POSTER DISCUSSION II

A995

TITLE: HALOTHANE MAY SUPPRESS THE ACTION OF THE G-PROTEIN, G_p, IN ENDOTHELIAL CELLS

AUTHORS: A. Robinson-White, Ph.D., Y. Kobayashi, D.D.S., Ph.D.
AFFILIATION: Department of Anesthesiology, USUHS, Bethesda, Maryland

The use of volatile anesthetics leads to vasodilation, yet the underlying cellular mechanisms remain unknown. Barbiturates were shown to inhibit the phosphatidylinositol (PI) pathway, in endothelial[1] cells and basophils, at the site of the GTP-binding protein, G_p,[2] that regulates PI breakdown. This study investigates the possibility that the volatile anesthetic, halothane, alters PI hydrolysis in human umbilical vein endothelial cells, by an action on G_p.

METHODS: Cells (Clonetics Corp., San Diego, CA), were > 95 % endothelial cell as shown by light and fluorescent microscopy. Cells were permeabilized[2] with streptolysin O, and viability assessed with ethidium bromide/fluorescein diacetate. Cells, loaded with [^{3}H]inositol, were incubated (37°C) in $LICL^+$ buffer for PI breakdown[1-2]. Air or halothane was passed in and out of inlet and outlet ports of two air-tight containers, with culture plates and stimulants. Reactions were halted with chloroform-methanol. [^{3}H]inositol phosphates were separated on Dowex-1 formate columns, and inositol phospholipids purified with methanol.

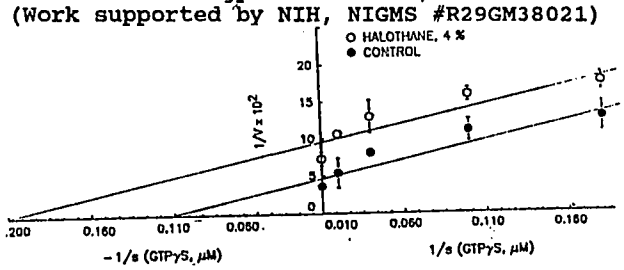
RESULTS: Hydrolysis was induced in intact cells with 1-100 µM histamine. 1-4 % halothane inhibited hydrolysis (10 to 40 %, to 40 min) by a competitive type inhibition, with no effect on basal hydrolysis or on control lipid levels. In permeabilized cells, G_p was directly stimulated with 1-100 µM $GTP\gamma S$. Halothane inhibited this hydrolysis (30-50 %; 4 %; 40 min). Kinetic analysis showed uncompetitive inhibition (K_m = 10 ng/ml reduced to 5 ng/ml) (figure).

DISCUSSION: Halothane alters stimulated hydrolysis. In intact cells, inhibition by halothane (1-2 %) was of the competitive type, which suggests that halothane caused a reduced affinity of the receptor for the agonist. The data with $GTP\gamma S$, however, showed uncompetitive inhibition. The data indicates that halothane may inhibit hydrolysis at a site distal to the receptor, on G_p, or on the G_p-phospholipase C complex, as has been shown with the barbiturates. The results may help to explain the action of volatile anesthetics on the vasculature.

REFERENCES:

1. Eur J. Pharmacol. 172:291-303, 1989.
2. Anesthesiology 72:996-1004, 1990

(Work supported by NIH, NIGMS #R29GM38021)



A996

TITLE: HALOTHANE AND ISOFLURANE INDUCED INHIBITION OF NATURAL KILLER CELL STIMULATION WITH INTERFERON α / β

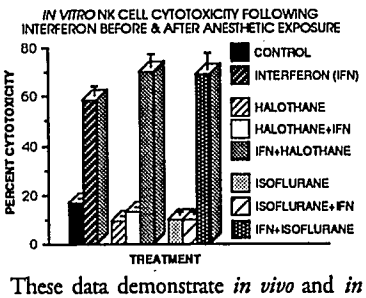
AUTHORS: S.N. Markovic, Ph.D., D.M Murasko, Ph.D., P.R. Knight, M.D., Ph.D.

AFFILIATION: Dept of Microbiol & Immunol, Medical College of Pennsylvania, Philadelphia, PA 19129, and Dept of Anesth, University of Michigan, Ann Arbor, MI 48109

Natural killer cells (NK cells) are a sub-population of lymphocytes which display spontaneous cytotoxicity against tumor cells and provide a first line of defense during an infection until an adoptive immune response can develop. NK cell activity is depressed following anesthesia and surgery in mice and men.[1,2] The current study was designed to compare two intravenous agents ketamine and tribromoethanol with two commonly used inhalational agents, halothane and isoflurane, *in vivo* and *in vitro*.

Mice were anesthetized for 2 hours with 1.5% halothane, 2.1% isoflurane, 0.1 g/kg ketamine, or 0.5 g/kg tribromoethanol on 10, 5, and 1 days prior to IFN treatment ($5 \cdot 10^4$U/mouse IP). One day after IFN treatment mice were sacrificed by cervical dislocation and the spleens removed. NK cell specific cytotoxicity was assessed by the release of ^{51}Cr from Yac-1 target cells into the culture media following 4 hours of co-activation. In vitro experiments were performed by exposing spleen cell removed from mice to the anesthetic for 2 hours. IFN ($1 \cdot 10^3$ U/ $1 \cdot 10^6$ cells) was added to the cultures before or after exposure to the anesthetic. Cytotoxicity was assayed as previously described. Variables were analyzed using Students T test for unpaired data.

All four agents administered to animals 24 hours prior to IFN treatment inhibited the IFN induced NK stimulation >90% ($p<0.01$), except halothane which decreased stimulation by 67% ($p<0.05$). IFN induced NK activation completely recovered in mice anesthetized with ketamine and tribromoethanol by 10 days post anesthesia. Mice anesthetized with halothane and isoflurane showed significant inhibition of NK stimulation at 10 days post anesthetic exposure 66% and 44% of control levels, respectively ($p<0.05$).



The inability of IFN to stimulate NK cytotoxicity when the cells were exposed to any of the anesthetic agents *in vitro* prior to exposure to IFN was <90% ($p<0.05$). When IFN was given before anesthetic exposure IFN stimulated NK activity was not suppressed.

These data demonstrate *in vivo* and *in vitro* suppression of IFN stimulated NK cell activity by two volatile, and two injectable, anesthetics. None of the anesthetics inhibited NK cells previously activated by IFN. Anesthetic inhibition of activation of NK cells may explain the mechanisms of clinically observed transient postoperative immunosuppression. The persistently enhanced NK activity when IFN is given prior to anesthesia suggests possible therapy to decrease infectious complications in patients who are immunosuppressed prior to surgery. In addition these results are important in the temporal application of treatment in the newly developing area of immunotherapy for solid tumors.

1. *Clin Immunol Immunopath* 56:202-209, 1990.
2. *Acta Anaesthesiol Scand* 28:654-660, 1984.