Anesthesiology
V 75, No 3A, Sep 1991

A993

TITLE: LIVER CYTOSOLIC PROTEINS FROM HALOTHANE TREATED MICE ARE RECOGNIZED BY ANTIBODIES IN HALOTHANE HEPATITIS PATIENTS' SERA.

AUTHORS: J.E. Lindsey, M.D., J.L. Martin, M.D., L.R. Pohl, Ph.D.

AFFILIATION: NHLBI, NIH, Bethesda, MD 20892, National Naval Medical Center, Bethesda, MD 20814 and The Johns Hopkins Medical Inst., Baltimore, MD 21205

Because the volatile anesthetic halothane ($CF_3CHBrCl$) is still widely used, the search for the etiology of the rare, but often fatal, form of hepatitis caused by this agent remains imperative. Current evidence suggests an immune basis for halothane hepatitis. The immune response appears to be caused by liver neoantigens formed by the covalent binding of the trifluoroacetyl chloride (TFA-Cl) metabolite of halothane to tissue proteins. Previous studies employing immunoblotting have shown that the serum antibodies of 42/68 (62%) of the halothane hepatitis patients react with the liver microsomal TFA-neoantigens of 100 kDa, 76 kDa, 59 kDa, 57 kDa, and 54 kDa[1]. In the present study, we investigated the possibility that antibodies from halothane hepatitis patients' sera may also be directed against liver cytosolic TFA-proteins.

Liver cytosolic proteins from halothane treated and control male mice were separated into constituent polypeptides using SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes. The membranes were then incubated with the sera from patients with a clinical diagnosis of halothane hepatitis (n=33) or sera from the following patient groups: primary biliary cirrhosis (n=5), serum hepatitis + halothane exposure (n=5), acute fulminant hepatitis (n=4), chronic active hepatitis (n=5), normal halothane exposed (n=5), subclinical halothane exposed (n=4), and normal unexposed (n=5). Immunoblots revealed that 4/33 (12%) of the halothane hepatitis patients' sera reacted with halothane induced cytosolic proteins. No sera from the control groups reacted with these proteins.

The results of this study indicate that halothane induced neoantigens are also formed in the cytosol fraction of the liver and may play a role in the development of halothane hepatitis in certain individuals.

Reference

1. J. Pharm. Exp. Ther. 245:1103-1109, 1988

A994

TITLE: PENTOBARBITAL INHIBITS CA^{2+} SENSITIVE SECRETION IN BASOPHILS

AUTHOR: A. Robinson-White, Ph.D.
AFFILIATION: Department of Anesthesiology, USUHS, Bethesda, MD 20814

Serotonin (5-HT) secretion in rat mast cells and is induced via a receptor coupled GTP-binding protein (G-protein), G_p, that regulates phosphatidylinositol (PI) hydrolysis, and by a Ca^{2+}-sensitive G-protein, G_e, that directs secretory vesicle movement.[1] Barbiturates inhibit PI hydrolysis at the level of G_p.[2] This study determines if pentobarbital (PB) also interferes with G_e activity, to inhibit 5-HT secretion in rat basophilic (RBL-2H3) cells. METHODS: RBL-2H3 cells were a gift of Dr. M.A. Beaven (National Institutes of Health, Bethesda, MD). 5-HT secretion was measured[2] by incubating cells with [^{3}H]5-HT (18 hrs, 37°C), washing, and stimulating[3] with inducers and PB. For PI hydrolysis, cells loaded with [^{3}H]inositol, were incubated in $LiCl^+$ buffer (10 min), and with stimulants PB or buffer (15 min). Reactions were halted with chloroform-methanol. [^{3}H]inositol phosphates were separated on Dowex-1-formate columns, and inositol lipids purified with methanol. Cells were loaded with $^{45}CA^{2+}$, stimulants, PB or buffer for $^{45}Ca^{2+}$ uptake. RESULTS: PB inhibited antigen ($DNP_{34}BSA$)-induced PI hydrolysis and [^{3}H]5-HT secretion, with a greater effect on secretion (see table). Secretion induced by Ca^{2+} mobilizing agents (A23187 and tharpsigargin; TG) was similarly inhibited. Inhibition occurred in the absence of PI hydrolysis, when hydrolysis was blocked by neomycin (NEO), or when secretion was induced with TG. Secretion was also restored by activating protein kinase C (with PMA), independent of PI breakdown. DISCUSSION: The data show that Ca^{2+} sensitive secretion is more susceptible to PB than the receptor coupled system (PI hydrolysis via G_p); and that PB inhibits the action of the G-protein G_e. However, other sites of PB action cannot be excluded since $^{45}Ca^{2+}$ uptake was also inhibited at submillimolar concentrations. The data reinforces the idea that PB interferes with PI and Ca^{2+} stimulating events at clinical concentrations. (NIH, NIGMS #R29GM38021)

1. J.C.B.105:2745-2750, 1987.
2. Anesthesiology 72:996-1004, 1990.
3. J.B.C 265:745-753, 1990.

EC_{50} of PENTOBARBITAL ON CELL ACTIVITIES

Response	Stimulants, at Optimal Concentrations	EC_{50} (mM)
PI Hydrolysis	DNP-BSA	0.7
[^{3}H] 5-HT Secretion	DNP-BSA	0.15
"	" + NEO	<0.10
$^{45}Ca^{2+}$ Uptake	DNP-BSA	0.11
[^{3}H]5-HT Secretion	A23187	0.10
"	" + NEO	<0.02
"	A23187 + PMA	1.0
"	TG	0.12