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A Laboratory Animal Model for Malignant Hyperpyrexia[1]

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ABSTRACT

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Standard laboratory rabbits which are not genetically susceptible to malignant hyperpyrexia were anesthetized with either halothane or pentobarbital. Administration of caffeine in 125 mg increments produced a syndrome strongly resembling malignant hyperpyrexia in rabbits anesthetized with halothane. All these animals became rigid, hyperpyrexic, acidotic and hyperkalemic, whereas caffeine-treated, pentobarbital-anesthetized animals developed only mild acidosis. Pentobarbital alone and halothane alone caused no changes in measured variables. This model for malignant hyperpyrexia resembles the naturally occurring disease more closely than several preceding pharmacologic models.

The syndrome of malignant hyperpyrexia (MH) is a rare but often fatal disease which occurs in susceptible patients on exposure to a variety of anesthetic drugs. The principle features of the syndrome are hyperpyrexia, respiratory and metabolic acidosis and skeletal muscle rigidity (Britt, 1974). A similar syndrome has been reported in other animal species (DeJong *et al.,* 1976; Bagshaw *et al.,* 1978; Rosenberg and Waldron-Mease, 1979). In particular, the porcine stress syndrome has been suggested as a model of the human syndrome (Jones *et al.,* 1973). Because of the requirement for large animal handling facilities, only a few centers have been able to use the porcine model. Attempts at producing the MH syndrome in standard laboratory animals have not been completely successful. A new small animal model of MH would be useful in studying the pathophysiology and treatment of MH.

Caffeine is known to cause contractures in isolated muscle strips *in vitro,* and these contractures are potentiated by halothane (Strobel and Bianchi, 1971). Muscle strips from MH-sensitive humans (and pigs) are more sensitive to caffeine (*i.e.,* they develop contractures at lower caffeine concentrations than normal muscle strips) and this forms the basis for one of the *in vitro* diagnostic tests for MH (Kalow *et al.,* 1970). By administering caffeine to halothane-anesthetized rabbits we have produced many of the classic features of MH. The characterization of this new model is presented in this paper.

Methods

Male New Zealand White rabbits weighing 2 to 3 kg were anesthetized with either halothane or pentobarbital. The femoral artery and vein were cannulated, a tracheostomy was performed and the animal was attached to a Harvard small animal ventilator equipped with an expiratory reservoir bag allowing for spontaneous ventilation. The ventilator was set to deliver 10 ml/kg 30 times/min. Mean arterial blood pressure was monitored with an aneroid manometer, and a precordial electrocardiogram lead was continuously recorded. Rectal temperature was monitored continuously with a Yellow Springs Telethermometer. To avoid environmental cooling, normal body temperature was maintained between 39.5 and 40.5°C (Altman and Ditmer, 1973) by intermittent use of a heating pad which was discontinued when the rectal temperature reached 40.0°C. Blood pressure and vascular volume were maintained with intermittent infusions of normal saline (maximum 100 ml/kg). Vital signs and arterial blood gases measured every 30 min were stable for at least 1 hr before the experimental period.

Four groups of animals were studied. In group I, animals ($n = 6$) were anesthetized with halothane (1.31 ± 0.02% S.E.) and received 125 mg of caffeine i.v. every 20 min until death. Immediately preceding each caffeine dose, arterial blood gases, serum K^+, Na^+ and ionized calcium were measured. In group II, animals ($n = 6$) were anesthetized and maintained with pentobarbital i.v. and received 125 mg of caffeine every 20 min and the same measurements were made as in group 1. The total cumulative dose of pentobarbital was 163 ± 31 mg. In group III, animals ($n = 4$) received only halothane (1.22 ± 0.06%) and measurements were made every 20 min for 3 hr. In group IV, animals ($n = 6$) received only pentobarbital i.v. and measurements were made every 20 min for 3 hr. The total cumulative dose was 204 ± 60 mg. As an objective measurement of muscle rigidity, one of the rabbit's hind limbs was immobilized by clamping the tibia. The foot was then passively flexed over a fixed range and the developed tension measured

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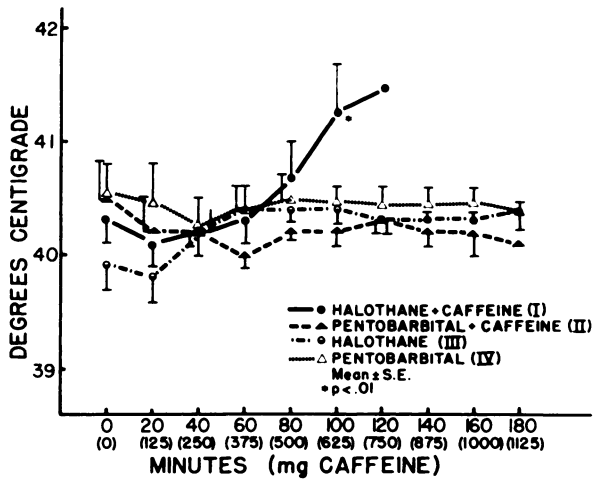

Fig. 1. Rectal temperature *vs.* time after stabilization in min or cumulative dose of caffeine (groups I and II). Mean ± standard error. Statistical significance was tested between group I and group II by Student's *t* test.

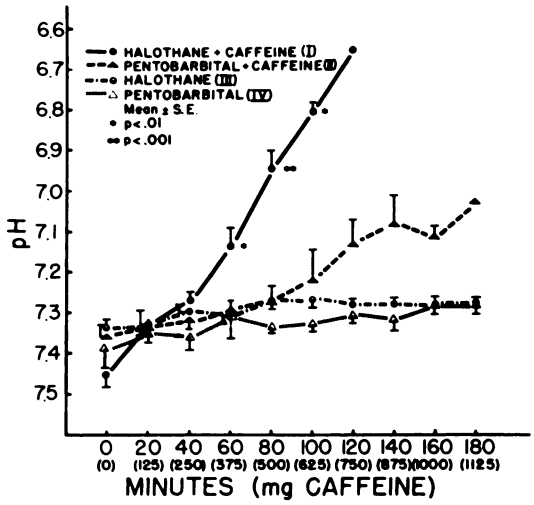

Fig. 2. pH *vs.* time after stabilization in minutes or cumulative caffeine dose (groups 1 and 2). Means of H^+ concentration ± standard error converted to pH. Significance was tested between group I and group II by Student's *t* test.

with a Grass FT-03 force transducer. Change in muscle compliance was expressed as the ratio of the tension required for foot flexion at the termination of the experiment compared to the tension required at the beginning of the experiment for the same degree of flexion. CO_2 production was not measured. However, minute ventilation was adequate to produce a pCO_2 less than 40 mm Hg during the stabilization period. Therefore, a rise in pCO_2 was interpreted as an increase in CO_2 production. pH values were converted to H^+ concentration for averaging and statistical analysis and then re-expressed as pH. Other values were treated arithmetically. Groups were paired and comparisons were made using Student's *t* test.

Results

There were no significant differences in initial temperature, serum K^+, Ca^{++}, pH or pCO_2 among the groups (table 1). All the animals not receiving caffeine (groups III and IV) survived 3 hr with no change in measured parameters (table 1). There were no cardiac arrhythmias in these rabbits.

All animals receiving caffeine developed significant cardiac arrhythmias ranging from premature atrial contractions to transient ventricular fibrillation. The arrhythmia usually began immediately after the caffeine injection and spontaneously reverted to sinus rhythm in several minutes. There was no difference in the incidence, severity or duration of the arrhythmias between those animals receiving halothane and caffeine (group I) and those receiving pentobarbital and caffeine (group II).

Compared to the precaffeine period in animals receiving pentobarbital and caffeine (group II), the only significant change was a decrease in pH.

Following caffeine injections, significant changes occurred in animals in group I in rectal temperature, pH, serum K^+ and pCO_2. Only animals in group I showed a rise in rectal temperature. The time course of the change in rectal temperature is shown in figure 1.

Figure 2 shows the time course of the change in pH. The earliest and most significant fall in pH is seen in group I. A fall in pH also occurred in animals in group II (pentobarbital and caffeine) which was significant when compared to animals in group IV (pentobarbital alone). This change occurred later and was less marked than in group I (table 1).

TABLE 1

Effects of halothane and pentobarbital ± caffeine in the rabbit *in vivo*†

Changes in rectal temperature, pCO_2, pH and serum K^+ and final-to-initial leg muscle tension for the four treatment groups. Means ± S.E.

Group	Temperature		Leg Muscle Rigidity:	pCO_2		pH		K^+		Ca^+	
	Initial	Final	Final/ Initial	Initial	Final	Initial	Final	Initial	Final	Initial	Final
	°C			mm Hg				meG/l			
Halothane + caffeine (I) (N = 6)	40.3 ±0.2	41.6** ±0.1	1.95 ±0.25	26 ±2	44* ±4	7.45 ±0.01	6.80** ±0.04	4.2 ±0.2	9.2** ±0.8	2.66 ±0.34	2.96 ±0.35
Pentobarbital + caffeine (II) (N = 6)	40.5 ±0.3	40.0 ±0.1	0.56 ±0.04	38 ±4	32 ±1	7.35 ±0.03	7.10* ±0.07	3.8 ±0.2	5.3 ±0.8	2.67 ±0.34	2.55 ±0.17
Halothane (III) (N = 4)	40.0 ±0.2	40.3 ±0.2	1.02 ±0.02	33 ±4	31 ±1	7.33 ±0.03	7.27 ±0.01	4.0 ±0.2	4.8 ±0.2	2.56 ±0.15	2.51 ±0.30
Pentobarbital (IV) (N = 6)	40.6 ±0.2	40.3 ±0.1	1.14 ±0.02	37 ±4	38 ±3	7.38 ±0.01	7.28 ±0.02	3.9 ±0.2	3.9 ±0.2	2.30 ±0.38	2.24 ±0.26

* $P < .01$, final compared to initial by Student's *t* test.

** $P < .001$.

† Initial refers to values after stabilization period. Final refers to values before the caffeine injection that resulted in death of the animal (groups I and II) or at the end of 3 hr (groups III and IV).

The rise in serum K^+ mirrors the fall in pH as shown in figure 3. The change is earliest and most significant in the halothane-caffeine group. Animals in group II show a late rise in K^+ which is statistically different compared to animals anesthetized with pentobarbital only. The fall in pH precedes the rise in rectal temperature in group I as shown in figure 4.

All animals in group I became rigid, as indicated by the decreased hind limb compliance in this group (table 1). Mortality occurred earlier in group I than in group II (table 2). In both groups, progressive hypotension, refractory to fluid administration and bradycardia were the terminal events.

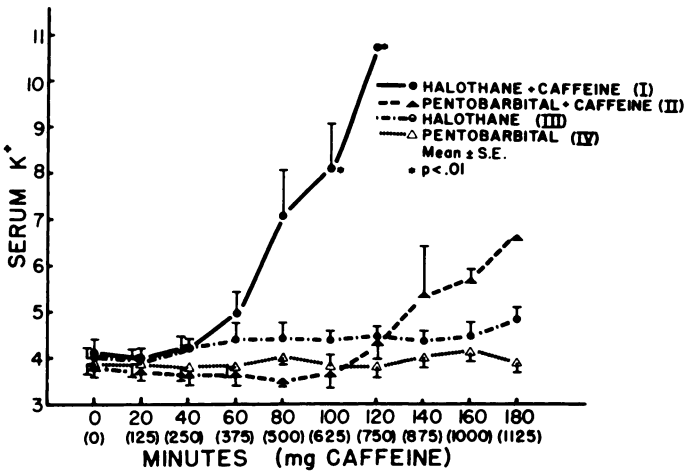

Fig. 3. Serum K^+ (milliequivalents per liter) *vs.* time after stabilization in minutes or cumulative caffeine dose (groups I and II). Mean ± standard error. Significance between group I and group II Student's *t* test.

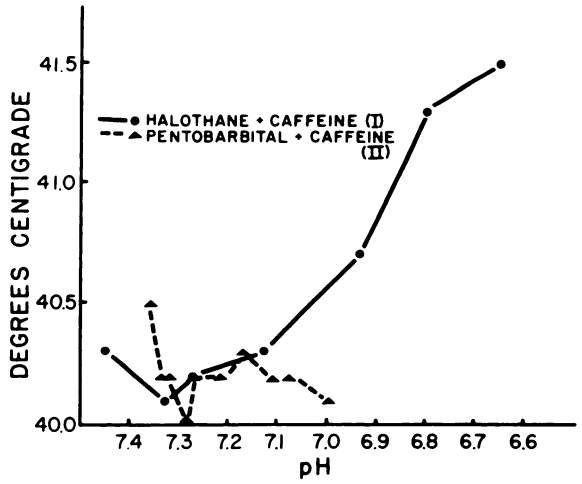

Fig. 4. Rectal temperature *vs.* pH in animals in groups I and II. The data in this graph are derived from figures 1 and 2. Only mean values are depicted, standard errors are as in figures 1 and 2.

Discussion

This study demonstrates that anesthetic agents may significantly alter the physiological and biochemical effects of caffeine *in vivo*. Furthermore, halothane-anesthetized, caffeine-treated rabbits display many of the cardinal symptoms and signs of malignant hyperpyrexia, *i.e.*, muscle rigidity, metabolic acidosis, hyperthermia, hyperkalemia and increased CO_2 production. Although acidosis and hyperkalemia also occurred in the caffeine and pentobarbital treatment group, the magnitude of changes was less compared to the halothane and caffeine group. The cause of death in this treatment group was most likely related to direct myocardial depression from the caffeine (Sollman and Pilcher, 1911). There is also a qualitative difference in the acidosis that developed in the halothane-caffeine group compared to the pentobarbital-caffeine group and not just a shift to the left in a dose-response curve: only halothane- and caffeine-treated animals showed signs of hypermetabolism as manifested by increased CO_2 production, hyperpyrexia and rigidity. Pentobarbital probably does not prevent the development of the syndrome, since *in vitro* pentobarbital does not inhibit halothane-potentiated caffeine contractures in mammalian skeletal muscle (H. Rosenberg, unpublished observation).

Several breeds of swine (*e.g.*, Pietrain, Landrace, Poland-China) develop a syndrome strikingly similar to MH upon exposure to halothane (Jones *et al.*, 1973). However, there are several dissimilarities between the porcine syndrome and the human form of MH. The same swine breeds that develop the MH-like syndrome upon halothane exposure will also develop hypermetabolism, muscle rigidity, acidosis and cardiac arrhythmias (as in MH) by exercise or stress in the *absence* of drug exposure. Rarely, if ever, do susceptible humans develop overt symptoms of MH in the absence of exposure to inhalation anesthetic agents or depolarizing relaxants.

Changes in serum Ca^{++} seem to be opposite those reported in human MH (Britt, 1975). The sympathetic nervous system is an important part of production of the syndrome in some breeds, as demonstrated by high levels of circulating catecholamines and the fact that adrenergic blockade is protective (Lister *et al.*, 1976; Lucke *et al.*, 1978). This has not been demonstrated in human MH. Creatine phosphokinase elevation is a reliable indicator of susceptibility in swine but not in humans (Eikelenboom and Minkema, 1974).

Various *in vivo* pharmacological models for MH have been suggested. Ryanodine, an alkaloid insecticide, was noted to cause an elevation in body temperature and rigidity in one of three cats after halothane and succinylcholine (Casson and Downes, 1973).

Dinitrophenol has been administered to rats (Hoch and Hogan, 1973) and dogs (Wilson *et al.*, 1966) in an attempt to reproduce MH. This uncoupler of mitochondrial oxidative metabolism causes hyperpyrexia, acidosis and death by itself.

TABLE 2
Number of animals surviving *vs.* dosage of caffeine

	No. of Animals Surviving Dose of Caffeine (mg)								
	125	250	375	500	625	750	875	1000	1125
Group I: Halothane + caffeine (n = 6)	6	6	6	6	3	1	0	0	0
Group II: Pentobarbital + caffeine (n = 6)	6	6	6	5	4	4	4	2	1

When halothane is added, these effects are seen at lower dinitrophenol doses and some animals became rigid. Many features of MH are missing, such as cardiac arrhythmias and elevation of creatine phosphokinase. Muscle rigidity is not always observed. Furthermore, isolated mitochondria from MH susceptibles are functionally normal, even in the presence of halothane (Britt *et al.*, 1975).

The etiology and pathogenesis of MH are not clearly understood at present. That MH is a skeletal muscle disorder is supported by such findings as extreme creatine phosphokinase elevations following a clinical episode of MH (Britt, 1975), elevation of muscle temperature that precedes the rise in body temperature (Gronert *et al.*, 1977) and abnormal contractile behavior of isolated skeletal muscle from MH susceptible swine and humans (Kalow *et al.*, 1970; Nelson, 1978).

In skeletal muscle biopsies from those who have survived an episode of MH, the threshhold for caffeine contractures is reduced and contraction tension increased in the presence and absence of halothane (Kalow *et al.*, 1970). The muscle is also atypical in that contractures frequently develop in the presence of halothane only and KCl contractures are greatly augmented (Moulds and Denborough, 1974). Structural abnormalities of muscle are not constant, however (Britt, 1975). Since a great deal of experimental evidence indicates that caffeine inhibits calcium uptake into the sarcoplasmic reticulum (SR) and high caffeine concentrates cause SR calcium release (Weber and Herz, 1968; Endo, 1977), it has been hypothesized that the primary defect in MH is an inability of the SR to regulate intracellular calcium concentrations. Although Ryan *et al.* (1976) have found that SR isolated from muscle of MH suceptibles exhibit a defect in calcium uptake, this has not been uniformly confirmed (Nelson *et al.*, 1972; Britt, 1975). However, the SR in skinned muscle fiber preparation from MH susceptibles also demonstrates enhanced Ca^{++} release (Wood, 1978).

It has recently been proposed that MH is a result of a defect in excitation contraction coupling (Nelson and Denborough, 1977). The rationale is as follows: dantrolene at low concentrations seems to inhibit steps in excitation contraction coupling since KCl contractures are blocked at a dose of the drug having no effect on caffeine contractures (Putney and Bianchi, 1974). Nelson and Denborough (1977) and Nelson (1978) found that in normal muscle low concentrations of dantrolene inhibited halothane-potentiated potassium contractures to a greater extent than halothane-potentiated caffeine contractures muscle. At higher dantrolene concentrations, the contracture response to caffeine and caffeine plus halothane was blocked in normal and MH susceptible muscle.

Disturbances of mitochondrial oxidative phosphorylation and calcium uptake are unlikely to be the primary defect in MH. Mitrochondria isolated from normal and MH muscle have behaved similarly *in vitro* (Britt, 1975).

In the present study it was especially interesting that caffeine in the presence of pentobarbital failed to produce the changes seen during halothane administration. Perhaps two (or more) intracellular lesions are needed to precipitate the MH-like syndrome. In this model, one such defect might be the accelerated calcium release from SR that occurs in normal muscle in the presence of halothane (Rosenberg, 1979; Endo *et al.*, 1975), and the second might be the increased intracellular calcium concentration caused by caffeine-induced inhibition of calcium uptake or enhanced calcium release (Endo, 1977). The elevated intracellular calcium would then cause muscle contraction and contracture. An increase in metabolic rate and possible functional mitochondrial uncoupling in an attempt to reduce the elevated Ca^{+} levels to normal might result. Cell death would ensue from acidosis and depletion of high energy phosphate compounds.

Further investigations of this model are needed to confirm the degree of similarity to human MH. These include the effects of succinylcholine, procaine amide and dantrolene, agents known to exacerbate or alleviate MH, as well as observations of other biochemical changes that take place in this model. Biochemical evaluations of the model may provide information regarding the pathogenesis and treatment of human MH.

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