CALCIUM UPTAKE INTO MUSCLE OF PIGS SUSCEPTIBLE TO MALIGNANT HYPERTHERMIA: IN VITRO AND IN VIVO STUDIES WITH AND WITHOUT HALOTHANE

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SUMMARY

MHS and normal pigs were anaesthetized with nitrous oxide and diazepam. Halothane 1% was then administered for 1 hr. Immediately before and immediately after the halothane inhalation, 10 g of vastus lateralis muscle was excised. SR was isolated from this muscle. Calcium accumulation by the MHS sarcoplasmic reticulum in the absence of halothane was greater than normal. Halothane in vitro produced a similar increase in calcium uptake into both the MHS and the normal SR. Halothane in vivo significantly reduced calcium accumulation by hyperthermic SR but had no significant effect on calcium uptake into the normal SR. Thus the action of halothane on the MHS sarcoplasmic reticulum is indirect, occurring only in the intact cell. It is possibly a result of the deleterious effect which lack of ATP, a low pH or a high temperature is likely to have on the calcium accumulating mechanism of the SR membrane. Our results suggest that the primary defects of porcine and human malignant hyperthermia are not entirely synonymous.

The immediate cause of a malignant hyperthermic (MH) crisis appears to be an excessively great concentration of calcium in the skeletal muscle myoplasm (Berman and Kench, 1973; Bernhardt and Schiller, 1973; Bianchi, 1973; Britt and Kalow, 1970; Britt, 1974; Denborough et al., 1973; Furniss, 1971; Harrison, 1973; Kalow et al., 1970; Nelson et al., 1972). The source of this calcium remains uncertain. The sarcoplasmic reticulum (SR) is a likely possibility, since its normal function is actively to accumulate calcium against a concentration gradient during relaxation, and then passively to release calcium to the myoplasm during contraction. However, inconsistent results have been reported for calcium uptake into SR isolated from malignant hyperthermia susceptible (MHS) swine. Denborough and colleagues (1973) described normal calcium binding, but increased calcium exchange and ATPase activity, for SR obtained from MHS Landrace pigs. Accelerated calcium uptake into the SR of MHS hogs was observed by Nelson and co-workers (1972) (Poland-China), and by Berman and Kench (1973) (Landrace). On the other hand, Brucker and colleagues (1973) reported that SR, isolated from MHS Poland-China pigs, manifested a reduction in calcium uptake which was proportional to the dose of halothane in vitro. In this latter study, however, the muscle was not excised from some (though apparently not all) of their animals until after a halothane-induced rigor had developed. Thus, this muscle was affected by halothane both in vivo and in vitro and, because of this experimental design, these two influences could not be differentiated.

The purpose of this paper is to describe our separate examination of the effects of halothane in vitro and in vivo on calcium uptake into the sarcoplasmic reticulum of porcine skeletal muscle.

METHODS

Pig colony.

The MHS swine were crossbred Poland-China/York hogs. They were all descendants of two purebred MHS Poland-China pigs obtained from the colony maintained by Jones and Nelson (1968) at Oklahoma State University. The normal animals were purebred York swine.

Anaesthetic and surgical techniques.

Premedication consisted of diazepam (Valium) 4 mg/kg body weight i.m. Anaesthesia was induced using further diazepam 2 mg/kg i.v. and nitrous
oxide in oxygen (10:4 litre/min). Anaesthesia was maintained with nitrous oxide and i.v. diazepam as required. A field block of procaine was infiltrated around, but not into, the surgical site. The muscle biopsy was from the vastus lateralis. All possible fascia and fat were removed from the muscle before severing its blood supply. Two muscle specimens were taken from each pig. The first was excised immediately following induction with diazepam and nitrous oxide. This sample was utilized for those experiments requiring halothane in vitro only. One percent halothane vapour was then added to the nitrous oxide/oxygen mixture via an endotracheal tube for 1 hr. In the MHS pigs, this procedure permitted a hyperthermic rigor to develop and to reach its final stages. At the conclusion of the hour of halothane administration, a second sample of muscle was excised and used for studying the influence of halothane in vivo on calcium accumulation by the SR.

**Biochemical techniques.**

Sarcoplasmic reticulum was isolated from 10 g of muscle and $^{45}$CaCl$_2$ uptake into the SR was measured in the presence of 0, 0.5, 2.0 or 5.0 vol% (v/v) of halothane by techniques described by us previously (Britt et al., 1973).

**RESULTS**

Halothane in vitro increased calcium uptake into both the normal and MHS sarcoplasmic reticulum (fig. 1) (for the significance of the separate slopes, $t=4.52$, d.f.=13, $P<0.001$ and $t=5.83$, d.f.=17, $P<0.001$, respectively). The slopes were not significantly different ($t=2.08$, d.f.=9). Thus, considering all halothane concentrations, calcium uptake into the MHS sarcoplasmic reticulum was greater than in the normal SR. This implies also that calcium accumulation by MHS sarcoplasmic reticulum in the absence of halothane was greater than normal ($t=3.37$, d.f.=9, $P<0.01$).

Halothane in vivo significantly lowered calcium accumulation by hyperthermic SR ($t=3.66$, d.f.=8, $P<0.01$), but slightly (although not significantly) increased calcium uptake into the normal SR ($t=0.48$, d.f.=10).

The statistical analysis of in vitro data is based on the square root of the halothane concentration which yields an approximately linear response and permits the inclusion of zero halothane concentration.

**DISCUSSION**

This experiment shows that calcium accumulation by the SR is less than normal during a hyperthermic rigor. The impairment, however, occurs only when halothane is administered to the intact muscle cell. Isolated SR retains fully and even improves its calcium accumulating capacity in the presence of halothane. Thus we see that the results of Nelson and colleagues (1972) and Berman and Kench (1973) on the one hand, and Brucker and colleagues (1973) on the other hand, are not necessarily contradictory. The SR defect, therefore, may be secondary to some deleterious influence emanat-
ing from beyond the confines of the SR. There are several possibilities. First, since adenosine triphosphate (ATP) concentrations decrease during terminal MH crises (Berman and Kench, 1973) probably as a result of both decreased production and increased utilization (Bernhardt and Schiller, 1973; Britt and Kalow, 1970; Britt, 1974; Denborough et al., 1972, 1973), there may be insufficient ATP available to provide the energy required for the active accumulation of calcium by the SR (Hasselbach, 1964; Carvalho, 1968). Second, the precipitous decrease in cellular pH which occurs during MH crises may inhibit the sarcoplasmic reticulum ATPase which normally catalyses calcium uptake into the SR (Sreter, 1969; Mitchelson, 1974).

The results we have reported here do not rule out any primary effect of halothane on porcine SR. It may be that halothane directly accelerates calcium release from the SR. This is a factor which we are studying at present and hope to report upon in the future.

We have found that MHS mitochondria isolated from porcine skeletal muscle take up less than normal amounts of calcium in the absence of halothane (Britt et al., 1975). Nevertheless, in the living animal in the absence of halothane, myoplasmic calcium must remain nearly within normal limits, since otherwise the pig would be in a continuous state of hyperthermic rigor, a situation incompatible with the relatively normal lives of MHS swine. It may be that other membranes such as the SR have developed an increased capacity to accumulate calcium in the face of chronic challenge by a very slightly higher than normal myoplasmic calcium concentration. In addition, MHS sarcolemma might be able to pump calcium out of the cell more efficiently than normal sarcolemma.

In previous studies, Britt and Kalow (1970) and Britt and others (1973) showed, using the same experimental technique as in this experiment, that radioactive calcium uptake into human MHS muscle was less than normal in the absence of halothane. The addition of halothane alone in vitro further reduced movement of calcium into the SR. This effect was not seen in normal SR treated similarly. In fact, at low and medium doses (0.5 and 2.0% v/v) halothane increased calcium accumulation by the normal SR. Thus, halothane has a direct effect on isolated human MHS sarcoplasmic reticulum which it does not have on isolated porcine SR. In this respect the muscle defect in the two species apparently differs.

Ryan and others (1974) reported recently that in vivo halothane administered to an MHS patient induced about a tenfold reduction in calcium uptake into the SR. The indirect or secondary effect of halothane on the SR is, therefore, qualitatively similar in both species.

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REFERENCES


Carvalho, A. P. (1968). Calcium-binding properties of sarcoplasmic reticulum as influenced by ATP, caffeine, quinine and local anesthetics. J. Gen. Physiol., 52, 622.


HALOTHANE AND MUSCLE CALCIUM IN MHS SWINE


MONTEE DE CALCIUM DANS LES MUSCLES DES PORCS SUSCEPTIBLES D'HYPERTHERMIE MALIGNE: ETUDES IN VITRO ET IN VIVO AVEC OU SANS HALOTHANE

RESUME

Des porcs normaux et des porcs susceptibles d'hypertension maligne ont été anesthésiés à l'oxyde azoteux et au diazépam. On leur a ensuite administré, pendant une heure, 1% d'halothane. On a excisé immédiatement avant et immédiatement après l'inhalation d'halothane 10 g du muscle vastus lateralis (vaste lateral) et on a isolé le réticulosarcoplasme de ce muscle. L'accumulation de calcium par le réticulosarcoplasme susceptible d'hypertension maligne a été, en l'absence d'halothane, plus importante que la normale. L'halothane in vitro a provoqué une augmentation similaire de la montée de calcium aussi bien chez les sujets susceptibles d'hypertension maligne que chez les réticulosarcoplasmes normaux. In vitro, l'halothane a réduit d'une manière tangible l'accumulation de calcium par le réticulosarcoplasme hyperthermique, mais n'a eu aucun effet significatif dans la montée de calcium sur le réticulosarcoplasme normal. Ainsi, l'action de l'halothane sur le réticulosarcoplasme susceptible d'hypertension maligne est une action indirecte, ne se produisant que dans la cellule intacte. C'est probablement le résultat de l'effet délétère que le manque d'Acide Adénosine Triphosphorique, le faible pH ou la forte température peut vraisemblablement avoir sur le mécanisme d'accumulation du calcium sur la membrane du réticulosarcoplasme. Nos résultats permettent de suggérer que les défauts primaires de l'hypertension maligne chez les humains et chez les porcs ne sont pas complètement synonymes.

KALZIUMAUFNAHME IN MUSKELN VON SCHWEINEN MIT ANFÄLLIGKEIT AUF MALIGNE HYPERTHERMIE: STUDIEN IN VITRO UND IN VIVO MIT UND OHNE HALOTHAN

ZUSAMMENFASSUNG


ASCENSION DEL CALCIO AL MUSCULO DE CERDOS SUSCEPTIBLE DE HIPERTERMIA MALIGNA: ESTUDIO IN VITRO E IN VIVO CON Y SIN HALOTANO

SUMARIO

Se anestesiaron cerdos normales y MHS con óxido nítrico y diazepam. Se administró luego halotano al 1% durante 1 h. Inmediatamente antes e inmediatamente después de la inhalación de halotano, se cortaron 10g del músculo vastus lateralis. Se aisló el SR de este músculo. La acumulación de calcio por el retículo sarcoplásico MHS en la ausencia de halotano fue mayor que la normal. El halotano in vitro produjo un aumento similar en la ascensión del calcio tanto en el MHS como en el SR normal. El halotano in vitro redujo significativamente la acumulación de calcio por el SR hipertérmico pero no tuvo efecto significativo en la ascensión de calcio en el SR normal. De este modo la acción del halotano sobre el retículo sarcoplasmático MHS es indirecta, teniendo lugar únicamente en la célula intacta. Es posible un resultado de efecto deletéreo cuya falta de ATP es posible que tenga sobre el mecanismo de acumulación de calcio de la membrana SR un bajo pH o una temperatura alta. Nuestros resultados sugieren que los defectos primarios de la hipertermia maligna humana y porcina no son completamente sinónimos.