Title: BACTERIAL GROWTH IN THE PRESENCE OF ANESTHETIC AGENTS.

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Introduction. Volatile anesthetic agents have been shown to inhibit cell division and incorporation of precursors into macromolecules of eucaryotic cells.[1] To further study the effects of these agents on cellular structure and function, E. coli was chosen in the present study as a simple model system. This organism is not only among the most commonly isolated etiologic agents of gram-negative bacteremias, but is also a fast growing cell, which is well characterized biochemically and genetically.

Methods. Escherichia coli B/r (ATCC 12407) was grown at 37° C in minimal C medium supplemented with either 0.2% glucose or 0.2% glycerol in the presence or absence of 20 µg of each of the standard 20 L-amino acids per ml. Growth was measured as the increase in the concentration of cell mass by following the absorption of light at 460 nm by the culture (1-cm light path). An optical density of 1.0 was found to correspond to 4.6×10^8 colony forming cells/ml. The cultures were aerated with air containing the indicated concentrations of volatile anesthetic which were delivered from the Verni-Trol vaporizer of an Ohio anesthesia machine. The rates of protein and RNA synthesis were determined from the kinetics of radioisotope incorporation into acid precipitable material: ^{14}C-leucine and ^{3}H-uridine were both added to an exponentially growing culture, from which aliquots were lysed, precipitated in the presence of carrier RNA with acid on nitrocellulose filters, and washed prior to counting in a liquid scintillation counter.

Results. When exponentially growing cultures of E. coli are exposed to volatile anesthetic agents, their growth rates decrease with increasing doses. Growth under these conditions is still exponential, however, and its inhibition is completely reversible by withdrawal of the agent. Fig. 1 shows that growing E. coli is more susceptible to isoflurane than to enflurane, and even more to halothane. In fact, all agents reduce the growth rate to the same degree if applied at doses of equal clinical potency (= same multiples of MAC), regardless of the carbon source used to sustain growth. Different carbon sources, however, increase the susceptibility to each of the 3 agents proportionately in the following order: glycerol + amino acids > glycerol > glucose + amino acids > glucose. Replacement of air by an 80% N_2O-20% O_2 mixture has no additional effect on cells grown in the presence of 5% halothane. Dose response curves obtained with droperidol and ketamine qualitatively differ from those obtained with volatile agents in that they show a steep decrease in growth rate beyond a certain threshold concentration. A 50% reduction is found when cells growing in glucose + amino acid medium are exposed to 2.6×10^{-4} molar droperidol or 4×10^{-3} molar ketamine, respectively. Again, withdrawal of the drugs results in resumption of the pre-treatment growth rate after a short lag-period. Since protein and nucleic acids comprise about 75% of the dry weight of a bacterial cell, it is of interest to compare the reduction in the accumulation of cell mass with possible changes in macromolecular synthesis: it is found that exposure of E. coli to 10% halothane reduces protein synthesis 40% and RNA synthesis 70%. The preferential reduction of RNA synthesis is clearly seen, when the cells are exposed to 1% halothane: the rate of leucine incorporation remains unchanged, whereas the rate of uridine incorporation decreases 32%.

Discussion. The data show that growth of E. coli is reversibly inhibited during exposure to halothane, isoflurane or enflurane. During anesthesia, this inhibition is probably masked by the simultaneous inhibition of the microbicidal activity of human phagocytic cells.[2] The decrease in bacterial growth rate is accompanied by a decrease in macromolecular synthesis, and RNA synthesis is more susceptible to halothane than is protein synthesis. This observation suggests that volatile anesthetic agents interfere with the synthesis and/or function of the ribosome.

References.

1. Conklin, KA, Lau, S-S: Enflurane effects on cell division and macromolecular synthesis in Tetrahymena pyriformis. Anesthesiology 53, 287-292, 1980.
2. Welch, WD: Halothane reversibly inhibits human neutrophil bacterial killing. Anesthesiology 55 650-654, 1981.

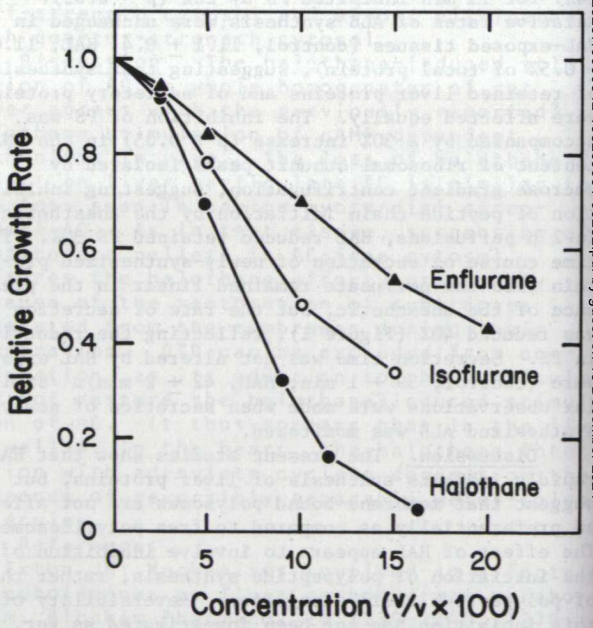

Figure 1: Response of growth rates of E. coli B/r exposed to different concentrations of halothane, isoflurane and enflurane. Rate of control: 1.94 doublings/hr in medium supplemented with glucose and amino acids. Each point represents the slope of a growth curve, obtained from 9 independent measurements (see Methods).