Title: *IN VITRO* ANALYSIS OF HALOTHANE INDUCED INHIBITION OF PROTEIN SYNTHESIS

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Introduction. Exposure of cells or tissues to volatile anesthetics results in an inhibition in protein synthesis.[1] This inhibition may result in potentially toxic side effects, especially in those tissues which are most rapidly dividing, are metabolically active, or which have the greatest rate of protein turnover, such as cells from the liver or the immune system. While it has been suggested that the initiation and/or elongation of peptide chains may be the site(s) of the anesthetic induced inhibition, the exact mechanism of inhibition has not yet been determined. In this report we describe the effect of halothane on an *in vitro* protein synthesizing system which retains many of the characteristics of the *in vivo* system. This model allows us to examine the molecular events which occur during anesthetic induced inhibition of protein synthesis. The effects of halothane exposure on *in vitro* translation are described below.

Methods. Protein synthesis assays were performed using a Promega *in vitro* translation kit. ^{35}S-methionine incorporation into polypeptide chains was monitored. One milliliter glass reaction tubes were sealed with a tight-fitting rubber septum and perfused for 20 min with a designated halothane concentration. Halothane was delivered by directing a 95% air, 5% CO_2 mixture through a Drager® vaporizer at 3 L/min. The vaporizer output was connected to Teflon tubing containing a rubber septum from which samples of anesthetic vapors were withdrawn. The delivered anesthetic concentrations were determined using a Gow Mac® gas chromatograph. Halothane vapors were delivered into each reaction tube via a 20 gauge needle placed into the septum; a second 20 gauge needle provided an outlet for each flask. Following the addition of halothane, the following reaction components were added: 35 µl reticulocyte lysate, 2 µl of a solution containing 19 non-labeled amino acids, 2 µl RNA primer, and 6 µl H_2O, bringing the reaction volume to 45 µl. Each tube was equilibrated for 10 min, after which time the reactions were initiated by the addition of 5 µl of ^{35}S-methionine.

Reactions were terminated by the addition of 0.5 ml 1N NaOH at 0 min, 20 min, and 40 min following the addition of radiolabel. Incorporation of ^{35}S-methionine into peptides was monitored by incorporation of the radiolabel into acid precipitable material, as well as analysis of protein products by polyacrylamide gel electrophoresis.[2]

Results. Inhibition of *in vitro* protein synthesis was observed following exposure to halothane (see *Figure*). While a 1% concentration of the anesthetic failed to inhibit *in vitro* translation of RNA, a 2% halothane concentration reduced protein synthesis levels to 74% and 87% of those of the non-exposed controls after 20 min and 40 min, respectively. Protein synthesis was inhibited by exposure to a 3% halothane concentration to 61% of control level at 20 min and 68% of control level at 40 min. Polyacrylamide gel electrophoresis of protein products synthesized during halothane exposure revealed that all protein species were inhibited similarly.

Discussion. The exact site(s) of inhibition of protein synthesis during volatile anesthetic exposure are not yet known, although the synthesis of messenger RNAs from DNA does not appear to be a sensitive step.[1] By using a system which enables us to control carefully each step of the translation process we hope to determine the exact site of protein synthesis inhibition. Halothane induced protein synthesis inhibition *in vitro* was detected more rapidly (within 20 min) than results obtained *in vivo* (4 - 6 hrs). The site of anesthetic action may not be as accessible in intact cells as in cell lysates, or a different site may be more readily affected in lysates than in whole cells. In intact cells the plasma membrane may be the primary site of anesthetic action, and protein synthesis inhibition a result following a cascade of changes in the cell. In cell lysates the anesthetic may directly alter the sensitive site(s).

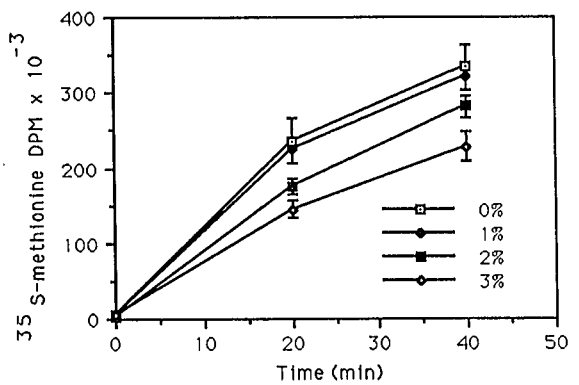
Inhibition of protein synthesis during anesthetic exposure may be particularly important in cells such as lymphocytes, which can be stimulated by mitogens,[3] and in metabolically active cells which exhibit a rapid rate of protein turnover, e.g., hepatocytes. It is expected that further studies will enable us to pinpoint exactly which molecular components interact to produce anesthetic induced inhibitory effects on protein synthesis.

References.

1 JA Hammer, DE Rannels, *Amer. Rev. Repir. Dis.* **124**, 50(1981); KE Flaim, LS Jefferson, JB McGwire, DE Rannels, *Mol. Pharmacol.* **24**, 277 (1983); H Aune, H Bessesen, A Olsen, J Morland, *Acta Pharmacol. Toxicol.* **53**, 363 (1983); Bedows, *et. al.*, *In Vitro Toxicol.* **2**, in press (1988).

2 E Bedows, PJ Cohen, PR Knight. in *Molecular Mechanisms of Anesthesia.* S Roth and KW Miller, Eds. (Plenum Press, New York, 1986) pp 365-378.

3 DL Bruce, *Anesthesiology* **36**, 201 (1975).



Concentration dependence for halothane induced inhibition of *in vitro* protein synthesis. Each point represents the mean of three experiments. [Bars indicate S.D.]