

TITLE: INCREASED PHOSPHOLIPID N-METHYLATION IN THE RAT BRAIN MYELIN MEMBRANES DURING ANESTHESIA WITH HALOTHANE

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INTRODUCTION: Anesthetic concentrations of halothane depress somatosensory, auditory and visual evoked potentials indicating that halothane interferes with electrical axonal impulse conduction and/or synaptic transmission. Phospholipid N-methylation plays a significant role in biosignal transfer with respect to particular receptor and cell systems.¹ There is an inverse relationship between phospholipid methylation and neurotransmitter release which might apply to central synapses during anesthesia.² Minimum effective dose (MED) of halothane (abolishing tailflick) increased phospholipid methylation in rat synaptosomes, indicating that it might interfere with synaptic transmission.³ Both synaptosomal and myelin membranes have phospholipid composition similar to that of plasma membranes. A question arises as to whether halothane influences phospholipid methylation in myelin membranes and thereby effects impulse conduction along axons and across nodes of Ranvier. Therefore, we have investigated methylation of myelin phosphatidylethanolamine (PE) during halothane anesthesia.

METHODS: Animal use was approved by the Animal Care Committee of Vanderbilt University. Male Sprague-Dawley rats (285-455g) were anesthetized with halothane in humidified air and oxygen FI_{O₂}. After 20 min of anesthesia, rats were decapitated, the brains were dissected and placed in 0.32M sucrose at 4°C. The excised brains were homogenized in 0.32M sucrose. The homogenate was subjected to differential centrifugation. The 15,000g fraction was separated by Ficoll gradients. Myelin membranes were obtained from 4% Ficoll. Methylation of myelin

PE to phosphatidyl-N-methylethanolamine (PME) was assayed with S-adenosyl-L-[³H]-methionine (SAM, 2μM) at 37°C in tris-glycylglycine buffer (50mM, 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 activity of PE-N-methyltransferase was expressed as fmol of PME formed/mg protein/30min. Delivered halothane concentrations were confirmed by gas chromatography.

RESULTS: The MED of halothane abolishing pain response in the rat was 1.4±0.1%. This concentration of halothane increased PME formation from 580±41 fmols (control) to 809±48 fmols (p<0.01) in myelin. Other concentrations of halothane (0.5, 0.92, 1.92 and 2.45%) did not increase PME formation in myelin. There was no increase in PME formation (412±13fmols) in myelin of rats anesthetized with one MED of halothane after recovery from anesthesia. PME formation also increased in myelin membranes exposed to halothane vapor (1.0%) for 30 min.

DISCUSSION: This study indicates that halothane increases phospholipid methylation both in vivo and in vitro. This may influence conduction of electrical potentials along the axon and nodes of Ranvier. In the axon, the resting potential is maintained by Na-K ATPase which acts as a pump moving Na⁺ out of the cells and K⁺ in. This pump is distributed along the axolemma, at the nodes as well as internodal membranes. Administration of SAM to rats increased liver plasma membrane Na-K ATPase activity and fluidity.⁴ Increased phospholipid methylation in myelin during halothane anesthesia might increase Na-K ATPase activity, hyperpolarize the axolemma and thereby may interfere with nerve impulse conduction.

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TITLE: RAPID DEVELOPMENT OF TOLERANCE DURING FENTANYL INFUSION

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Introduction. The pharmacodynamics of fentanyl in neonates have not been well delineated although fentanyl is used widely in neonatal anesthesia and intensive care. We have used fentanyl by continuous infusion to sedate infants undergoing extracorporeal membrane oxygenation (ECMO) and have previously reported infusion rates of 30-40 μg/kg/hr to provide adequate sedation.¹ In order to define the plasma levels that produce clinical sedation and elucidate the pharmacodynamics of fentanyl in the newborn, we measured plasma fentanyl concentrations in a group of infants undergoing ECMO sedated with a continuous infusion of fentanyl.

Methods. We prospectively studied five infants undergoing ECMO between July and September, 1989 after obtaining informed consent in accordance with the Institution's Committee on Clinical Investigation. Fentanyl infusions were initiated within the first twenty-four hours of life in all infants and titrated to achieve a uniform level of sedation. All infants were asleep but arousable and frequently breathing spontaneously during the period of study. Plasma fentanyl concentrations were determined by gas chromatography using nitrogen-phosphorus detection with a lower limit of detection of 0.5 ng/ml and a coefficient of variation of 6.9% at a concentration of 1.0 ng/ml. Using unpaired, two-tailed t-testing, we compared fentanyl concentrations on days 2 through 7 with the day 1 value.

Results. Plasma fentanyl levels increased steadily during the course of fentanyl infusion (Figure). Fentanyl levels were significantly greater on days 5, 6 and 7 when compared with day 1 (p=.03, .05, .004, respectively).

Discussion. There are limited data regarding the pharmacodynamics of fentanyl in the neonate. Our study population offered a unique opportunity to assess these parameters as infusion rates were titrated carefully to provide a constant level of sedation. Plasma fentanyl levels in our study neonates were consistently above 1-2 ng/ml which has been identified as the apneic threshold in neonates² and the minimal analgesic concentration in adults.³ Furthermore, these infants appear to develop tolerance to the sedating effects of fentanyl within several days of the initiation of fentanyl infusion. The rapid development of tolerance may limit the usefulness of fentanyl as an agent for continuous sedation in the newborn population.

References.

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