**TITLE** SUPEROXIDE PRODUCTION BY STIMULATED ENDOTHELIAL CELLS EXPOSED TO VARYING HALOTHANE CONCENTRATIONS

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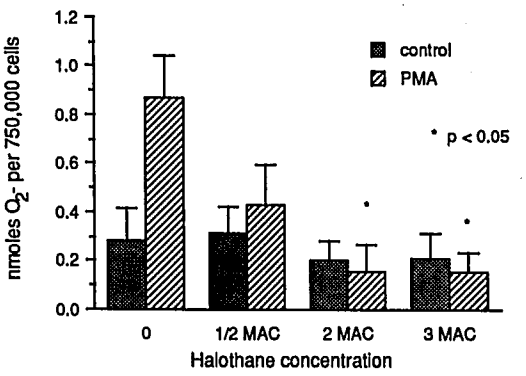
Vascular endothelium produces superoxide anion ($O_2^-$) when exposed to soluble or particulate stimuli. Halothane (Hal) has been known to inhibit $O_2^-$ production by stimulated neutrophils.[1] In contrast, volatile anesthetics have been found to enhance oxidant–induced endothelial injury.[2] Given this finding, and the assumption that endothelial cell production of $O_2^-$ is related to injury, we expected to find that Hal enhanced $O_2^-$ production.

Rat pulmonary artery endothelial cells (RPAECs) were isolated as described elsewhere,[3] and cultured into monolayers on microcarriers (μc) in Minimal Essential Media containing 10% fetal calf serum. Ten million RPAECs added to two million μc took 5–6 days to grow to confluence. The final culture contained 160 million cells. At confluence, the μc were washed in Hanks Balanced Salt Solution, 0.2% BSA, and aliquoted to test tubes so that each contained 750,000 cells. Tubes were equilibrated with either carrier gas (5% $CO_2$ in air) or Hal (0.5, 2, or 3 MAC) for 10 minutes. Half of each group of cells were stimulated with phorbol myristate acetate (PMA), 1 μg/cc. All tubes were then incubated for 1 hour at 37°C. $O_2^-$ production was measured by reduction of ferricytochrome C, as described elsewhere, using an extinction coefficient of 18.5/cm•mM.[4]

Values for $O_2^-$ production were analyzed by 2-way ANOVA. If the F-ratio was significant ($p<0.05$), between–group comparisons were made using 1-way ANOVA and Dunnett's test. The $O_2^-$ content in the medium was decreased significantly for PMA–stimulated cells exposed to Hal 2 and 3 MAC. Values shown in the figure represent mean ± s.e. for 12 experiments.

In conclusion, Hal was found to inhibit the release of $O_2^-$ into the medium by PMA–stimulated cells. This could be a result of inhibited $O_2^-$ release or production. Possible mechanisms for inhibited $O_2^-$ production are inhibition of the xanthine dehydrogenase to xanthine oxidase conversion or inhibition of purine metabolism.

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[3] *J Tissue Cult Methods* 10:9–13, 1986. [4] *J Clin Invest* 52:741–745, 1973.

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**TITLE:** EFFECTS OF HALOTHANE ON PHOSPHOLIPID N-METHYLATION IN RAT BRAIN SYNAPTOSOMES

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**INTRODUCTION:** The mechanism of action of inhalational anesthetics is unknown, but neuronal membrane alteration is a favored hypothesis. Axelrod, Hirata and others[1] have demonstrated that enzymatic conversion (methylation) and translocation of inner membranal phosphatidylethanalamine (PE) to outer membranal phosphatidylcholine (PC) facilitates transduction of receptor-mediated signals through cell membranes. We recently have shown that PE methylation is doubled in brain synaptosomes taken from rats anesthetized with 1.4% halothane, returning to normal in rats allowed to recover.[2] We report here the effect of in vitro exposure of isolated synaptosomes to varying concentrations of halothane.

**METHODS:** Animal use was approved by the Animal Care Committee of Vanderbilt University. Male Sprague-Dawley rats weighing 285 to 460g were used. Synaptosomes were isolated from brain homogenates by differential centrifugation, as described by Cotman.[3] Methylation was measured by the incorporation of tritiated methyl groups from S-adenosyl-L-[$^3$H-methyl]methionine (SAM) into PE.[4] The incubation mixture, consisting of 0.2mg of synaptosomal protein, buffers and 2μM $^3$H-SAM, was exposed to varying concentrations of halothane for 30 min in a Dubnoff shaker. (Delivered halothane concentrations were confirmed by gas chromatography.) The methylated phospholipids were extracted with chloroform:methanol:HCl (2:1:0.02, v/v) and separated by thin layer chromatography. The activity of PE-N-methyltransferase, the rate limiting enzyme in transmethylation, was expressed by the amount of phosphatidyl-N-methylethanolamine (PME) formed in fmol/mg protein/30 min.

**RESULTS AND DISCUSSION:** Halothane in concentrations of 1.0% and 1.4% increased PME ($P<0.01$) formation from 495±27 fmols (control, N=8) to 1594±107 fmols (N=6) and 1562±68 fmoles (N=7), respectively. Thus, halothane at 1.0-1.4% produced a three-fold increase in phospholipid methylation. Halothane concentration of 0.5% did not increase PME formation. PME formation was 1006±47 fmols (N=7) and 821±57 fmols (N=7), at halothane concentrations of 1.9 and 2.4%, respectively. These high concentrations of halothane caused significant but smaller increases in PME formations. These observations indicate that halothane exhibits a biphasic effect on PME formation. Halothane concentrations higher than 1.4% do seem to retard PME formation.
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