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Acute calcium homeostasis in MHS swine

To elucidate a pathogenesis for the reduction in bone calcium content observed in MHS individuals, we studied the acute calcium homeostasis of MHS swine. This was achieved by the serial measurement, with a calcium selective electrode, of calcium transients in Landrace MHS (five) and control Landrace/large white cross MH negative (five) swine following IV bolus injection of calcium gluconate $0.1 \text{ mmol} \cdot \text{kg}^{-1}$ – a dose which induced an acute 45 per cent increase in plasma ionised calcium. Experimental animals were anaesthetised with ketamine $10 \text{ mg} \cdot \text{kg}^{-1}$ IM, thiopentone (intermittent divided doses) $15\text{--}25 \text{ mg} \cdot \text{kg}^{-1}$ (total) IV and $\text{N}_2\text{O}/\text{O}_2$ (FiO_2 0.3) by IPPV to maintain a normal blood gas, acid/base state. The plasma ionised calcium decay curve observed in MHS swine did not differ from that of control normal swine. Further it was noted that the induced acute rise in plasma ionised calcium failed to trigger the MH syndrome in any MHS swine. It is concluded that the mechanisms of acute calcium homeostasis in MHS swine are normal. An explanation for the reduction in bone calcium content observed in MHS individuals must be sought, therefore, through study of the slow long-term component of the calcium regulatory process. In addition, the conventional strictures placed on the use, in MHS patients, of calcium gluconate are called in question.

Key words

HYPERTHERMIA: malignant hyperthermia, MHS swine;
MALIGNANT HYPERTHERMIA: calcium homeostasis, bone physiology.

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Although the primary site of the functional lesion responsible for malignant hyperthermia (MH) resides in the skeletal muscle organelles, there is evidence which suggests a more widespread and general membrane defect.¹ Inter alia, reduction in bone calcium content and, by inference, abnormalities in osteocyte function have been reported in patients susceptible to MH.² These observations, considered in the light of the central role of bone in the homeostasis of plasma calcium concentration,³ led us to investigate the acute calcium homeostasis of MHS swine.

Methods

The experimental animals, five especially bred Landrace MHS swine, weight 30–50 kg, selected initially based on a positive MH response to brief “barnyard” exposure to mask administration of halothane⁴ were formally retested under anaesthesia in the laboratory before use in the definitive experiments and the onset of MH (for criteria see later) in response to halothane was timed.

Five large white/Landrace crossbred swine which displayed a negative reaction to similar testing served as controls.

Anaesthesia

For all experiments, animals were anaesthetised with ketamine $10 \text{ mg} \cdot \text{kg}^{-1}$ IM followed by IV thiopentone via an ear vein and then tracheal intubation. Anaesthesia thereafter was maintained with $\text{N}_2\text{O}/\text{O}_2$ (FiO_2 0.3) delivered by mechanical ventilation via a non-return circuit, the volume of ventilation being adjusted to maintain PaCO_2 in the range 40–45 mmHg. Intermittent supplemental doses of thiopentone were administered at any sign of lightening anaesthesia. Total doses of thiopentone ranged from $15\text{--}25 \text{ mg} \cdot \text{kg}^{-1}$. Mean pH during the control period of the experiments was 7.41 ± 0.05 ($n = 30$). PaO_2 ranged from 180–210 mmHg. Catheters inserted into the carotid artery and internal jugular vein via cut-down in the neck provided access to blood samples for calcium, blood gas and acid/base assay (Radiometer), arterial and venous pressure monitoring (Hellige) and the route for fluid and drug administration.

The ECG was monitored via skin electrodes and temperature was recorded from a thermistor needle probe (Ellab) placed deep in the thigh muscle mass.

FIGURE 1 Transient response of MHS and normal swine to the bolus injection of calcium gluconate $0.1 \text{ mmol} \cdot \text{kg}^{-1}$. Plot is of difference from control values (Mean $\pm$ SD).

Protocol

Following establishment of steady state conditions under anaesthesia and three control blood samples, a bolus of calcium gluconate ($0.1 \text{ mmol} \cdot \text{kg}^{-1}$) was administered via the jugular vein infusion. Thereafter blood was sampled serially for 60 minutes at the following time intervals: one minute for the first ten; five minutes for the next 20, and ten minutes for the last 30.

The dose of calcium gluconate chosen was such as would induce an appreciable increase in plasma ionised calcium, without invoking cardiac arrhythmia.

Calcium concentration was measured by calcium selective carrier disc electrodes,⁵ all samples being read in duplicate and the mean result recorded. Values expressed are of ionized calcium.

Additionally, the possible onset of MH was monitored for, the following criteria of the syndrome being sought: >20 per cent increase in heart rate and/or mean arterial pressure, development of combined respiratory and metabolic acidosis (>20 per cent increase in PaCO_2), increase in muscle core temperature of $>0.5^\circ\text{C}$ and extension of the hind legs.

Results

Data points on the constructed plasma ionised calcium concentration decay curve (change from control values) (Figure) reflect mean and SD of values at each time interval in experimental and control groups. The significance of differences observed between MHS and control animals at individual data points was tested with Student's *t* test after equivalence of variance had been tested.

Following bolus injection of 0.1 mmol calcium gluconate $\cdot \text{kg}^{-1}$, peak concentration in plasma ionised calcium was reached within the first minute, the mean rise above control values being 34 per cent ($1.40 \text{ mmol} \cdot \text{L}^{-1} - 1.88 \text{ mmol} \cdot \text{L}^{-1}$) and 45 per cent ($1.33 \text{ mmol} \cdot \text{L}^{-1} - 1.90 \text{ mmol} \cdot \text{L}^{-1}$) respectively in normal and MHS swine. This was followed by a fast transient response which restored the ionised calcium concentration to control levels in 25 and 35 minutes respectively, followed by an overshoot below control levels. This decay curve was in every way analogous to our previously published observations on normal swine.⁵

No difference of statistical significance was demonstrated between the normal and MHS pig plasma ionised calcium decay curves.

The acutely induced rise in plasma ionised calcium concentration failed to trigger the MH syndrome in any animals.

Discussion

The mechanisms involved in the control of plasma ionised calcium are arranged in a hierarchical fashion from the minute-to-minute to long term.³ Our investigation examines the initial calcitonin-dependent fast transient response ($t_{1/2}$ of 15 minutes) of MHS swine, a response mediated by the cells lining the fluid compartment of bone.

Our observations show this response in MHS swine to be no different from normal controls and to differ in no way from our previously published data on the normal response.⁵

These findings provide no explanation for the reduced bone calcium content of MHS patients reported by Britt, Harrison and MacNeill² which must be related therefore to a possible dysfunction of the slow long-term component of the calcium regulatory process.

Our incidental observation of the failure of an acutely induced rise in plasma ionised calcium concentration to trigger the onset of MH in susceptible swine, in line with similar observations recently reported,⁶ calls in question the strictures placed in the past on the use of calcium gluconate in MHS patients.⁷

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Résumé

Afin d'élucider pathogénèse pour la réduction dans le contenu osseux en calcium observé chez les personnes SHM, nous avons étudié l'homéostasie aiguë du calcium chez le porc SHM. On a mesuré de façon sériée, à l'aide d'une électrode sélective de calcium, les changements de calcémie chez cinq porcs SHM Landrace et chez cinq porcs témoins Landrace/crois-blanc-large HM négatif, l'effet d'une injection de 0.1 mmol·kg⁻¹ de gluconate de calcium IV – dose qui a provoqué une augmentation aiguë de 45 pour cent dans le calcium ionisé plasmatique. On a anesthésié les animaux expérimentaux avec de la kétamine 10 mg·kg⁻¹ (total), du thiopental IV (dose intermittente 15–25 mg·kg⁻¹) et du N₂O/O₂ (FIO₂ 0.3) par VPPI afin de maintenir un état de gaz sanguin acido-basique normal. La courbe décroissante du calcium plasmatique ionisé que l'on a remarquée chez le porc SHM ne différerait pas de celle du porc-témoin normal. De plus, on a remarqué que la provocation d'une augmentation aiguë dans le calcium ionisé plasmatique n'a pas réussi à déclencher le syndrome HM chez aucun des porcs SHM.

On en conclut donc que les mécanismes d'homéostasies aigus de calcium chez les porcs SHM sont normaux. On doit donc chercher à expliquer la réduction du contenu osseux en calcium remarqué chez les personnes SHM en étudiant la composante lente et à long terme du processus régulateur du calcium. De plus, on remet en question les mises en gardes conventionnelles concernant l'utilisation du gluconate de calcium chez les patients SHM.