Title: DANTROLENE ANTAGONIZES THE SYNERGISTIC HEMOLYSIS OF HUMAN RED BLOOD CELLS BY HALOTHANE AND PHOSPHOLIPASE A_2: MODEL FOR MALIGNANT HYPERTHERMIA.

Authors: J.E. Fletcher, Ph.D., P. Kistler, B.S., H. Rosenberg, M.D., K. Michaux, B.S. and L. Tripolitis, B.S.

Affiliation: Department of Anesthesiology, Hahnemann University, Philadelphia, Pennsylvania 19102

Introduction. Elevated phospholipase A_2 (PLA_2) activity has been suggested as a possible cause of porcine[1] and human[2] malignant hyperthermia (MH). PLA_2 and halothane act in synergy to induce contractures in skeletal muscle[2] and hemolysis of red blood cells (RBCs).[3] The present study examines whether dantrolene antagonizes the synergistically-induced hemolysis of RBCs by halothane and PLA_2 to determine the usefulness of the RBC model in understanding the action of dantrolene in skeletal muscle.

Methods. Human RBCs that had not reached their expiration date were obtained from the Hahnemann University blood bank from partially used bags. A saturated halothane solution (SHS) was the aqueous phase of a two-phase system comprised of 500 ml HEPES buffer (NaCl 130 mM, $CaCl_2$ 2 mM, HEPES 20 mM at pH 7.4) and 25 ml halothane.[2] This solution was maintained at 37°C with constant stirring for 3 hrs prior to use. Packed RBCs were obtained by three washings of the cell with HEPES buffer, as previously described.[3] Packed RBCs (10 µl) were added to test tubes of 10 ml capacity containing either 5 ml HEPES buffer, or 2.5 ml HEPES buffer plus 2.5 ml SHS. Bee venom PLA_2 (Sigma Chemical Co.), dantrolene, arachidonic acid (AA), or lysophosphatidylcholine (LPC) were added to final concentrations of 1 and 10, 1 and 2.5 µM, respectively (see Table). The tubes were capped and incubated at 37°C for 30 min. After the incubation the tubes were centrifuged (1000 x g, 8 min) and the supernatant read at 540 nM against a distilled water blank using a spectrophotometer. The absorbance was compared to 100% hemolysis for each preparation of packed RBCs (10 µl RBCs in 5 ml distilled water) to yield percent hemolysis. The data in the Table were analyzed with a one-way analysis of variance and Duncan's multiple-range test ($p<.05$).

Results. As is shown in the Table, 50% SHS induced about 9% hemolysis in the absence of PLA_2 and about 72% hemolysis in the presence of PLA_2. Hemolysis induced by halothane alone or halothane in the presence of PLA_2 was antagonized by dantrolene. Halothane also exhibited a synergistic hemolysis with the major products of PLA_2 activity (AA and LPC). To determine if dantrolene antagonized PLA_2 activity, the effects of dantrolene on the interactions of AA and LPC with halothane were also examined. Dantrolene was most effective in antagonizing hemolysis due to the interaction of halothane with AA.

Discussion. PLA_2 and AA at concentrations inducing less than 2% hemolysis greatly potentiate hemolysis due to halothane. This synergism is antagonized by dantrolene. Dantrolene appears to stabilize the membrane to halothane lysis by an unknown mechanism. We propose that halothane acts at the sarcoplasmic reticulum of muscle in a manner analagous to that inducing hemolysis of RBCs, i.e. causing calcium release by membrane leakage, as opposed to activation of specific Ca^{2+} channels.[4] In patients susceptible to MH the enhanced Ca^{2+} release by halothane is potentiated by the presence of elevated PLA_2 activity.[1,2] RBC hemolysis is a convenient model in which to study the mode of action of dantrolene and the interaction of halothane with PLA_2.

References.

1. Cheah KS, Cheah AM: Skeletal muscle mitochondrial phospholipase A_2 and the interaction of mitochondria and sarcoplasmic reticulum in porcine malignant hyperthermia. Biochim biophys Acta 638:40-49, 1981
2. Fletcher JE, Rosenberg H: In vitro muscle contractures induced by halothane and suxamethonium: II. Human skeletal muscle from normal and malignant hyperthermia susceptible patients. Br J Anaesth 58:1433-1439, 1986
3. Tolpin EI, Fletcher JE, Rosenberg H, Michaux K: Effects of anaesthetic agents on erythrocyte fragility: Comparison of normal and malignant hyperthermia susceptible patients. Can J Anaesth, in press
4. Ohnishi ST, Waring AJ, Fang SRG, Horiuchi K, Flick, JL, Sadanaga KK, Ohnishi T: Abnormal membrane properties of the sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia: Modes of action of halothane, caffeine, dantrolene, and two other drugs. Arch Biochem Biophys 247:294-301, 1986.

Acknowledgements. This study was supported by the Hahnemann Anesthesia Research Foundation and USNIH grant #34872.

TABLE. Effects of dantrolene on hemolysis of human RBCs induced by halothane and PLA_2, or halothane and the products of PLA_2 activity.

Agents Incubated with RBCs	% Hemolysis (mean±SD)
Halothane (50% of saturated soln)	9.0±0.9
Halothane + Dantrolene (10 µM)	5.4±0.5
PLA_2 (1 µM)	1.9±0.1
Halothane + PLA_2	71.5±1.7
Halothane + PLA_2 + Dantrolene	25.2±1.3
Arachidonic acid (AA; 1.0 µM)	0.5±0.7
Halothane + AA	32.9±1.7
Halothane + AA + Dantrolene	17.9±2.2
Lysophosphatidylcholine (LPC; 2.5 µM)	15.0±0.4
Halothane + LPC	58.6±1.0
Halothane + LPC + Dantrolene	44.1±0.7

All differences greater than 2.2% hemolysis are significant ($p<.05$). For all conditions n=3.