PORCINE MALIGNANT HYPERTHERMIA:

STUDIES ON ISOLATED

MUSCLE STRIPS

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PREFACE

This study examines isometric twitch tension and contracture responses of isolated muscle strips, from normal and malignant hyperthermia susceptible swine, to various pharmacological treatments. Microelectrode studies on normal and malignant hyperthermia porcine muscle provide basic measurements of electrophysiological characteristics which identify pre-existent myogenic abnormalities and aid interpretation of drug induced changes in malignant hyperthermia muscle.

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CHAPTER I

INTRODUCTION

Background

Malignant hyperthermia (MH) is a pharmacogenetic disease, characterized by fever and muscle rigidity, which is observed in susceptible individuals exposed to certain triggering anesthetic agents. Although of rare occurrence, (1 in 15,000 anesthetics) MH is a serious complication of general anesthesia in man. It may occur in otherwise healthy individuals in whom no anesthetic abnormality is expected. The mortality rate is 60 to 70 percent, and no simple reliable diagnostic test has yet been established (1).

Inheritance

An inherited syndrome which is similar if not identical to MH in man has been described in several breeds of swine including Landrace (2, 3), Pietrain (4), and Poland China (5). Until recently, MH has been considered to be inherited in man and swine as an autosomal dominant trait with reduced penetrance and variable expressivity (1, 4). Recent studies suggest the inherited basis of MH in man and swine may be multi-factorial or bigenic (6, 7, 8), while MH in Dutch Landrace swine is reported to be controlled by one major recessive gene with complete penetrance (9).

Clinical and Biochemical Features

Malignant hyperthermia is characterized clinically by tachycardia, tachypnea, skeletal muscle rigidity, blotchy cyanosis of the skin, a rapid progressive increase in core temperature, cardiac arrhythmias, acute cardiac failure, late neurological deterioration, coma and death (1, 5).

Concomitant biochemical changes may include hypercarbia, lactacidosis, hyperkalemia, hypocalcemia, elevated serum enzymes including SGOT, CPK, and LDH, hemoglobinuria, myoglobinuria and elevated serum inorganic phosphates (1, 5, 10).

Triggering Anesthetic Agents and Factors

The syndrome has been triggered in man and swine by most inhalation anesthetic agents including halothane, chloroform, enflurane, methoxyflurane, isoflurane, diethyl ether, cyclopropane and nitrous oxide (1, 3, 11). The muscle relaxants succinylcholine and pancuronium alone or in combination with the inhalation agents also appear capable of initiating the syndrome (2, 12). In swine a similar if not identical syndrome, the porcine stress syndrome, may be induced in MH susceptible swine by exercise, fighting, sexual activity and high ambient temperatures (4, 13).

Recent evidence suggests a similar hyperthermic "stress" syndrome exists in some MH susceptible humans (14, 15) and more rarely, some MH susceptible humans may develop a normothermic "stress" cardiomyopathy (16).

Etiological Mechanisms

Numerous etiological mechanisms and theories for MH have been proposed, but as yet the precise location and nature of the primary defect remains incompletely defined.

Considerable evidence exists which suggests skeletal muscle is the target tissue in anesthetic induced MH. Whether a muscle defect exists as the primary abnormality or arises secondarily to a neurogenic defect is unclear, since La Cour et al. (17) have demonstrated the presence of degenerating axons, regenerating axons and multiple sprouting of new normal and abnormal nerve endings in MH muscle.

However, the evidence favoring a peripheral myogenic etiology is convincing. Skeletal muscle rigidity is observed in nearly every case of porcine MH (2, 4, 5) and approximately 70 percent of human MH cases (1). Porcine MH muscle when incubated in vitro has a rapid decline in ATP content which is enhanced by exposure to halothane (3, 18). Isolated hind limb perfusion experiments in swine clearly indicate that biochemical and metabolic features of the syndrome develop in skeletal muscle independent of central thermoregulatory and adrenal control (19, 20).

Malignant hyperthermia susceptible humans and swine frequently have pre-existent elevated serum CPK levels suggestive of a subclinical myopathy (21, 22, 23). During and immediately following a MH episode, serum levels of CPK as well as SGOT and LDH are grossly elevated (5, 22).

Many human MH patients and some of their relatives have localized muscle abnormalities such as strabismus, ptosis, kyphoscoliosis, spontaneous hernias and joint dislocations (1). Non specific myopathies characterized by muscle weakness, cramps after exercise, areas of muscle

atrophy interspersed with areas of pseudo-hypertrophy and loss of deep tendon reflexes have been observed in some human MH patients (17, 24).

Morphological changes in human MH muscle observed by light and electron microscopy are generally non specific and include internal nuclei, variation in fiber size, "splitting", atrophied fibers and "moth-eaten" core-targetoid fibers (25). Most muscle changes are observed in type I fibers and are not seen in muscle from susceptible young children suggesting the structural abnormalities are secondary to biochemical alterations within MH muscle cells. Other defects observed in human MH muscle include loss of myofibrils, distension of mitochondria (26), degenerative and regenerative alterations of the intramuscular nerve endings, and double Z-lines (27).

Porcine MH muscle prior to initiation of the syndrome shows occasional areas of supercontracted sarcomeres alternating with overstretched sarcomeres, evidence of muscle fiber degeneration, and the presence of myosattelite cells between the glycocalyx and plasmalemma of regenerating muscle cells (28).

Human and porcine isolated MH muscle strips develop contractures when exposed to halothane at 37^{o}C (29, 30), are abnormally sensitive to caffeine-induced contractures (6, 29, 31, 32), and demonstrate even greater sensitivity to combined treatment with halothane and caffeine (6, 29). Accumulation of calcium by sarcoplasmic reticulum (SR) isolated from human MH muscle is inhibited by halothane (31, 32). Although some reports indicate calcium uptake by SR from porcine MH muscle is similarly affected by halothane (33, 34), other reports demonstrate the reverse effect (10, 18).

The hypermetabolic state, expressed by rigidity, ATP depletion and heat production which occurs in MH muscle exposed to specific triggering anesthetic agents, may be due to persistent elevation of free calcium in the myoplasm. Several mechanisms have been proposed including increased calcium influx (30), increased permeability of the SR membrane to calcium (1), impaired uptake of calcium by SR (1) and decreased calcium uptake by mitochondria (35).

Most current hypotheses accept an elevated myoplasmic calcium concentration as a key event in the pathogenesis of MH, however there is some disagreement as to whether this represents the primary event. Some reports suggest cytoplasmic calcium levels rise due to a primary depletion of ATP perhaps brought about by inhibition of mitochondrial respiration (32, 35), uncoupling of oxidative phosphorylation (35, 36), or "futile" substrate cycling (37). An ATP deficit in skeletal muscle, by inhibiting active transport of calcium into SR and extracellular fluid, results in elevated free myoplasmic calcium. Activation of the contractile process occurs with elevated free calcium and in the presence of low ATP levels, contraction rapidly progresses to rigor.

Recent reports suggest that a "vicious cycle" mechanism implicating skeletal muscle hypermetabolism and catecholamine secretions may operate in MH (38, 39). Circulating catecholamines are elevated during porcine MH (39) and the syndrome can be prevented by pretreatment of susceptible swine with phentolamine, an adrenergic blocking drug (38). Catecholamines enhance skeletal muscle contraction in animals and man possibly through cyclic AMP mediated effects on SR membranes (40, 41). The progressive cyclic reaction is believed to be initiated by the triggering effects of anesthetic agents on MH susceptible muscle to cause myoplasmic

calcium excess. The resultant muscle hypermetabolism and acidosis induce catecholamine secretion which enhances further the calcium release mechanisms.

Therapy

Clinical management of human MH crises has largely been symptomatic with generally unsatisfactory results. A wide range of regimens have been used including hyperventilation, active surface cooling, peritoneal and gastric lavage with ice-cold electrolyte solutions, intraveneous administration of ice-cold balanced electrolyte and sodium bicarbonate solutions, massive doses of corticosteroids, procaine, and procainamide intravenously, insulin therapy, and the use of vasodilators (1, 42, 43). Successful therapy has been attributed to the particular drug regimen employed, however, it is now clearly evident that the earlier the syndrome is recognized and treatment initiated, the greater are the chances of a successful outcome. This is particularly apparent in porcine MH where following early recognition of the syndrome, and immediate withdrawal of the triggering inhalation anesthetic agent, hyperventilation alone without other concomitant therapy will ensure near 100 percent recovery (44).

Although no definitive study has been made of the relative value of therapeutic drugs used in MH, those drugs capable of decreasing free intracellular calcium levels appear to be most effective in alleviating clinical signs of MH. Procaine and procainamide prevent caffeine-induced release of calcium from SR and when given in high concentrations apparently stabilize intracellular calcium fluxes during MH (45). However, cardiac arrhythmias are frequently observed at these dose rates

necessitating careful electrocardiographic monitoring of patients during procaine and procainamide therapy (42, 45). The use of procaine and procainamide in porcine MH has produced variable results (46, 47).

Dantrolene sodium, a relatively new muscle relaxant which interrupts excitation-contraction (EC) coupling, prevents development of MH in susceptible swine and reverses metabolic and functional alterations which occur in anesthetic-induced porcine MH (48, 49, 50).

Rationale

The basic assumption in this study is that MH occurs in susceptible individuals due to the effect of triggering anesthetic agents on defective calcium storage sites within skeletal muscle.

Pharmacological studies of twitch tension and contracture responses in isolated muscle strips from MH susceptible humans and swine suggest the defect in skeletal muscle involves some essential step in the EC coupling process. Studies on isolated muscle strips utilizing drugs which potentiate (caffeine, halothane) or inhibit (dantrolene sodium) EC coupling attempt to a) clarify the site and nature of the inherent defect and b) evaluate the prophylactic and therapeutic potential of dantrolene sodium in MH.

Microelectrode studies on individual muscle cells provide new basic information on electrophysiological characteristics of porcine MH and normal muscle. These data enable further characterization of possible muscle defects and assist interpretation of drug-induced changes in MH muscle.

CHAPTER II

SELECTED LITERATURE REVIEW

Excitation-Contraction Coupling in Skeletal Muscle

Introduction

Excitation-contraction coupling in skeletal muscle is defined as the relationship between electrical changes (depolarization or propagated action potentials) associated with the muscle surface membrane and subsequent mechanical contraction of the muscle through activation of contractile elements within the fiber (51).

In brief, EC coupling involves several steps: a) the regenerative action potential at the sarcolemma is actively conducted to the interior of the muscle-fiber through the transverse tubular system (T-system or T-tubule), b) depolarization of the T-system results in release of stored calcium from the terminal cysternae of SR, c) the elevated sarcoplasmic calcium concentration removes the inhibitory effect of calcium-free troponin on actin-myosin interaction, cross-bridge formation occurs and contraction ensues accompanied by ATP hydrolysis and d) during the relaxation phase, calcium is actively reaccumulated within the SR and cross-bridges detach from the thin filaments.

The steps above of direct relevance to MH are step (b) which includes the transmission of excitatory signal down the T-system,

transfer of signal to the SR and calcium release from the SR, and step (d) which involves ATP dependent uptake of calcium by SR.

T-System Structure and Excitability

The T-system, a direct extension of the sarcolemma, in general is arranged transversely to the muscle fibers' longitudinal axis (52). However, recent electronmicroscopic studies indicate a small proportion of the T-system is longitudinally arranged, while the major proportion of the T-system follows a spiral course with small slope around the axis of the whole fiber (53). The T-system has qualitatively similar electrophysiological properties to the surface membrane including delayed rectification (54) and the ability to produce regenerative conduction (55). However, quantitatively, the properties of the T-system membranes are distinctly different from those of the sarcolemma. The T-system has a very much lower chloride conductance, a lower potassium conductance, and there are fewer sodium and potassium channels in the T-system (56). Spread of the excitatory action potential along the T-tubule by an active regenerative process is essential for physiological contraction (57).

T-System-Sarcoplasmic Reticulum Coupling

The coupling between the T-system and the terminal cysternae of the SR is the least understood step in EC coupling. Unfortunately, it appears to be the most likely site of action of many of the drugs which trigger MH.

Morphologically, the triadic junction is a unique low-resistance gap junction. The two membranes are separated by a sizeable gap of

approximately 120A^{o}. At about 300A^{o} intervals, the SR sends out projections or "feet" which come to within 50A^{o} of the T-tubule membrane and which are joined to the T-tubule by an amorphous material of unknown composition. The junctional feet cover about 30 percent of the T-tubule membrane but only constitute about three percent of the SR surface (58, 59, 60, 61).

Three general mechanisms have been considered to operate in the transmission of an excitatory signal from the T-system to the SR. Firstly, a direct electrical coupling between T-tubule and SR has been suggested, in which SR permeability to calcium is assumed to be voltage dependent (62). Secondly, chemical linkage between the two membranes has been proposed, in which the so called "trigger" calcium effects release of SR calcium (63). Thirdly, the recent concept of voltage dependent charge movement in excitable membranes has been investigated as an important step in EC coupling. The charge movement concept refers to a group of charged particles in the T-tubule membranes with extensions which span the T-tubule SR space. When the charged particles move across the T-tubule membrane in response to a change in membrane potential, the extensions alter calcium permeability of SR either by directly opening calcium channels located at the feet of the terminal cysternae or by inducing electrical depolarization of the SR membrane (64).

A voltage dependent charge movement that is confined to the T-system and which is different to the gating current of delayed rectification has been demonstrated in amphibian and mammalian skeletal muscle (64).

The relationship between charge movement and calcium release from SR appears unequivocal. There is close agreement between them relative to their time course and threshold. Both the charge movement and contractility are eliminated in the inactivated state during prolonged depolarization. There are also close correlations between the time course and temperature dependence of repriming of the contractile response and recovery of voltage dependent charge movement (65). Thus recovery of contractility requires a threshold amount of charge movement.

Available evidence favors the hypothesis that the voltage dependent charge movement is the manner in which excitatory signals traverse the T-tubule-SR gap in the EC coupling process.

Calcium Release From the Sarcoplasmic Reticulum

Physiological calcium release from the SR is incompletely understood. Two mechanisms have been demonstrated including calcium-induced calcium release (63, 66, 67) and depolarization-induced calcium release (68, 69).

Calcium-induced calcium release does not appear to be an important physiological mechanism in EC coupling, since many of the conditions necessary for calcium-induced calcium release, including SR maximally preloaded with calcium, low sarcoplasmic magnesium concentrations, and high sarcoplasmic calcium concentrations, are not present under physiological conditions (66, 67, 70). Similarly, the high concentration of free calcium (> than 3×10^{-4} M) necessary to induce a net release of SR calcium by calcium under physiological conditions, can not be attained during depolarization by calcium influx and/or by release of calcium bound to the T-tubule membrane (70). However, calcium-induced release

may play an important role under certain pharmacological (71) or pathological conditions (6).

Unlike calcium-induced calcium release, depolarization-induced calcium release does not require preloading of SR to a specific level, nor is it affected by free magnesium concentration. The properties of depolarization-induced calcium release are compatible with the possibility of its role in physiological calcium release from SR (68, 69).

Calcium Uptake During Relaxation

Relaxation of skeletal muscle occurs when myoplasmic calcium concentration is reduced below 3×10^{-7} M (72). Calcium released from the terminal cysternae of SR during excitation is actively reaccumulated by the whole surface of SR in white, fast skeletal muscle (73). However, while the calcium accumulating capacity of the SR can account for the relaxed state of muscle at steady state, even the fastest rate of uptake measured is not fast enough to adequately explain the rate of relaxation of living muscle (74). This discrepancy may be explained by rapid binding of calcium to high affinity sites on the calcium pump ATPase (75) which is present in a very high concentration and/or to unidentified high-affinity calcium-binding proteins of the SR (76). Mitochondrial uptake of calcium is not a physiologically important mechanism during relaxation of white, fast, skeletal muscle; however, its role in red, slow, skeletal muscle is less clear (77). Mitochondrial calcium uptake may be more important when myoplasmic calcium concentrations are elevated (78).

For more rigorous reviews on EC coupling, the reader is directed to several recent publications (67, 78, 79).

Pharmacology of Excitation-Contraction Coupling

Caffeine

In amphibian and mammalian skeletal muscle, caffeine potentiates direct and indirect twitch tensions at low concentrations and induces contractures at high concentrations (80). The primary site of action of caffeine is the SR membrane (80, 81). Low concentrations reportedly decrease SR reaccumulation of calcium while higher concentrations stimulate calcium release from SR and thus activate contraction and metabolism in muscle (81). Recently, Endo (71) has demonstrated that skinned fibers in the presence of higher caffeine concentrations, undergo reversible contracture and fragmented SR accumulates calcium almost to its full extent. The amount and rate of calcium uptake by skinned fibers at low calcium concentration is not affected by caffeine. These observations suggest caffeine facilitates calcium release from SR with little or no effect on the capacity of SR to accumulate calcium. Since calcium release from SR due to caffeine is a regenerative process, the likely action of caffeine is labilization of calcium-induced calcium release (67).

Calcium release by caffeine is inhibited by lowering mypolasmic calcium, raising free magnesium, and by the presence of procaine. Threshold preloading of SR with calcium is also necessary for caffeine-induced calcium release from SR (71).

A second site of caffeine action has been proposed. In the presence of high concentrations of manganese ion (10 mM or more) caffeine

contractures in intact fibers are significantly depressed while depolarization-induced contractures are not affected (78). Since manganese is believed to uncouple excitation-contraction at the T-system-SR step, the manganese-attenuation of the caffeine response may be due to blocking of a caffeine effect at this site.

In general, amphibian muscle is less sensitive than mammalian skeletal muscle to the effects of caffeine (82). Reported differences in sensitivity may also be explained in part by the variable potency of different caffeine solutions, depending on the manner of their preparation (83).

Local Anesthetic Agents

Caffeine-induced twitch potentiation and contraction in amphibian and mammalian skeletal muscle are antagonized by the local anesthetics procaine and tetracaine, whereas, lidocaine potentiates caffeine-induced contractures (63, 84). Procaine and tetracaine apparently stabilize or inhibit the calcium-induced calcium release mechanism (78). Procaine and tetracaine at higher doses also retard the repriming of the contractile response of fibers inactivated by prolonged depolarization (85).

Differences among the local anesthetic agents in their effects on release of calcium from SR may be explained in terms of the ratio of charged to uncharged forms of the various drugs at physiological pH. Because of its low pK_a, at physiological pH, a large proportion of lidocaine molecules exist in the uncharged form which have predominately caffeine potentiating properties. The charged form of lidocaine and other local anesthetics blocks the SR effect of caffeine (63, 84).

Halothane

Halothane potentiates twitch tension (86, 87, 88) and caffeine-induced contractures (89) in amphibian and mammalian skeletal muscle. The relaxation phase of twitch and tetanus responses in amphibian muscle is prolonged by 0.5 to 1.0 percent halothane (89, 90) while calcium uptake by isolated SR from human and guinea pig muscle is inhibited by halothane (91). Halothane also causes calcium release from SR by enhancement of the calcium-induced release mechanism, in a manner similar to the action of caffeine (92).

Dantrolene Sodium

In 1967, Synder and coworkers (93) reported the synthesis of dantrolene sodium, 1- [5- {(p-nitrophenyl) furfurylidene} amino] hydantoin sodium, a compound with skeletal muscle relaxant properties which has proved beneficial in the treatment of muscle spasticity. Unlike other clinically available muscle relaxants, dantrolene sodium acts directly and specifically on skeletal muscle and has no effects on the central nervous system.

Dantrolene sodium has no effects on transmission of nerve impulses to muscle, on release of acetyl choline or on the development of trans-membrane potential at the myoneural junction (82, 94, 95, 96). Post junctional development of action potentials and their conduction over the muscle fiber membrane are unaffected by dantrolene sodium (82). It also has no effect on calcium stimulated ATPase activity of the myofibrils, suggesting dantrolene sodium does not act at the level of the contractile filaments (97).

Active calcium uptake by fragmented SR from rabbit skeletal and canine cardiac muscle is unaffected by dantrolene sodium (97), and normal calcium uptake kinetics are observed in dantrolene sodium treated, skinned frog sartorius muscle fibers (98).

Several reports indicate dantrolene sodium inhibits intracellular release of calcium during contraction. In frog semitendinosus muscle, dantrolene sodium inhibits twitch force and causes a shift in the dose-response curve for potassium contractures without affecting maximum potassium contracture or significantly reducing tetanus force (99). Single barnacle muscle fibers injected with the calcium sensitive bioluminescent protein aequorin, when electrically stimulated, have decreased transient luminescence in the presence of dantrolene sodium (100). Isolated SR from rabbit and cat skeletal muscle treated with dantrolene sodium, demonstrate normal ATP dependent calcium binding but impaired calcium release (101).

In frog, rat, and goat skeletal muscle, dantrolene sodium raises the rheobase of the strength duration curve for mechanical threshold of contraction and also raises the threshold of activation to short duration pulses. Dantrolene sodium also competitively antagonizes a calcium ionophore believed to act specifically on SR membranes, and the time course of the peak-to-off phase of voltage dependent charge movement is shortened by dantrolene sodium (102). These observations suggest dantrolene sodium decreases calcium release from SR by firstly restricting the period of charge movement, and thereby attenuating the excitatory signal at the T-tubule to SR step in EC coupling and secondly by inhibiting natural calcium ionophores in SR membrane, thus decreasing SR permeability to calcium.

Isolated Muscle Contracture and Twitch Tension Studies

Introduction

Screening procedures for identification of individuals susceptible to MH have comprised a battery of tests including in vitro pharmacological testing of isolated muscle specimens (6, 29, 32, 103, 104). This procedure utilizes known hypersensitivity of MH muscle to a wide range of physico-chemical stimuli. The technique has also enabled the in vitro identification of potentially new triggering anesthetic agents as well as the evaluation of potential prophylactic and/or therapeutic drugs in MH (29, 43, 103, 104). Since many of the drugs utilized in the test system have known sites and actions, pharmacological assays using these drugs in isolated muscle, have provided additional insight into the etiological mechanisms operating in MH (29, 30, 104, 105, 106).

The most common drugs tested in vitro on MH muscle are halothane, caffeine, and these two in combination. Other drugs tested include potassium chloride, succinylcholine, procaine, dantrolene sodium and hydrocortisone. Most studies have investigated both the ability of drugs such as halothane, caffeine, potassium chloride and succinylcholine to induce contractures in isolated MH muscle, and the ability of drugs such as procaine, dantrolene sodium and hydrocortisone to prevent or reverse these contractures. Test conditions have varied considerably with differences in temperature, selection of test muscle, anesthetic technique used during biopsy, the use of simultaneous periodic twitch, incubating solution and resting length/tension conditions reported from the various laboratories (6, 29, 30, 103).

Contracture Studies

Halothane consistently induces contractures in human and porcine MH muscle in vitro at 37°C (29, 30, 103, 104), but rarely produces contractures at 25°C (31); whereas normal muscle is usually unreactive to halothane (29, 30, 103). Halothane-induced contractures in MH muscle are clearly dependent on normal extracellular fluid calcium concentrations (29, 30). Malignant hyperthermia muscle is also abnormally sensitive to chemical membrane depolarization by 80 mM potassium chloride, and to the effects of large doses of succinylcholine (29).

Human and porcine MH muscle is abnormally sensitive to caffeine at 25°C or 37°C, and simultaneous exposure of MH muscle to halothane and caffeine results in potentiated contracture responses (6, 29, 104). These contractures in isolated human MH muscle are significantly attenuated by 10mM procaine pretreatment (107). Some contractures induced by halothane alone in human MH muscle are prevented by procaine while halothane-induced contractures may be partially reversed by 5 mM procaine treatment (29, 108). However in some human MH muscle, procaine actually induces contractures and potentiates halothane-induced contractures (109). Procaine also inhibits the abnormal contractures in MH muscle by succinylcholine (29).

Dantrolene sodium significantly reduces halothane-induced contractures in porcine MH muscle, and reverses halothane-induced contractures in porcine MH muscle (106). Hydrocortisone treatment (0.029 mM) reverses halothane-induced contractures in human MH muscle (11). However, dexamethasone, 0.05 mM, does not prevent the development of halothane contractures in isolated human MH muscle strips (43).

Twitch Tension Studies

Although many investigators electrically stimulate MH muscle specimens routinely during pharmacological testing, there are few reports of the effects of drugs such as halothane, caffeine, and dantrolene sodium on the twitch tension response of MH muscle.

The twitch response of porcine MH muscle in the presence of 0.5, 1.0, and 2.0 mM caffeine and various concentrations of dantrolene sodium (zero, 0.25 μ g/ml, 0.5 μ g/ml, 1 μ g/ml) has been investigated (106). Caffeine potentiates twitch tension in a dose related manner in the absence of dantrolene sodium. As the concentration of dantrolene sodium is increased, caffeine twitch tension potentiation is also increased. This twitch-potentiating effect of dantrolene sodium on caffeine treated MH muscle is presumed due to its inhibition of the caffeine-induced contractures normally observed at these concentrations of caffeine.

Electrophysiological Studies

Most electrophysiological studies on MH muscle appear as clinical reports on electromyographic (EMG) observations in human MH patients. Generally EMG changes in MH muscle are minor, non specific and inconsistent (17, 32, 110). One microelectrode study on porcine MH muscle cells reports resting membrane potentials in the range of -75 mV to -85 mV which were not altered by subsequent exposure to halothane (110).

Mechanical threshold of contraction has been studied in isolated human MH muscle, using stepwise chemical depolarization of muscle cells by increasing concentrations of potassium chloride in the extracellular fluid. Contractures were observed at normal concentrations of potassium

chloride (between 20 and 30 mM) suggesting the threshold linking membrane depolarization to contractile response is normal in human MH muscle (29).

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Malignant hyperthermia susceptible muscle was obtained from an inbred line of Poland China swine, bred and maintained under standard conditions of husbandry and nutrition at Oklahoma State University. Normal muscle came from unrelated Hampshire, Hampshire-Yorkshire or Duroc swine. Animals were classified according to their susceptibility to MH as positive or negative based on their reactions to in vitro pharmacological testing of isolated muscle strips and/or their subsequent in vivo reaction to a standard halothane-succinylcholine challenge (30). Experimental animals of both sexes were 3 to 6 months of age and weighed between 25 kg and 75 kg.

Experimental Design

Twitch tension, contracture and electrophysiological characteristics of isolated normal and MH, gracilis and external intercostal, muscle strips were studied under varying pharmacological conditions. Gracilis muscle was selected due to its accessability and as being representative of fast, white, skeletal muscle. External intercostal muscle was selected for microelectrode studies because dissection enabled preparation of intact tendon-to-tendon muscle fibers. External intercostal

muscle is representative of mixed but predominantly slow, red, skeletal muscle.

In Experiment 1, the actions and interactions of halothane, caffeine and dantrolene sodium on the twitch tension response of MH gracilis muscle strips were compared with responses of similarly treated normal gracilis muscle strips.

Experiment 2 compared the contracture responses of MH and normal gracilis muscle strips when treated with halothane or caffeine. Interactions between halothane and dantrolene sodium, caffeine and dantrolene sodium, caffeine and halothane and among dantrolene sodium, caffeine and halothane relative to contracture responses were also studied in MH and normal gracilis muscle.

Experiment 3 examined twitch and contracture responses of normal and MH external intercostal muscle. The effect of halothane and halothane plus dantrolene sodium on the time course of twitch tension was studied to identify pre-existent and drug-induced abnormalities in calcium release and/or uptake during single twitch responses.

In Experiment 4, electrophysiological characteristics of individual muscle fibers from MH and normal external intercostal preparations were studied. Basic electrophysiological data such as resting membrane potentials and mechanical threshold of activation of contraction in porcine MH and normal muscle have not been previously recorded.

Twitch and Contracture Studies

Gracilis Muscle

Biopsy Procedure. Gracilis muscle strips were obtained from swine

during nitrous oxide, ketamine[1], and thiopental[2] anesthesia (111). Bundles of parallel muscle fibers approximately 30 mm X 3 mm X 3 mm in size and weighing 200 to 300 mg were tied in situ at resting length to an applicator stick, excised and immediately placed in bicarbonate buffered Krebs-Ringer (KRB) solution (Table I) which was continually aerated with a 95 percent O_2, 5 percent CO_2 gas mixture (carbogen) at 37°C (30). Each surgical biopsy produced between eight and sixteen muscle strips.

Isometric Tension Measurements. Each muscle strip was prepared for isometric tension studies by firmly tying around the muscle specimen, two stainless steel rings 25 mm apart. The distal ring was attached to a suspension rod by a rigid glass hook and the proximal ring was attached by a 7.5 cm stainless steel wire (size 00) to the microscale accessory of a force-displacement transducer.[3] The mounted muscle preparation was then placed in a water-jacketed muscle bath containing carbogen-gassed KRB at 37°C. The transducer signal was amplified and recorded by an ink writing oscillograph.[4] Each muscle strip was equilibrated in the muscle bath at a tension of 1.0 gm for a period of 15 to 20 minutes before experimental treatments commenced.

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[1]Ketalar, Park Davis Co., Ann Arbor, Michigan.

[2]Pentothal, Abbot Laboratories, North Chicago, Illinois.

[3]Statham VC2, Statham Instruments Inc., Oxnard, California.

[4]Beckman Type R Dynagraph, Beckman Instrument Co., Schiller Park, Illinois.

TABLE I

KREBS-RINGER SOLUTION USED FOR
GRACILIS MUSCLE STUDIES

Salt	gm/l	mM
Dextrose	2.000	11.1
NaCl	6.844	117.0
$NaHCO_3$	2.066	24.6
KCl	0.343	4.6
$CaCl_2.2H_2O$	0.256	2.0
KH_2PO_4	0.105	0.8
$MgSO_4$	0.091	0.7

During twitch studies, depolarizing currents capable of producing supramaximal twitches were delivered from a square wave stimulator,[5] to the muscle preparation every five seconds via directly contacting silver wire electrodes.

Preparation of Drug Treatment Solutions. A stock solution of 0.11 M caffeine was prepared by dissolving 2.173 gm caffeine in sufficient KRB to make a final volume of 100 ml. Selected test concentrations of caffeine in the muscle bath (0.35 mM to 22.4 mM) were achieved by addition of aliquots of the stock solution.

The stock solution of dantrolene sodium was prepared by dissolving 50 mg pure dantrolene sodium powder[6] in 100 ml of an alkaline solution of 4 percent mannitol to give a final concentration of 0.5 mg/ml (48). Dantrolene sodium concentrations in the muscle bath ranging from 2.5 mg/l to 15.0 mg/l were obtained by the addition of aliquots of the stock solution.

Halothane vapor 2 to 4 percent in carbogen was delivered to the muscle chamber from a calibrated vaporizer[7] via a gas delivery system which was independent of the delivery system used to provide anesthetic-free gases. To ensure reasonably consistent halothane concentrations, the carbogen flow rate over the vaporizer was maintained at 2 to 3 l/min. The actual flow rate to the muscle bath was significantly less than this due to a system of escape valves.

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[5]Grass SD 9, Grass Instruments, Quincy, Mass.

[6]Dantrium, Eaton Laboratory, Norwich, New York.

[7]Fluomatic, Forreger, Rosalyn Heights, New York.

Experiment 1: Twitch Studies. The effects of the following treatments on twitch tension were studied:

a) dantrolene sodium 2.5 mg/l on normal and MH susceptible muscle

b) increasing concentrations of dantrolene sodium (2.5 mg/l to 15.0 mg/l) on MH muscle

c) halothane four percent on normal and two percent on MH muscle

d) dantrolene sodium 2.5 mg/l on halothane-induced twitch potentiation in normal and MH muscle

e) caffeine 0.35 mM to 2.8 mM on normal muscle

f) caffeine 0.35 mM to 0.7 mM on MH muscle

g) dantrolene sodium 2.5 mg/l on caffeine-induced twitch potentiation in normal and MH muscle

h) caffeine 2.8 mM on dantrolene sodium inhibited twitch in normal and MH muscle.

Drug-induced alterations of twitch tension during a period of ten minutes immediately post-treatment were expressed as percent increase or decrease of basal (pretreatment) twitch height. When necessary, basal twitch height was corrected for spontaneous twitch decay observed in some muscle strips. Differences among means were examined for significance using Student's "t" test for unpaired data.

Experiment 2: Contracture Studies. The following treatments were evaluated:

a) halothane four percent on normal and MH muscle (Group I)

b) halothane four percent on MH muscle then treated with dantrolene sodium 2.5 mg/l at the time of maximum contracture tension (Group II)

c) halothane four percent on MH muscle equilibrated in KRB plus dantrolene sodium 2.5 mg/l (Group III)

d) caffeine 1.4 mM to 22.4 mM on normal muscle

e) caffeine 1.4 mM to 11.2 mM on MH muscle

f) caffeine 1.4 mM to 22.4 mM on normal muscle equilibrated in KRB plus dantrolene sodium 2.5 mg/l

g) caffeine 1.4 mM to 11.2 mM on MH muscle equilibrated in KRB plus dantrolene sodium 2.5 mg/l

h) caffeine-halothane induced contractures in MH muscle

i) dantrolene sodium-halothane-caffeine contractures in MH muscle

j) dantrolene sodium-caffeine-halothane contractures in MH muscle

k) halothane-dantrolene sodium-caffeine contractures in normal muscle.

Where appropriate, results in the contracture studies were expressed as maximal change in tension (Δ G) from the initial resting tension of 1.0 gm. The interval, from the time of maximal contracture until the muscle tension decreased to a value midway between the initial tension and peak tension (one-half relaxation time), was also recorded. Differences among means were examined for significance using Student's "t" test for unpaired data.

External Intercostal Muscle

Biopsy Procedure. External intercostal muscle was removed from normal and MH susceptible swine during ketamine, thiopental and nitrous oxide anesthesia, by methods previously described for goat external intercostal muscle biopsy (112). From the biopsy, bundles of intact muscle fibers running from tendon-to-tendon (average wet weight of

approximately 80 mg) were dissected in an aerated, circulating water-jacketed muscle chamber at 25°C. The composition of the electrolyte bathing medium during dissection is shown in Table II.

Experiment 3: Isometric Twitch Tension Studies. The muscle preparation was mounted in a water-jacketed muscle chamber containing electrolyte solution which was continuously aerated by 95 percent O_2, 5 percent CO_2 at 27°C and pH 7.20.

Measurements of isometric twitch tension were made by attaching the distal tendon of a muscle bundle to a rigid glass hook projecting from the bottom of the glass muscle chamber. The proximal tendon of the muscle bundle was then tied by a fine stainless steel wire to the arm of a pieso-resistive strain sensor.[8]

Muscles were field stimulated once every 20 seconds through platinum plates with a square wave pulse of 200 μ seconds duration and magnitude between one and two amperes. After placing a muscle in the muscle chamber, the supramaximal stimulus and muscle length that maximized the peak tension (L_o) were determined and these were then kept constant throughout the experiment.

The amplified transducer signal was displayed on a storage oscilloscope[9] and on an ink writing oscillograph.[10] Stored traces were photographed with a kimeograph camera[11] on 35 mm film. Rest tensions were

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[8]DSC-3, DSC Inc., Bellvue, Washington.

[9]Tektronix 7313, Tektronix Inc., Beaverton, Oregon.

[10]Grass Instruments, Quincy, Massachusetts.

[11]Ibid.

TABLE II

PHYSIOLOGICAL SALT SOLUTION USED FOR EXTERNAL INTERCOSTAL MUSCLE STUDIES

Salt	gm/l	mM
Dextrose	1.000	5.55
NaCl	8.649	148.00
$NaHCO_3$	1.008	12.00
KCl	0.336	4.50
$CaCl_2.2H_2O$	0.294	2.00
$MgCl_2.\ 6H_2O$	0.203	1.00
$Na_2HPO_4.\ H_2O$	0.061	0.44

measured directly by a digital voltmeter and also on the ink writing oscillograph.

Analysis of Data. Individual twitch responses were examined by projecting the developed 35 mm film at about 500X magnification. Each twitch curve was digitized and analyzed using an ID digitizer[12] and computer.[13] Measurements of the time-course of each photographed twitch response were made by the computer, identifying the times from beginning of contraction to 10, 25, 50, 75, 90, and 100 percent of the maximal twitch tension and, the times from the maximal twitch tension to 10, 25, 50, 75, 90, and 100 percent of full relaxation.

For descriptive purposes, the time-course of twitch responses were related to the temporal phases of the halothane-induced and dantrolene sodium treated contracture response.

Twitch responses were examined pre-halothane (control), early after initiation of halothane treatment (halothane early), near maximal response (halothane late), soon after dantrolene sodium treatment (halothane and dantrolene early), and 10 to 15 minutes after dantrolene sodium treatment (halothane and dantrolene late).

Drug Treatments. The effects of halothane on twitch response and isometric tension were determined by layering halothane into the bottom of the muscle chamber such that it covered the fritted glass gas delivery system, and allowing the carbogen gas to bubble through the halothane

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[12]ID Digitizer, Summagraphics Inc., Fairfield, Conn.

[13]Modcomp II, Modular Computer Systems, Fort Lauderdale, Florida.

layer. Dantrolene sodium was added to the bath as a supersaturated solution to make a final concentration of 15 mg/l.

Threshold of Mechanical Activation Studies

External Intercostal Muscle Biopsy

External intercostal muscle was removed from normal and MH susceptible swine as described for twitch and contracture studies. Thin muscle bundles of approximately 1000 muscle fibers intact from tendon-to-tendon were carefully dissected at 38°C with the aid of a dissecting microscope.

Experiment 4: Mechanical Threshold Measurements

Muscle preparations were spirally mounted around a 2 mm diameter clear plastic rod in a shallow illuminated temperature controlled muscle bath at 38°C. The carbogen-gassed bathing electrolyte solution (Table II) contained 1.3×10^{-6} M tetrodotoxin (TTX) to block sodium movements and eliminate surface electrical activity (54, 113).

Microelectrodes were prepared from 1 mm O.D. capillary tubing. The voltage recording electrodes were filled with 3 M KCl and had tip resistances of 8 to 12 megohms while the current electrodes were filled with 2 M potassium citrate and had tip resistances of 4 to 10 megohms.

Thresholds of mechanical activation were studied using the two-point voltage clamp method described by Adrian et al. (54). The voltage recording electrode was first inserted into a superficial muscle fiber using micromanipulators and a stereomicroscope at 100X magnification.[14]

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[14]Carl Zeiss Inc., New York, New York.

Successful entry was identified by a sudden change in voltage which was continuously displayed on a dual beam oscilloscope[15] and a digital voltmeter. Only fibers with resting membrane potentials of -60 mV or greater degrees of hyperpolarization were selected for measurement of mechanical threshold.

The current electrode carrying a hyperpolarizing current pulse (which was also continually displayed on the oscilloscope) was then inserted into the same muscle fiber approximately 0.05 mm from the voltage electrode. Successful entry was confirmed when the voltage signal displayed an electrotronic hyperpolarizing voltage pulse coincident with the current pulse.

The TTX-blocked fibers were voltage clamped to a holding potential of -90 mV by a two-electrode voltage clamp. The fibers were then depolarized in steps from their holding potential by depolarizing current pulses of varying duration. The membrane potential necessary to produce a microscopically visible threshold contraction near the electrodes was recorded for several pulse durations (2, 5, 20, 50, 100, and 500 milliseconds). Membrane potential was read directly from the calibrated oscilloscope display or digital voltmeter while contractions were identified by direct observation with a binocular microscope at a magnification of 100X.

Analysis of Data

Mechanical threshold data obtained by the voltage-clamp method were analyzed by examination of the "strength-duration" curves obtained by

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[15]Tektronix Inc., Beaverton, Oregon.

plotting membrane potential (Y axis) against the corresponding pulse duration (X axis). Typically, increasingly more positive potentials were needed to produce threshold contractions as pulse duration was decreased and the threshold potential approached a constant rheobasic value for pulses of long duration (Figure 26).

CHAPTER IV

RESULTS

Twitch and Contracture Studies

Experiment 1: Gracilis Muscle

Twitch Tension Studies

Effect of Dantrolene Sodium on Basal Twitch Height. Dantrolene sodium 2.5 mg/l inhibited twitch height in normal and MH muscle to 45 percent and 38 percent of basal twitch height respectively, ten minutes post-treatment (Figures 1 and 2). These differences were not significant ($P > 0.05$). Increasing concentrations of dantrolene sodium up to 10.0 mg/l in MH muscle resulted in a dose related inhibition of twitch tension ten minutes post-treatment (Figure 3). Significant differences were demonstrated between 2.5 mg/l and 10.0 mg/l ($P < 0.005$) and between 2.5 mg/l and 15.0 mg/l ($P < 0.001$). At the highest concentration tested (15.0 mg/l), twitch tension was decreased to 15.5 percent of its pretreatment level.

Effect of Halothane on Basal Twitch Height. Exposure of normal muscle to four percent halothane caused slight (4 to 25 percent) potentiation of twitch response. In several muscle preparations, twitch potentiation was temporary, after which twitch decreased to 70 to 80 percent of pretreatment height (Figure 4). No significant concomitant

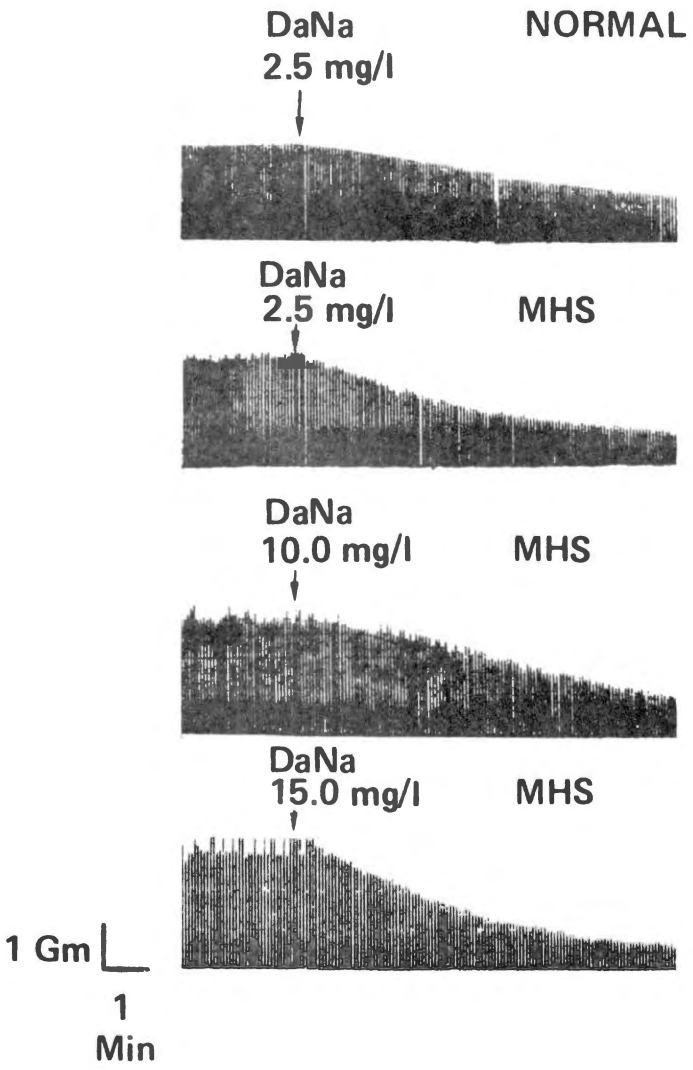

Figure 1. Gracilis Muscle: Effect of dantrolene sodium (DaNa) on twitch tension in normal and malignant hyperthermia susceptible (MHS) muscle. Representative tracings from normal pig number 12-3 and MH pig number 42-7. Twitch tension in gm (Y axis) versus time in minutes (X axis).

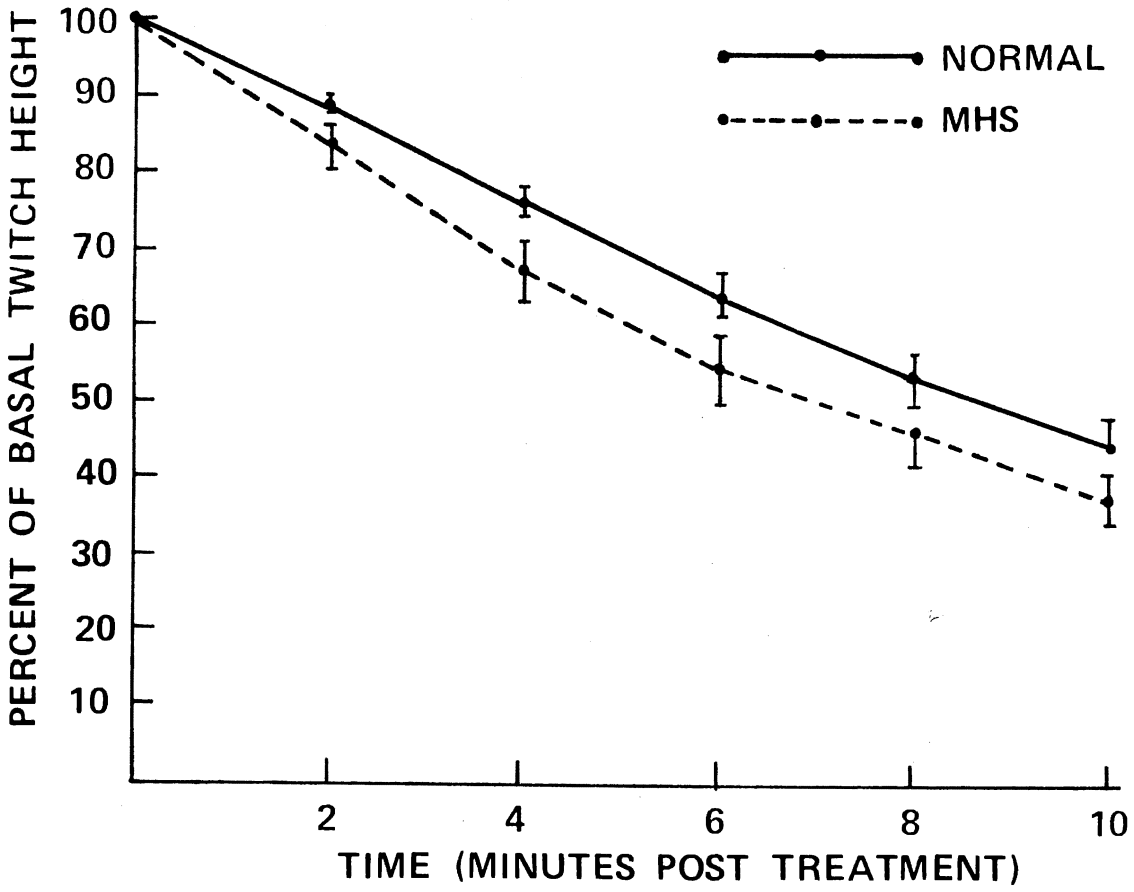

Figure 2. Gracilis Muscle: Effect of dantrolene sodium 2.5 mg/l on twitch tension in normal and MHS muscle during ten minutes immediately post-treatment. Values plotted represent means $\pm$ standard errors of the means (SEM) for 9 muscle strips from 3 normal pigs and 11 muscle strips from 7 MHS pigs.

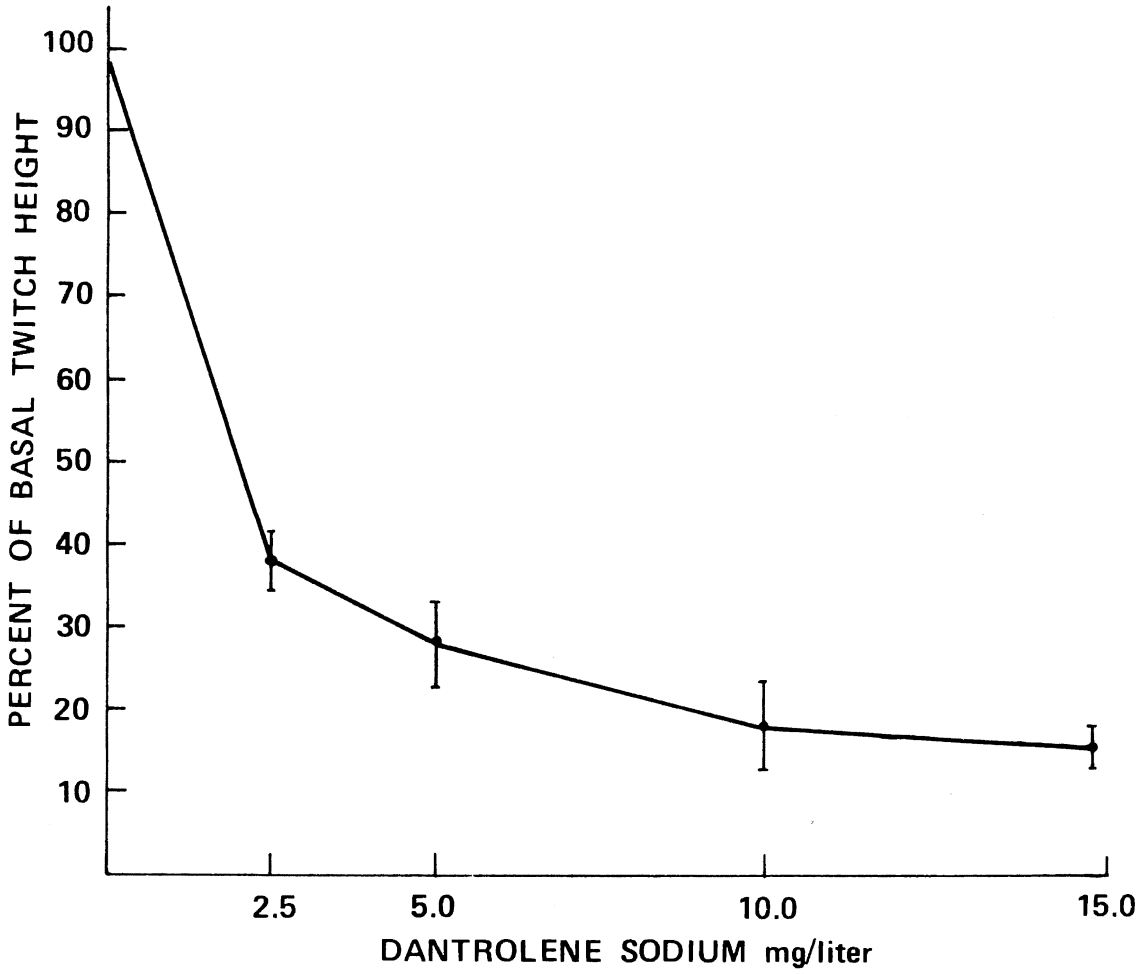

Figure 3. Gracilis Muscle: Effects of increasing concentrations of dantrolene sodium on basal twitch tension ten minutes post-treatment in porcine malignant hyperthermia muscle. Means $\pm$ SEM for 10 muscle strips from 5 MHS pigs.

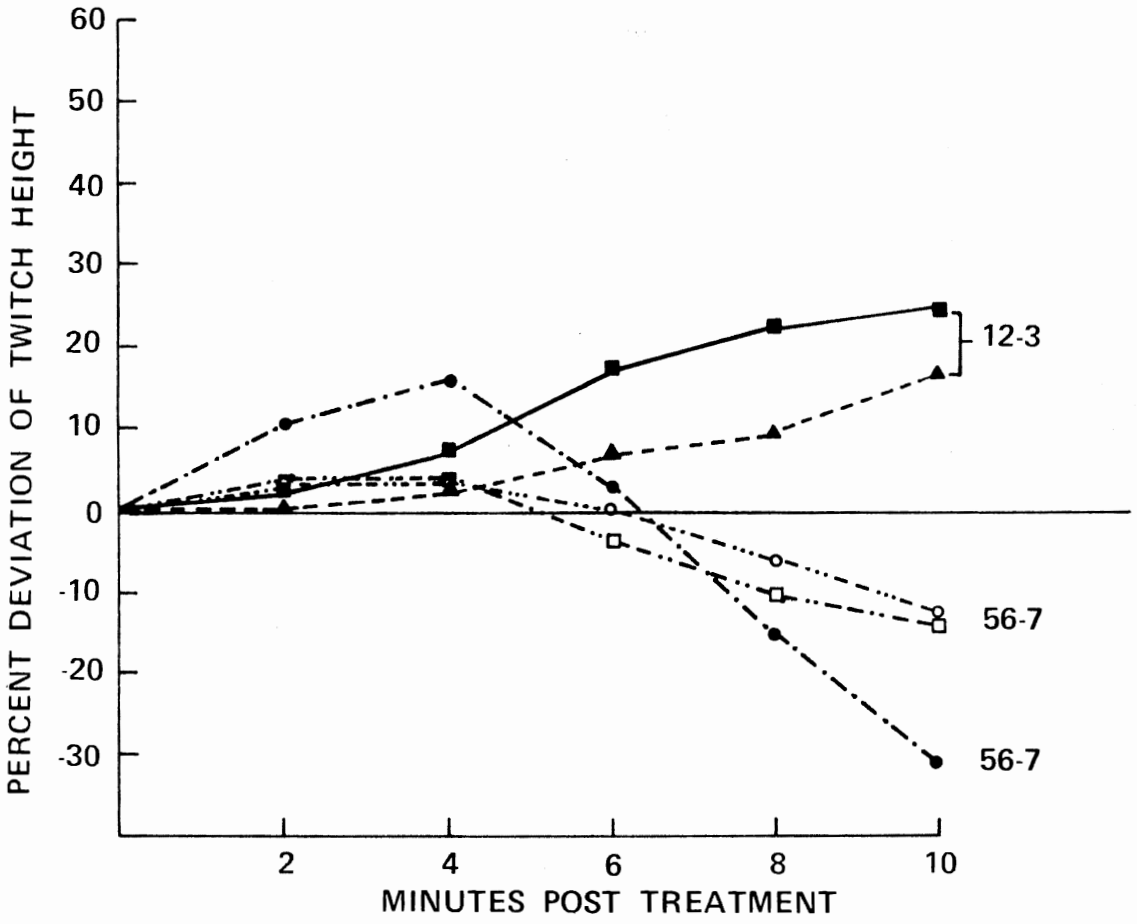

Figure 4. Gracilis Muscle: Effects of halothane on twitch tension in normal porcine muscle. Percent deviation of basal twitch height is plotted against time for 5 muscle strips from 2 normal pigs (12-3 and 56-7).

contractures were observed in normal muscle exposed to four percent halothane.

The twitch height in MH muscle was consistently potentiated (7 to 110 percent) in spite of concomitant halothane-induced contractures in all strips tested (ΔG = 0.3 to 2.2 gm). A representative tracing from MH pig number 9-2 is shown in Figure 5.

Effect of Dantrolene Sodium on Halothane-Induced Twitch Potentiation. The addition of dantrolene sodium 2.5 mg/l to the muscle bath in which normal muscle strips were exposed to four percent halothane, resulted in a rapid inhibition of twitch height to 20 to 50 percent of the pre-dantrolene sodium twitch within ten minutes after treatment (Figure 6).

In MH muscle, halothane-induced twitch height potentiation and contractures were reversed by dantrolene sodium 2.5 mg/l (Figure 5).

Effect of Caffeine on Basal Twitch Height. In normal muscle, 0.35 to 2.80 mM caffeine, potentiated basal twitch height in a dose related manner (Figures 7 and 8). At these concentrations, caffeine did not cause contractures and the twitch height generally was potentiated maximally ten minutes after initiation of the treatment. Concentrations greater than 2.8 mM induced contractures and maximal twitch potentiation coincided with the time of maximal tension. Subsequently, twitch height slowly decayed but at ten minutes post-treatment was usually still potentiated above the basal twitch height (Figure 7).

The twitch height in muscle from MH swine was potentiated by low concentrations (0.35, 0.70 mM) of caffeine (Figures 9 and 10) and

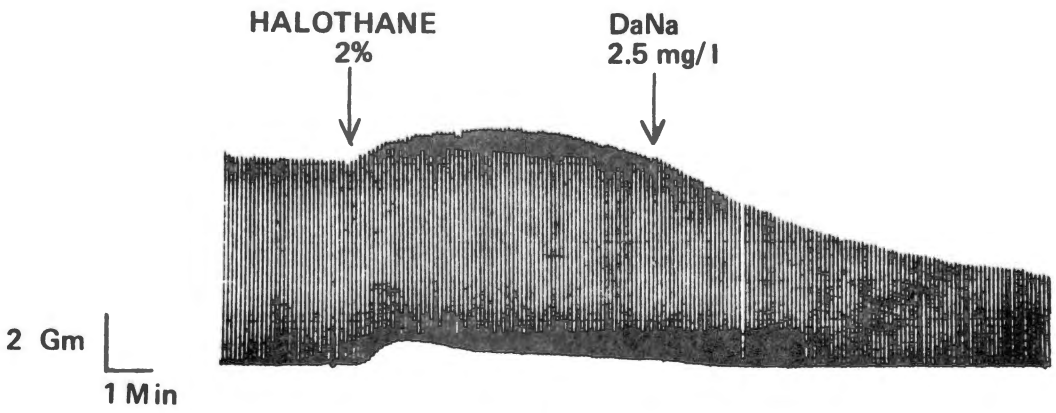

Figure 5. Gracilis Muscle: Effects of 2 percent halothane on isometric twitch and rest tension in malignant hyperthermia muscle and dantrolene sodium (DaNa) 2.5 mg/l on halothane-induced twitch potentiation and contractures. Representative tracings from MHS pig number 9-2.

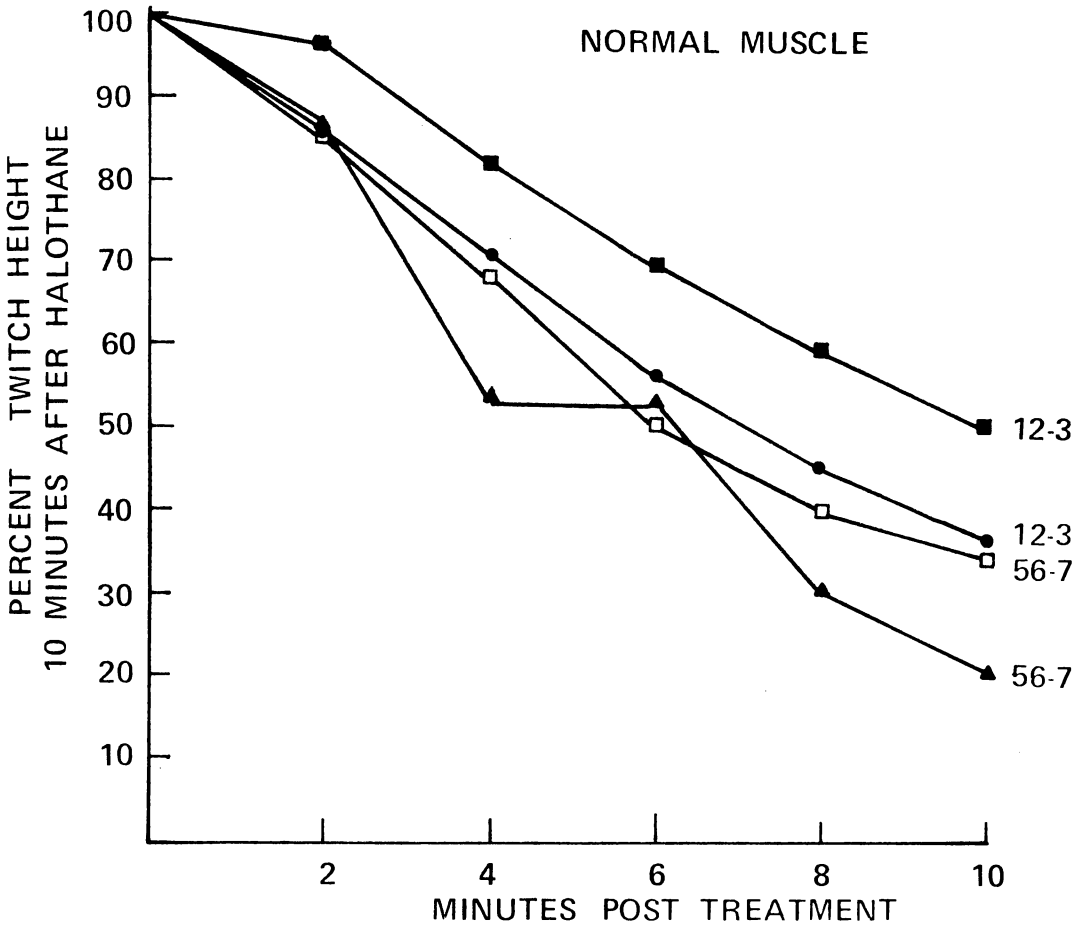

Figure 6. Gracilis Muscle: Effect of dantrolene sodium 2.5 mg/l on halothane-potentiated twitch response in normal muscle. Twitch tension responses to dantrolene sodium for 4 muscle strips from normal pigs (12-3, 56-7) which had halothane-potentiated twitch tensions.

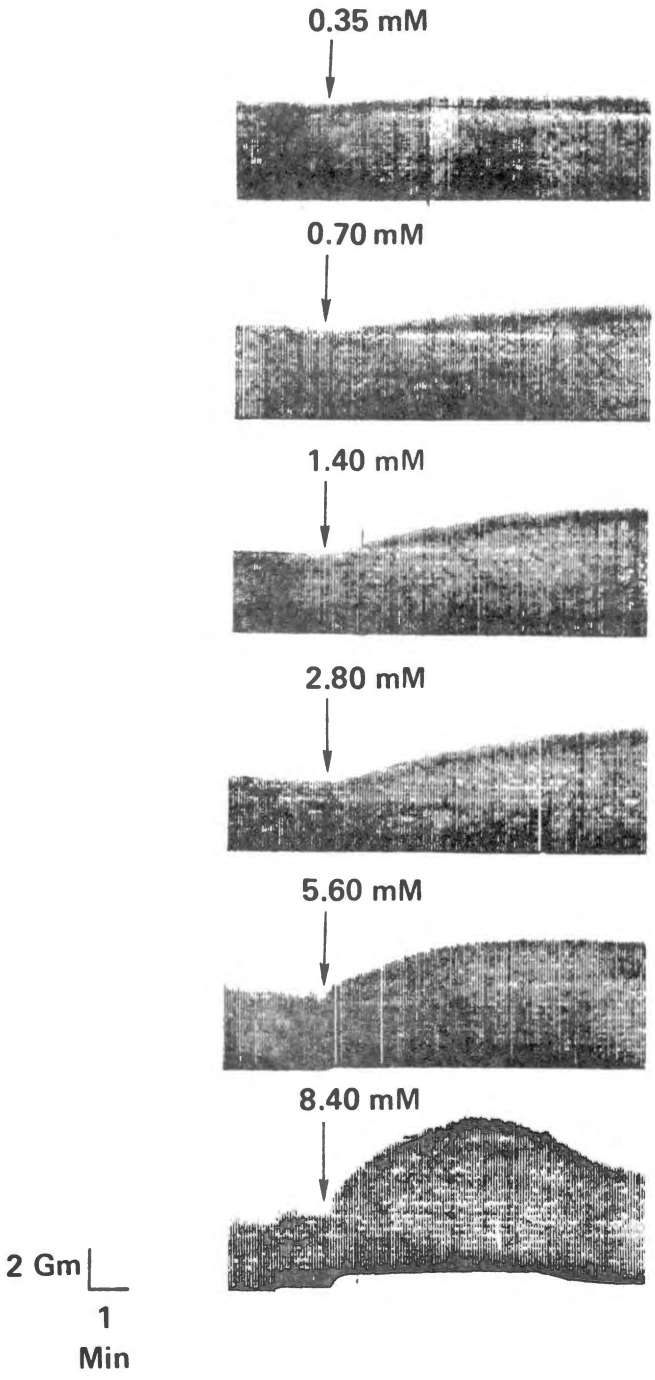

Figure 7. Gracilis Muscle: Effect of caffeine 0.35 - 8.40 mM on isometric twitch and resting tension in normal porcine muscle. Representative tracings from normal pig number 56-7. Twitch tension in gm (Y axis) versus time in minutes (X axis).

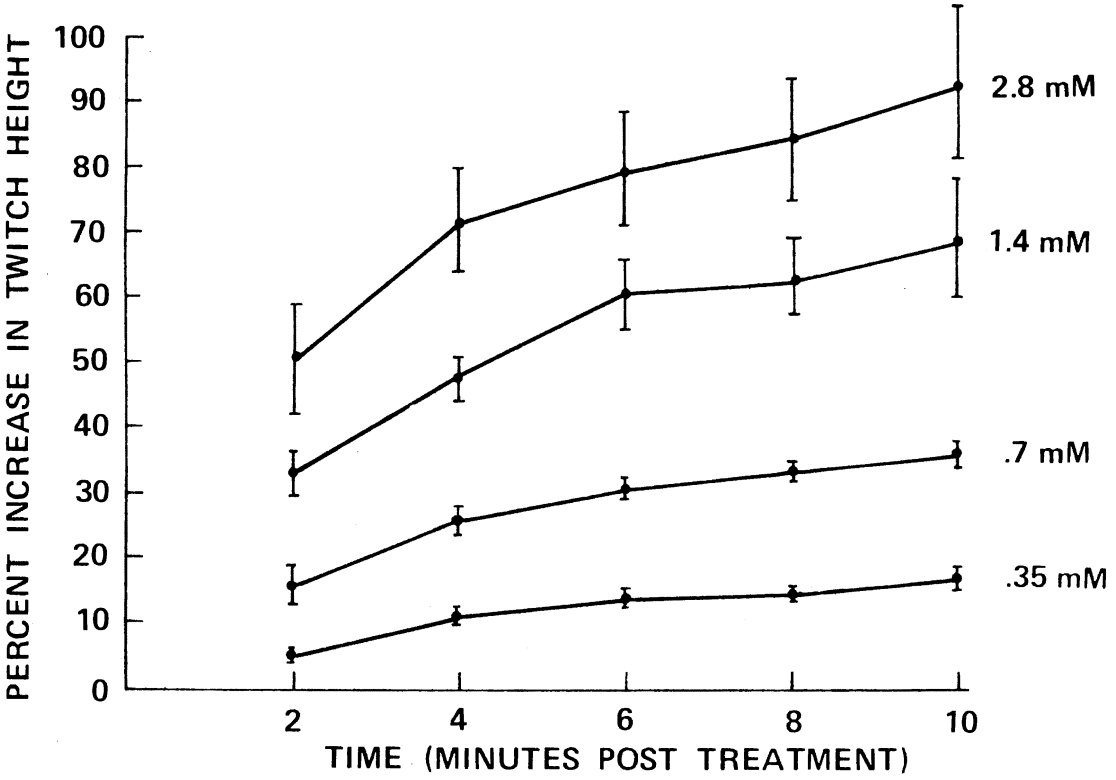

Figure 8. Gracilis Muscle: Effect of low concentrations of caffeine (0.35 - 2.8 mM) on basal twitch tension in normal porcine muscle. Means $\pm$ SEM for 9 muscle strips from 3 normal pigs (12-3, 12-4, 56-7).

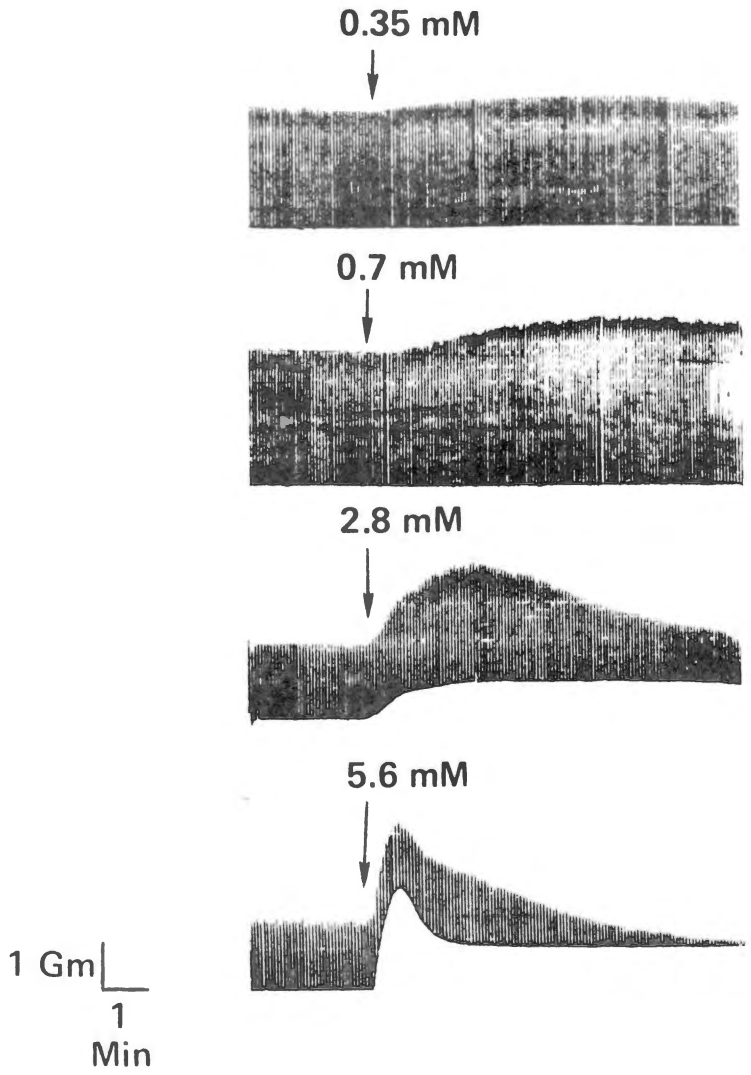

Figure 9. Gracilis Muscle: Effect of caffeine 0.35 - 5.60 mM on isometric twitch and resting tension in malignant hyperthermia muscle. Representative tracings from MHS pig number 43-4. Twitch tension in gm (Y axis) versus time in minutes (X axis).

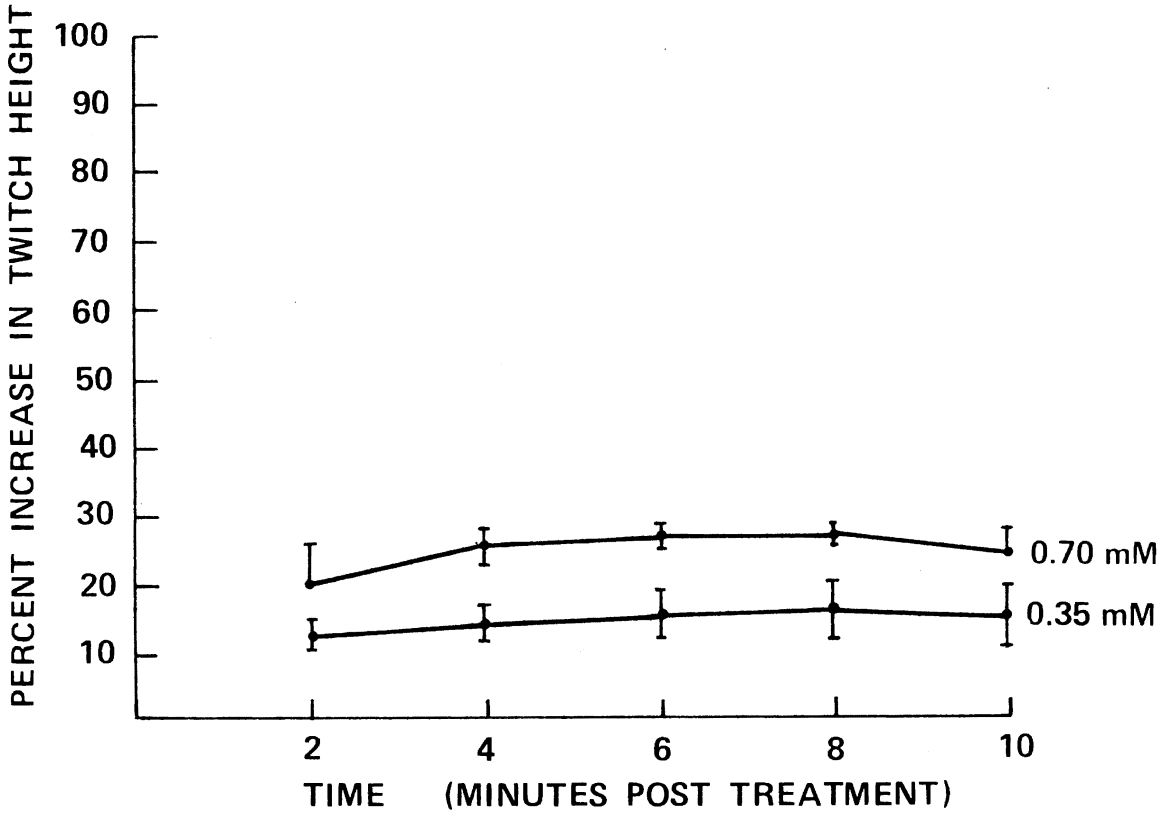

Figure 10. Gracilis Muscle: Effect of low concentrations of caffeine (0.35 - 0.70 mM) on basal twitch tension in malignant hyperthermia muscle. Means $\pm$ SEM from 5 muscle strips from 2 MHS pigs (43-3, 43-4).

contractures were consistently observed at caffeine concentrations greater than 0.7 mM.

Twitch potentiation by caffeine in MH muscle was maximal 2 to 4 minutes after treatment. The twitch tension consistently decayed rapidly and often completely within ten minutes in muscle strips undergoing simultaneous caffeine induced contractures (Figures 9 and 11). This effect was never observed in normal muscle.

Effect of Dantrolene Sodium on Caffeine-Induced Twitch Potentiation. In normal muscle strips treated ten minutes with caffeine (0.7 mM to 2.8 mM), dantrolene sodium 2.5 mg/l caused inhibition of twitch height to approximately 45 percent of the pre-dantrolene sodium height which appeared to be independent of caffeine concentration (Figures 12 and 13). An apparently greater inhibition of caffeine-potentiated twitch by dantrolene sodium was observed in MH muscle (Figures 12 and 13).

Effect of Caffeine on Dantrolene Sodium-Induced Twitch Inhibition. In both normal and MH muscle, the twitch inhibition caused by dantrolene sodium 2.5 mg/l was wholly or partly reversed by 2.8 mM caffeine (Figure 14). In MH muscle, caffeine potentiation was reduced by increased concentrations of dantrolene sodium (Figure 15).

Experiment 2: Gracilis Muscle Contracture Studies

Halothane-Induced Contractures. Muscle from normal swine rarely showed contractures in the presence of four percent halothane. When contractures were observed in normal muscle, they were always slight

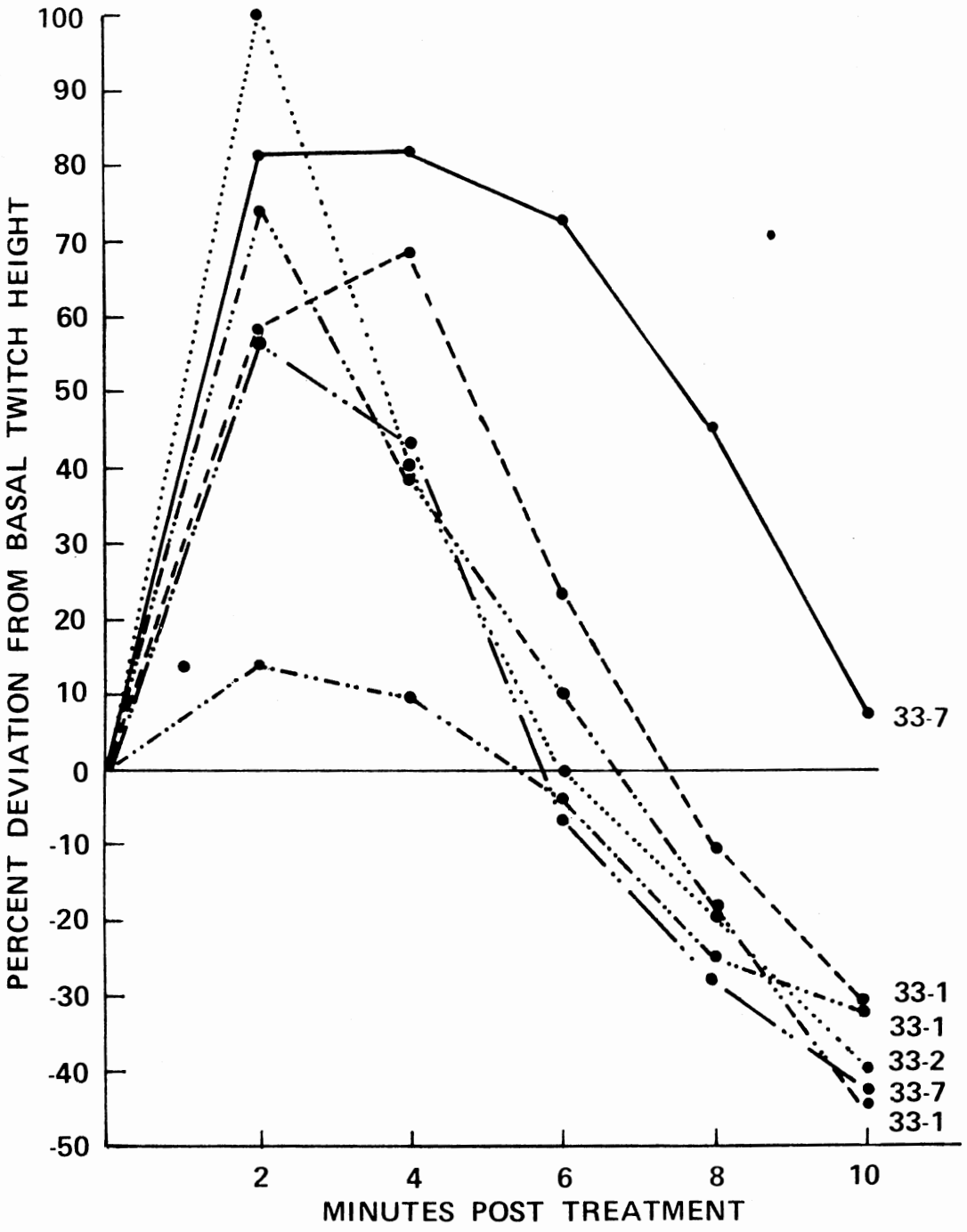

Figure 11. Gracilis Muscle: The effect of 2.8 mM caffeine on twitch tension in malignant hyperthermia muscle. Individual responses of 6 muscle strips from 3 MHS pigs (33-1, 33-2, 33-7).

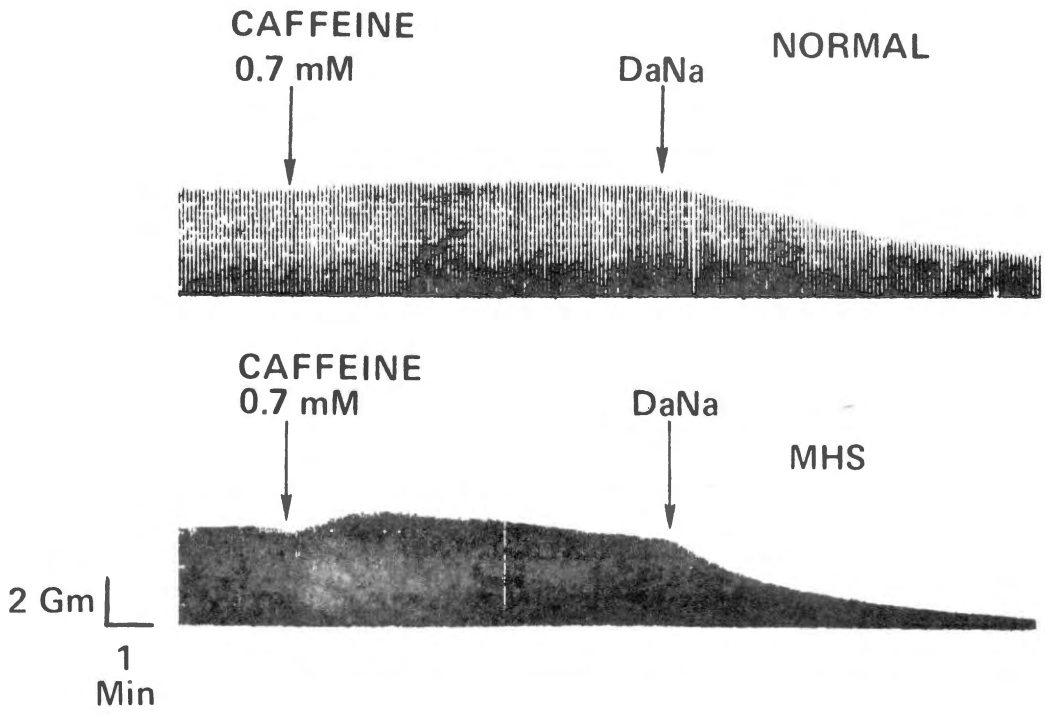

Figure 12. Gracilis Muscle: The effect of dantrolene sodium (DaNa) 2.5 mg/l on the twitch responses in caffeine-treated normal (upper tracing) and malignant hyperthermia susceptible (lower tracing) muscle. Representative tracings from normal pig number 12-4 and MHS pig number 43-4.

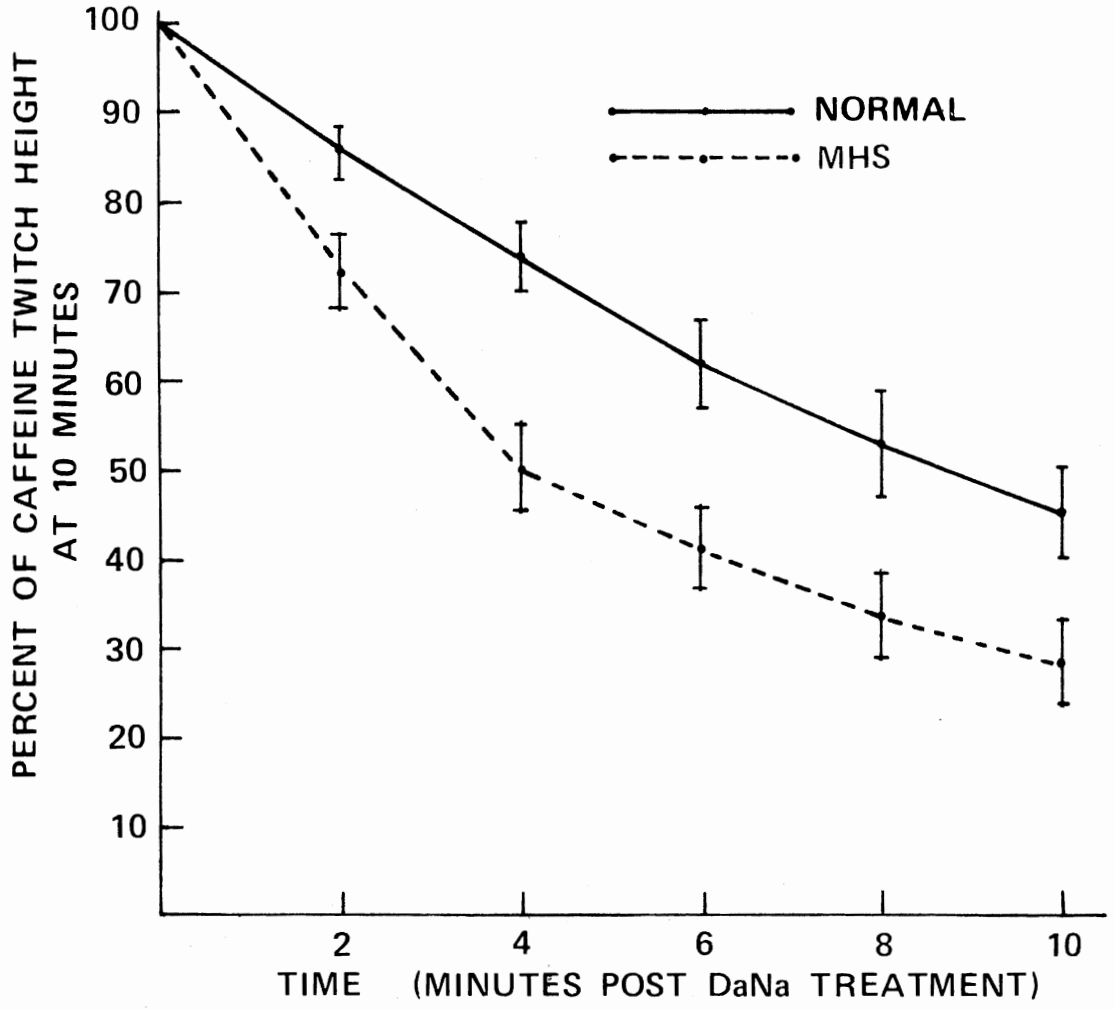

Figure 13. Gracilis Muscle: Effect of dantrolene sodium 2.5 mg/l on caffeine-potentiated twitch. Means ± SEM for 8 muscle strips from 2 normal pigs (12-4, 56-7) and 8 muscle strips from 2 MHS pigs (43-3, 43-4).

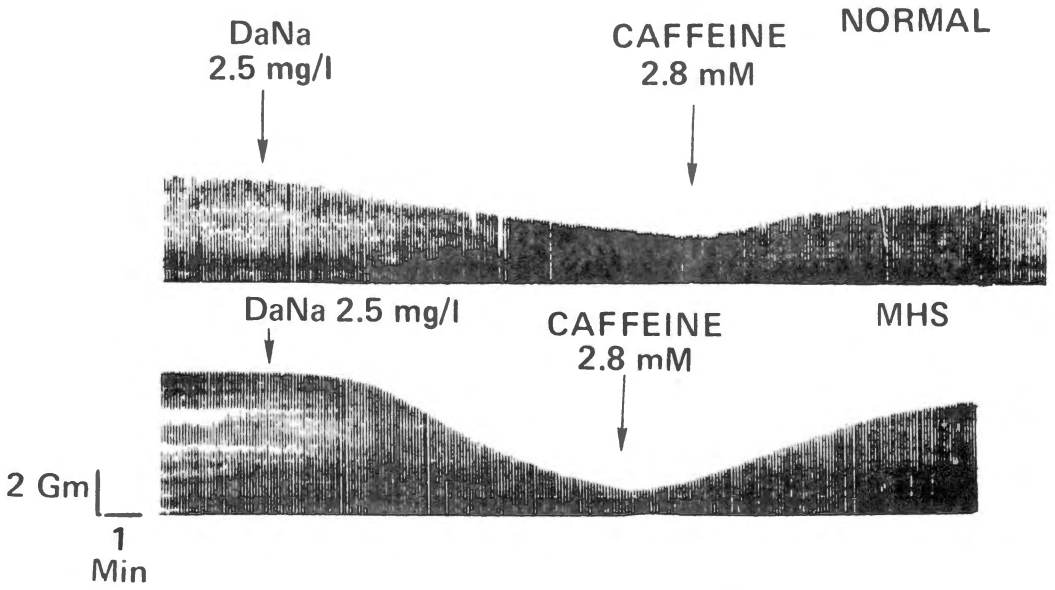

Figure 14. Gracilis Muscle: Effect of 2.8 mM caffeine on twitch response in normal (upper tracing) and malignant hyperthermia susceptible (lower tracing) muscle previously treated with dantrolene sodium (DaNa) 2.5 mg/l. Representative tracings from normal pig number 12-4 and MHS pig number 33-1.

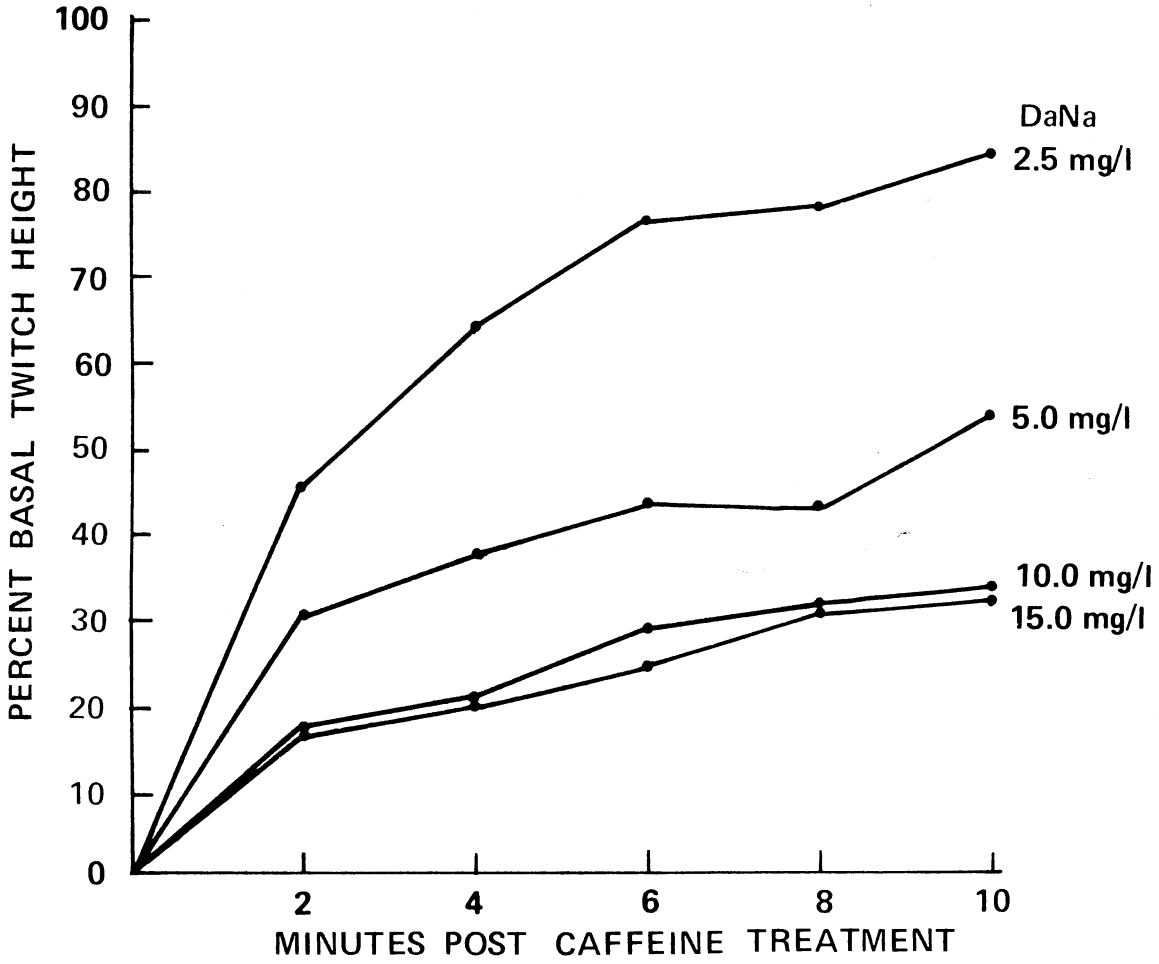

Figure 15. Gracilis Muscle: Effect of 2.8 mM caffeine on twitch response in malignant hyperthermia muscle strips previously treated with various concentrations of dantrolene sodium (2.5 - 15.0 mg/l). Means of 4 muscle strips from 4 MHS pigs.

($\triangle G < 0.1$ gm), delayed and spontaneously relaxed to pretreatment tensions in the continued presence of halothane.

Halothane treatment of MH muscle strips consistently caused contractures (mean $\triangle G$ 1.6 gm, range 0.5 to 3.5 gm) which usually reached maximum tension 2 to 3 minutes after halothane exposure, and in the presence of halothane only slowly relaxed, (Figure 16, Table III, Group I).

Effect of Dantrolene Sodium on Halothane-Induced Contractures. When MH muscle strips were incubated in KRB plus dantrolene sodium 2.5 mg/l, and subsequently exposed to four percent halothane (Table III, Group III) contractures were significantly reduced ($P < 0.01$) compared with contractures observed in Groups I and II. Dantrolene sodium 2.5 mg/l treatment of fibers at the time of maximum halothane contracture (Table III, Group II), significantly ($P < 0.001$) reduced the one-half relaxation time when compared with fibers not treated with dantrolene sodium.

Caffeine-Induced Contractures. Normal muscle demonstrated weak contractures when exposed to increasing concentrations of caffeine. The mean increases in tension for normal muscle in 1.4, 2.8, 5.6, 11.2 and 22.4 mM caffeine were 0, 0.01, 0.05, 0.30 and 0.90 grams respectively. By comparison, MH muscle was much more sensitive to caffeine-induced contractures. The mean increases in tension for MH muscle in 1.4, 2.8, 5.6, and 11.2 mM caffeine were 0.54, 1.13, 2.60, and 6.11 grams respectively (Figure 17).

Effect of Dantrolene Sodium on Caffeine-Induced Contractures. Dantrolene sodium 2.5 mg/l, virtually abolished the caffeine-induced contractures in normal muscle at all concentrations of caffeine tested

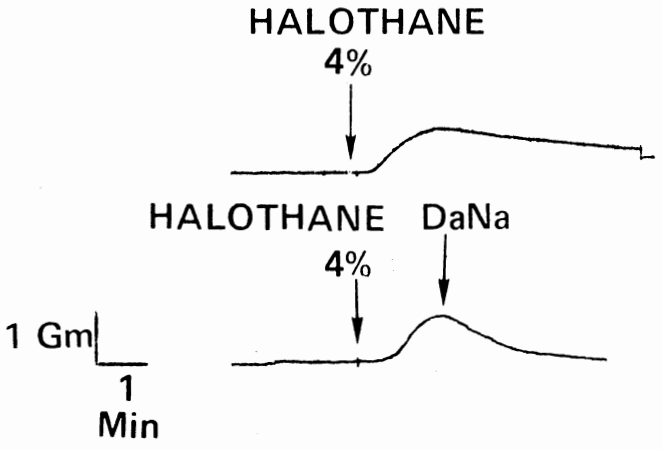

Figure 16. Gracilis Muscle: Effect of halothane 4 percent on isometric rest tension and dantrolene sodium treatment of halothane-induced contractures in porcine malignant hyperthermia muscle. Representative tracings from MHS pig number 26-4.

TABLE III

EFFECTS OF DANTROLENE SODIUM PRETREATMENT ON HALOTHANE-INDUCED CONTRACTURES AND DANTROLENE SODIUM TREATMENT OF HALOTHANE-INDUCED CONTRACTURES

Group		Δ G (gm)	One-Half Relaxation Time (Seconds)
[a]Group I	$\bar{x}$	1.36**	290.6*
	SEM	0.11	17.5
	n	26	26
[b]Group II	$\bar{x}$	1.81**	79.7*
	SEM	0.16	7.4
	n	25	25
[c]Group III	$\bar{x}$	0.22**	
	SEM	0.03	
	n	17	

[a]Group I KRB + four percent Halothane.

[b]Group II KRB + four percent Halothane. Dantrolene sodium 2.5 mg/l at peak tension.

[c]Group III KRB + Dantrolene sodium 2.5 mg/l then four percent Halothane.

**Groups I and II vs. Group III ($P < 0.01$).

*Group I vs. Group II ($P < 0.001$).

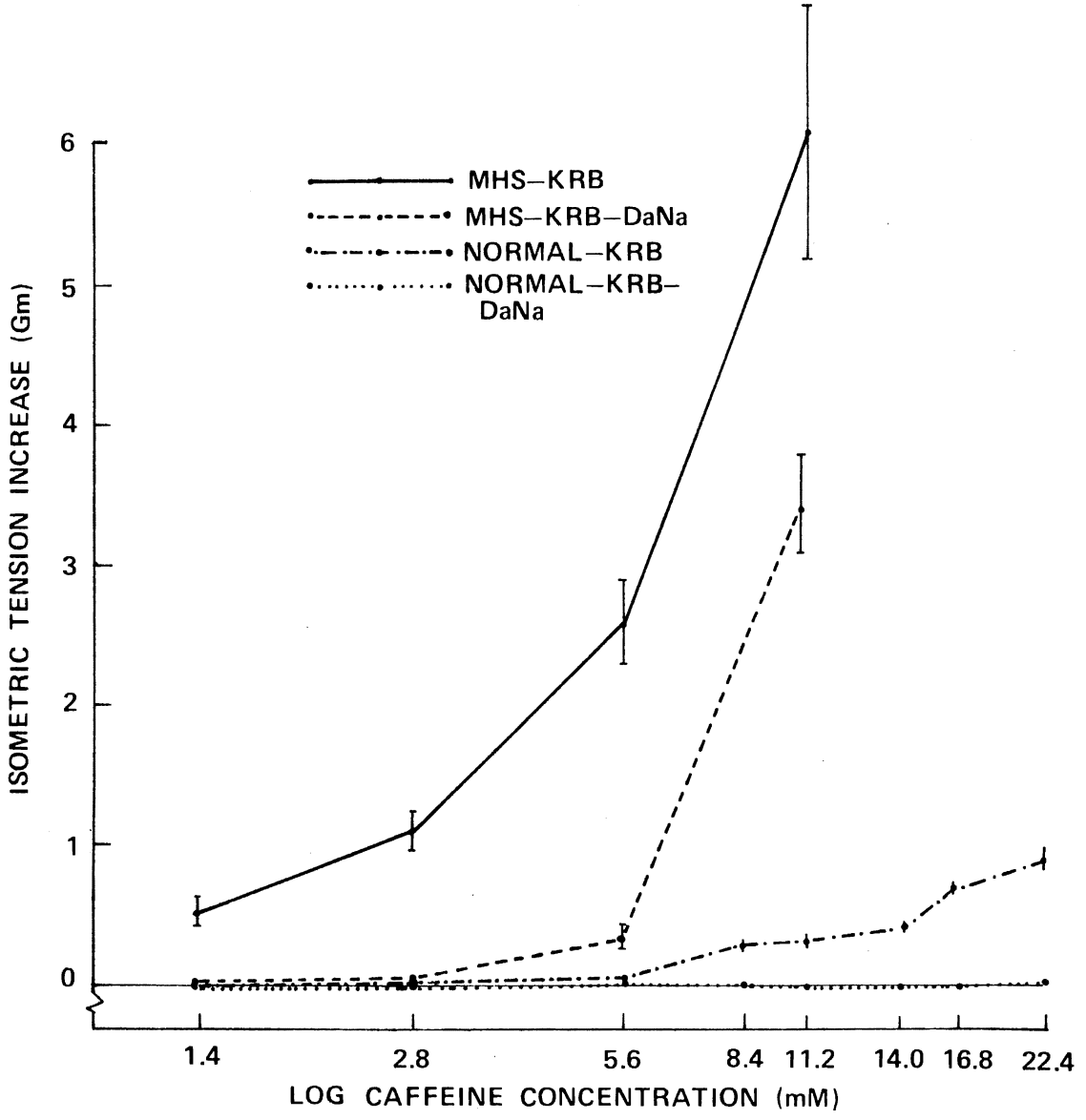

Figure 17. Gracilis Muscle: Effects on isometric tension of caffeine (1.4 - 22.4 mM) in normal porcine muscle (normal - KRB) and dantrolene sodium pretreatment of normal muscle on caffeine-induced contractures (normal-KRB-DaNa). Effects on isometric tension of caffeine (1.4 - 11.2 mM) in malignant hyperthermia susceptible muscle (MHS-KRB) and dantrolene sodium pretreatment of MHS muscle on caffeine-induced contractures (MHS-KRB-DaNa). Means $\pm$ SEM of 8 muscle strips from 4 normal pigs and 20 muscle strips from 10 MHS pigs.

(Figure 17). In MH muscle, the 1.4 mM caffeine induced contractures were prevented completely by dantrolene sodium 2.5 mg/1, while the contractures observed at 2.8, 5.6, and 11.2 mM concentrations of caffeine were all significantly reduced (Figure 17, Table IV).

Caffeine-Halothane Induced Contractures. Muscle from susceptible swine contracted vigorously when exposed to 1.4 mM caffeine and four percent halothane (Figure 18). Pretreatment with dantrolene sodium 2.5 mg/1, did not appear to alter the response to the combined effects of halothane and caffeine irrespective of the order in which they were applied (Figures 19 and 20).

Halothane-Dantrolene Sodium-Caffeine-Induced Contractures in Normal Muscle. Caffeine 2.8 mM treatment of normal muscle which had been exposed to four percent halothane and ten minutes later treated with dantrolene sodium 2.5 mg/1, produced slight (1.4 gm) contracture and obvious twitch potentiation (Figure 21).

Experiment 3: External Intercostal Muscle Isometric Twitch Tension Studies

Halothane-Induced Contractures. External intercostal muscle from susceptible swine demonstrated marked contractures when exposed to halothane (Figure 22). From a resting tension of 1.0 gm, halothane caused an increase in tension of over 7.0 gm during the first 20 minutes. Dantrolene sodium (15 mg/1) rapidly reversed the contracture.

Normal external intercostal muscle exposed to halothane developed slight contractures ($\Delta G < 0.5$ gm) which spontaneously reversed in the continued presence of halothane.

TABLE IV

EFFECTS OF DANTROLENE SODIUM PRETREATMENT
OF MALIGNANT HYPERTHERMIA MUSCLE STRIPS
ON CAFFEINE-INDUCED CONTRACTURES

Caffeine Concentration	Muscle Bath Solution	KREBS Ringer Δ G (gm)	KREBS Ringer + Dantrolene Sodium 2.5 mg/l Δ G (gm)	
1.4 mM	$\bar{x}$	0.54	0	P< 0.01
	SEM	0.13	-	
	n	11	11	
2.8 mM	$\bar{x}$	1.13	0.06	P< 0.001
	SEM	0.16	0.03	
	n	20	17	
5.6 mM	$\bar{x}$	2.60	0.36	P< 0.001
	SEM	0.29	0.08	
	n	16	16	
11.2 mM	$\bar{x}$	6.11	3.46	P< 0.01
	SEM	0.91	0.35	
	n	12	11	

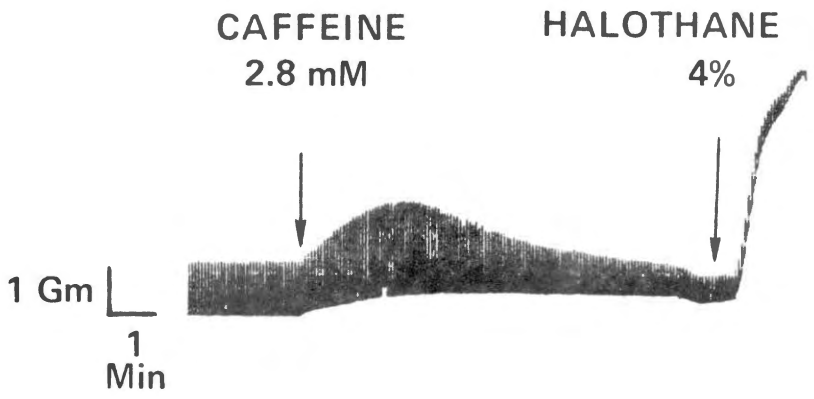

Figure 18. Gracilis Muscle: Effect of 2.8 mM caffeine and 4 percent halothane on isometric twitch and rest tension in porcine malignant hyperthermia muscle. Representative tracing from MHS pig number 33-1.

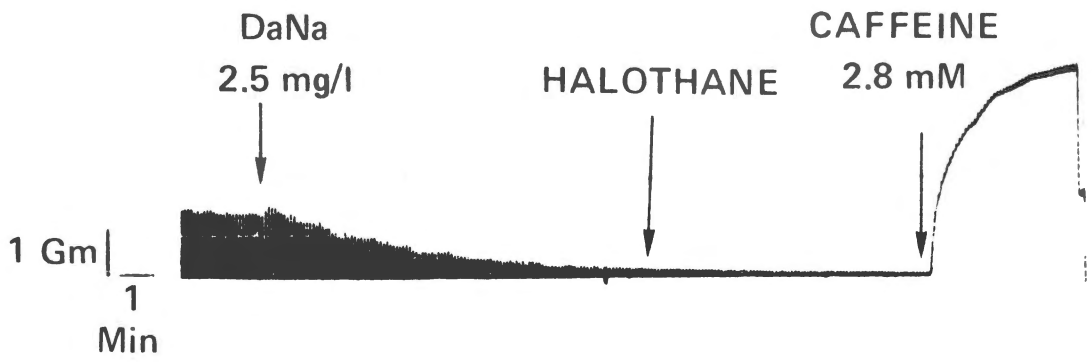

Figure 19. Gracilis Muscle. Effect of 4 percent halothane and 2.8 mM caffeine on isometric twitch and rest tension in malignant hyperthermia muscle previously treated with dantrolene sodium (DaNa) 2.5 mg/1. Representative tracings from MHS pig number 33-7.

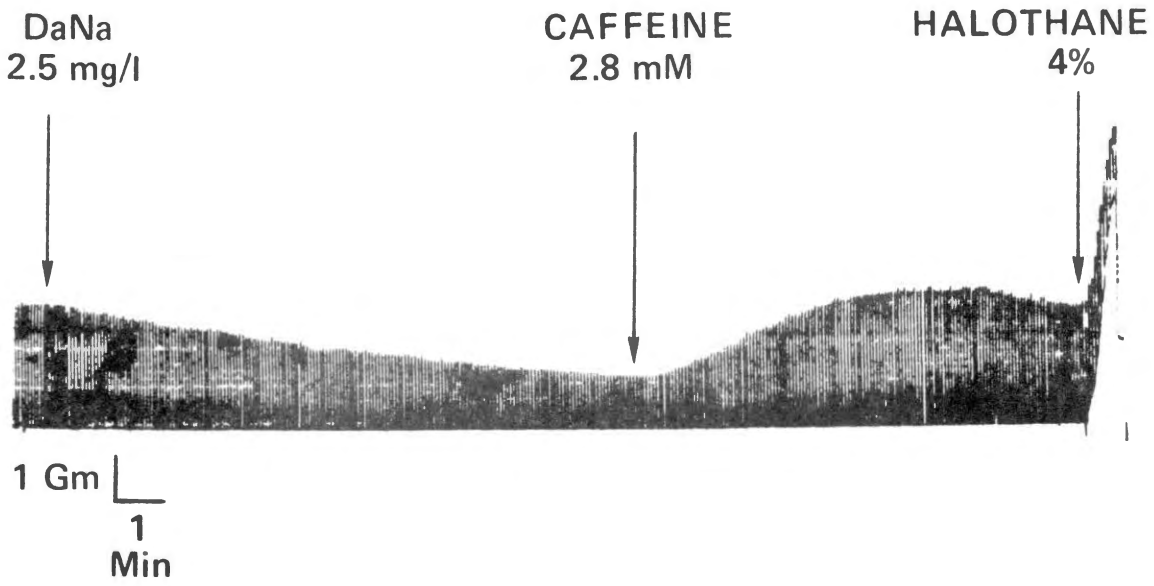

Figure 20. Gracilis Muscle: Effect of 2.8 mM caffeine and 4 percent halothane on isometric twitch and rest tension in malignant hyperthermia muscle previously treated with dantrolene sodium (DaNa) 2.5 mg/l. Representative tracing from MHS pig number 33-1.

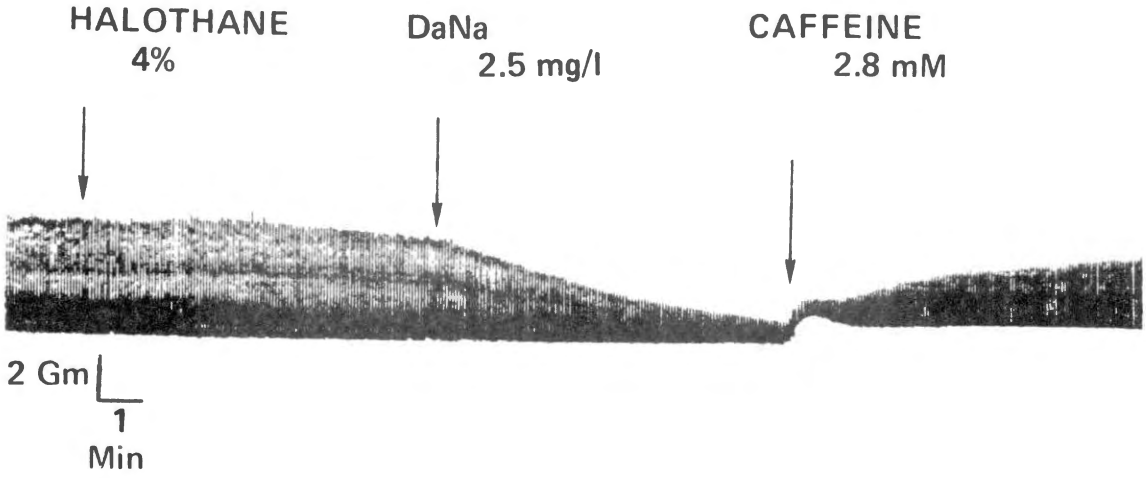

Figure 21. Gracilis Muscle: Effect of 2.8 mM caffeine on isometric twitch and rest tension in normal muscle previously treated with 4 percent halothane and dantrolene sodium (DaNa) 2.5 mg/1. Representative tracing from normal pig number 56-7.

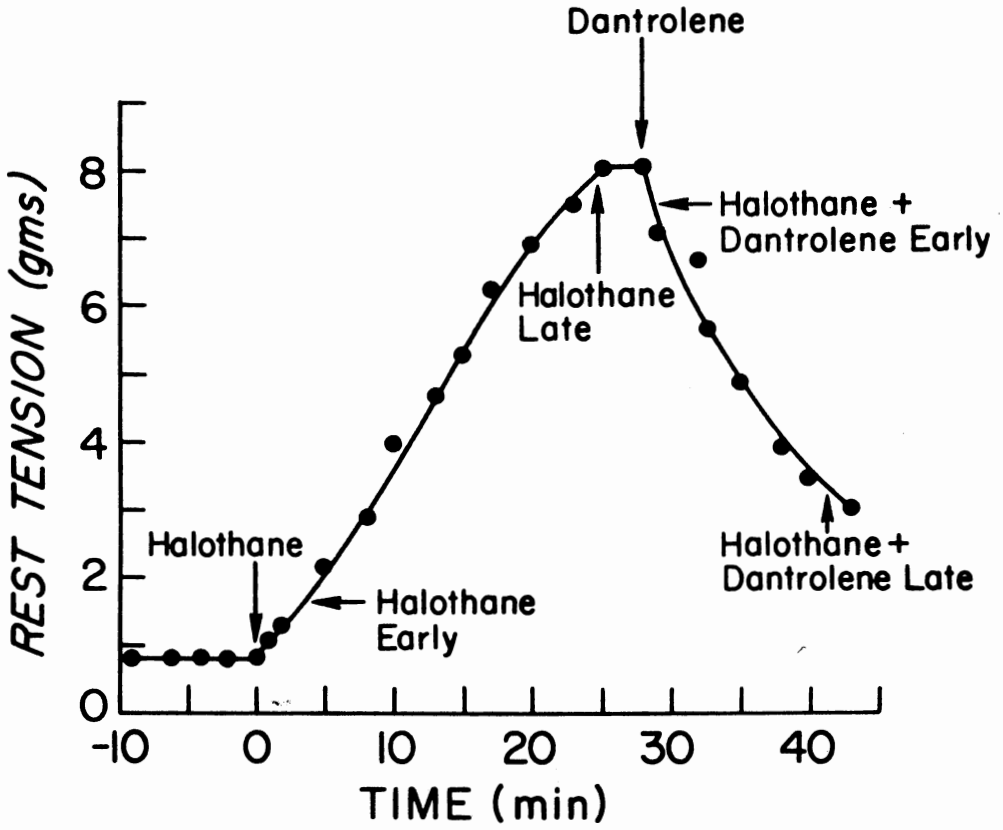

Figure 22. External Intercostal Muscle: Halothane-induced contractures and dantrolene sodium relaxation in porcine malignant hyperthermia muscle. Isometric rest tension is plotted as a function of time for a representative muscle preparation from pig number 33-2. The notations halothane early, halothane late, halothane plus dantrolene early and halothane plus dantrolene late refer to periods during which individual twitch responses were examined.

Time-Course of Twitch Tension Studies. The time courses of twitch tension observed in normal and MH intercostal muscle prior to halothane exposure are shown in Figure 23. Alterations of the time-course of the twitch can best be determined if the curves to be examined have the same maximal (peak) force. The curves were superimposed (normalized) by taking the ratio of peak tensions (normal divided by MH) and multiplying each digitized MH point by this ratio. The resultant normalized curve for the MH control twitch is the dotted line in Figure 23. There was a small, but significant alteration of the relaxation phase in MH muscle. The 50 percent and 75 percent relaxation times in control MH twitch responses were significantly prolonged ($P < 0.05$) when compared with the normal muscle as demonstrated in Table V.

The effects of halothane on the twitch tension, time-course in muscle from MH swine are shown in Figure 24. The time-course of the control twitch tension (prior to the addition of halothane) is shown by the solid line. Early after the addition of halothane, the twitch was slightly potentiated, the time-course of the development of tension and time to peak tension were relatively unchanged as was the time-course of early relaxation. However, the terminal phases of relaxation were prolonged. Twitch responses during the late halothane period had a slightly greater time to peak tension and the entire relaxation phase was markedly slowed.

The typical effects on the time-course of twitch tension apparent in halothane treated MH muscle (Figure 24) and in normal muscle are quantitatively summarized in Table V. The relevant observations from Table V are: a) the 50, 75 and 90 percent relaxation times for MH muscle in the presence of halothane were increased 2.3, 3.8, and 5.6 times respectively, b) the halothane effect on relaxation time was

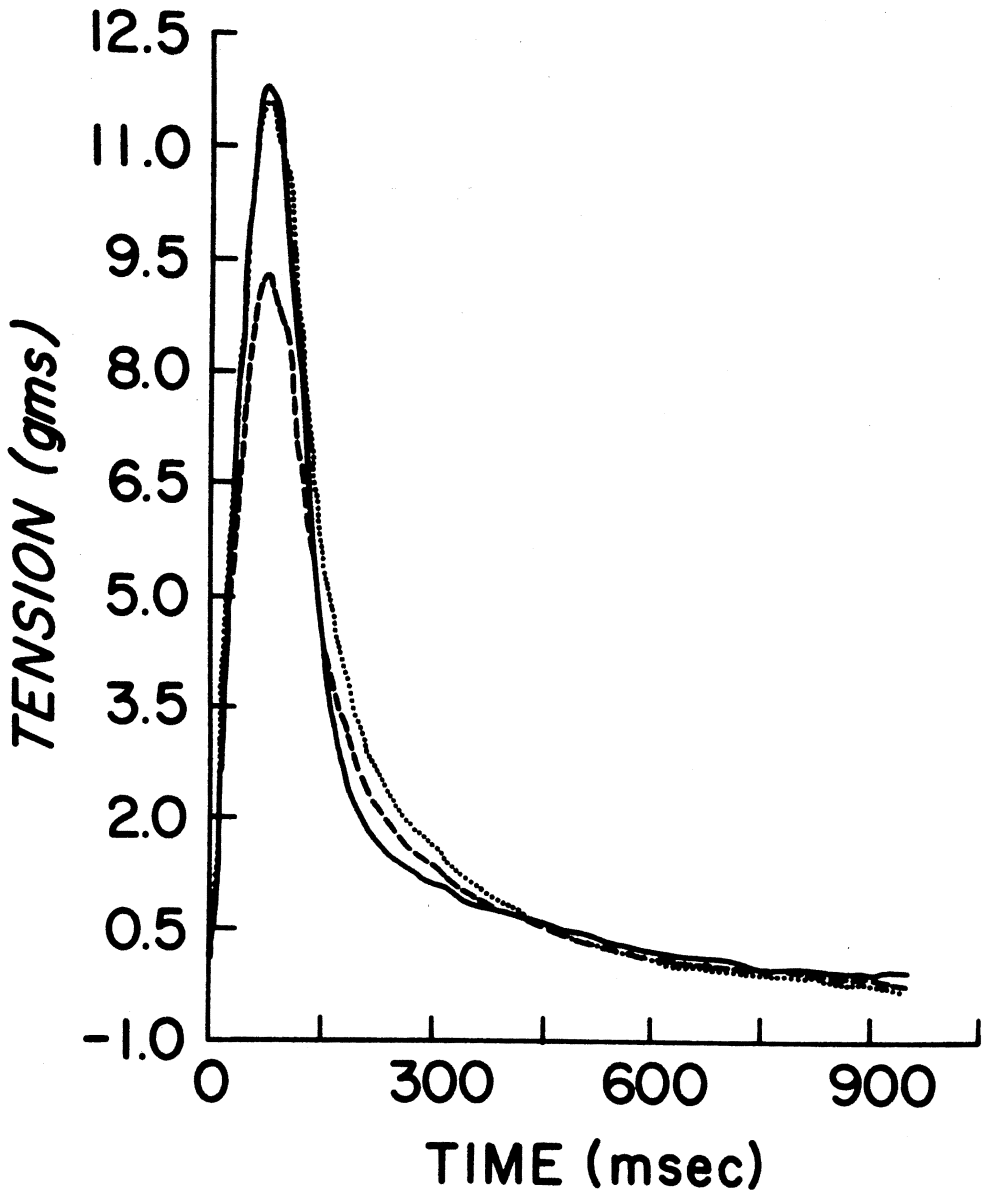

Figure 23. External Intercostal Muscle; Normal versus malignant muscle twitch time course. The time course of a typical twitch from normal muscle (solid line) is compared to the time course of a typical twitch from malignant hyperthermia muscle (dashed line). The dotted line is the normalized twitch for malignant hyperthermia muscle.

TABLE V

EXTERNAL INTERCOSTAL MUSCLE: TWITCH TENSION TIME COURSE IN NORMAL AND MHS PORCINE MUSCLE PRETREATMENT (CONTROL), DURING HALOTHANE AND HALOTHANE-DANTROLENE SODIUM TREATMENT

			TWITCH TENSION TIME COURSE (MSEC)										
Muscle Source	Treatment		Developing Tension					Peak	Declining Tension				
			10%	25%	50%	75%	90%	100%	10%	25%	50%	75%	90%
Normal[a]	Control	N[c]	19	19	19	19	19	16	16	16	16	15	12
		Mean	5.8	13.2	25.0	38.5	50.3	71.8	26.1	45.3	77.0*	135.0*	271.0
		SEM	0.6	0.9	1.1	1.5	1.7	2.1	0.7	1.4	2.6	6.2	9.0
MHS[b]	Control	N	28	28	28	28	28	28	26	26	26	21	21
		Mean	8.1	14.3	25.0	37.4	49.1	71.5	26.1	45.6	82.0*	168.0*	313.0
		SEM	0.7	0.6	0.5	0.6	0.7	1.1	0.8	1.3	2.3	10.5	13.7
Normal	Halothane	Early	16.8	13.6	26.2	40.7	53.1	73.5	33.4	56.4	97.0	171.0	392.0
		Late	5.9	11.7	26.4	45.8	56.5	83.0	27.4	47.3	92.0	196.0	437.0
MHS	Halothane	Early	12.4	21.4	27.7	46.2	58.0	77.2	24.6	43.1	76.0	184.0	406.0
		Late	14.2	18.9	36.6	58.5	71.1	109.3	56.1	90.0	175.0	507.0	1518.0
		After wash	8.7	14.6	29.3	45.7	58.3	85.1	28.9	55.7	105.0	217.0	-
MHS	Dantrolene Sodium	Early after Dantrolene	14.8	25.2	39.3	63.1	86.4	129.5	77.6	214.0	548.0	1162.0	-
		Late after Dantrolene	13.8	24.7	39.8	59.7	87.2	135.5	72.1	158.0	369.0	745.0	1219.0

[a] Twitch measurements from four intercostal preparations from two normal pigs (C_1, C_2).

[b] Twitch measurements from five intercostal preparations from three MHS pigs (33-1, 33-2, 34-6).

[c] N refers to the number of individual twitches examined.

* Normal versus MHS significantly different ($P < 0.05$).

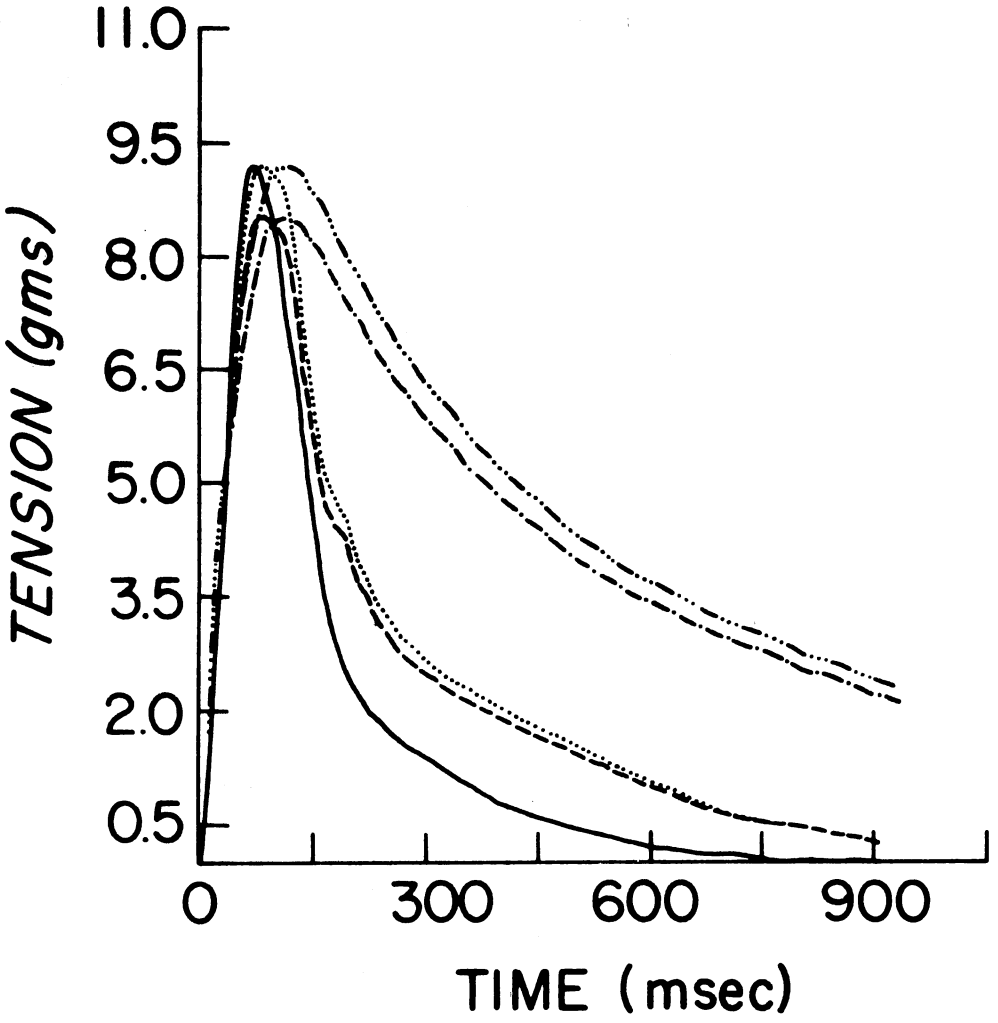

Figure 24. External Intercostal Muscle: Effect of halothane on twitch time course in malignant hyperthermia muscle from MHS pig number 33-2. The solid line is a reproduction of digitized data for a twitch prior to exposure to halothane. The dashed line is the time course of a twitch early in the halothane effect and the dotted line is that same twitch normalized so that the peak twitch magnitude is the same as the control magnitude. The dashed line separated by one dot is a twitch time course at the maximum halothane effect, and the dashed line separated by three dots is that twitch normalized to the control peak twitch magnitude.

reversible when halothane was removed from the bath, and c) a qualitatively similar halothane effect on relaxation time occurred in muscle from normal swine, however over equivalent periods of halothane exposure, the effect in normal muscle was quantitatively less.

Dantrolene sodium 15 mg/l reversed the halothane-induced contractures (Figure 22) and twitch tension potentiation, but did not abolish the halothane-induced delayed relaxation phase of the twitch response in MH muscle (Figure 25).

Threshold of Mechanical Activation Studies

Experiment 4: Mechanical Threshold Measurements

The mean rheobase for 30 normal muscle fibers from two pigs was -65 mV with the curve leveling off between 100 and 500 msec, whereas the mean curve for 45 MH fibers from three pigs was markedly shifted in an approximately parallel manner to a rheobase of near -80 mV. The local contractions produced in the MH fibers around the microelectrodes often induced an abnormal, slowly propagating wave of contraction that could result in destruction of the fiber interior.

Dantrolene sodium 2.5 mg/l added to the bath shifted the mean strength-duration curve of 15 MH fibers from two pigs toward control values with a rheobase of -55 mV (Figure 26).

There was no apparent difference in resting potentials between MH and normal fibers in a small sample, although a controlled study of resting membrane potentials was not undertaken.

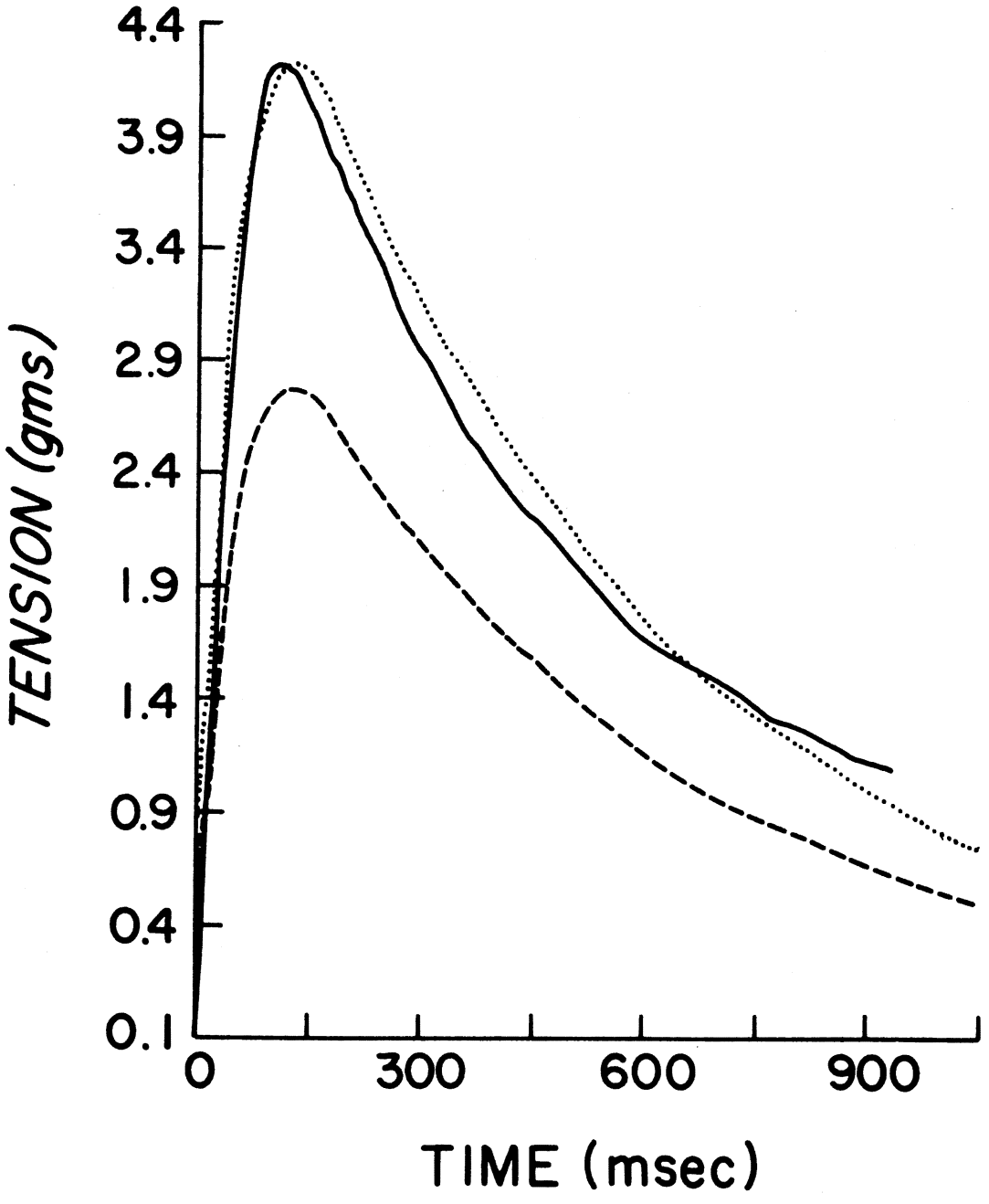

Figure 25. External Intercostal Muscle: The effects of dantrolene sodium 15.0 mg/l on the time course of twitch tension during halothane exposure in malignant hyperthermia muscle. The time course of a twitch response 10-15 minutes after dantrolene sodium treatment (the dashed line) is compared with the time course of a twitch response in the late halothane period (the solid line). The dotted line is the normalized late halothane plus dantrolene twitch response.

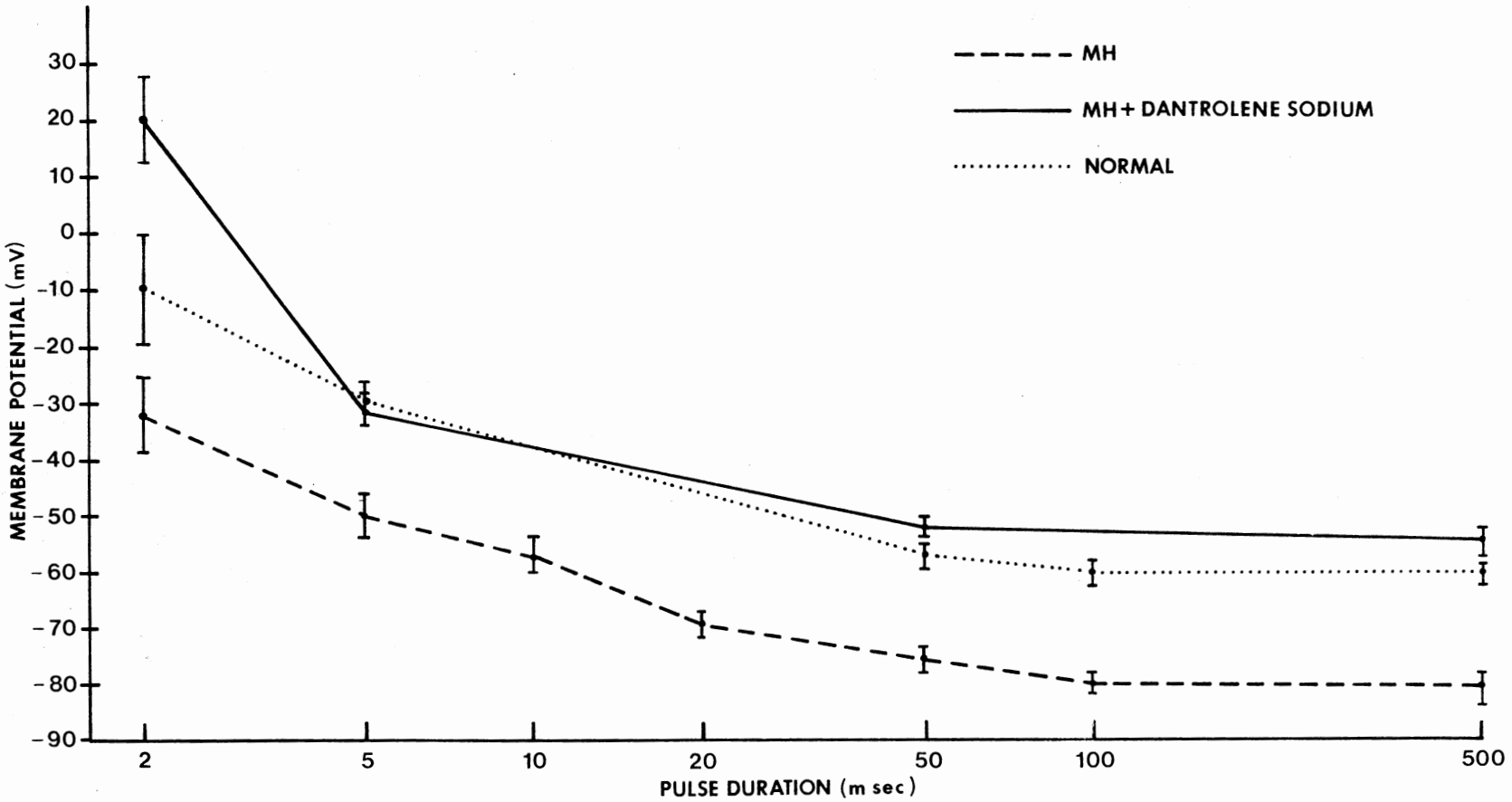

Figure 26. External Intercostal Muscle: Strength-duration curve for mechanical threshold of contraction in normal, untreated malignant hyperthermia and dantrolene sodium treated malignant hyperthermia muscle. Means $\pm$ SEM of 30 fibers from two normal pigs (C_1, C_2); 45 untreated fibers from three MH pigs (33-1, 33-2, 34-6); 15 dantrolene sodium fibers from two MH pigs (33-2, 34-6).

CHAPTER V

DISCUSSION

Introduction

Interpretation of data from pharmacological testing of isolated muscle strips is complicated by the inherent variability of responses. Whereas malignant hyperthermia was previously considered to be inherited through a single dominant gene (1, 4) the wide spectrum of phenotypes recognized relative to MH susceptibility (6, 7, 8) is strong evidence for a multifactorial genetic basis for MH. The previously accepted screening procedures for MH susceptibility should then be re-examined in light of these variable degrees of susceptibility to MH. Variation in responses of isolated muscle strips from MH positive swine to specific treatments is probably due in part to differences in inherited susceptibility.

The use of the porcine MH syndrome as an animal model for MH in humans is widely accepted. Except for minor differences, the two syndromes appear nearly identical and much knowledge of the MH syndrome in man has resulted directly from investigations of swine susceptible to MH.

This study utilized an inbred colony of MH susceptible swine in which almost 100 percent of each litter was MH positive. However, considerable variation was still observed among MH positive pigs in their reactions to in vivo halothane-succinylcholine challenge and/or

in vitro responses of isolated muscle strips to various pharmacological treatments.

Characteristics of Untreated Malignant Hyperthermia Muscle

Results from this study suggest that untreated porcine MH muscle has significantly lower mechanical threshold of activation than normal porcine muscle (Figure 26). Malignant hyperthermia muscle requires a very minor depolarizing signal of reasonable duration ($>$100 msec) to initiate contraction. No apparent abnormality in mechanical threshold of activation was observed in one investigation of human MH muscle fibers (29). However, the stepwise chemical depolarization used in the human study produces much slower changes in membrane potential compared with an electrical depolarization technique used in this porcine study. These apparently conflicting results may represent actual differences in mechanical threshold for human and porcine MH muscle fibers. They may also indicate an adaptation process which is associated with repriming of charge movements and which is more evident in fibers subjected to slower depolarization (114).

The wide range of triggering stimuli in MH (inhalation anesthetic agents, depolarizing neuromuscular blocking drugs, excitement, exercise, heat stress) may initiate the MH syndrome by causing sufficient depolarization of the sarcolemma to reach threshold of mechanical activation with subsequent muscle contraction and hypermetabolism.

The abnormal, slowly propagating waves of contraction seen around the microelectrodes in porcine MH muscle have been previously observed

in human dystrophic myotonic fibers (114). However in most other respects MH and dystrophic myotonic muscle fibers are dissimilar (115).

Malignant hyperthermia susceptible muscle and normal porcine muscle had similar resting membrane potentials which were similar to values reported elsewhere for porcine MH muscle (110).

The lowered mechanical threshold in untreated porcine MH muscle fibers is consistent with the hypothesis that the inherent abnormality is associated with defects in the T-tubule-SR coupling system for calcium release and/or the SR system for calcium uptake within the muscle fiber (54).

Malignant hyperthermia susceptible muscle also demonstrated a slightly delayed relaxation phase of the twitch response which was not observed in normal porcine muscle (Figure 23, Table V). These observations suggest that the calcium uptake process is impaired in untreated MH muscle, and may provide an explanation for the abnormally low mechanical threshold observed in porcine MH muscle.

Effects of Dantrolene Sodium

This study demonstrates quantitatively similar twitch tension inhibition by dantrolene sodium in normal and MH porcine muscle (Figure 2). These effects are similar to those reported for dantrolene sodium in other mammalian skeletal (105) and amphibian muscle (99). In porcine MH muscle, maximum twitch inhibition by dantrolene sodium occurred at concentrations of 10.0 to 15.0 mg/l, although significant effects were noted at all concentrations tested (Figure 3). The evidence from this study and previous reports (82, 99) indicates that for maximum effect, dantrolene sodium must be present in a concentration

which is close to its maximum solubility in physiological solutions (15 mg/l) and even at this concentration, complete twitch inhibition is still not observed.

Dantrolene sodium had no effect on isometric rest tension in untreated normal and MH porcine muscle, which confirms earlier reports on its apparent inactivity in muscle with normal tonus (82, 94).

The strength-duration curves for mechanical threshold of contraction in porcine MH muscle fibers were shifted towards normal values by dantrolene sodium (Figure 26). This parallel elevation of the rheobase of the strength-duration curve by dantrolene sodium has been previously observed in normal goat, rat and frog skeletal muscle (102).

Recent studies by Morgan and Bryant (102) suggest dantrolene sodium acts at two steps in EC coupling. In single fiber mechanical threshold studies conducted under voltage clamping conditions, they demonstrated competitive antagonism between dantrolene sodium and a specific calcium ionophore and concluded that dantrolene sodium may act by decreasing the mobility of natural ionophores in the SR membrane. Van Winkle has also recently presented evidence that dantrolene sodium suppresses release of calcium from isolated SR (101). The second site of action for dantrolene sodium suggested by Morgan and Bryant is the transmission of signal between T-tubule and terminal cysternae of the SR. Their evidence for action at this site was based on the effect of dantrolene sodium on voltage dependent charge movements. Earlier studies by Putney and Bianchi (96) demonstrated that dantrolene sodium caused a decreased calcium influx during a twitch response and also during potassium-induced depolarization, which suggested dantrolene sodium has a superficial site of action, possibly interfering with T-tubule to SR

transmission of impulses. Nelson and Denborough (105) have also proposed this step in the EC coupling process as a possible site of dantrolene sodium action.

Effects of Halothane

This study also demonstrates potentiation of twitch tension by halothane in both normal and MH susceptible muscle (Figures 4 and 5). Similar twitch potentiation by halothane in normal human muscle has been reported previously (86, 105). Halothane also significantly delayed the relaxation phase of twitch tension response in MH muscle and to a lesser extent in normal muscle (Figure 24, Table V). This effect has been previously described in frog muscle (90). The delayed relaxation phase observed in untriggered MH muscle suggests an initial defective calcium uptake mechanism which is grossly exaggerated in the presence of halothane. Studies on isolated SR from MH susceptible humans indicate that they have decreased rates of calcium uptake in the presence of halothane (31, 32). Similar porcine studies have proved inconclusive but some reports demonstrate halothane depression of calcium uptake by isolated SR (33, 34). However direct comparison of calcium uptake data from isolated SR experiments with twitch tension time-course events may not be valid due to the vast differences in rates of reactions monitored (minutes versus milliseconds).

Halothane induced contractures in MH gracilis and external intercostal muscle (Figure 16, Table III) while insignificant contractures were observed in halothane-treated normal muscle. The marked halothane-induced contractures observed in MH muscle confirms reports of this response in human and porcine MH muscle at 37^{o}C (29, 30, 103, 104).

The absence of halothane-induced contractures in some MH muscle tested at lower temperatures (31) may be explained by temperature dependent alterations in mechanical threshold rheobase values reported by Morgan and Bryant (102). In general, mechanical threshold rheobase values become more positive as temperature decreases. Since at 37°C the porcine MH fibers appear to have mechanical thresholds very close to their resting membrane potentials, the effect of lowering temperature could be to render previously sensitive fibers less so.

The halothane-induced effects observed in MH muscle (twitch tension potentiation, delayed relaxation of twitch tension and marked sustained contractures) reflect a persistent elevation of free myoplasmic calcium concentration. This could result from normal calcium release and defective calcium uptake, from abnormal calcium release and normal uptake or from both abnormal calcium release and impaired uptake. Isolated SR studies tend to support halothane-induced impairment of calcium uptake while Endo et al. (92) have suggested halothane may have a direct caffeine-like action on SR permeability to calcium. Nelson and Denborough (105) have proposed that halothane amplifies the T-tubule-SR signal probably through increased voltage dependent charge movement with increased numbers of calcium channels opened in the terminal cysternae of SR.

Effects of Caffeine

Caffeine in low concentrations potentiated twitch height in both normal and MH muscle without producing contractures (Figures 7, 8, 9 and 10). At equivalent caffeine concentrations, normal muscle twitch potentiation was at least as great as that observed in MH muscle

(Figures 8 and 10), suggesting that with respect to twitch responses MH muscle and normal muscle are equally sensitive to low concentrations of caffeine. Low concentrations of caffeine are reported to potentiate twitch tension by delaying calcium uptake (81).

The rapid decay of twitch response observed in MH muscle undergoing simultaneous caffeine-induced contractures (Figures 9 and 11) is difficult to explain. Physiologically a decreased twitch response at submaximal contracture tension suggests a reduced amount of activator calcium is released with each successive electrical depolarization or alternatively there is a progressive depletion of available energy. The latter alternative can be eliminated since treatment of these fibers with halothane results in marked potentiation of contracture response (Figure 18) which must be energy dependent. A reasonable explanation for this phenomenon is currently unavailable (114).

Malignant hyperthermia muscle demonstrated greater sensitivity to higher concentrations of caffeine. Contractures occurred in MH muscle at lower concentrations of caffeine, and at equivalent caffeine concentrations, MH muscle developed significantly greater contractures (Figure 17). These observations confirm previous reports of MH muscle hypersensitivity to caffeine-induced contractures (6, 29, 32).

Caffeine at higher concentrations induces calcium release from SR thus initiating contraction. Endo (71) has clearly demonstrated the labilization of calcium-induced calcium release from SR by higher concentrations of caffeine. The low concentrations of caffeine required to initiate contractures in MH muscle, suggest the conditions required for calcium-induced calcium release i.e. fully calcium loaded SR, low free myoplasmic magnesium and/or high free myoplasmic calcium, may exist

in untriggered MH muscle. It is currently believed, calcium-induced calcium release is not a physiologically important mechanism in EC coupling in normal muscle (67), which may explain the relative refractoriness of normal muscle to caffeine-induced contractures.

Caffeine-Halothane Interactions

Muscle from MH susceptible swine developed significant contractures when exposed simultaneously to halothane and caffeine (Figure 18), confirming earlier reports of this effect (6, 24, 104). Since caffeine and halothane have similar effects on calcium release mechanisms, and possibly also on uptake processes, reciprocal enhancement of each drug's effect on skeletal muscle is not surprising. Nelson and Denborough (105) have proposed that halothane and caffeine have agonistic effects at two separate but unidentified sites involved in transmission of the excitatory signal from the T-tubule to SR.

Effect of Dantrolene Sodium on Halothane-Induced Responses

Dantrolene sodium in this study reversed halothane-induced twitch potentiation in normal and MH muscle (Figures 5, 6, and 25), significantly prevented and reversed halothane-induced contractures in MH muscle (Figures 16 and 22 and Table III) but had no effect on halothane-induced delayed relaxation of twitch tension in MH external intercostal muscle (Figure 25). The halothane-induced delay in twitch tension relaxation is apparently then not the only factor contributing to the twitch potentiation and contractures observed in MH and normal muscle. Halothane appears to act at more than one site in the EC coupling

process in both normal and MH muscle, however quantitative differences in halothane responses of normal and MH muscle are apparent.

Halothane must affect both calcium release and calcium uptake during muscle contraction. The action of dantrolene sodium in skeletal muscle is to reduce activator calcium release from SR either directly by decreasing SR permeability to calcium or indirectly by attenuation of the T-tubule to SR excitatory signal (102).

These observations are consistent with the presence of both uptake and release defects in MH muscle which are sensitive to halothane. The halothane potentiated uptake defect appears insensitive to the antagonistic effects of dantrolene sodium, while the release process is significantly attenuated by dantrolene sodium.

This study indicates that low concentrations of dantrolene sodium confer significant protection against halothane-induced contractures in isolated MH muscle. It also demonstrates the apparent effectiveness of dantrolene sodium in reversing existing halothane-induced contractures in isolated MH muscle. This evidence supports the report by Harrison (48) of the apparent effectiveness of this drug in reversing and preventing the development of halothane-induced malignant hyperthermia in known susceptible swine.

This present study demonstrates that dantrolene sodium prevents or at least attenuates and reverses the functional responses occurring in halothane-induced malignant hyperthermia. There is no direct evidence from this study to indicate that the metabolic changes present in malignant hyperthermia are prevented or reversed by dantrolene sodium. However Gronert et al. (49) have recently demonstrated dantrolene sodium reversal of metabolic and biochemical changes in porcine MH.

Effect of Dantrolene Sodium on Caffeine-Induced Responses

Dantrolene sodium inhibited twitch tension in caffeine-treated normal and MH muscle (Figures 12 and 13) which confirms Nelson and Denborough's observations in normal human skeletal muscle (105). In a limited number of swine, dantrolene sodium appeared to inhibit caffeine-potentiation of twitch to a greater extent in MH muscle (Figure 13). This effect may be related to the unexplained spontaneous twitch tension decay observed in caffeine-treated MH muscle.

Pretreatment of normal and MH muscle with dantrolene sodium however did not prevent subsequent caffeine-potentiation of twitch tension (Figure 14). These observations suggest a sequential effect for combinations of dantrolene sodium and caffeine. In one case (caffeine followed by dantrolene sodium), predominantly twitch inhibition was observed while in the other case (dantrolene sodium followed by caffeine), twitch potentiation or pretreatment twitch tensions were observed.

In contrast to Nelson's observations of increased caffeine-potentiation of twitch tension with increasing concentrations of dantrolene sodium in an undefined number of MH muscle strips and animals (106), this study demonstrated decreased caffeine-induced twitch potentiation with increasing concentrations of dantrolene sodium (Figure 15). Although this study involved a limited number of porcine muscle strips, it also demonstrated a dose related effect of dantrolene sodium on caffeine-induced twitch tension up to 10 to 15 mg/l. This provides additional evidence of maximum dantrolene sodium effect at concentrations

near saturation in physiological solutions. A similar effect was observed for 5.6 mM caffeine and dantrolene sodium 2.5 mg/l.

Dantrolene sodium significantly attenuated caffeine-induced contractures in normal and MH muscle (Figure 17 and Table IV). Dantrolene sodium has been reported both to affect (94) and not to affect (99) caffeine-induced contracture responses in isolated amphibian muscle preparations. As suggested by Ellis and Carpenter (82), these conflicting reports can be explained by variations in dantrolene sodium and caffeine concentrations and also by variation in the sensitivities of different muscle preparations to caffeine.

Caffeine-induced contractures in normal human skeletal muscle were delayed and significantly reduced in magnitude by dantrolene sodium (105). Similar attenuation of caffeine-induced contractures by dantrolene sodium in porcine MH muscle has recently been reported (106).

Attenuation of caffeine-induced contractures in normal and MH muscle by dantrolene sodium can be explained if the current theories on each drug's mechanism of action are assumed to be correct. Endo (71) has clearly demonstrated labilization of calcium-induced calcium release from isolated SR by caffeine while Van Winkle (101) and Morgan and Bryant (102) have demonstrated dantrolene sodium decreased SR permeability to calcium. These two antagonistic actions probably involve common sites in SR membranes. Twitch tension potentiation by caffeine at sub-contracture concentrations occurs due to decreased rates of calcium uptake by SR (81). Dantrolene sodium would not therefore prevent caffeine potentiation of twitch responses since Brocklehurst (98) and Van Winkle (101) have clearly demonstrated normal rates of uptake for calcium in dantrolene sodium treated SR.

Effect of Dantrolene Sodium on Caffeine-Halothane-Induced Responses

Although dantrolene sodium significantly attenuated individually caffeine or halothane-induced contractures in MH muscle, it did not prevent marked contractures induced by combinations of halothane and caffeine (Figures 19 and 20). The synergistic actions of halothane and caffeine on calcium-induced calcium release from SR, and apparent competitive antagonism by dantrolene sodium could explain these observations.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Microelectrode measurements of electrophysiological characteristics were made of isolated external intercostal muscle fibers from normal and MH susceptible swine. Isometric twitch tension and contracture responses to varying pharmacological treatments were also studied in isolated porcine gracilis and external intercostal muscle strips.

Untreated porcine MH muscle fibers had significantly lower mechanical thresholds of contraction than normal porcine muscle. A slightly prolonged relaxation phase of twitch tension was also observed in untreated porcine MH muscle.

Halothane produced marked sustained contractures in porcine MH muscle while normal muscle was usually unreactive to halothane. Malignant hyperthermia muscle was also more sensitive to caffeine-induced contractures than was normal porcine muscle.

Halothane potentiated twitch tension responses in both normal and MH porcine muscle and exaggerated the delayed relaxation phase of twitch response especially in MH muscle.

Dantrolene sodium elevated the rheobase of MH muscle fiber's strength-duration curve for mechanical threshold, reversed and prevented halothane-potentiation of twitch tension in MH and normal muscle. but had no effect on halothane-induced twitch relaxation delay.

Dantrolene sodium also prevented and reversed halothane-induced contractures in MH muscle and significantly attenuated caffeine-induced contractures in normal and MH muscle.

Caffeine potentiated twitch tension equally in normal and MH muscle, and induced contractures in MH muscle at lower concentrations than those observed in normal muscle.

These observations are consistent with the hypothesis that MH arises in susceptible animals due to persistent elevation of free myoplasmic calcium concentration. Muscle from MH susceptible swine has a pre-existent defect in calcium uptake which is exaggerated by exposure to halothane. It is proposed that the calcium uptake defect accounts for the lower mechanical threshold of activation observed in MH and renders this muscle extremely sensitive to depolarizing physicochemical stimuli. In particular the calcium uptake defect creates favorable conditions for activation of calcium-induced calcium release from SR by halothane and/or caffeine. Dantrolene sodium has antagonistic effects on these processes possibly by stabilizing SR permeability to calcium and/or by attenuating excitatory signals at the T-tubule to SR step in EC coupling.

This study indicates possible prophylactic and therapeutic value of dantrolene sodium in malignant hyperthermia and suggests that the previously reported effectiveness of dantrolene sodium in preventing and treating halothane-induced contractures may be due, at least in part, to its direct effect on muscles.

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