Effects of Halothane on Spinal Neuronal Responses to Graded Noxious Heat Stimulation in the Cat

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This study was undertaken to examine the dose–response effects of central concentrations of halothane on activity of wide-dynamic-range (WDR) neurons in the dorsal horn of the spinal cord of the decerebrate, spinal cord-transected cat. All cells (n = 40) responded maximally to high-intensity (greater than 45°C) noxious heat stimulation. Following administration of halothane, 0.5, 1.0, and 1.5 per cent, the mean spontaneous discharge frequency was significantly decreased (P < 0.01) by 44, 74, and 87 per cent, respectively. The mean evoked discharge frequencies were also significantly decreased at all temperatures (46, 48.5, 51°C) by all concentrations of halothane. The slope of the regression line relating heat intensity and evoked neuronal discharge frequency was significantly decreased (P < 0.01) with both 1.0 and 1.5 per cent halothane by 46 and 75 per cent, respectively. Since the spinal cord was transected, these results indicate that these effects were the result of a direct action at the level of the spinal cord. The neuronal activity that was suppressed was evoked by stimuli that were exclusively noxious. This substantiates the ability of halothane to modify the transmission of noxious information at the spinal cord level, and thus explains a mechanism by which halothane may induce analgesia. (Key words: Anesthetics, volatile; halothane; pain; heat stimulation; threshold. Spinal cord: Rexed laminae; wide-dynamic-range neurons.)

In 1967, Wall suggested that the analgesic action of various anesthetics and analgesics may be due, in part, to their effects at the spinal level. This has since been experimentally demonstrated for halothane, ether, nitrous oxide, thiopental, ketamine, Althesin, morphine, and morphine-like analgesics. Kitahata et al. demonstrated, in the decerebrate, spinal-cord-transected cat, that the spontaneous activity of cells in laminae I, V, VI, and VII of the dorsal horn was suppressed by halothane, 0.5, 1.0, and 1.5 per cent, while activity in lamina IV did not change. Using noxious mechanical stimuli, they also delineated the suppressive effects of halothane on evoked activity of wide-dynamic-range (WDR) and high-threshold (HT) neurons located in laminae I, V and VII. Contrary to these results, de Jong et al. reported that halothane depressed activity of lamina IV cells in monkey and cat.

Wide-dynamic-range neurons are characterized by their response profile to increasing stimulus intensity. Such neurons will respond to low-threshold stimuli (e.g., light brushing of the receptive field), but their firing frequency increases as the strength of the stimulus increases until they respond maximally to stimuli in the noxious range. These neurons have also been referred to as lamina V type because they are found most often (but not exclusively) in Rexed lamina V of the dorsal horn. High-threshold neurons respond exclusively to stimuli of high intensity. High-threshold neurons have been found in laminae I and VII.

Among neurons in the dorsal horn, lamina V cells are suppressed to the greatest extent following the administration of various anesthetic agents. These neurons play an important role in pain processes, since they are activated by small-diameter (A delta and C) cutaneous afferent fibers, and respond maximally to stimuli in the noxious range.

In the present series of experiments the use of a decerebrate, spinal-cord-transected preparation permitted us to examine the effects of halothane on spinal sites in the absence of both descending, supraspinal influences and basal narcosis. This enables us to eliminate the potential influences of the potent hypnotic properties of halothane. In addition, the use of noxious radiant heat permits a quantitative, dose–response study of the effects of halothane on wide-dynamic-range neuronal activity in the spinal cord.

Methods

Forty cats of either sex, each weighing 2.5 to 4.8 kg, were used. While anesthetized with halothane–nitrous oxide–oxygen, each animal was prepared with jugular-vein cannulation for the administration of drugs and intravenous fluids, carotid arterial cannulation to monitor arterial pressure, and tracheostomy for controlled ventilation. The carotid arteries were ligated bilaterally for ischemic partial decerebration. The cat was then placed in a stereotactic frame, and electrolytic lesions were made in the midbrain reticular formation to provide an electrolytic decerebration. A laminectomy was performed to expose the lumbar and sacral cord segments, which were then covered with warm (37°C) paraffin oil. The spinal cord was transected by electrocautery at Th12. Anesthesia was then discontinued, and the animal was

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immobilized with gallamine triethiodide (2.5–3.5 mg/kg/hr, iv) and artificially ventilated with a non-rebreathing system. End-tidal CO₂ was kept at 32 to 34 torr as measured by an infrared gas analyzer. The mean arterial blood pressure was always maintained above 80 torr. If mean blood pressure fell below 80 torr, the experiment was terminated. Rectal temperature was maintained at 37 ± 0.1 °C with a servo-controlled warm-water mattress and infrared heating lamp. If necessary, pneumothorax was performed to reduce movement of the spinal cord due to respiration. The receptive fields (located on the foot pads of the hind limbs) were blackened with India ink to provide a uniform surface for maximal heat absorption.

Neural recording was begun approximately four hours after the end of anesthesia. A tungsten microelectrode was inserted, by a hydraulic microdrive, into the lumbar spinal cord near the L7 root entry zone. Signals from single cells, amplified by a differential AC preamplifier and isolated by a differential amplitude discriminator, were recorded on magnetic tape and simultaneously monitored on cathode-ray oscilloscopes and a polygraph. The cell type was identified by the depth of the electrode, by the spontaneous firing pattern, and by characteristic evoked responses to peripheral stimuli of the following types: 1) touch—air puff, light stroking of hair or skin with a camel-hair brush; 2) pressure—compression of the skin by deep squeezing; 3) pinch—compression of the skin with a forceps; 4) noxious radiant heat—skin temperature above 50 °C; 5) cold—application of ethyl chloride. All cells included in this study responded maximally to high-threshold noxious heat stimulation.

Heat stimuli were produced by a Hardy-Wolff-Goodell dolorimeter with a 3.5 cm² aperture which was directed at the receptive field of the cell under study. A small thermistor (YSI-409) was placed adjacent to the center of the receptive field to monitor the skin temperature changes. A series of heat stimuli of three different intensities, 46, 48.5, and 51 °C, separated by one-minute delays were used to evoke single-unit activity. This series of stimuli was repeated every 5 minutes.

Following control determinations, halothane (0.5, 1.0 or 1.5 per cent) was administered to the animal for 25 minutes by use of calibrated Fluotec Mark 3 vaporizer. This was followed by a 60-minute recovery period, during which the animal breathed 100 per cent oxygen. Each animal served as its own control and

**Fig. 1.** Examples of polygraph tracings of the firing frequencies in impulses per second of spontaneous activity and activity evoked by three different intensities of noxious heat stimulation. *Top row:* control study. *Second row:* after 15 minutes after administration of 1 per cent halothane. *Third row:* after 30 minutes of administration of 100 per cent oxygen at the end of the halothane administration. *Bottom row:* heat stimuli of 46, 48.5, and 51 °C. This neuron, like all other cells included in this study, increased its firing frequency as the level of noxious heat stimulation was raised. Halothane administration significantly reduced both spontaneous and evoked activity. There was significant recovery of activity 30 minutes after the end of halothane administration.
received only one of the three concentrations of halothane.

At the end of each experiment an electrolytic lesion was made by passing a DC current through the recording microelectrode. The lesions were later verified histologically. Data recorded on magnetic tape were processed off-line with a digital computer (DEC PDP 11/40).

The spontaneous discharge frequency of each cell was determined by averaging the discharge frequencies observed for 20 seconds immediately before each exposure to heat. The stimulus-evoked activity was analyzed in the following manner. The variability of stimulus duration at each temperature for each neuron necessitated normalizing the data relative to time. This was accomplished by determining, for each neuron, the duration of the stimulus-evoked activity during the control state for each of the three temperatures (i.e., the total time that the control stimulus maintained the neuronal activity above the spontaneous firing rate). This control stimulus duration for each temperature was the value used to determine the duration of activity to be analyzed during the drug studies. This analysis enabled us to normalize all of the activity to impulses per second and thus compare effects across temperatures and neurons. In addition to a statistical analysis of the firing frequencies under the various conditions of the experiment, a linear relationship between the intensity of heat and the frequency of neuronal firing was calculated by a least-squares method. Data were evaluated for statistical significance using Student’s $t$ test for paired data (for comparison of changes from the control in each experiment) and Student’s $t$ test for unpaired data (for comparison of effects at each halothane concentration).

**Results**

All cells included in this study ($n = 40$) were of the WDR type, i.e., although each cell responded to non-noxious stimuli, its activity increased with increasing stimulus intensity but was maximal only when the stimuli were within the noxious range. Lesions of 29 cells were histologically verified: 19 were in lamina V, seven were in lamina VI, and three were in lamina IV. The mean values ($\bar{x} \pm 1$ SE) of the spontaneous and evoked activities in impulses per second of all cells studied in the control period were $15.4 \pm 1.4$ (spontaneous), $40.9 \pm 2.6$ (46 C), $63.0 \pm 3.5$ (48.5 C) and $86.5 \pm 4.4$ (51 C). Following halothane administration the spontaneous and evoked activities of several cells were increased slightly by 0.5 per cent halothane at 4–6 minutes. However, in all cells studied activity was suppressed by 10 minutes, and reached a maximum suppression within 15 minutes, irrespective of the halothane concentration. Complete suppression occurred in one of 15 cells with 1 per cent halothane and in seven of 12 cells with 1.5 per cent halothane.

Typical changes in the spontaneous and evoked activities of a WDR cell before and after administration of 1 per cent halothane are depicted in figure 1. The firing frequencies at all three intensities of stimulation, as well as the spontaneous activity, were greatly decreased by the drug administration. There was significant recovery of both types of activity following 30 minutes of administration of 100 per cent oxygen.

### Table 1. Effects of Halothane on Mean Neuronal Activity (Mean ± 1 SE)*

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous Discharge Frequency</th>
<th>Heat-evoked Discharge Frequency</th>
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<tbody>
<tr>
<td></td>
<td>At 46 C</td>
<td>At 48.5 C</td>
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<tr>
<td>Halothane, 0.5 per cent (n = 15)</td>
<td></td>
<td></td>
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<tr>
<td>Control (impulses/sec)</td>
<td>17.4 ± 2.4</td>
<td>43.2 ± 5.0</td>
</tr>
<tr>
<td>After administration (impulses/sec)</td>
<td>11.9 ± 3.2</td>
<td>31.8 ± 3.2</td>
</tr>
<tr>
<td>Per cent suppression</td>
<td>44.3 ± 11.0</td>
<td>35.3 ± 10.9</td>
</tr>
<tr>
<td>Halothane, 1 per cent (n = 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (impulses/sec)</td>
<td>14.8 ± 1.9</td>
<td>40.1 ± 3.5</td>
</tr>
<tr>
<td>After administration (impulses/sec)</td>
<td>4.0 ± 0.9</td>
<td>11.5 ± 2.2</td>
</tr>
<tr>
<td>Per cent suppression</td>
<td>74.9 ± 6.3</td>
<td>68.2 ± 6.7</td>
</tr>
<tr>
<td>Halothane, 1.5 per cent (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (impulses/sec)</td>
<td>15.0 ± 2.7</td>
<td>38.8 ± 4.4</td>
</tr>
<tr>
<td>After administration (impulses/sec)</td>
<td>2.1 ± 1.3</td>
<td>5.1 ± 2.1</td>
</tr>
<tr>
<td>Per cent suppression</td>
<td>87.1 ± 5.6</td>
<td>86.2 ± 5.2</td>
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* Halothane-induced percentage suppression was consistently significantly different from the control value, $P < 0.01$; with 0.5 per cent halothane at 51 C, $P < 0.05$.  

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Table 2. Effects of Halothane on the Slope of the Temperature–Response Regression Line (Mean ± SE)

<table>
<thead>
<tr>
<th>Impulse/Sec/Degree C</th>
<th>Per Cent Depression</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Halothane, 0.5 per cent (n = 15)</td>
<td>9.79 ± 1.21</td>
</tr>
<tr>
<td>Halothane, 1 per cent (n = 13)</td>
<td>9.43 ± 0.75</td>
</tr>
<tr>
<td>Halothane, 1.5 per cent (n = 12)</td>
<td>9.37 ± 0.94</td>
</tr>
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</table>

* Significantly different from control value, P < 0.01.

Each concentration of halothane caused a greater, statistically significant depression of neuronal activity (table 1). For each drug concentration, there was no significant difference in the percentages of suppression of neuronal activity among the three intensities of stimulation, although increasing concentrations of halothane resulted in significantly greater depression of neuronal activity.

Evaluation of the recovery of neuronal activity following the end of halothane administration in 27 cells showed that activity returned to within 80 per cent of the control value by 30 minutes after the discontinuation of halothane administration irrespective of the concentration used.

As shown in both table 2 and figure 2, 1.0 per cent and 1.5 per cent halothane resulted in statistically significant decreases in the slope of the temperature–response regression line. Halothane, 0.5 per cent, did not cause a significant reduction of the slope of the regression line.

Discussion

During the past decade, many reports have emphasized the importance of WDR neurons of the dorsal horn of the spinal cord in the transmission and integration of nociceptive information.15–17,18,19 These WDR cells respond to a wide range of stimuli, from light touch to heavy pinch, but they respond maximally to high-threshold mechanical and noxious stimulation.20–21 Moreover, they increase their discharge frequency as the magnitude of stimulus intensity increases.16–17 Neurons included in this study were classified as WDR because they fit the above description when their electrophysiologic responses were evaluated. Histologic studies verified that most of the cells in this study were located in Rexed lamina V, with the remainder in the deeper portions of lamina IV and the higher areas of lamina VI.

Following initial reports by de Jong et al.3,4 that halothane could depress activity of spinal neurons, Kitahata et al.5,4 reported that in decerebrate, spinal-cord-transected cats, halothane induced laminar-specific suppression of the activities of laminae I and V evoked by noxious mechanical stimuli, while activity of neurons responding solely to non-noxious cutaneous stimuli was not suppressed. The important difference in the present study, which adds to the previously reported work, is a demonstration of a dose–response effect of halothane upon the graded response of WDR neurons activated by noxious radiant heat.

The three intensities of stimuli used in this study were all above the commonly accepted threshold for pain in man. Hardy et al.22 reported that the threshold for pricking pain in human subjects during radiant heating of the forehead (blackened with ink) was 44.3 ± 0.5 C. Stoll et al.23 reported that in man, threshold pain occurred at skin temperatures of 45 C. In addition, cats react to temperatures above 44–46 C as if they were noxious (nociceptive).24

In this study tissue damage was avoided by not using excessively high levels of noxious heat.25 In addition, by employing adequate inter-stimulus intervals we were able to stimulate neuronal activity without any apparent sensitization or fatigue of neural elements.

Two aspects of the procedures used in this study had the potential for altering the neuronal responses irrespective of the direct halothane effect. Although spinal cord transection can alter neuronal activity,19,24,25 in the present study, during the time after the transection when the drug effects were normally evaluated, control studies indicated no significant fluctuation in neuronal activity with time.

The second potential problem is associated with the hypotensive action of halothane. In the present study we experienced large changes in blood pressure following administration of halothane, especially 1.5 per cent. These changes made neuronal recording extremely difficult, not because of changes in firing frequency but because of a decrease in the signal-to-noise ratio. Any data obtained from animals with mean blood pressures below 80 torr were not included in this study. The changes, however, could be avoided..
by careful attention to hemostasis during the surgical procedure and the infusion of appropriate volume expanders to maintain adequate blood volume prior to the administration of halothane. These precautions enabled us to obtain stable recordings for the duration of the drug studies. In two control studies, lowering the blood pressure with sodium nitroprusside to the level comparable to that produced by halothane did not result in a change in neuronal firing frequency.

The data presented in this study further substantiate important aspects of the analgesic action of halothane. It is apparent that these effects were the result of a direct action at the level of the spinal cord, because descending influences were removed by spinal cord transection and general anesthesia does not usually affect primary afferents. The present study demonstrates that information about purely noxious stimuli can be modified, at spinal sites, by halothane.

The importance of the action of anesthetics within the spinal cord remains to be determined. However, a comparison of the dose–response data from this study with clinical and experimental reports from other studies indicates the potential for a significant contribution of drug actions within the spinal cord. The minimum alveolar concentrations (MAC) of halothane in humans and dogs are 0.77 per cent and 0.87 per cent, respectively. Although the MAC of halothane in cats has not been measured, de Jong et al. reported that "... intact cats no longer responded to pinching of the foot when the partial pressure of halothane in arterial blood was 0.9 per cent or greater." Heavner and de Jong reported that 50 per cent suppression of evoked activity of dorsal horn cells correlated roughly with light surgical anesthesia in intact cats. These facts fit well with the findings of this study that 1 per cent halothane (presumably above MAC) caused a greater than 50 per cent reduction in neuronal activity, while 0.5 per cent halothane (presumably less than MAC) caused less than a 50 per cent reduction of evoked activity.

An important point to remember when considering halothane effects is that halothane is a potent hypnotic. In human studies of experimental pain, halothane (0.5 per cent) and nitrous oxide (25–30 per cent) both increased the thermal pain threshold, but halothane resulted in the subjects’ being much closer to unconsciousness than did nitrous oxide. The hypnotic effect of halothane must play an important role in the production of surgical analgesia and anesthesia.

Zimmerman presented a scheme to explain the difference seen in encoding functions of spinal neurons that have been subjected to modulatory influences. Carstens, Yokota and Zimmerman used this scheme to evaluate the mechanism of action by which periaqueductal gray stimulation (PAGS) inhibits spinal cord neuronal activity. The PAGS has been shown to cause behavioral analgesia in animals. According to their interpretation, a decrease in slope of the response function of spinal cord neurons could represent presynaptic inhibition. They concluded that PAGS mediates its action on dorsal horn neuronal activity primarily through presynaptic mechanisms.

In the present study the slopes of the intensity function curves were decreased by 1.0 and 1.5 per cent halothane. In keeping with the scheme presented by Zimmermann, this would suggest that halothane altered the neuronal activity, at least in part, by a presynaptic mechanism.

The significance of the present study is the demonstration that halothane can suppress, in a dose-dependent fashion, the activity of WDR dorsal horn neurons elicited by noxious heat stimuli. This suppression of the transmission of information about a noxious stimulus, in the absence of descending supraspinal input, indicates that spinal cord sites are potentially of great importance to surgical analgesia and anesthesia.

References