Low Concentrations of Halothane Increase Response to a Noxious Thermal Stimulus and Attenuate the Antinociceptive Effect of Intraventricular but Not Intrathecal Morphine

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Background: Classically, the first plane of anesthesia is known as the stage of analgesia. Nonetheless, clinical evidence suggests that low doses of inhaled agents might enhance pain perception. The present experiments test the hypothesis that low concentrations of halothane increase response to a noxious thermal stimulus and attenuate the antinociceptive effect of intraventricular morphine via disruption of descending inhibition.

Methods: In the first experiment, the temperature at which rats withdraw their tails from a heat source was measured in animals breathing various concentrations of halothane. In the second experiment, the effect of intraventricular or intrathecal morphine on tail-flick latency was assessed in rats breathing either oxygen or 0.23% halothane.

Results: Low concentrations of halothane decreased the temperature threshold for tail-flick with a maximum effect at 0.06% atmospheres. Halothane attenuated the antinociceptive potency of intraventricular morphine but enhanced the efficacy of intrathecal morphine.

Conclusions: Subanesthetic concentrations of halothane may enhance response to a noxious stimulus. The differential effect on intraventricular and intrathecal morphine suggests that this enhancement results from disruption of descending inhibition.

CLASSICALLY, the first stage of anesthesia, extending from the onset of anesthetic administration to loss of consciousness, is known as the stage of analgesia.1 Despite this nomenclature, there is human experimental evidence to suggest that low concentrations of inhaled anesthetics might actually increase pain perception, i.e., produce hyperalgesia.2 In addition, laboratory data indicate that inhaled anesthetics may reduce the antinociceptive effect of systemic narcotics.3 Descending pain modulatory systems project from the brainstem to the spinal cord and are capable of inhibiting nociceptive input.4,5 The present experiments test the hypothesis that low concentrations of halothane may increase response to a noxious thermal stimulus and attenuate the antinociceptive effect of intraventricular morphine via disruption of descending inhibition.

Materials and Methods

The studies were approved by the Committee on Animal Research of the University of California at San Francisco. All experiments were conducted on male Sprague-Dawley rats weighing 200–350 g.

Experiment 1

The effect of halothane on the tail-flick reflex was examined in 18 rats. The tail-flick test was performed by placing the tail of each rat over the slit of an apparatus through which a beam of light from a projection lamp was focused. To control for changes in initial tail temperature and rate of tail heating, tail temperature was monitored by a small thermistor in contact with the heated portion of the tail. Voltage output to the projection lamp was controlled by feedback from the thermistor to produce a 57°C “holding” temperature before the stimulus, a maximum temperature of 52°C, and a rate of heating of 1.5°C/s. The occurrence of the tail flick was detected by a transducer attached to the tail; temperature curves and occurrence of the tail flick were displayed on a strip chart recorder.

Animals were restrained in cloth holders and then placed in individual clear plastic cylindrical chambers. Each chamber contained an opening for anesthetic gases, a port for exhaust gases, an opening for the tails, and a small hole for gas sampling. Baseline tail-flick temperature was assessed using three determinations at 5-min intervals while animals were exposed to 100% oxygen. Halothane was then administered in oxygen and directed into the chambers with an average flow into each chamber of approximately 1 l/min. Anesthetic concentrations in the cylinders were analyzed using a Perkin-Elmer Model 1100 Mass Spectrometer (Norwalk, CT). The initial concentration was adjusted to 0.03% halothane and maintained for 12 min, after which the tail-flick temperature was determined. The anesthetic concentration was then doubled, and this sequence was repeated until the animal failed to respond by the cutoff temperature of 52°C.

Experiments 2 and 3

Thirty-two rats were anesthetized with methohexitol (40–60 mg/kg intraperitoneally) to permit insertion of intraventricular guide cannulae or intrathecal catheters. The intraventricular cannulae were placed stereotactically above the caudal third ventricle of 16 rats. Intra-
Intrathecal catheters, composed of PE-10 tubing, were introduced into the subarachnoid space of an additional 16 rats using the method of Yaksh and Rudy: catheters were passed through a slit in the atlanto-occipital membrane and positioned to lie with their tip just caudal to the lumbosacral enlargement. Animals were allowed to recover for at least 7 days before starting experimental trials.

Animals were restrained in cloth holders and placed in individual cylindrical chambers. The tail-flick test was performed as in experiment 1 except that heating was not under feedback control and latency to movement was the measured end point. Animals were divided into two groups to receive either halothane in oxygen or oxygen alone. Baseline tail-flick temperature was assessed in both groups using three determinations at 5-min intervals while animals were exposed to 100\% oxygen. (However, because of a methodologic error, only a single baseline determination was made for the initial five animals in the intrathecal morphine experiment: two animals were in the halothane group, and three were in the oxygen group. The tail-flick latencies for these animals [3.3 ± 0.27] were similar to those that had three determinations [3.4 ± 0.36].) For animals in the halothane group, anesthetic was then introduced into the chamber to maintain a concentration of 0.23\%; animals in the control group continued to be exposed to 100\% oxygen. After 30 min, 1 µg of intraventricular or intrathecal morphine was administered. Intraventricular and intrathecal injections were performed using a volume of 2 or 5 µl in normal saline, respectively. Injections were made over approximately 30 s using a 10-µl (intraventricular) or 25-µl (intrathecal) syringe attached to a length of calibrated PE-50 tubing. The injection was monitored by observing the movement of a small air bubble within the tubing. Fifteen minutes later, three tail-flick determinations were made, again at 5-min intervals. To prevent tissue damage, the heat was shut off if there was no response by 10 s (cutoff). Analgesia was defined as a lack of response to the heat stimulus on two consecutive trials. A 2-µg dose was then administered to animals that did not develop analgesia. This sequence was repeated once more using a 6-µg dose. The following week, all animals with intact cannulae or catheters were tested again while receiving the alternative experimental treatment, i.e., those that initially received halothane were given oxygen, and vice versa.

Statistics
The data from experiment 1 were analyzed using repeated-measures analysis of variance, with post hoc comparison using the Dunnett test. The data for experiments 2 and 3 were analyzed using paired t tests with Bonferroni correction for multiple comparisons. A P value less than or equal to 0.05 was considered significant.

Results

Experiment 1
There were significant differences in tail-flick latency at different halothane partial pressures (fig. 1). Low concentrations of halothane increased sensitivity to the noxious thermal stimulus: animals breathing 0.06 and 0.12\% atm halothane had significantly lower tail-flick latencies than control animals. The greatest reduction in tail-flick temperature was observed during the administration of 0.06\% halothane.

Experiments 2 and 3
All animals with intrathecal catheters were studied twice. However, six of the animals with intraventricular cannulae did not complete the crossover because the cannula became nonfunctional before the second week (four of these animals had initially received halothane and oxygen; two had received oxygen alone).

There was no significant difference in baseline tail-flick latency between groups. The presence of halothane attenuated the antinociceptive effect of intraventricular morphine, which reached statistical significance at the 3- and 9-µg data points (fig. 2). Conversely, the presence of halothane increased the potency of intrathecal morphine, and this effect was statistically significant at all data points (fig. 3).

Discussion
Nociceptive information is conducted primarily along afferent nerve fibers that terminate in the dorsal horn of the spinal cord and is transmitted either directly or via interneurons to cells that project to higher centers. In...
addition, nociceptive processing involves descending components that project from the brainstem and may inhibit spinal pain transmission via an action on primary afferents, ascending neurons, or interneurons. Facilitation of the tail-flick reflex could therefore be the result of an action on any of these components (i.e., dorsal horn neurons, nociceptive primary afferents, or descending systems). A direct action on dorsal horn neurons is unlikely, because halothane suppresses the spontaneous and evoked activities of these cells. Although halothane can have a direct sensitizing effect on nociceptive primary afferents, the results of the present experiments suggest that facilitation more likely results from disruption of tonic descending inhibition.

Descending nociceptive modulation may be promoted by a variety of factors, both physiologic and nonphysiologic. For example, stress can decrease an animal’s response to a noxious stimulus. Thus, the tonic activity of this system may vary, and the effect observed with disruption of the system should depend on the resting state of the animal. This likely accounts for some of the inconsistencies reported in the literature.

Studies using electrical stimulation or microinjection to alter activity of nociceptive neurons or attenuate nocifensive reflexes have identified components of the pain modulatory system in the midbrain, medulla, and spinal cord that are interconnected, with three regions of particular relevance: the periaqueductal grey, the rostral ventral medulla, and the spinal cord dorsal horn. The system is, to a large extent, opiate-mediated, and administration of exogenous opiates in these regions presumably mimics the effect of endogenous opiates such as enkephalin.

Predictably, manipulations that remove or reduce descending inhibition may increase response to noxious stimuli and reduce the antinociceptive effect of systemic morphine. Thus, the tail-flick reflex in the rat is facilitated by spinal cord transection, and the dose of systemic morphine required to block this nocifensive reflex is significantly increased if descending systems are disrupted. In contrast, the effect of intrathecal morphine is potentiated by these manipulations. A similar pattern of effects was produced in the present study by subanesthetic concentrations of halothane. These data therefore suggest that, with respect to descending pain modulatory systems, halothane might be considered rather simplistically to induce a pharmacologic transection of the spinal cord.

The present results may help interpret data from previous animal studies examining the interaction of halothane and morphine on reaction threshold to tail clamping in rats. In these experiments, morphine elevated the reaction threshold and suppressed the increase in heart rate in response to tail clamp. This effect was antagonized by the presence of halothane and, as would be predicted from the results of the present study, antagonism was most apparent with the combination of low concentrations of halothane and high doses of morphine.

The results of the present study may also help to reconcile the fragmented and often contradictory data concerning the effects of halothane on human pain processing. Studies by Dundee et al. suggested that halothane could lower the pain threshold as measured by...
tibial pressure. However, Robson et al.\textsuperscript{14} noted only elevations of threshold in response to tibial pressure or to thermal stimulation. Siker et al.,\textsuperscript{15} using electric current as the test stimulus, reported both elevations and depressions of pain threshold in patients exposed to halothane. Houghton et al.\textsuperscript{16} applied the submaximum tourniquet test to determine the effects of 0.25 and 0.35% halothane on experimental ischemic muscle pain and found significant analgesia at the higher concentration but no effect at the lower concentration. However, none of these studies systematically examined the effect of low concentrations of anesthetic. Dundee et al.\textsuperscript{17} were the only investigators to report a consistent reduction in pain threshold, but there was minimal, if any, change while the patients were breathing 0.5% halothane; consistent reductions occurred only during the recovery phase. In the study by Siker et al.,\textsuperscript{15} significant reductions in pain thresholds were noted on three occasions during inhalation of halothane and on two occasions during recovery. Houghton et al.\textsuperscript{16} studied 0.25 and 0.35% halothane but did not test patients during the recovery phase; however, they observed a suggestion of hyperalgesia in the 0.25% series.

Interestingly, the effects observed with halothane in the present experiment are similar to those reported with barbiturates. In rats, barbiturates have been demonstrated to increase sensitivity to noxious thermal stimuli and to antagonize the effect of systemically administered narcotics.\textsuperscript{17} In the presence of barbiturates, pain-related spinal cord neurons respond to stimuli to which they either would not have responded or responded only poorly.\textsuperscript{18} Most significantly, pentobarbital has been reported to decrease the inhibition of the tail-flick reflex produced by morphine injected directly into the peri-aqueductal grey but not alter the antinociceptive potency of intrathecal morphine.\textsuperscript{17} In addition, limited human experimental data suggest that low doses of barbiturates may increase, and high doses decrease, perception of pain.\textsuperscript{19,20}

The results of the present experiment have important implications to postoperative pain management. Reductions in tail-flick latency were observed during administration of 0.06 and 0.12% halothane, which are in the range of those occurring during the early postoperative period.\textsuperscript{21} Moreover, based on the solubility of halothane, the short period of equilibration, and the lack of controlled ventilation, the actual alveolar concentration should have been significantly less than the chamber concentration. Thus, the presence of residual anesthetic in the postoperative period might increase pain perception and decrease the response to systemic narcotics. This could produce agitation, hypertension, or tachycardia. Furthermore, narcotics titrated to effect during this state might represent a relative overdose after further elimination of the inhaled anesthetic. It is possible that this effect might account for some episodes of respiratory depression occurring in the immediate postoperative period.

The procedure for assessing the effect of halothane on the tail-flick reflex used a controlled temperature ramp, with tail temperature rather than tail-flick latency as the measured end point. This was used because, in contrast to analgesia, the maximum change in latency associated with hyperalgesia is relatively small. Such changes might be difficult, if not impossible, to detect using the more standard method of testing tail-flick latency.

Six of the animals in the intraventricular experiment did not complete the crossover and were excluded from analysis: four of these animals had received halothane and three had received only oxygen. The effect of halothane in this subset was similar (but of greater magnitude) than the effect observed in the group completing the crossover. Thus, exclusion of these animals could not have affected the present findings.

There are three issues that limit extrapolation of these results to clinical practice. First, this study examined a spinal reflex, and caution must be exercised when extrapolating to clinical pain, which involves more significant supraspinal components. Second, the study used a thermal stimulus, which is not equivalent to surgical injury. Finally, the effects of only a single volatile anesthetic were examined, and there is evidence that important differences might exist among these compounds.\textsuperscript{22,23} Such differences have obvious practical as well as important theoretical implications.

In summary, these results demonstrate that subanesthetic concentrations of halothane decrease the temperature threshold for tail-flick reflex. Furthermore, the presence of halothane increases the dose of intraventricular morphine required to block the tail-flick reflex but enhances the efficacy of intrathecal morphine. These data are consistent with the hypothesis that, with respect to descending inhibition, halothane induces a “pharmacologic transection of the spinal cord.”

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