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# *Synergistic Interaction of Morphine and Halothane in the Guinea Pig Ileum*

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**The present study describes the effects of halothane on morphine activity in the myenteric plexus-longitudinal muscle preparation of the guinea pig ileum. Morphine and halothane produced a dose-related inhibition of the electrically induced muscle contractions with $IC_{50}$ of $1.9 \times 10^{-7}$ and 1.7 V/V%, respectively. The effects of morphine, but not halothane, were antagonized by naloxone. The $IC_{50}$ of morphine was decreased in the presence of halothane (0.8–3.0 V/V%). Hill coefficients derived from dose-response curves were less than one for morphine or halothane alone, while it was 1.4 for the combination. The $pA_2$ values (a measure of affinity of the antagonist for the opioid receptor) for naloxone in the absence and presence of halothane (1.6%) were 9.4 and 9.1, respectively. These results indicate that 1) halothane increases the potency of morphine in the guinea pig ileum at clinically relevant concentrations, 2) the interaction between the agents is synergistic, and 3) halothane does not modify the binding of naloxone to opioid receptors, but may affect membrane or intracellular processing of the receptor signal. (Key words: Anesthetics intravenous: morphine. Anesthetics, volatile: halothane. Guinea pig ileum. Interactions. Drug: halothane; morphine. Receptors: opioid.)**

IT IS WELL ESTABLISHED that opiates decrease the minimal alveolar contraction (MAC) of inhalation anesthetics in experimental animals and humans.[1–3] However, the effects of inhalation anesthetics on opiate activity, opioid-receptor interactions, and intracellular processing of the receptor signal have not been well defined. To address these problems, we have used the electrically stimulated myenteric plexus-longitudinal muscle (MPLM) from the guinea pig ileum. In this preparation, the binding of opioids to their receptors leads to a reduction in the ganglionic release of acetylcholine.[4] This, in turn, produces an inhibition of the electrically induced smooth muscle contractions. The guinea pig ileum preparation is particularly suited to the study of the interaction between halothane and opioids because: 1) it contains a high concentration of mu-opioid receptors, the subtype predominantly involved in opiate analgesia; 2) the inhibitory effects of opiates on the electrically induced contractions of the MPLM are reversed by naloxone; 3) the potency of opiates in this preparation correlates closely with their analgesic potency in humans[5]; and 4) halothane produces a dose-related inhibition of the twitch response.[6]

The present study describes the effects of halothane on morphine activity in the guinea pig MPLM preparation and the characteristics of the interaction of both drugs at the opioid receptor sites.

## Materials and Methods

The experimental protocol was approved by our local animal studies committee. All experiments were carried out on the guinea pig ileum MPLM preparation. Male, albino guinea pigs (English Short Hair), weighing 300–400 g were stunned by a blow to the head and decapitated immediately. Their abdomens were opened and the MPLMs were prepared as described by Puig *et al.*[7] Each strip of tissue, weighing between 25 and 30 g, was suspended in a 10-ml organ bath containing Krebs-bicarbonate solution and gassed with 95% $O_2$/5% $CO_2$. When halothane was used, the $O_2/CO_2$ gas mixture was passed through a Fluotec® Mark II vaporizer and the delivered concentration of halothane verified by gas chromatography (Hewlett Packard® 5830A Gas Chromatograph). The strips were placed under a resting tension of 0.3 g and were allowed to equilibrate for 60 min before starting experiments.

The MPLM preparation was stimulated with platinum ring electrodes placed at the top and bottom of the muscle strip and separated by a distance of 6.0 cm. Symmetrical, biphasic stimuli of 1-ms duration and supramaximal voltage (35 V) were generated by a Grass® S-88 stimulator, mixed with the aid of two Grass® stimulus isolation units (SIU 5), fed into a Crown® DC 300 audio amplifier and then applied to the electrodes. The voltage and duration of the stimuli were monitored constantly on a Tektronix® R5103N oscilloscope. The strips were stimulated at a frequency of 0.1 Hz. Isometric contractions of the muscle were registered by means of a Grass® force transducer (model FT03C) coupled to a Grass® polygraph recorder. Bath temperature was maintained at 37° C.

Halothane was delivered to the organ bath at concentrations of 0.4 to 3 V/V%. After the effect of halothane was established, the preparation was equilibrated

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TABLE 1. 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 ($P < 0.05$).

for an additional 15 min in the presence of the anesthetic. The delivered halothane concentration remained unchanged throughout each experiment. Morphine solutions were made daily with glass distilled water and added to the bath in a volume of 0.1 ml. The tissue was exposed to the opiate for a period of 3 min while its effect was measured. The preparation then was washed with the drug-free Kreb's-bicarbonate solution. In some experiments, naloxone (dose range, $1–5 \times 10^{-8}$ M) was added directly to the bath during the initial equilibration period and remained present throughout the entire experiment. Dose-response curves to morphine with and without naloxone were obtained in both the absence (control) and presence of different halothane concentrations.

When the maximal effect of the drug (halothane or morphine) was attained, the height of the contraction was compared to its pretreatment values, and the per cent inhibition then was plotted against the log dose. When the effects of morphine were determined in the presence of halothane, the percent inhibition was calculated, using the height of the contractions after halothane at point M as the control. The slopes of the curves and their 95% confidence limits (CL) were compared as a means of testing for parallelism. Log dose-response curves were used to determine the $IC_{50}$ of morphine and halothane[8]; this value was defined as the dose that produces a 50% inhibition of the amplitude of the contraction. The analysis of the interaction between halothane and morphine was performed with a computer using the method of Chou *et al.*[9,10] This procedure compares the characteristics of three different, log dose-response curves: morphine alone, halothane alone, and a combination curve where the concentrations of each agent increases but their proportion remains the same. The $IC_{50}$ and Hill coefficient are calculated for each of the log dose-response curves. Furthermore, the procedure compares the combination curve with each of the other two, and indicates if the effect of the combination of agents is additive, synergistic, or antagonistic.

Schild plots[11] were used to determine the affinity of naloxone for opioid receptors. This procedure involves determining dose ratios for each concentration of antagonist used, and from these, $pA_2$ values are calculated. This value is a measure of the dissociation constant of a competitive antagonist, which is the reciprocal of its affinity. Schild plots were constructed for control experimental conditions and in the presence of 1.6% halothane.

Calculation of $IC_{50}$, $pA_2$, and the slopes of the log dose-response curves were performed on a microcomputer by the methods of Tallarida and Murray.[8] Values were considered to be significantly different if their 95% confidence limits did not overlap.

## Results

### Effects of Halothane and Morphine on the Twitch Response in the MPLM Preparation

Exposure of the MPLM to either morphine or halothane produced a dose-related inhibition of the electrically induced muscle contraction. The $IC_{50}$ of morphine alone was $1.9 \times 10^{-7}$ M (CL, $1.6–2.1 \times 10^{-7}$; n = 25), and the slope of the log dose-response curve was 43.8 (CL, 39–48). Fifty per cent inhibition of contractions occurred at a halothane concentration of 1.7 V/V% (CL, 1.4–1.9%; n = 21). The slope of the halothane curve was 47.7 (CL, 40–55).

These experiments were repeated in the presence of $1 \times 10^{-7}$ M naloxone, a competitive opioid antagonist. Under these conditions, the $IC_{50}$ of morphine increased to $2 \times 10^{-5}$ M, while the $IC_{50}$ of halothane remained unchanged, 1.5 V/V% (CL, 1.1–2.2; n = 5). Naloxone did not change the slope of the dose-response curves in either case.

### Effects of Halothane on the Potency of Morphine

Morphine dose-response curves were obtained in the presence of halothane at 0.4, 0.8, 1.0, 1.6, and 3.0 V/V%. At each of these concentrations, the $IC_{50}$ of morphine (Table 1) was significantly reduced from control values obtained without halothane. Furthermore, the slopes of the individual dose-response curves of morphine remained unchanged. When halothane concentrations were plotted against the corresponding $IC_{50}$ values of morphine, the resulting line had a correlation coefficient of 0.99 (fig. 1). These results demonstrate that halothane increases the potency of morphine in a dose-related manner.

Hill plots were obtained for each drug alone and combined in a fixed ratio. The resulting Hill coefficient is a measure of the interaction between functional sites. The Hill coefficients for morphine and halothane were 0.91 and 0.92, respectively, while the coefficient for the

combination was 1.4. A positive Hill coefficient greater than one indicates a cooperative interaction between sites. Furthermore, the analysis shows that combinations of these drugs that produce levels of response greater than 50% have synergistic effects.

Figure 2 shows results from a typical experiment. The top tracing demonstrates the inhibition of the twitch response produced by halothane (0.8 V/V%) and the further inhibition caused by the addition of morphine in the organ bath. In the lower tracing, the muscle was not exposed to halothane, and the same concentration of morphine produced a smaller response. In both instances, naloxone antagonized the inhibitory effect of morphine. Moreover, the upper tracing shows that naloxone did not reverse the inhibitory effects of halothane.

In another series of experiments, the $pA_2$ of naloxone, a measure of the affinity of the antagonist for the opioid receptor, was determined. Morphine dose-response curves were obtained in the presence of increasing concentrations of naloxone (1, 2, and $5 \times 10^{-8}$ M). When naloxone was present in the bath, the morphine dose-response curves shifted to the right in a parallel manner. From these experiments, the $pA_2$ values were calculated. Under control conditions (morphine without halothane), the $pA_2$ of naloxone was 9.4 ± 0.2 (mean ± SE; n = 15), and in the experiments performed in the presence of 1.6% halothane, this value remained unchanged ($pA_2$ = 9.1 ± 0.9; n = 15). These observations demonstrate that halothane does not alter the affinity of naloxone for the opioid receptors in the guinea pig ileum.

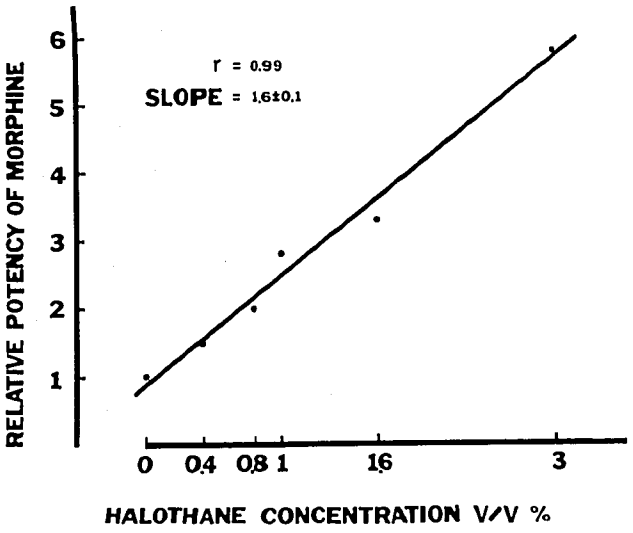


FIG. 1. Relative potency is defined as the ratio of the $IC_{50}$ of morphine alone and in the presence of the indicated concentration of halothane.

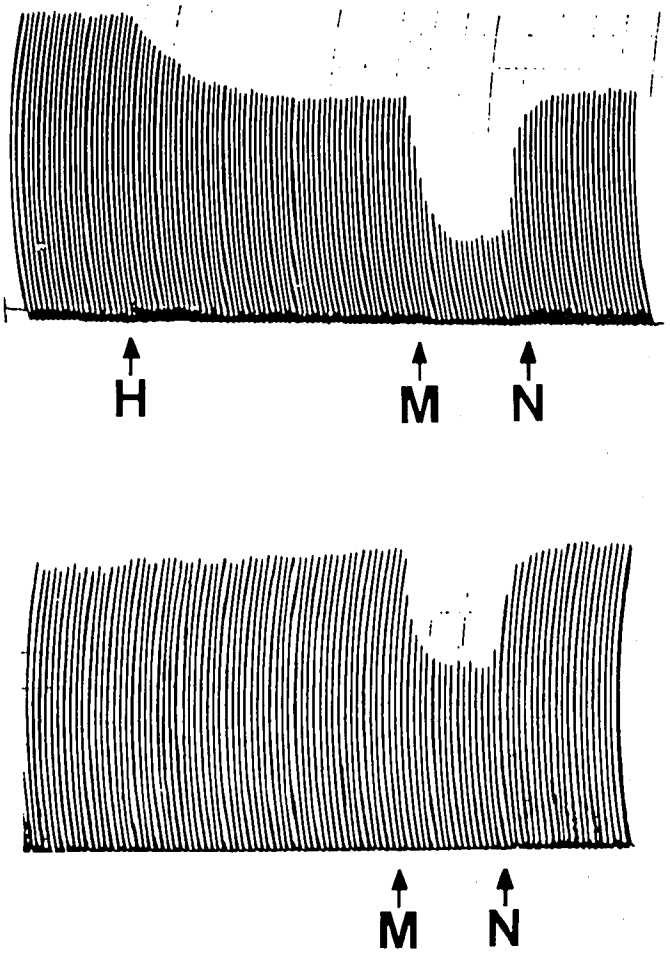


FIG. 2. Effects of morphine and halothane on the electrically stimulated MPLM preparation. The arrows indicate the addition of halothane (H, 0.8 V/V%), morphine (M, $1 \times 10^{-7}$ M), or naloxone (N, $2 \times 10^{-7}$ M) to the organ bath. Horizontal bars indicate 1-min intervals. Tracings were obtained from two MPLM strips from the same guinea pig.

## Discussion

The MPLM preparation has been used extensively to define the interactions of opiates with mu-opioid receptors.[5] In this preparation, morphine produces an inhibition of the electrically induced contractions which is reversed by naloxone.[5,7] Halothane also produces inhibition of the electrically induced contractions in a dose-dependent manner, which is not affected by naloxone[6] (fig. 2). It is worth noting that both the effects of morphine and halothane are produced within clinically relevant dose ranges. Thus, in addition to its known usefulness in opiate studies, the MPLM preparation also may be appropriate to study effects of inhalation anesthetics such as halothane.

Based on the effects of naloxone, the data indicate that inhibition of the electrically induced contraction by morphine and halothane is mediated through different

mechanisms. Naloxone blocks the effect of morphine by a competitive antagonism at opioid receptors. However, since the effect of halothane is not altered by naloxone, its inhibitory activity is not mediated directly by an interaction with opioid receptors. Our studies show a potentiation of the morphine-mediated effects in the presence of halothane. They also indicate that both the effects of the individual agents alone, as well as in combination, follow the law of mass action, and that halothane and morphine interact in a cooperative manner. The effect of the combination of drugs is complex and synergistic at high levels of response (above 50% inhibition).

The results are consistent with those of other investigators[12,13] who reported that the interaction between halothane and morphine *in vivo* can be antagonistic or synergistic at different doses. Our results showing synergism *in vitro* indicate that this interaction may occur at the cellular level, and it is not a consequence of factors which often obscure interpretation of *in vivo* data. Moreover, we have confirmed the nature of this interaction by the use of a different method of analysis (Hill coefficients *vs.* isobolograms). Because naloxone antagonized the enhanced effects of morphine in the presence of halothane (fig. 2), it may be suggested that the potentiation is related to events occurring at the opioid receptor complex. However, the inability of halothane to alter the $pA_2$ of naloxone demonstrates that the affinity of opiates for the receptor remains unchanged. This supports the hypothesis that halothane may modify post-binding events related to membrane or intracellular transduction of the receptor signal.[14,15]

It has been suggested from *in vivo* experiments that the effects of inhalation anesthetics could be mediated, in part, by the endogenous opioid system.[16,17] This neurochemical system is composed of putative transmitters (endogenous opioid peptides, or EOP) and opioid receptors (OR). In the present *in vitro* model, which contains both EOP and OR, we have demonstrated that naloxone does not modify the effects of halothane at doses that antagonize the effects of morphine. Consequently, the inhibitory effects of halothane do not appear to be related to EOP release, but rather to an increase in sensitivity of the OR to both exogenous and endogenous opioids.

Our results also indicate that the increased effects of opiates in the presence of halothane, can still be completely reversed by opioid antagonists such as naloxone.

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