Title: SYNERGISTIC INTERACTION OF MORPHINE AND HALOTHANE IN-VITRO

Authors: MM Puig MD PhD, W Warner PhD*, CK Tang MD, M Lovitz BS, and H. Turndorf MD

Affiliation: Department of Anesthesiology, New York University Medical Center. 550 First Avenue, New York, NY 10016 and *Department of Pharmacology, NYU School of Dentistry, New York, NY

Introduction. It is well established that opiates decrease the MAC of inhalation anesthetics in experimental animals and humans. However, the effects of inhalation anesthetics on opiate activity,opioid-receptor interactions and intracellular processing of the receptor signal have not been defined. The present study describes the effects of halothane on morphine activity in the guinea pig ileum preparation and the characteristics of the interaction at the opioid receptor sites.

Methods. We have used the electrically stimulated myenteric plexus-longitudinal muscle guinea pig ileum preparation (MPLM) which is widely accepted as a model to study the interaction of opiates with mu-opioid receptors. MPLM strips were mounted in Krebs-bicarbonate solution at 37°C, equilibrated with 95% O_2-5%CO_2 and stimulated at supramaximal voltage and 0.1 Hz. When halothane was used, the O_2/CO_2 mixture was passed through a Fluotec Mark II vaporizer and the delivered concentration of halothane verified by gas chromatography. Dose response curves for morphine and halothane were determined under control conditions and in the presence of naloxone. Moreover, dose response curves of morphine were also determined in the presence of halothane (0.8-3.0 V/V %). The slopes of the individual curves and their 95% confidence limits (CL) were compared for parallelism and the IC_{50}'s of morphine and halothane under the different experimental conditions determined. pA_2's were calculated from the Schild plots and Hill coefficients used for the analysis of the interaction between halothane and morphine.

Results. Exposure of the MPLM to either morphine or halothane produced a dose related inhibition of the muscle contraction with an IC_{50} of $1.9X10^{-7}$ M (CL $1.6\text{-}2.0 \times 10^{-7}$M; n=25) for morphine and of 1.7 V/V % (CL 1.4-1.9 V/V %; n=21) for halothane. The IC_{50} of halothane was unchanged in the presence of naloxone. Morphine dose response curves were also obtained in the presence of halothane (0.8-3.0 V/V %). At each concentration tested (see table), the IC_{50} of morphine was significantly reduced from control values. When halothane concentrations were plotted against the corresponding IC_{50}'s of morphine, the resulting line had a coefficient correlation of 0.99. These results demonstrate that halothane increases the potency of morphine in a dose related manner. Hill coefficients for morphine and halothane were 0.91 and 0.92 respectively, and the coefficient for the combination was 1.4, indicating a cooperative (synergistic) interaction between binding sites. Under control conditions (morphine without halothane), the pA_2 of naloxone was 9.4 ± 0.2, while in the presence of 1.6 V/V % halothane, this value remained unchanged ($pA_2=9.1\pm0.9$) demonstrating that halothane does not alter the affinity of naloxone for the opioid receptors.

Conclusions. Our results show that 1) halothane increases the potency of morphine in the guinea pig ileum, at concentrations which are within the range used to produce clinical anesthesia, 2) the interaction between halothane and morphine is synergistic and 3) that halothane does not modify the binding of opiates to opioid-receptors, but seems to affect the membrane events which lead to the intracellular processing of the receptor signal. The present study, which address(es) the mechanism(s) of morphine halothane synergy in-vitro, could be useful for a better understanding of drug interactions in clinical anesthesia. Moreover, the sensitization of opioid receptors by halothane could explain the mechanism by which halothane may interact with the endogenous opioid system.

EFFECTS OF HALOTHANE ON THE POTENCY OF MORPHINE

	Halothane Concentration V/V%				
	0	0.8	1.0	1.6	3.0
Morphine $IC_{50} \times 10^{-8}$ M	19.0	11.0	9.6	5.8	2.6
95% Confidence Limits	16-21	7.2-16	7.6-12	4.0-8.2	1.6-4.0
Number of Experiments	25	5	5	5	5

The IC_{50} of morphine alone is significantly different from each of the values obtained with halothane ($p<0.05$).