Halothane and putative second messenger agents enhance the release of intracellular Ca^{2+} in hepatocytes prepared from swine susceptible to malignant hyperthermia

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SUMMARY

The effects of halothane and several agents involved in intracellular signal transducing pathways on the release of Ca^{2+} were monitored in primary cultures of hepatocytes from normal swine and those susceptible to malignant hyperthermia (MH).

In general, halothane in a dose-dependent manner, induced the release of intracellular Ca^{2+}. This release was increased in hepatocytes prepared from swine susceptible to MH compared to those from the control group.

It was also observed that 1,4,5-inositol trisphosphate (IP_3), guanosine-5-trisphosphate (GTP) and arachidonic acid (AA) all induced a significant release of $^{45}Ca^{2+}$ from permeabilized swine hepatocytes, only the quantities of $^{45}Ca^{2+}$ released by IP_3 were significantly higher for the hepatocytes prepared from the susceptible animals.

These data indicate an abnormal Ca^{2+} homeostasis in hepatocytes isolated from swine susceptible to MH which supports the hypothesis that membrane systems from several organs may be affected in MH.

INTRODUCTION

Episodes of MH can be initiated in genetically predisposed humans and swine by volatile anesthetics such as halothane. It has been reported that the Ca^{2+} release channel of the sarcoplasmic reticulum in skeletal muscle is abnormal in MH (MICKELSON et al. 1988, MCLENNAN et al. 1990, MCCARTHY et al. 1990) and that the phospholipid and fatty acid profiles of membranes from both cardiac and skeletal muscle may be altered, perhaps secondarily (SEEWALD et al. 1991). However, there are numerous reports suggesting that the regulation of $[Ca^{2+}]_i$ is abnormal in cells from tissues other than muscle (CHEAH et al. 1989, GRONERT et al. 1988, ERVASTI et al. 1989, KLIP et al. 1987, NIEBROJ-DOBOSZ et al. 1984, OHNISHI et al. 1988, RUPPERSBERG et al. 1988, THATTE et al. 1987). In this study, we investigated Ca^{2+} mobilization in hepatocytes prepared from swine susceptible to MH and compared it to their normal counterparts.

METHODS

Swine were obtained from a special breeding program at the University of Minnesota. Normal swine were mongrels,

and swine susceptible to MH were purebred Pietrains. Animals were anesthetized with sodium thiopental (30 - 50 mg/kg) and fresh specimens of skeletal muscle were obtained for in vitro contracture testing in accordance with the protocol for the investigation of MH susceptibility (EUROPEAN MALIGNANT HYPERPYREXIA GROUP 1984). The livers were removed and immediately perfused with cold Dulbecco's PBS containing heparin (6000 units/l). Hepatocytes were prepared by a two-step perfusion: 1) for 10 min at 37 °C with 2 liters of Ca_{2+}-free PBS containing 25 mM $NaHCO_3$, 12.5 mM HEPES and 0.5 mM EGTA: and 2) for 20 min at 37 °C with PBS containing 25 mM $NaHCO_3$, 12.5 mM HEPES (pH 7.4), 4 mM $CaCl_2$ and 1 g/l collagenase B (Boehringer Mannheim, Mannheim, Germany). The hepatocytes were isolated by filtration and centrifugation and washed once with Krebs buffer (viability > 80%) (IAIZZO et al. 1990).

After washing, hepatocytes were incubated at 37 °C for 20 min in uptake buffer containing 0.005 % saponin for permeabilization. The method used to load the cells with $^{45}Ca^{2+}$ is reported elsewhere (SEEWALD et al. 1990). Cells were removed in 100 µl aliquots at various times and the rate of $^{45}Ca^{2+}$ uptake was determined using liquid scintillation counting. Following maximum uptake after approximately 8 minutes (Figure 1) the effects of various agents on $^{45}Ca^{2+}$ release were determined .

Changes in $[Ca^{2+}]_i$ in intact hepatocytes were measured with the Ca^{2+} -sensitive photoprotein aequorin (OLSEN et al. 1988). An estimate of $[Ca^{2+}]_i$ was made by recording the light emission from serum-deprived aequorin loaded cells. When the resting levels of aequorin luminescende were considered stable, the effect of halothane on $[Ca^{2+}]_i$ was determined. Halothane was prepared by dilution of a halothane-saturated aqueous buffer. The final concentrations of halothane in the incubation solutions were determined by gas chromatography.

RESULTS

The uptake of $^{45}Ca^{2+}$ by saponin-permeabilized swine hepatocytes was maximal after incubation for 7 to 10 min (Figure 1).

In general, halothane in a dose-dependent manner, induced the release of $^{45}Ca^{2+}$ from intracellular stores. This release was increased in hepatocytes prepared from swine susceptible to MH compared to those from the control group (Figure 2). The putative second messengers IP_3, GTP and arachidonic acid induced significant release of $^{45}Ca^{2+}$ in each type of hepatocyte. (Figure 3). The $^{45}Ca^{2+}$ release induced by IP_3 was greater in the hepatocytes prepared from the animals susceptible to MH ($p<0.05$).

The estimated levels of resting $[Ca_{2+}]_i$ were the same in intact hepatocytes: 162 + 37.8 nM (n = 12) in hepatocytes from normal swine and 145.4 + 74.7 nM (n = 14) in hepatocytes of MH swine (mean+SD). Halothane caused transient increases in $[Ca^{2+}]_i$ in each preparation. The amplitude and duration of the Ca^{2+} transients were dose-dependent and were larger for the hepatocytes prepared from MH animals (Figure 4).

DISCUSSION

Our results support the hypothesis that the mobilization of intracellular Ca^{2+} is abnormal in organ systems other than the skeletal muscles in swine susceptible to MH. The exact mechanism by which halothane causes the release of Ca^{2+} within swine hepatocytes is not known, but several possibilities exist: 1) halothane may have a direct

effect on the endoplasmic reticulum e.g. on Ca^{2+} channels (WEILAND et al. 1989) or 2) halothane may modify the function of the surface membrane and associated second messenger systems e.g. IP_3 (IAIZZO et al. 1989, RUPPERSBERG et al. 1988). It has been recently reported that the genetic defect in MH is specifically related to the calcium release channel located in the sarcoplasmic reticulum of skeletal muscle (MICKELSON et al. 1988, MACLENNAN et al. 1990, MCCARTHY et al. 1990). It is also reported that this ryanodine-sensitive calcium release channel exists only in muscle (GILL 1989, MCGREW et al. 1989). Therefore, in the light of these findings, if they are validated in all cases of MH, the changes which we obseved within the hepatocytes are likely to be secondary and difficult to place in perspective in the absence of the ryanodine receptor in hepatocytes.

REFERENCES

CHEAH, K.S, CHEAH, A.M., FLETCHER, J.E. and ROSENBERG, H. (1989): Skeletal muscle mitochondrial respiration of malignant hyperthermia-susceptible patients. Ca^{2+} -induced uncoupling and free fatty acids. Int J Biochem 21:913-920.

GILL, D.L. (1989): Receptor kinships revealed. Nature 342:16-18

GRONERT, G.A., MOTT, J. and LEE, J. (1988): Aetiology of malignant hyperthermia. Br J Anaesth 60:253-267.

ERVASTI, J.M., CLASSENS, M.T., MICKELSON, J.R. and LOUIS, C.F. (1989): Altered transverse tubule dihydropyridine receptor binding in malignant hperthermia. J Biol Chem 264:2711-2717.

EUROPEAN MALIGNANT HYPERPYREXIA GROUP (1984): A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. Br J Anaesth 56:1267-1269.

IAIZZO, P.A., LEHMANN-HORN, F., TAYLOR, S.R. and GALLANT, E.M. (1989): Effects of halothane on the surface membrane. Muscle Nerve 12:173-179.

IAIZZO, P.A., OLSEN, R.A., SEEWALD, M.J., POWIS, G., STIER, A. and VAN DYKE, R.A, (1990): Transient increases of intracellular Ca^{2+} induced by volatile anesthetics in rat hepatocytes. Cell Calcium 11:515-524.

KLIP, A., BRITT, B.A., ELLIOTT, M.E., PEGG, W., FRODIS, W. and SCOTT, E. (1987): Anaesthetic-induced increase in ionized calcium in blood mononuclear cells from malignant hyperthermia patients. Lancet 8531:463-466.

MCCARTHY, T.V., HEALY, J.M.S., LEHANE, M., HEFFRON, J.J.A., DEUFEL, R., LEHMANN-HORN, F., FARRALL, M. and JOHNSON, K. (1990): Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. Nature 343:562-563.

MACLENNAN, D.H., DUFF, C., ZORZATO, F., FUJII, J., PHILLIPS, M., KORNELUK, JR.G., FRODIS, W. and BRITT, B.A. (1990): Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature 343:559-561.

MCGREW, S.G., INUI, M., CHADWICK, C.C., BOUCEK, R.J. JR., JUNG, C.Y. and FLEISCHER, S. (1989): Comparison of the calcium release channel of cardiac and skeletal muscle sarcoplasmic reticulum by target inactivation analysis. Biochem 28:1319-1323.

MICKELSON, J.R., GALLANT, E.M., LITTERER, L.A., JOHNSON, K.M., REMPEL, W.E. and LOUIS, C.F. (1988): Abnormal sarcoplasmic reticulum ryanodine receptor in malignant hyperthermia. J Biol Chem 263:9310-9315.

NIEBROY-DOBOSZ, I., KWIATKOWSKI, H. and MAYZNER-ZWADZKA, E. (1984): Experimental porcine malignant hyperthermia: macromolecular characterization of muscle plasma membrane. Med Biol 62:250-254.

OLSEN, R.A., SANTONE, K., MELDER, D., OAKES, S.G., ABRAHAM, R. and Powis, G. (1988): An increase in intracellular free Ca^{2+} associated with serum-free growth stimulation of Swiss 3T3 fibroblasts by epidermal growth factor in the presence of bradykinin. J Biol Chem 263:108030-108035.

OHNISHI, S.T. and OHNISHI, T. (1988): Halothane induced disorder of red cell membranes of subjects susceptible to malignant hyperthermia. Cell Biochem Funct 6:257-261.

RUPPERSBERG, J.P. and RÜDEL, R. (1988): Differential effects of halothane on adult and juvenile sodium channels in human muscle. Pflügers Arch 412:17-21.

SEEWALD, M.J., OLSEN, R.A., SEHGAL, I., MELDER, D.C., MODEST, E.J. and POWIS, G. (1990): Inhibition of growth factor-dependent inositol phosphate Ca^{2+} signaling by antitumor ether lipid analogues. Cancer Research 50:4458-4463.

SEEWALD, M.J., EICHINGER, H.M. and IAIZZO, P.A. (1991): Malignant hyperthermia: an altered phospholipid and fatty

acid composition in muscle membrane. Acta Anaesthesiol Scand (in press).

THATTE, H.S., MICKELSON, J.R., ADDIS, P.B. and LOUIS, C.F. (1987): Erythrocyte membrane ATPase and calcium pumping activities in porcine malignant hyperthermia. Biochem Med Metab Biol 38:355-365.

WEILAND, S.J., FLETCHER, J.E., ROSENBERG, H. and QI-HUA, G. (1998): Malignant hyperthermia: slow sodium current in cultured human muscle cells. Am J Physiol 257:C759-C765.

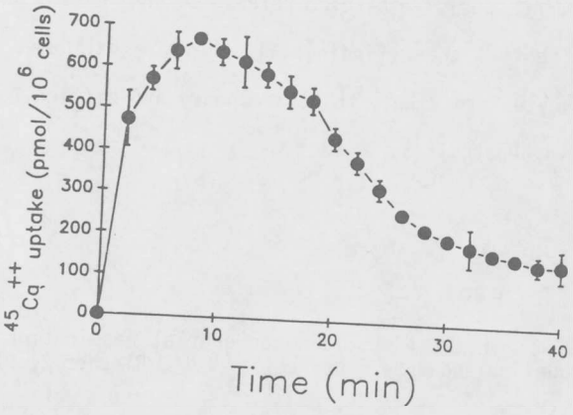

Figure 1. Uptake of $^{45}Ca^{2+}$ by saponin-permeabilized hepatocytes from swine. Values from five samples were averaged at each time point (X + SD).

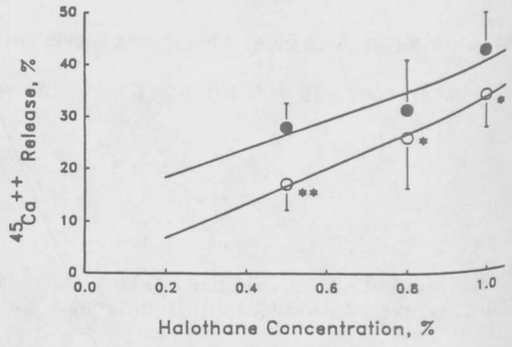

Figure 2. The effects of halothane on $^{45}Ca^{2+}$ release in hepatocytes prepared from normal swine (o) and swine susceptible to MH (o). The mean values (bars indicate SD) of anesthetic- induced release (n = 15 for 3 animals from each group) were plotted against the halothane concentration. At each halothane concentration significantly more $^{45}Ca^{2+}$ was released from the hepatocytes prepared from the animals susceptible to MH (* = $p < 0.05$, ** = $p < 0.01$).

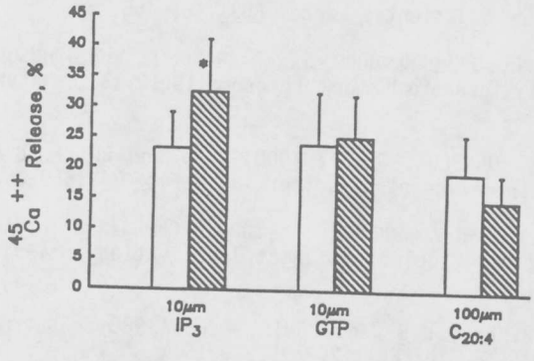

Figure 3. The release of $^{45}Ca^{2+}$ from hepatocytes prepared from normal swine and those susceptible to MH, induced by several agents considered important in intracellular second messenger systems. Mean values (and SD) were derived from data obtained from hepatocytes prepared from three normal animals (open bars) and three animals susceptible to MH (hatched bars). Only the release of $^{45}Ca^{2+}$ induced by IP_3 (10 M) was significantly different (*) between the two types of preparation ($P<0.05$).

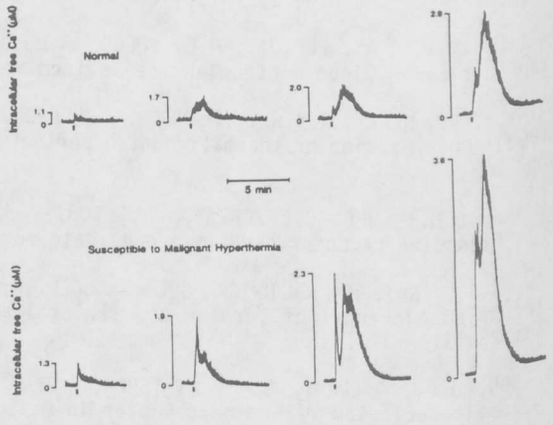

Figure 4. Transient increases in $[Ca^{2+}]_i$ induced by halothane in intact hepatocytes prepared from swine susceptible to MH. Swine hepatocytes were loaded with aequorin using a low Ca^{2+} centrifugation method and plated in culture dishes for at least 20 hours before study. The halothane was administered by adding dilutions of saturated solutions to the culture dishes. The upper records show the response of hepatocytes prepared from a normal animal to increasing concentrations of halothane; the lower set of responses was recorded from cells prepared from an animal susceptible to MH. The increasing halothane concentrations were approximately 0.5, 1, 2, and 6 %.