of succinylcholine to MH-susceptible (MHS) swine causes a severe arterial hypotension before the animals actually display hypermetabolism.[3] The hypotension has been observed to occur immediately (within 2 min of succinylcholine administration), even when clinically used doses of pancuronium are given as prior treatment. Thus, the cardiovascular depression was not considered to be caused by the effects of succinylcholine on the skeletal muscle itself (*e.g.*, due to hyperemia and consecutive vasodilation after muscle fasciculations); rather, it was considered to be due to direct effects on the cardiovascular system. In other words, the MHS phenotypic response to succinylcholine is not easily explained by the genotypic defect of the skeletal muscle calcium release channel. Several studies have concluded that tissues other than the skeletal muscle may be altered in MH,[4–10] with described effects on the heart being controversial.[4,6,11,12] Therefore, it was of interest to reinvestigate the effects of succinylcholine on the cardiovascular system *in vivo* and *in vitro*. The purpose of the present study was to gain new insights into the hypotensive response mechanism caused by succinylcholine in MHS swine. To do so, both *in vivo* and *in vitro* experiments were performed. The latter were undertaken to deter-

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mine whether observed effects of succinylcholine were due to either direct effects on the heart or arteries, or due to their responses to either a secondarily released substance or reflex mechanism.

Methods

In Vivo *Effects of Succinylcholine*

After obtaining approval from the Institutional Animal Care and Use Committee of the University of Minnesota, the following experiments were conducted on purebred Pietrain swine with the homozygeous mutation of arginine 615 in the ryanodine receptor (*RYR1*). These MHS animals, as well as nonsusceptible normal mongrel swine, were obtained from the University of Minnesota Rosemount Animal Facility (weight of MHS swine: 42.1 ± 5.1 kg; normals: 47.5 ± 7.6 kg, mean ± SD).

Malignant hyperthermia-susceptible swine (n = 10) and normal swine (n = 5) were initially anesthetized with 20 mg/kg intramuscular ketamine (KetaVed; Phoenix Scientific, Inc., St. Joseph, MO). An 18-gauge ear vein catheter (Jelco; Johnson & Johnson, Arlington, TX) was placed, and thiopental 20–25 mg/kg (Gensia Pharmaceuticals, Inc., Irvine, CA) was administered intravenously. The animals were intubated with a 6.5- or 7-mm ID endotracheal tube and mechanically ventilated with 65% N_2O and 35% O_2 to maintain an arterial carbon dioxide partial pressure (Pa_{CO2}) of 40 ± 2 mmHg. End-tidal carbon dioxide was monitored using a gas analyzer (Nellcor, Hayward, CA). No muscle relaxants were administered at this time. A thiopental infusion was then titrated to effect at a rate of approximately 100–200 mg/min. A femoral artery cannula (A. femoralis superficialis) was used for monitoring invasive blood pressure, and an axillary artery cannula was used for blood sampling. Arterial oxygen partial pressure (Pa_{O2}), Pa_{CO2}, *p*H, base excess, K^+, and Ca^{2+} were determined from fresh arterial samples using a blood gas analyzer with temperature compensation (Model 1304; Instrumentation Laboratories, Inc., Lexington, MA). Mikro-Tip catheter transducers (5 French, Model MPC-500; Millar Instruments, Inc., Houston, TX) were placed in the right and left ventricles *via* access through the right external jugular vein and the right carotid artery, respectively. In addition, a balloon-tipped pulmonary artery thermodilution catheter (Swan-Ganz catheter model 93A-131-7F; American Edwards Laboratories, Santa Ana, CA) was inserted *via* the right external jugular vein and advanced into the pulmonary artery to measure pulmonary artery pressures, blood temperatures, and thermodilution cardiac outputs (Cardiac output monitor model 9520A; American Edwards Laboratory). A five-electrode lead configuration was used to monitor electrocardiograms (Spacelabs model 1020; SpaceLabs Inc., Chatsworth, CA). The common femoral artery and veins were surgically exposed, and femoral artery and vein blood flow was monitored using ultrasonic transit-time flow meters (Transonic Systems Inc., Ithaca, NY). In one MHS animal (MHS 3), flow artery data were not collected because of a technical problem. Core temperatures were measured using axillary, rectal, and pulmonary artery probes.

A specially designed pressure bulb was positioned between the upper and lower incisors to assess the relative force output of the muscles of mastication, especially the masseter muscle. The signal from this unit was converted to voltage using a pressure transducer connected to the SpaceLabs monitor model 1020.

An additional two normal and three MHS animals were used for the isolated heart studies only and not for the *in vivo* protocol.

The following data were automatically collected using data acquisition hardware and LabVIEW software (National Instruments, Austin, TX): heart rates, arterial blood pressures, femoral artery and vein blood flows, and end-tidal carbon dioxide levels. Blood gas data, core temperatures, and cardiac outputs were recorded manually.

Epochs of the following data were collected at a sampling frequency of approximately 670 Hz by an ATCODAS analog-to-digital data acquisition system (Dataq Instruments Inc., Akron, OH): pulmonary artery pressures, central venous pressures, right ventricular pressures, and left ventricular pressures. The first derivative of left and right ventricular pressures (dP/dt) was determined by off-line differentiation of the pressure signal. Maximum positive (+dP/dt) measurements were taken from the differentiated left and right ventricular pressure waves. Furthermore, systemic vascular resistance was calculated using the following simplified formula: systemic vascular resistance = mean arterial pressure/cardiac output.

For these investigations, all animals were maintained normothermic (38.05 ± 0.2°C) using convective-air warming as needed (Bair Hugger; Augustine Medical Inc., Eden Prairie, MN). The animals received NaCl 0.9% for intravenous fluid administration *via* the ear vein catheter (approximately 600–800 ml/h during the control periods).

After the control periods, (Pa_{CO2} 40 ± 2 mmHg, tem-

Table 1. Time to Trigger Malignant Hyperthermia, Malignant Hyperthermia Diagnosis, Masseter Bulb Pressures, and Epinephrine Administration in Malignant Hyperthermia–Susceptible Animals Exposed to Succinylcholine 2 mg/kg *In Vivo*

	MHS 1	MHS 2	MHS 3	MHS 4	MHS 5	MHS D1	MHS D2	MHS V1	MHS V2	MHS V3
Triggering time MH (min, Pa_{CO_2} >70 mmHg)	20	5	22	7	*	No	No	No	No	No
MH diagnosis	*In vivo*	*In vivo*	*In vivo*	*In vivo*	IVCT	IVCT and *in vivo*†	IVCT	IVCT	*In vivo*‡	*In vivo*§
Maximal developed masseter bulb pressure (mmHg)	—	254	—	42	89	30	49	0	0	0
Epinephrine Intravenous	No	No	No	50 μg at 5 min	3 mg at 5 min	No	100 μg at 7 min	100 μg at 12 min	—	—

* Died at 5 min.

† 30 min after first dose of succinylcholine, second dose of succinylcholine and halothane 2 minimum aveolar concentration; 10 minutes until Pa_{CO_2} >70 mmHg.

‡ 90 min after first dose of succinylcholine, second dose of succinylcholine; 15 minutes until Pa_{CO_2} > 70 mmHg.

§ 70 min after first dose of succinylcholine, 10 min after second dose of vecuronium; exposure to halothane 1 minimum aveolar concentration (0.87%); 30 minutes until Pa_{CO_2} > 70 mmHg.

MH = malignant hyperthermia; MHS = malignant hyperthermia–susceptible (no pretreatment); MHS D = pretreatment with dantrolene 2.5 mg/kg intravenous; MHS V = pretreatment with vecuronium 1 mg/kg intravenous; IVCT = *in vitro* contracture test.

perature 38 ± 0.5°C) and just before succinylcholine was given, all data were collected using the methods previously described (indicated as t = 0 min). Succinylcholine (Quelicin; Abbott Laboratories, North Chicago, IL), as a bolus dose of 2 mg/kg, was administered intravenously by injection into the central port of the pulmonary artery catheter; all data were sampled for a subsequent 15 min (manually recorded data were obtained at t = 1.5, 3, 5, 10, and 15 min). Five MHS animals and five normal swine did not receive any pretreatment (table 1). Two MHS animals received an intravenous pretreatment of 2.5 mg/kg dantrolene (Norwich-Eaton Pharmaceuticals, Norwich, NY) in 250 ml mannitol 1% 30 min before the bolus succinylcholine injection. Similarly, three MHS animals received a 1-mg/kg intravenous pretreatment with vecuronium (Norcuron; Organon Pharmaceuticals, West Orange, NJ) 10 min before succinylcholine was administered (table 1).

In pilot studies, two MH animals were pretreated with either: (1) 1 mg/kg ranitidine intravenously (Zantac; Glaxo Pharmaceuticals, Research Triangle Park, NC) and 1 mg/kg diphenhydramine intravenously (Elkins-Sinn, Inc., Cherry Hill, NJ); or (2) atropine 20 μg/kg intravenously (Elkins-Sinn, Inc.). The ranitidine/diphenhydramine and atropine were administered a minimum of 30 min before the bolus dose of succinylcholine was injected.

Treatment of severe, succinylcholine-induced arterial hypotension included intravenous fluids (NaCl 0.9%) as needed, initiated not before 5 min after the succinylcholine administration. If hypotension persisted beyond 5 min, administration of sympathicomimetic agents, such as epinephrine, was also provided (table 1).

Susceptibility to MH was verified by the initiation of MH *in vivo* (Pa_{CO2} > 70 mmHg at constant ventilation) and/or by *in vitro* contracture testing. For the latter, bundles of rectus abdominis muscle were used for testing with the protocol recommended by the North American Malignant Hyperthermia Registry, on equipment previously described (table 1).[13,14]

Effects on an Ex Vivo *Isolated Heart Model*

After median sternotomy was performed, a cardioplegia cannula (9 French double lumen, Medtronic, Minneapolis, MN) was introduced into the aorta, and refrigerated modified St. Thomas' cardioplegia (NaCl 110 mM, KCl 16 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 16 mM, $NaHCO_3$ 10 mM) was prepared for antegrade flow through the coronary vessels. A total of 10,000 USP units of heparin and 25 mg of adenosine were administered intravenously. Immediately after cardioplegia introduction, the excised hearts were placed in an iced saline slurry while transported to the apparatus, as well as during the (re)cannulation process. After removal of excess tissue and isolation of the great vessels, an aortic cannula (24 French) was directly inserted distal to the cardioplegia cannula. In addition, cannulas were inserted into the pulmonary artery (28 French), pulmonary vein (28 French), and directly into the right atrium (36 French). The cannulas were secured

into place, with remaining apertures into the heart either tied or sutured closed.[15] The Millar pressure catheters were placed *in vitro* as they were used in the *in vivo* preparations described previously. The flow of cardioplegia was then stopped, and warm (38°C) perfusate (NaCl 118.0 mM, mannitol 16.0 mM, glucose 11.5 mM, $NaHCO_3$ 20.0 mM, EDTA 0.32 mM, KCl 4.5 mM, $MgCl_2$ 1.2 mM, NaH_2PO_4 1.2 mM, Na pyruvate 2.27 mM, $CaCl_2$ 2.5 mM, insulin 10 U/l) was supplied to the cardiac chambers. If there was no spontaneous atrial-ventricular rhythm, the heart was defibrillated using an electrocardiac monitor unit (9790C Vitatron, Medtronic). The heart was allowed to function in a Langendorff perfusion mode for recuperation from ischemia until atrial-ventricular rhythm stabilized and the heart became self sustaining. Both sides of the heart were then allowed to work by supplying fluid pressure heads into the preload and afterload chambers.[15] The preload was held constant throughout the experiment (mean right atrial pressure 11 mmHg, range 5–12 mmHg; mean left atrial pressure 14 mmHg, range 12–15 mmHg); the afterload was defined as the diastolic aortic root pressure (mean, 24 mmHg; range, 14–30 mmHg).

Isolated hearts from six MHS animals and five normals were investigated. In MHS hearts that had been exposed to succinylcholine *in vivo*, the time between *in vivo* and *in vitro* administration was 90, 150, and 200 minutes, respectively. From an additional three MHS and two normal animals, the hearts were removed without having been exposed to succinylcholine *in vivo*. For these MHS animals, which were therefore not used for *in vivo* effects of succinylcholine, MH susceptibility was verified by *in vitro* contracture testing as previously described.

After a control phase, *i.e.*, stabilized function with a normal sinus rhythm, succinylcholine 2 mg/kg was added to the Krebs solution, and the effects on left and right ventricular pressures and contractility (dP/dt) were analyzed at 1.5, 3, 5, and 10 min.

In Vitro *Effects on Femoral Artery Rings*

The common femoral arteries (opposite side from where the femoral cannula was placed) were removed from normal (n = 6) and MHS (n = 4) swine before succinylcholine exposure. Subsequently, no ischemia in the limbs was observed. The femoral arteries were prepared under a stereo-microscope as vessel rings without removing the endothelium. Care was taken to remove as much surrounding tissue as possible without damaging the intimal surface. The presence of endothelium was later verified by histologic assessment of selected preparations. The vessel rings (15 MH, 32 normals) were approximately 0.5–1 mm in diameter and weighed between 10 and 20 mg. The experiments were performed in organ chambers (42 ml) containing Krebs solution (NaCl 118.3 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM, glucose 11.1 mM, Ca^{2+} EDTA 0.026 mM) that was gassed with 95% O_2/5% CO_2. Temperature of the bathing solution was kept constant at 37°C. The vessel rings were suspended between a fixed clip and a force transducer by two stainless-steel wires inserted into the lumen. Isometric tensions were recorded continuously. After this procedure, the preparations were allowed to equilibrate at their optimal length for approximately 30 min before drug administration. After a stable baseline reading was obtained, the following agents were studied: (1) phenylephrine added as incremental doses (0.5 μM per dose, up to 2 μM); (2) succinylcholine at a final bath concentration of 0.06 mM; and (3) acetylcholine at a 0.1-μM concentration (Sigma Chemical Co., St. Louis, MO). Vessel rings that did not elicit a response to phenylephrine or acetylcholine were excluded.

Statistical Analysis

Statistical analyses were performed using either repeated measures analysis of variance with a Bonferroni multiple comparison posttest or the two-tailed Mann—Whitney test, as appropriate. A P value < 0.05 was considered significant. The data were presented on an animal-by-animal basis or as mean ± SD unless otherwise indicated.

Results

In Vivo *Study*

The MH phenotype was verified by *in vivo* triggering (Pa_{CO_2} > 70 mmHg at constant minute ventilation) and/or by *in vitro* muscle contracture testing as previously described (table 1).

Hemodynamic Data

Succinylcholine (2 mg/kg intravenously) caused a significant, severe decrease in arterial blood pressure in the MHS animals, including those pretreated with dantrolene (fig. 1A). In two MHS animals, epinephrine was necessary for blood pressure support 5 min after succinylcholine administration; for statistical comparisons between groups, hemodynamic data were provided only at 0, 1.5, and 3 min. In animals pretreated with 1 mg/kg

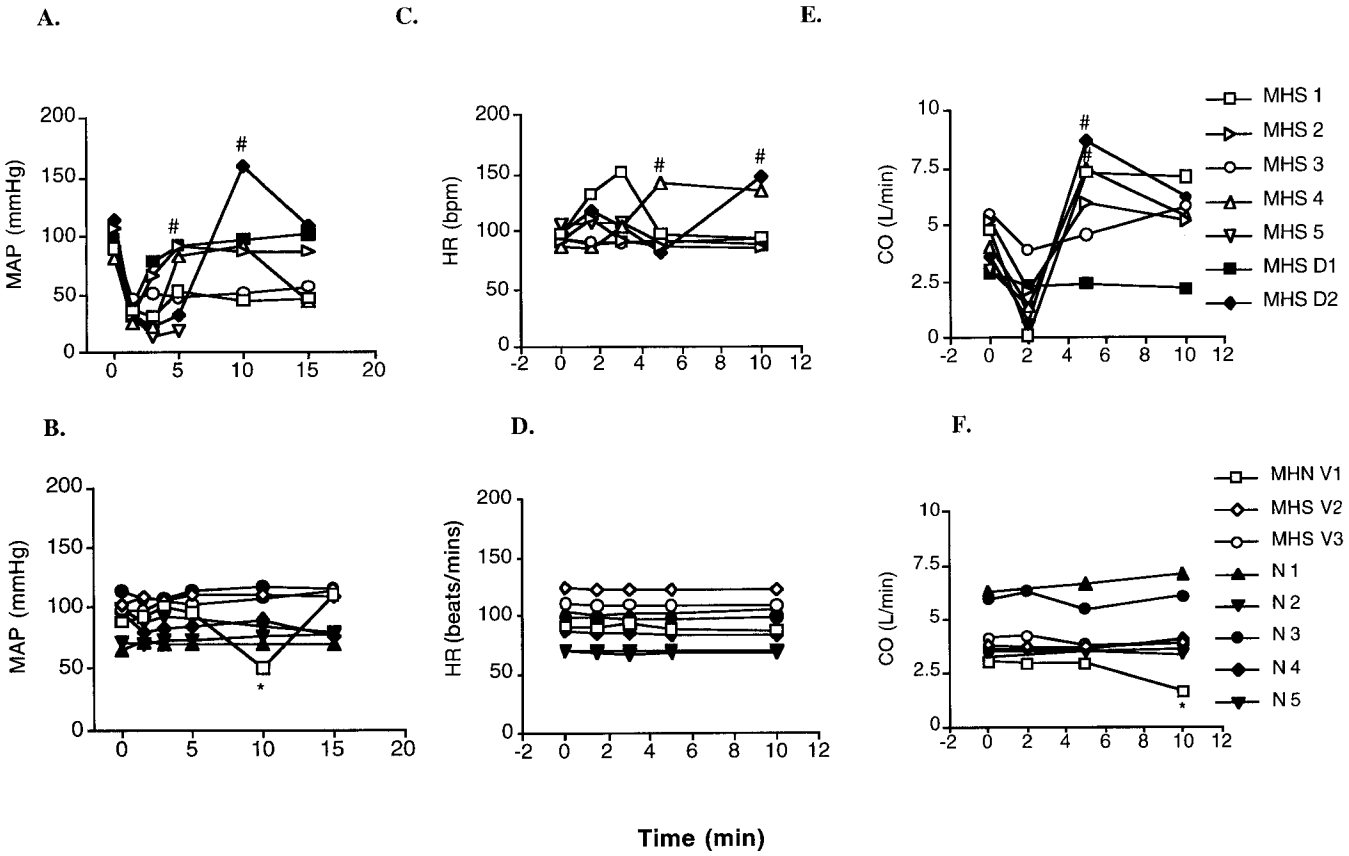

Fig. 1. ***In vivo*** **mean arterial blood pressure (MAP), heart rate (HR), and cardiac output (CO) after administration of 2 mg/kg succinylcholine at time = 0 min for (1) individual MHS animals (MHS 1–5); (2) MHS animals pretreated with 2.5 mg/kg dantrolene (MHS D1 and 2); (3) MHS animals pretreated with 1 mg/kg vecuronium (MHS V1–3); and (4) normals (N 1–5). It can be observed that MAP and CO decreased significantly in MHS and MHS D, but not in MHS V or normals (N). MHS 4 and MHS D2 both required epinephrine therapy at 5 and 7 min, respectively (indicated by number sign). A decrease of MAP and CO in MHS V1 was considered to be caused by an air embolus (indicated by asterisk).**

vecuronium (n = 3) and in normal swine, severe arterial hypotension was not observed (fig. 1B). However, one animal in this group developed a fatal hypotension 10 min after succinylcholine administration, probably caused by an air embolism, and died at 20 min; no acute effects of succinylcholine on the cardiovascular system were detected.

Another MHS animal without pretreatment died shortly after succinylcholine administration during the hypotensive period (t = 5 min). Neither of these animals displayed signs of a MH episode (Pa_{CO2} > 70 mmHg at constant ventilation); their MH susceptibility was confirmed by *in vitro* contracture testing.

In swine with the MH mutation, the mean arterial pressure just before succinylcholine administration was 96 ± 11 mmHg and subsequently decreased to 35 ± 7 mmHg at 1.5 min ($P < 0.001$; intragroup comparison), and to 41 ± 25 at 3 min ($P < 0.01$). In normal swine, the mean arterial pressure before succinylcholine administration was 89 ± 20 mmHg, and was 84 ± 15 mmHg and 86 ± 15 mmHg at 1.5 and 3 min posttimes, respectively. Importantly, the mean arterial pressures were statistically different between the genotypes at both 1.5 min ($P < 0.001$) and 3.0 min ($P < 0.01$; intergroup comparison). Interestingly, pretreatment with vecuronium abolished the succinylcholine-induced hypotension completely (fig. 1B).

In three of the seven MHS animals, heart rates increased; however, these effects were relatively inconsistent and thus not significant (fig. 1C). No changes in heart rates were observed in MHS animals pretreated with vecuronium or in normals (fig. 1D).

Cardiac output decreased for only the MH animals (fig. 1E) after succinylcholine administration (from 4.1 ± 1

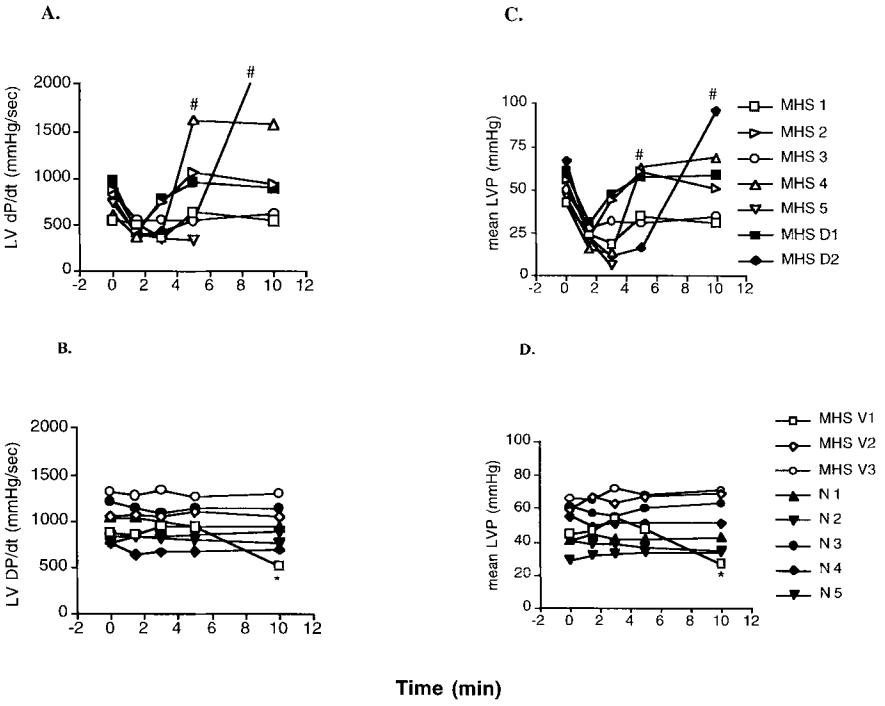

Fig. 2. *In vivo* left ventricular contractilities (LV dP/dt) and mean left ventricular pressures (LVP) are shown in the first 10 min after administration of 2 mg/kg succinylcholine at time = 0 min for the same animals as in fig. 1. Succinylcholine caused a significant decrease of LV dP/dt and mean LVP in MHS, MHS D (dantrolene), but not in MHS V (vecuronium) or normals. The number sign indicates the administration of epinephrine, and the changes in cardiac function in MHS V1 was considered to be caused by an air embolus (*).

l/min to 1.7 ± 1.3 l/min at 2 min; $P < 0.01$ *vs.* normals and $P < 0.05$ *vs.* MHS pretreated with vecuronium; fig. 1F).

The succinylcholine-induced arterial hypotension in the MH genotype was associated with decreased left ventricular contractility (+dP/dt) at 1.5 min as well as 3 min after succinylcholine administration ($P < 0.01$ *vs.* normals and MHS with vecuronium; fig. 2A). Pretreatment with vecuronium abolished such decreases in left ventricular contractility in the MHS animals. Contractility was stable throughout in normals (fig. 2B). Associated with the abnormal changes in contractility in the MHS animals were concomitant and significant changes in left ventricular pressures (both at 1.5 and 3 min postadministration; $P < 0.05$) compared with preadministration values (fig. 2C); no contractility changes were observed in MHS swine pretreated with vecuronium or in normals (fig. 2D).

There was a significant decrease in systemic vascular resistance in MHS swine only at 5 min after succinylcholine injection, but not at 1.5 or 3 min (when excluding one animal pretreated with dantrolene; $P < 0.05$, intragroup comparison; table 2).

No genotype-specific changes were observed in either right ventricular pressures or contractilities (table 2). The mean pulmonary artery pressures before succinylcholine administration did not change significantly and were 22 ± 4 mmHg in normals and 26 ± 3 mmHg in MHS animals; at 1.5 min, they were 25 ± 5 mmHg in normals and 29 ± 4 mmHg in MHS animals; and at 3 min, they were 24 ± 5 mmHg in normals and 24 ± 9 mmHg in MHS animals. In particular, pulmonary diastolic pressures (table 2) and central venous pressures (data not shown) remained stable for both genotypes after succinylcholine was administered.

In pilot studies, pretreatment with either atropine or diphenhydramine/ranitidine did not prevent immediate cardiovascular depression or MH in susceptible animals (data not shown).

In Vivo *Study: Metabolic Data*

No significant changes of K^+ and Ca^{2+} were detected during the hypotensive period in MHS swine (table 2). The mean time to trigger significant episodes of MH in susceptible swine (*i.e.*, $Pa_{CO2} > 70$ mmHg, fixed ventilation) was 13.5 min (table 1). Significant decreases in *p*H were observed at 5 and 10 min after succinylcholine administration ($P < 0.05$). Statistically significant changes in mean Pa_{CO2} did not occur within the first 10 min postsuccinylcholine, but a clear trend of increased Pa_{CO2} was observed (table 3). Changes in individual end-tidal carbon dioxide values are presented in figures 3A and B. Core temperatures were significantly elevated postsuccinylcholine in these MH animals, but not until

Table 2. Changes in Cardiovascular Variables and Metabolic Parameters after the Administration of Succinylcholine 2 mg/kg Intravenous (t = 0) in MHS Swine,* MHS Swine with Vecuronium Pretreatment† and Normal Swine

Time (min)	Right Ventricular dP/dt (mmHg/s)	Mean Right Ventricular Pressure (mmHg)	Peripheral Vascular Resistance (mmHg · min^{-1} · l^{-1})	Pulmonary Diastolic Pressure (mmHg)	K^+ (mM/l)‡	Ca^{2+} (mM/l)
MHS swine (n = 7)						
Control	259 ± 65	14 ± 5	25 ± 7	21 ± 7	4.15 (3.8–4.4) ± 0.2	1.2 (0.96–1.5) ± 0.2
1.5 min	295 ± 45	19 ± 3	22 ± 13	26 ± 6	—	—
3 min	267 ± 102	15 ± 6	—	21 ± 8	4.2 (3.6–4.7) ± 0.5	1.2 (0.84–1.29) ± 0.2
5 min	323 ± 152	20 ± 5	16 ± 14	21 ± 5	4.1 (3.4–4.7) ± 0.5	1.1 (0.77–1.32) ± 0.2
10 min	423 ± 196	23 ± 8	23 ± 14	28 ± 14	4.5 (3.8–5.3) ± 0.6	1.2 (0.92–1.31) ± 0.2
MHS swine with vecuronium pretreatment (n = 3)						
Control	352 ± 34	19 ± 1	27 ± 3	24 ± 6	3.9 (3.6–4.3) ± 0.4	1.3 (1.19–1.44) ± 0.1
1.5 min	367 ± 34	20 ± 1	28 ± 4	29 ± 4	4 (3.8–4.2) ± 0.2	1.3 (1.25–1.3) ± 0
3 min	405 ± 17	22 ± 2	—	25 ± 12	4 (3.8–4.3) ± 0.3	1.3 (1.17–1.38) ± 0.1
5 min	375 ± 35	21 ± 2	30 ± 3	30 ± 6	3.9 (3.5–4.3) ± 0.4	1.2 (1.04–1.39) ± 0.2
10 min	395 ± 1	23 ± 4	29 ± 2	33 ± 10	3.8 (3.4–4) ± 0.3	1.1 (0.92–1.25) ± 0.2
Normal swine (n = 5)						
Control	265 ± 47	11 ± 3	21 ± 7	13 ± 7	4.1 (3.7–4.4) ± 0.3	1.2 (1.01–1.39) ± 0.2
1.5 min	265 ± 41	12 ± 2	20 ± 4	16 ± 8	4 (3.6–4.6) ± 0.5	1.1 (0.99–1.32) ± 0.2
3 min	261 ± 33	12 ± 1	—	14 ± 7	—	—
5 min	268 ± 55	12 ± 2	20 ± 6	15 ± 8	4.4 (4.1–4.6) ± 0.2	1.2 (1.01–1.34) ± 0.1
10 min	265 ± 42	12 ± 2	18 ± 6	15 ± 7	4.3 (3.9–4.6) ± 0.3	1.1 (1.06–1.33) ± 0.1

* Including swine with dantrolene pretreatment.

† 1 mg/kg.

‡ Pooled data from MHS 1–5 and MHS D1,2 (see table 1).

MHS = malignant hyperthermia susceptible; dP/dt = contractility.

several minutes later. For example, the esophageal temperatures before succinylcholine were 37.9 ± 0.3°C in susceptible swine (n = 4), and at 10 min after succinylcholine administration, they increased to 38.3 ± 0.3°C ($P < 0.05$, intragroup comparison; table 3).

The initial administration of succinylcholine did not induce episodes of MH in animals pretreated with either dantrolene (n = 2) or vecuronium (n = 3). However, MH was triggered subsequently in two animals pretreated with vecuronium and in one MHS animal pretreated with dantrolene. The readministration of succinylcholine in one vecuronium animal 90 min after the first dose induced a cardiovascular reaction very similar to that without pretreatment; the mean arterial pressure decreased from 95 to 45 mmHg (table 1).

The maximum change in masseter force after succinylcholine administration (within 90 s) was 92 mmHg in MHS swine (n = 5) and 2 mmHg in normal animals (n = 4; $P < 0.05$). No change in masseter force was observed in MHS animals with vecuronium pretreatment (n = 3; table 1).

Femoral artery blood flow of MHS animals decreased at 3–4 min after succinylcholine administration and increased significantly at 8–11 min ($P < 0.05$, intragroup

Table 3. Changes in Metabolic Parameters after the Administration of Succinylcholine 2 mg/kg Intravenous (t = 0) in MHS and Normal Swine

Time (min)	Pa_{CO_2} (mmHg)	pH_a	Esophageal Temperature (°C)
MHS swine (n = 4)*			
Control	40.3 ± 2	7.5 ± 0	37.9 ± 0.3
5 min	62 ± 14	7.3 ± 0.1	37.9 ± 0.4
10 min	63 ± 8	7.2 ± 0.1	38.3 ± 0.3
MHS and dantrolene (n = 2)			
Control	40 ± 2	7.5†	38.1 ± 0.6
5 min	21 ± 11	7.6†	38 ± 0.4
10 min	46 ± 9	7.3†	37.9 ± 0.5
MHS and vecuronium (n = 3)			
Control	39 ± 2	7.5 ± 0	38 ± 0.2
5 min	38 ± 3	7.5 ± 0	37.9 ± 0.2
10 min	36 ± 3	7.4 ± 0.1	37.9 ± 0.2
Normal swine (n = 5)			
Control	42 ± 4	7.5 ± 0.03	38.1 ± 0.2
5 min	41 ± 2	7.5 ± 0.02	38 ± 0.2
10 min	41 ± 2	7.5 ± 0.05	37.9 ± 0.2

* One animal died 5 min after succinylcholine administration.

† Data from one animal.

MHS = malignant hyperthermia susceptible.

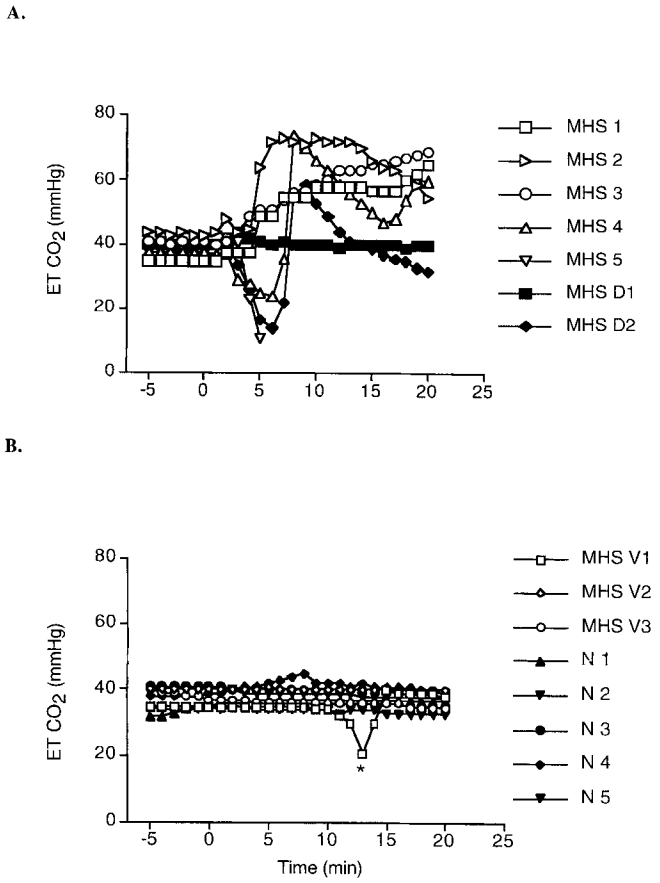

Fig. 3. End-tidal carbon dioxide (ET CO_2) increased after intravenous succinylcholine 2 mg/kg at time = 0 min in MHS animals but not in those pretreated with dantrolene (MHS D), vecuronium (MHS V), or in normals (N). A temporary decrease of ET CO_2 was considered to be caused by an air embolus in this animal (*). This animal required bolus doses of epinephrine to maintain normal blood pressure (table 1).

comparison, fig. 4). In the susceptible swine, a similar decrease of venous blood flows at 3 and 4 min and significant increases were observed at 9, 10, 11, and 12 min ($P < 0.05$, analysis of variance). Arterial and venous flows were stable throughout in normals and in the vecuronium-pretreated MH animals.

Ex Vivo *Isolated Heart Studies*

Administration of succinylcholine at an equivalent dose to 2 mg/kg to the Krebs perfusate had no effect on either right and left ventricular contractility or pressures, or on heart rates in hearts isolated from either genotype. The left ventricular contractility and left ventricular pressures of isolated MH and normal swine hearts are shown in figure 5.

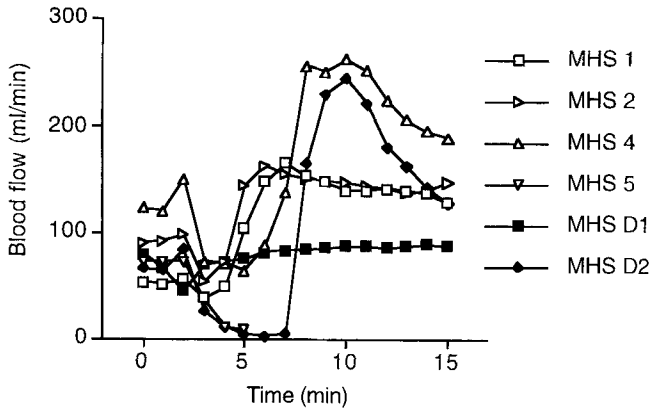

Fig. 4. Femoral artery blood flows in MHS animals with no pretreatment and those with 2 mg/kg dantrolene pretreatment (MHS D). After the administration of 2 mg/kg succinylcholine, femoral artery blood flow of MHS and MHS D swine decreased insignificantly, then increased significantly compared with baseline values. One animal died during the hypotensive response (MHS 5).

Isolated Femoral Artery Rings

The results from these investigations are summarized in figure 6. The baseline force was 0.88 ± 0.32 g in rings isolated from normal swine and 0.91 ± 0.3 g from the MHS swine. After an average dose of 1.65 ± 0.37 μM phenylephrine in normal swine and 1.85 ± 0.39 μM in MHS swine, the mean force was 3.68 ± 3.02 g in normal swine and 2.93 ± 2.46 g in rings from MHS swine, which for both cases were significantly different when compared with baseline values ($P < 0.001$, repeated measures analysis of variance and Bonferroni posttest). When comparing means of normal *versus* MHS animals, no differences could be detected, although the mean forces of the vascular rings from normal animals tended to be higher after phenylephrine administration (fig. 6).

The administration of 0.06 mM succinylcholine caused a small but insignificant reduction in mean force in rings isolated from either genotype: 0.25 ± 0.41 g for MHS swine, 0.63 ± 0.72 g for normal animals. In addition, these changes of force were then normalized by calculating the succinylcholine-induced changes as the percent of the maximal contraction induced by phenylephrine. In vascular rings from MHS swine, the succinylcholine-induced relaxation was 8.4 ± 13.8% compared with 22.9 ± 25.8% in normal animals ($P > 0.05$, Mann—Whitney test; a *t* test was not used because of unequal SDs).

The mean acetylcholine-induced reduction in measured force was 1.73 ± 2.34 g in MHS swine and 2.96 ± 2.81 g for normal animals ($P < 0.05$ for MHS swine and $P < 0.001$ for normal animals *vs.* phenylephrine). The mean normalized relaxation induced by acetylcholine

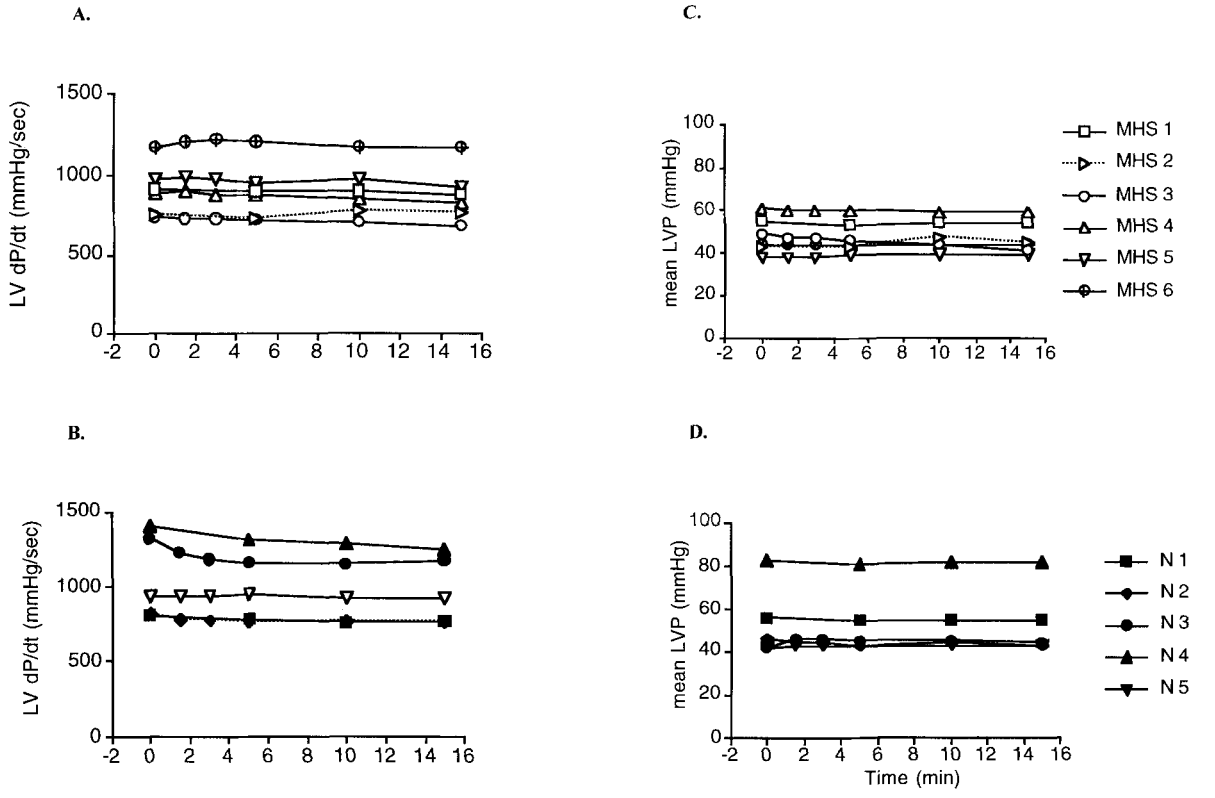

Fig. 5. Succinylcholine administration caused no significant changes in *in vitro* left ventricular contractilities (LV dP/dt) or mean left ventricular pressures (LVP) in hearts isolated from MHS animals. Control data were obtained from hearts isolated from normal animals (N; n = 5).

was 49 ± 34.4% in MHS swine and 78 ± 29.7% for normal swine ($P < 0.001$, unpaired two-tailed t test).

Discussion

Despite advances in the understanding of the molecular basis of MH, further knowledge of the genotype-phenotype association is desirable. Although abnormal increases of myoplasmic calcium of isolated MHS skeletal muscle fibers in response to halothane exposure is routinely used for the *in vitro* diagnosis, succinylcholine rarely causes abnormal contractions or contractures *in vitro*.[16] Nevertheless, succinylcholine *in vivo* can readily induce episodes of MH in susceptible swine.[3] Somewhat surprising, the pathologic mechanism by which succinylcholine initiates MH remains largely un-

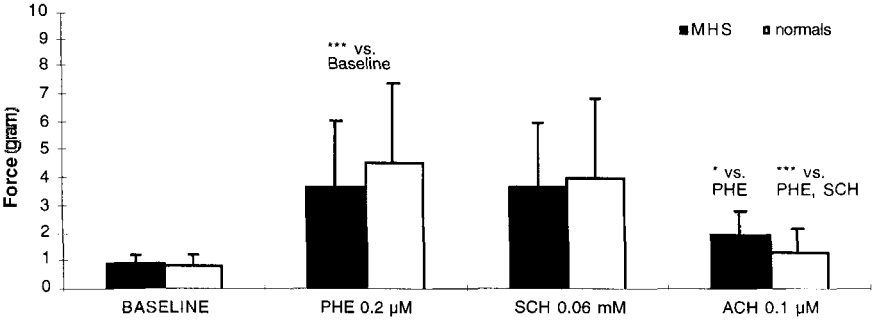

Fig. 6. Isometric forces of femoral artery vascular rings obtained from MHS (black columns, n = 15 rings) and normal swine (white columns, n = 32 rings). At baseline, no significant differences in forces were detected between the groups. The addition of 0.2 μM phenylephrine (PHE) increased force development significantly in all preparations. The addition of succinylcholine 0.06 mM, the equivalent dose to 2 mg/kg intravenously, did not lead to a significant relaxation in rings from either group. Finally, 0.1 μM acetylcholine increased relaxation in both groups but more significantly in preparations from normal animals.

known. Furthermore, bolus succinylcholine administration causes severe hypotension in MH animals, and this response is also poorly understood.[3] It was previously reported that pancuronium pretreatment at clinical doses greatly attenuated the succinylcholine-induced skeletal muscle fasciculations but did not prevent either arterial hypotension or subsequently induced MH episodes.[3] In addition, increases in circulating catecholamines were not responsible for either the hypotensive response or subsequent initiation of a MH episode; yet, the pancuronium pretreatment that did not prevent these responses was associated with a suppressed catecholamine release.[3]

Dantrolene pretreatment has been described to prevent myocardial necrosis by suppressing catecholamine release that is normally associated with succinylcholine administration.[17] It was speculated that succinylcholine may have additional effects such as an altered calcium release in the sympatho-adrenergic system. Pancuronium pretreatment prevented the succinylcholine-induced increase in catecholamines in MHS swine.[3]

Several reports support the hypothesis that an altered calcium metabolism may be found in tissues other than the skeletal muscle in humans or swine.[5,7-10] For example, abnormal action potential responses to halothane in cardiac muscle, increased inositol phosphate concentrations, and disordered catecholamine release and consecutive myocardial necrosis after succinylcholine administration have been described in susceptible swine.[4,6,17] Inotropic effects of isoproterenol on force of contraction were considered more pronounced in heart muscle isolated from the right ventricles of susceptible swine, and this effect was potentiated by succinylcholine.[18] However, in other reports, no differences between heart muscle isolated from normal swine or those with MH genotype could be found. For example, no significant differences in the effects of β- and α-adrenergic agonists, adenosine, and carbachol were shown by the same investigators in heart muscles isolated from the left ventricle of normal and MHS swine.[11] Altered myocardial oxygen consumption or lactate production has not been associated with MH swine.[12] In one study investigating the potential for cardiomyopathy in MH, nine endomyocardial biopsy results from susceptible humans did not provide a morphologic explanation for abnormal cardiac function, which was present in three of the nine patients.[19]

Our study clearly demonstrated that the *in vivo* administration of succinylcholine to MHS swine produces a significant and early onset (after 1.5 min) decrease in arterial blood pressure, which confirms an earlier study by one of the present investigators.[3] This arterial hypotension occurs before classically described features of a MH episode.[3] This hypotension was associated with: (1) a severe decrease in left ventricular contractility; (2) a decrease in cardiac output; (3) little or no significant changes in systemic vascular resistance; and (4) unchanged preload. Thus, succinylcholine administered *in vivo* has a negative inotropic effect in Pietrain swine with the *RYR1* MH genotype. Interestingly, no effects of similar bolus doses of succinylcholine on either *ex vivo* isolated hearts or on isolated femoral artery rings from these same swine could be demonstrated. This suggests an indirect or secondary effect of succinylcholine on the heart in susceptible animals. However, pretreatment with mega doses of vecuronium not only completely abolished the cardiovascular depression associated with the administration of succinylcholine to MHS swine, but also prevented subsequent signs of a MH crisis. Thus, it is hypothesized that the succinylcholine-induced cardiovascular depression is directly associated with a primary defect within the skeletal muscle and that the interaction of succinylcholine with junctional and extrajunctional nicotinic acetylcholine receptors is required. For example, succinylcholine could release a transmitter by a mechanism yet to be identified; such cardiodepressant could be directly released from skeletal muscle.

Although the finding that vecuronium prevented cardiovascular depression and MH may not necessarily have primary clinical significance, hypotensive responses after succinylcholine administration can be observed clinically. Such responses are commonly caused by an anaphylactic or anaphylactoid reaction leading to histamine release.[20] Yet, pretreatment with antihistamines in the present study did not attenuate the cardiovascular depression induced by succinylcholine in MHS swine (a pilot study). On the other hand, an abnormal cardiovascular reflex could lead to the observed cardiovascular depression. However, it was noted that the hypotension was not associated with increased vagal activities (*i.e.*, bradycardia). Furthermore, pretreatment with atropine, a blocker of the muscarinic receptors, did not prevent succinylcholine-induced cardiovascular depression in one MH swine (pilot study). Altered potassium and calcium levels were ruled out as possible sources of cardiovascular depression. In addition, in a recent pilot study, no associated changes in magnesium, histamine, or bradykinin levels were detected during succinylcholine-induced hypotension in susceptible swine (unpublished observation by our laboratory). Interestingly, pretreat-

ment with clinical doses of dantrolene did not prevent the succinylcholine-induced hypotension, although it was effective in preventing any subsequent episodes of MH. This may support the notion that the initial acetylcholine receptor activation is more important than secondary effects on intracellular [Ca^{2+}] for induction of hypotensive responses.

Potential limitations of our study may include the following: the slope of the left and right ventricular pressure curves that determines the change of pressure over time (dP/dt) is subject to confounding factors. One could speculate that hypotension *per se* could artificially produce a decrease in left and right ventricular pressures by changing the slope of the curves. To test this limitation, severe arterial hypotension was induced by vasodilators (nitroglycerine and nitroprusside) in several control swine and did not induce changes in contractility (results not shown). Furthermore, we have no clear explanation why only the left ventricle showed a significant decrease in contractility; the sustained pulmonary pressures support the hypothesis that the right ventricle was less affected by succinylcholine. It may be that significant changes were easier to detect for left ventricular contractility simply because slopes were larger.

It is unclear why a clinical dose of pancuronium did not prevent the succinylcholine-induced hypotension, whereas the megadose of vecuronium pretreatment did.[3] Our present opinion is that this is a dose-dependent phenomenon, and in our first study, an incomplete blocking of the acetylcholine receptors had occurred. This argument may be further substantiated by the notion that fasciculations were reduced but not completely prevented in the MHS animals receiving pancuronium pretreatment.[3]

To eliminate the argument that the isolated heart studies were performed after the animals were exposed to succinylcholine *in vivo*, and that this lead to the null result, five animals (three MHS, two normal) were exposed to succinylcholine only *in vitro*, and yet the results obtained were similar.

In the current study, the femoral artery was chosen for the investigation of the effects of succinylcholine on isolated vascular rings. Succinylcholine could have differential effects on any kind and diameter of vessels. In preliminary studies, femoral vein preparations turned out to be too fragile to use for analyses. Thus, even though one cannot rule out an effect of succinylcholine on capacitance vessels (veins), we did not observe an initial significant reduction in systemic vascular resistance that should have been detected in case of a massive vasodilation. However, in several cases, a slight decrease of systemic vascular resistance was detected in both MHS and normal animals; this was considered to contribute to the hypotension in the MHS animals but was not the primary cause. Acetylcholine induced a significantly greater amount of relaxation in vascular rings from normal animals compared with those from MH animals. One possible explanation for this phenomenon is that the MHS bundles might have been more responsive to acetylcholine, for example, by an upregulation of acetylcholine receptors. However, there was no difference in response to succinylcholine between the genotypes.

Succinylcholine causes an early severe, sometimes fatal, cardiovascular depression in MHS swine. This was associated with decreased left ventricular function, a normal preload, and no significant changes in systemic vascular resistance. This cardiovascular depression can be completely prevented by pretreatment with a megadose of vecuronium, but not with dantrolene, yet both agents prevented the initiation of MH episodes. The cardiovascular depression cannot be explained by direct effects of succinylcholine on the heart or vasculature of these MHS swine. These findings suggest a primary involvement of acetylcholine receptors in triggering MH and causing cardiovascular depression. However, whether the receptors responsible for this reaction are located solely within the skeletal muscle and/or extrajunctional remains unknown. Our laboratory is conducting further investigations to determine the exact cause of succinylcholine-induced hypotension in MHS swine.

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References

1. MacLennan DH, Phillips MS: Malignant hyperthermia. Science 1992; 256:789–94

2. Fujii J, Otsu K, Zorzato F, de Leon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH: Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 1991; 253:448–51

3. Iaizzo PA, Wedel DJ: Response to succinylcholine in porcine malignant hyperthermia. Anesth Analg 1994; 79:143–51

4. Röwer N, Greim C, Rumberger E, Schulte am Esch J: Abnormal action potential responses to halothane in heart muscle isolated from malignant hyperthermia-susceptible pigs. ANESTHESIOLOGY 1995; 82:947–53

5. Iaizzo PA, Seewald MJ, Olsen R, Wedel DJ, Chapman DE, Berggren M, Eichinger HM, Powis G: Enhanced mobilization of intracellular Ca^{2+} induced by halothane in hepatocytes isolated from swine susceptible to malignant hyperthermia. ANESTHESIOLOGY 1991; 74:531–8

6. Scholz J, Steinfath M, Röwer N, Patten M, Troll U, Schmitz W, Scholz H, Schulte am Esch J: Biochemical changes in malignant hyperthermia susceptible swine: Cyclic AMP, inositol phosphates, alpha 1, beta 1- and beta 2-adrenoceptors in skeletal and cardiac muscle. Acta Anaesthesiol Scand 1993; 37:575-83

7. Klip A, Ramlal T, Walker D, Brt BA, Elliott ME: Selective increase in cytoplasmic calcium by anesthetic in lymphocytes from malignant hyperthermia-susceptible pigs. Anesth Analg 1987; 66:381-5

8. Klip A, Brt BA, Elliott ME, Pegg W, Frodis W, Scott E: Anaesthetic-induced increase in ionised calcium in blood mononuclear cells from malignant hyperthermia patients. Lancet 1987; 1:463-6

9. Fink HS, Maak S, von Lengerken G, Till U: Abnormalities in the regulation of blood platelet free cytosolic calcium in malignant hyperthermia. II. Pig platelets. Cell Calcium 1992; 13:157-62

10. Fink HS, Hofmann JG, Hentschel H, Till U: Abnormalities in the regulation of blood platelet free cytosolic calcium in malignant hyperthermia. I. Human platelets. Cell Calcium 1992; 13:149-55

11. Bohm M, Röwer N, Schmitz W, Scholz H, Schulte am Esch J: Effects of beta- and alpha-adrenergic agonists, adenosine, and carbachol in heart muscle isolated from malignant hyperthermia susceptible swine. ANESTHESIOLOGY 1988; 68:38-43

12. Gronert GA, Ahern CP, Milde JH, White RD: Effect of CO_2, calcium, digoxin, and potassium on cardiac and skeletal muscle metabolism in malignant hyperthermia susceptible swine. ANESTHESIOLOGY 1986; 64:24-8

13. Iaizzo PA, Wedel DJ, Gallagher WJ: In vitro contracture testing for determination of susceptibility to malignant hyperthermia: A methodologic update. Mayo Clin Proc 1991; 66:998-1004

14. Larach MG: Standardization of the caffeine halothane muscle contracture test: North American Malignant Hyperthermia Group. Anesth Analg 1989; 69:511-5

15. Chinchoy E, Soule CL, Houlton AJ, Gallagher WJ, Hjelle MA, Laske TG, Morissette J, Iaizzo PA: Isolated four chamber working swine heart model. Ann Thorac Surg 2000; (in press)

16. Ørding H, Skovgaard LT: In vitro diagnosis of susceptibility to malignant hyperthermia: Evaluation of tests with halothane-caffeine, potassium chloride, suxamethonium and caffeine-suxamethonium. Acta Anaesthesiol Scand 1987; 31:462-5

17. Haggendal J, Jonsson L, Johansson G, Bjurstrom S, Carlsten J: Disordered catecholamine release in pigs susceptible to malignant hyperthermia. Pharmacol Toxicol 1988; 63:257-61

18. Scholz J, Röwer N, Rum U, Schmitz W, Scholz H, Schulte am Esch J: Effects of caffeine, halothane, succinylcholine, phenylephrine and isoproterenol on myocardial force of contraction of malignant hyperthermia susceptible swine. Acta Anaesthesiol Scand 1991; 35: 320-5

19. Mambo NC, Silver MD, McLaughlin PR, Huckell VF, McEwan PM, Brt BA, Morch JE: Malignant hyperthermia susceptibility: A light and electron microscopic study of endomyocardial biopsy specimens from nine patients. Hum Pathol 1980; 11:381-8

20. Moss J: Muscle relaxants and histamine release. Acta Anaesthesiol Scand Suppl 1995; 106:7-12