Anesthesiology
V 75, No 3A, Sep 1991

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TITLE: ENHANCEMENT OF ENZYMATIC PHOSPHOLIPID N-METHYLATION IN RAT BRAIN SYNAPTOSOMES DURING ANESTHESIA WITH ISOFLURANE

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INTRODUCTION: Isoflurane, a potent volatile anesthetic, may affect synaptic transmission. S-Adenosyl-L-methionine (SAM) mediated methylation of membrane phosphatidylethanolamine (PE) to phosphatidyl-N-methylethanolamine (PME) by phospholipid-N-methyltransferase (PMT1) plays a significant role in biosignal transfer across membranes.[1] There is an inverse relationship between PME formation and neurotransmitter release which might be applicable to central synapses during anesthesia.[2] Halothane has been shown to enhance PMT activity and PME formation in rat brain synaptosomes at anesthetic concentrations.[3] In the present study, the effects of isoflurane were studied on synaptosomal PMT in vivo and in vitro.

METHODS: Male Sprague-Dawley rats of average weight 370 g were anesthetized with isoflurane in warmed, humidified air and oxygen (FI_{O2} 0.03). The concentration of isoflurane was adjusted so that the dose delivered was just above the level where a tailflick could be induced by clamping, thus establishing the minimum effective dose (MED) for each rat. After 20 min of anesthesia, rats were decapitated, the brains were dissected, and synaptosomes were prepared by differential centrifugation of the homogenates in isotonic sucrose. Methylation of synaptosomal PE was assayed using ^{3}H-SAM (2 µM) and an incubation period of 30 min at 37°C in Tris-glycylglycine buffer (50 mM, pH 8.0). The methylated phospholipids were extracted with chloroform:methanol:HCl (2:1:0.02, v/v) and separated by thin layer chromatography. The assay conditions were optimum for measuring PMT1. Therefore, all values were expressed as the product of PMT1, ^{3}H-PME. Isoflurane concentrations (%) ranging between 0.635 and 3.070% were used. Delivered isoflurane concentrations were confirmed by gas chromatography.

RESULTS AND DISCUSSION: The MED of isoflurane for abolishing the pain response was 1.9 ± 0.07%. The total ^{3}H-PME formed/mg protein/30 min increased from 245 ± 22 (control, N=12) to 432 ± 42 (N=10) fmol at one MED. Other concentrations of isoflurane (0.6%, 2.5%) also increased ^{3}H-PME formation. ^{3}H-PME formation returned to control levels in synaptosomes of rats anesthetized with isoflurane and recovered from anesthesia. Phospholipid methylation in synaptosomes increased with exposure to isoflurane concentrations (0.64-1.92%) in vitro. The increase in ^{3}H-PME by isoflurane was inhibited by S-adenosyl-L-homocysteine (2 µM), an inhibitor of phospholipid methylation. Some of the PME was converted to phosphatidyl-dimethylethanolamine (PDE) and phosphatidylcholine (PC). All three products of transmethylation, PME, PDE, and PC, showed a similar pattern of increase at one MED of isoflurane exposure. All of these observations indicate that PMT may be a site of action for the anesthetic effect of isoflurane.

Supported by the Study Center for Anesthesia Toxicology.

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TITLE HALOTHANE-OXIDANT INTERACTIONS IN THE *EX VIVO* PERFUSED RABBIT LUNG: EDEMA FORMATION AND EICOSANOID PRODUCTION

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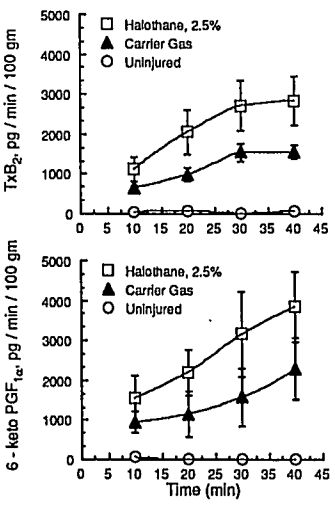
Isolated, perfused rabbit lungs ventilated with inhalational anesthetics give an enhanced vasopressor response to the organic oxidant *tert*-butyl-hyroperoxide (t-bu-OOH), which is related to the appearance of higher concentrations of thromboxane B_2 (TxB_2) in the effluent perfusate compared with lungs ventilated with 5% CO_2 in air (carrier gas).[1,2] We hypothesized that, when change in pulmonary vascular resistance is eliminated as a variable, an increased rate of pulmonary edema formation and increased production of TxB_2 will be seen accompanying t-bu-OOH infusion in lungs ventilated with halothane compared to lungs ventilated with carrier gas.

After appropriate animal use committee approval, 27 New Zealand White rabbits (2-3 kg) were used for these experiments. The pulmonary artery and left atrium were cannulated, the lungs ventilated via a tracheostomy, and perfused at 35 mL/min, in a recirculating fashion, at 37-38°C, with Ca^{2+}-free Krebs-Henseleit buffer. T-bu-OOH was infused for 1 min directly into the pulmonary artery at a final concentration of 200 µM 4 times in each experiment, each infusion separated from the previous one by a 15 min recovery phase. The filtration coefficient for perfusate (K_f) 10 min after each t-bu-OOH infusion was calculated as $(\Delta W_L/\Delta P_{pa})(0.3)$, where ΔP_{pa} is the difference in P_{pa} at high (105 mL/min) and low flow rates (with left atrial pressure at 5 mmHg); ΔW_L, the change in lung weight over the last min of high flow; and 0.3, the ratio of pulmonary capillary to pulmonary arterial pressure. At the end of the experiment, the lungs were excised *en bloc*, weighed, then dried and reweighed. Samples of perfusate were obtained just before each t-bu-OOH infusion and 90 sec after the start of the t-bu-OOH infusion for determination of [TxB_2] and [6-keto-$PGF_{1\alpha}$]. Eicosanoid production was expressed as pg/min/100 gm dry lung tissue.



TxB_2 (top) and 6-keto-$PGF_{1\alpha}$ (bottom) production as a function of t-bu-OOH infusion.

Three groups of lungs were studied: Uninjured, ventilated with carrier gas (n=9); Injured, ventilated with carrier gas (n=9); and injured, ventilated with 2.5% halothane in carrier gas (n=9). Data, expressed as mean±SE, were analyzed by ANOVA, using Scheffé's or Dunnett's tests for multiple comparisons where appropriate.

In the absence of a pulmonary vascular response to t-bu-OOH, the injured lungs ventilated with halothane exhibited a greater K_f (109±21 gm/min/mmHg/100 gm dry tissue) than the injured (43±39) or the uninjured lungs ventilated with carrier gas (14±7) ($P<0.04$ by the fourth t-bu-OOH infusion). TxB_2 production was also greater in the injured lungs ventilated with halothane compared to the injured or the uninjured lungs ventilated with carrier gas (*see* Figure). Lung wet/dry ratios were greater in both groups of injured lungs compared to the uninjured group ($P<0.04$).

With the elimination of capillary hydrostatic pressure change as a factor contributing to pulmonary edema formation in this model, these results indicate t-bu-OOH injury of lungs ventilated with halothane results in a higher rate of pulmonary edema formation as measured by the K_f (but not by the wet/dry ratio). Also the increase in TxB_2 (but not in 6-keto-$PGF_{1\alpha}$) production is consistent with earlier findings and indicates that halothane may enhance phospholipase A activation, possibly by a Ca^{2+}-mediated mechanism.

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