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Increasing Isoflurane from 0.9 to 1.1 Minimum Alveolar Concentration Minimally Affects Dorsal Horn Cell Responses to Noxious Stimulation

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Background: **The spinal cord appears to be the site at which isoflurane suppresses movement that occurs in response to a noxious stimulus. In an attempt to localize its site of suppressant action, the authors determined the effect of isoflurane on dorsal horn neuronal responses to supramaximal noxious stimulation at end-tidal concentrations that just permitted and just prevented movement.**

Methods: **Rats (n = 14) were anesthetized with isoflurane, and after lumbar laminectomy, the minimum alveolar concentration (MAC) for each rat was determined using a supramaximal mechanical stimulus. In these same rats, after extracellular microelectrode placement in the lumbar spinal cord, dorsal horn neuronal responses to the supramaximal stimulus were determined at the concentrations of isoflurane that bracketed each rat's MAC (0.1% higher and lower than MAC). The MAC of isoflurane was then re-determined.**

Results: **Dorsal horn neuronal response was 1,757 ± 892 impulses/min at 0.9 MAC and 1,508 ± 988 impulses/min at 1.1 MAC, a 14% decrease ($P < 0.05$). Cell responses varied, with some cells increasing their response at the higher concentration of isoflurane. The MAC of isoflurane was 1.38 ± 0.2% before and 1.34 ± 0.2% after determination of dorsal horn neuronal responses.**

Conclusions: **Isoflurane, at concentrations that bracket MAC, has a variable and minimal depressant effect on dorsal horn cell responses to noxious mechanical stimulation. These data suggest that the major action of isoflurane to suppress movement evoked by a noxious stimulus might occur primarily at a site other than the dorsal horn. (Key words: Anesthetic requirements; pain.)**

RECENT evidence suggests that the spinal cord is the site at which anesthetic agents suppress movement that occurs in response to noxious stimulation.[1,2] The exact site, however, is unknown. Three decades of classic studies have documented the effects of anesthetic agents on dorsal horn cells.[3–8] The critical role of these cells in transmission and modulation of noxious input[9–11] makes the dorsal horn a potential site of anesthetic action. Closer review of these studies,[3–8] however, reveals that although there is a dose-dependent suppression of dorsal horn nociceptive transmission, little evidence supports the notion that anesthetic agents suppress noxious-induced movement *via* action at the spinal dorsal horn. This is in part because these studies 1) examined wide ranges of anesthetic agents, as opposed to the narrow range (0.9–1.1 minimum alveolar concentration [MAC]) in which all animals move or do not move in response to a supramaximal stimulus; 2) used stimuli that were either nonnoxious or were not likely supramaximal; and 3) examined cells that were not involved in the modulation of noxious stimuli (*e.g.*, joint afferents). In the current study, we determined the effect of isoflurane on dorsal horn cell responses to a supramaximal stimulus in the narrow concentration range that just permitted (0.9 MAC) and just prevented (1.1 MAC) gross, purposeful movement in response to the noxious stimulus. We hypothesized that isoflurane would have minimal effect on the response.

Methods

The local animal care and use committee approved this study. Fourteen male Sprague-Dawley rats (mean ± SD weight, 475 ± 19 g) aged 6 months were anesthetized in an acrylic box with isoflurane 5% and were maintained on isoflurane 2–3% *via* mask during the initial surgical procedures. A midline anterior cervical incision permitted cannulation of the jugular vein and trachea (14-gauge catheter). Saline (0.9%) was infused at 3 ml/h. The ani-

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mals were ventilated with a Harvard rodent ventilator. A calibrated agent analyzer (Capnomac; Datex Instrumentarium Corp., Helsinki, Finland) sampled expired and inspired gases from a small tube with its tip at the attachment to the 14-gauge tracheal catheter. Expired carbon dioxide was maintained at 32 ± 4 mmHg. In six animals, a carotid artery cannula was placed for measurement of blood pressure and determination of arterial blood gases, the latter confirming that the expired carbon dioxide accurately reflected the arterial carbon dioxide. Rectal temperature was measured with a thermistor probe and maintained at 37.9 ± 0.7°C using a heating pad and heating lamp. The animal was turned prone, and a laminectomy was performed to access the lumbar enlargement of the spinal cord.

After these procedures, expired isoflurane was stabilized at 1.4% for ≥15 min, and a noxious stimulus was applied to a hindpaw. This stimulus was an A clamp, which delivered a force of 55–60 N over a 0.5-cm^2 area.[12] The clamp was secured to a magnetic holder that ensured repeated accurate application of the stimulus to the same site on the paw. The stimulus was applied for 10 s, and the animal was observed for gross, purposeful movement, which usually consisted of movement of the contralateral hindpaw or head. Stiffening and coughing were considered negative responses, as was withdrawal of the stimulated paw. The presence or absence of movement dictated the next step; the concentration of isoflurane was increased or decreased 0.2% and equilibrated for 15 min, and the clamp was applied for 10 s as before. This process was continued until two concentrations were found that just permitted and that just prevented movement. The MAC was the average of these. In a few instances when an equivocal response was found, the clamp was re-applied.

Next, the spine was secured using two custom made clamps and the dura incised. Agar was poured onto the cord to provide stabilization, and a small window was made to permit access to the cord. A hydraulic microdriver (D. Kopf, Inc., Tujunga, CA) advanced a tungsten electrode (~10 MΩ; F. Haer, Inc., Bowdoinham, ME) into the dorsal horn. Receptive fields were searched until a cell was found that reliably responded to noxious stimulation of the hindpaw. Cell spikes were amplified and fed into a personal computer for counting.[13] The hindpaw not used for the initial determination of MAC was used for determination of neuronal responses, except in a few animals, in which no cells could be found on the contralateral side that reproducibly responded to noxious stimulation.

The animal's paw was secured to a stage with modeling clay. The clamp was braced open and the paw inserted into the open clamp. This secure arrangement ensured that the clamp was applied to the same site on the paw. Pancuronium was given (0.1–0.2 mg/kg every 30–60 min) to prevent movement during the electrophysiologic studies. The isoflurane was equilibrated at the concentration (for each individual rat) that had been found to just prevent movement (*e.g.,* ~1.1 MAC) or just permit movement (*e.g.,* ~0.9 MAC), with the order alternating experiment to experiment. Spontaneous dorsal horn neuronal activity was determined for 60 s before application of the clamp and for 60 s after the beginning of application of the clamp, which lasted for 10 s. The clamp was applied to the same site on the receptive field of the hindpaw, usually two more times (5 min apart). The cell's response was the average of these three determinations. In some cases, the clamp was applied more than three times to obtain reproducible responses, resulting in elimination of < 3% of responses. The concentration of isoflurane was adjusted to those that bracketed MAC (*e.g.,* if the MAC was 1.5%, and the first concentration studied was 1.4%, the isoflurane was increased to 1.6%). After equilibration for ≥15 min, the cell's responses to application of the clamp were recorded once again. This process was generally repeated, with another set of three responses determined at 0.9 and 1.1 MAC.

At the end of the determination of the dorsal horn neuronal response, the effects of pancuronium were allowed to dissipate, and full neuromuscular strength (full train-of-four and tetanus) was documented using a twitch monitor with needle electrodes placed percutaneously near the sciatic nerve. The MAC of isoflurane was determined again using the same methods as before, except that the clamp was applied to the paw used for determination of neuronal responses. The animal was then killed with isoflurane and KCl. We did not make an electrolytic lesion to determine the exact location of the electrode tip, as such a lesion might have damaged the cord to an extent that the poststudy MAC would have been affected.

Statistical Analysis

All data are expressed as mean ± SD. The dorsal horn neuronal responses for each animal were averaged for each concentration (*e.g.,* 0.9 and 1.1 MAC). A paired *t*

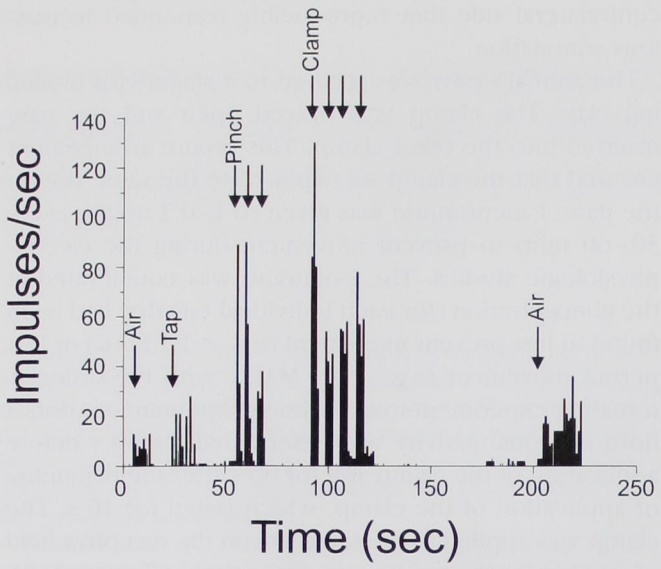

Fig. 1. Response of a wide-dynamic range cell to increasing stimulation (blowing air, tapping, pinch, and supramaximal noxious clamping using an A clamp).

test was performed to determine significant differences between the responses. A paired *t* test also was used to determine any differences in MAC of isoflurane before and after the study. A probability value < 0.05 was considered significant.

Results

Fifteen cells were studied in 14 rats. Cell depth was 546 ± 224 μm. Two cells had spontaneous activities that were 15% and 4%, respectively, of their evoked activity. The remaining cells had little to no spontaneous activity. Five of the cells were wide-dynamic range cells in that they responded with increased firing to increasing stimulation. The remaining cells were high-threshold cells that responded only to noxious stimulation. Examples of a wide-dynamic range and high-threshold response are shown in figures 1 and 2, respectively. An individual example shown in figure 3 demonstrates that there was little variation in the response with successive stimuli or with changing the concentration of isoflurane from 1.2% (0.9 MAC) to 1.4% (1.1 MAC). For all cells combined, there was a 14% decrease in the response when isoflurane was increased from 0.9 MAC to 1.1 MAC ($P < 0.05$; fig. 4). There was variation, however, with some cells increasing their response whereas others were depressed. We compared successive responses at each concentration and found no significant increase or decrease, suggesting that sensitization, if it occurred, was present before the neuronal responses were determined (fig. 5).

The MAC of isoflurane was 1.38 ± 0.2% before and 1.34 ± 0.2% after dorsal horn neuronal responses were determined (not significant). Mean blood pressure was 115 ± 19 mmHg, arterial oxygen tension was > 300 mmHg, and carbon dioxide was 32 ± 4 mmHg. During the experiment, both hindpaws developed inflammation, with redness, swelling, and exudation of fluid. The hindpaw used for neurophysiologic testing had much greater inflammation than the other, however.

Discussion

Since the demonstration that anesthetic agents influence dorsal horn cells, this area has been the focus of considerable research *vis-à-vis* mechanisms of anesthesia. The recent finding that the spinal cord is a major site of anesthetic action has underscored the potential role of the dorsal horn.[1,2] Scrutiny of prior studies reveals that they were conducted with a slightly different but important conceptual perspective from that of the current study. The landmark work by de Jong and Wagman first demonstrated that a potent inhaled anesthetic agent

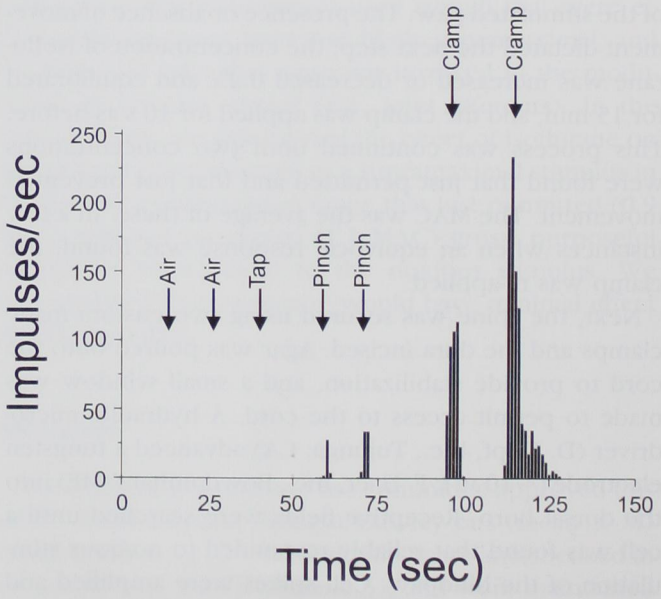

Fig. 2. Response of a high-threshold cell to light and high-threshold stimulation (blowing air, tapping, pinch, and supramaximal noxious clamping using an A clamp). Note that the cell does not fire when nonnoxious stimulation is used but that a noxious mechanical clamp causes the cell to fire.

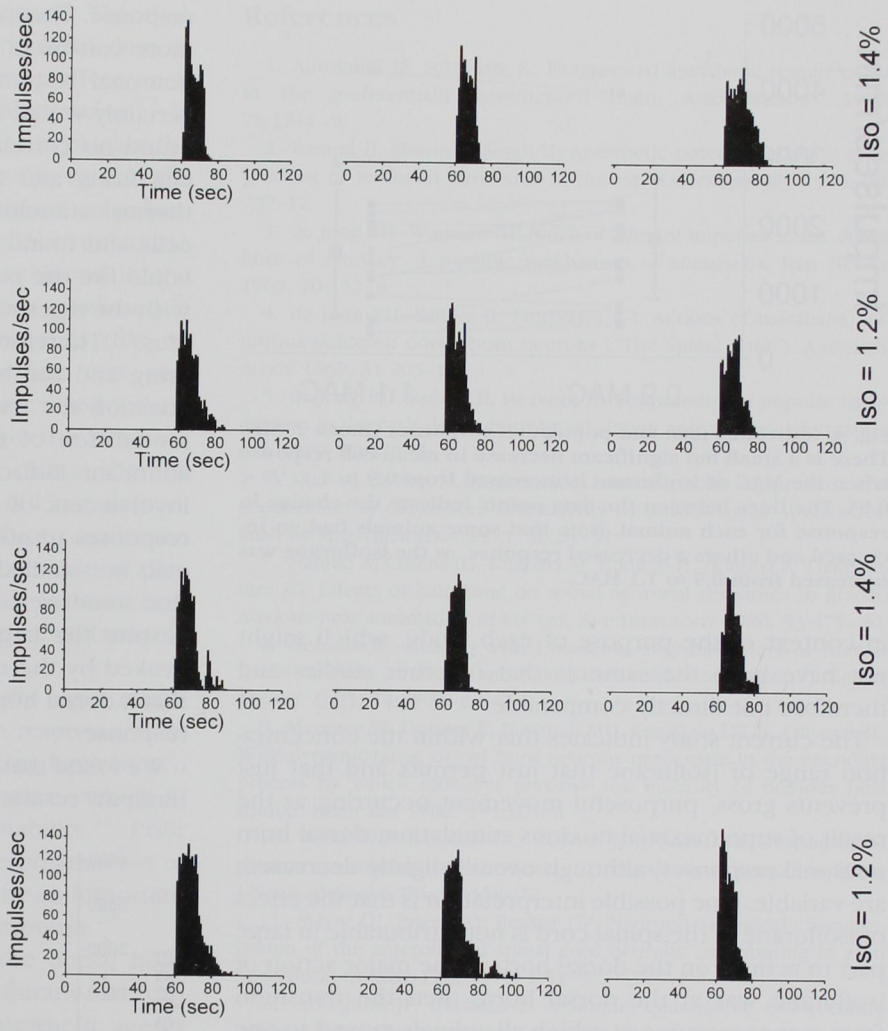

Fig. 3. Responses of an individual cell to repeated clamping. The clamp was applied at the 60-s mark for 10 s. Twelve responses are shown, with three responses determined at 1.4% and 1.2% each, and repeated. The MAC in this animal was 1.3%. The interval between clamp applications was ≥ 5 min, and the interval between different anesthetic concentrations was ≥ 15 min.

(halothane) depressed dorsal horn neuronal responses.[3] These investigators examined primarily receptive fields and used nonnoxious stimuli in addition to noxious stimulus of "pinching." They did not, however, describe the length of time and with what force pinching was applied. Further, they administered halothane at 1% or 2% and followed the cell's response over the ensuing 20–30 min as the concentration of halothane gradually increased. The varied amount of stimulation, particularly the use of innocuous stimuli, and the non-steady state use of halothane in a broad concentration range, make it difficult to interpret their findings regarding the effect of an inhaled anesthetic agent on dorsal horn neuronal responses to supramaximal stimuli.

Nakimi *et al.*[7] determined the effect of halothane on dorsal horn neuronal responses to a noxious heat stimulus. As with earlier studies, a wide anesthetic concentration range and a stimulus (thermal) not normally used for MAC studies were used. At the time, it was unclear whether a thermal stimulus could be considered supramaximal. More recent evidence now suggests that a thermal stimulus (52°C), as was used in the Namiki *et al.*[7] study, is likely supramaximal based on rabbit studies.[14] Because they used nonequilibrium concentrations of halothane, interpretation is difficult. Similar studies investigating halothane, N_2O, and thiopental examined a wide range of anesthetic concentrations and used various stimuli.[6,8,15] In particular, when a noxious stimulus was used, the exact nature was not described[4] or quantitative results were not reported.[5] In some cases, the noxious stimulus was not likely supramaximal and was applied for ~40 min.[15] These limitations must be taken

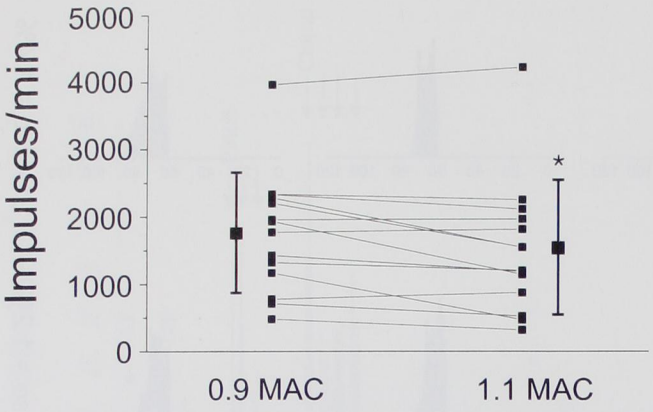

Fig. 4. Individual data and population responses (mean ± SD). There is a small but significant decrease in mean cell response when the MAC of isoflurane is increased from 0.9 to 1.1. *$P < 0.05$. The lines between the data points indicate the change in response for each animal. Note that some animals had an increased and others a decreased response, as the isoflurane was increased from 0.9 to 1.1 MAC.

in context of the purpose of each study, which might not have been the same as that of other studies and therefore not directly comparable.

The current study indicates that within the concentration range of isoflurane that just permits and that just prevents gross, purposeful movement occurring as the result of supramaximal noxious stimulation, dorsal horn neuronal responses, although overall slightly decreased, are variable. One possible interpretation is that the effect of isoflurane in the spinal cord is not attributable in large part to actions on the dorsal horn. If the major action of isoflurane was at the dorsal horn, then the transition from a concentration at which all animals moved to one at which none moved should have been accompanied by a consistent, substantial decrease in dorsal horn neuronal response to the noxious stimulus. Small anesthetic effects at multiple sites of complex neural circuits, however, could summate to a large behavioral change (*e.g.*, immobility in response to a noxious stimulus). This would support the contention that not all anesthetic action is at the dorsal horn. We cannot exclude the possibility, however, that a 14% decrease in dorsal horn neuronal responses might have been sufficient to cause a behavioral change.

The all-or-none movement response is somewhat artifactual, because, during a MAC study, some movements are arbitrarily defined as negative (*e.g.*, coughing, straining, or withdrawal of a hindlimb[2]). Thus, a quantitative measure of all movement during the transition from 0.9 to 1.1 MAC probably would not feature an all-or-none response. The gradual decrease in movement would be more consistent with the slight decrease in dorsal horn neuronal responses seen in the current study, but it certainly would not explain the variation. Nishioka *et al.* found no correlation between the absolute amount of cell firing and the movement response to a noxious thermal stimulus.[16] They studied wide-dynamic range cells and found that an innocuous stimulus (brushing) could fire the cell to the same degree as that occurring with the threshold movement response to the heat stimulus.[16] Therefore, there is a discrepancy between cell firing and the behavioral response, which brings into question the exact role of the dorsal horn cell in the modulation of responses to noxious stimuli. There is abundant indirect evidence that supports the critical involvement of dorsal horn cells in the modulation of responses to noxious stimuli.[9-11] It is possible that the cells we studied were functionally heterogeneous, and that some were not critical to the movement response, despite the large increase in dorsal horn cell activity evoked by the noxious stimulus. It is difficult to prove that a dorsal horn cell is involved in a nocifensive reflex response.

We found that repeated application of the clamp to the hindpaw resulted in clinically obvious tissue damage and

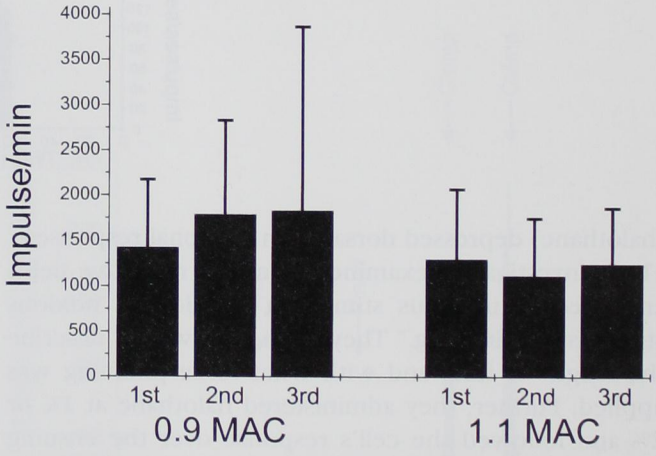

Fig. 5. Each set of three columns indicates the mean neuronal responses of the first, second, and third stimulus application at 0.9 and 1.1 MAC (mean ± SD). Therefore, the 0.9 MAC data are from those animals in which responses were determined first at 0.9 MAC, and the 1.1 MAC data are from those animals in which responses were determined first at 1.1 MAC. At 0.9 MAC, there was a trend for the response to increase, but this was not statistically significant. There were no statistically significant changes in the responses over successive stimuli at 1.1 MAC. The second sets of responses at 0.9 and 1.1 MAC were numerically higher than the first set, but the differences were not statistically significant (data not reported).

inflammation; however, MAC was unchanged. This finding is consistent with our previous work that demonstrated that tissue inflammation does not affect anesthetic requirements for a supramaximal stimulus.[17] In many MAC studies, investigators try to avoid placing the clamp on the same site. In our prior study, we did the same thing to a certain degree and relied on an inflammatory substance (carrageenan) to induce inflammation.[17] The amount of tissue damage achieved in the current study was clearly greater than that produced in our prior one.[17] During the search for cells that responded to noxious stimulation, pinching was performed using our fingers, and this alone caused inflammation. Therefore, it is possible that by the time we measured the neuronal response to the clamp, the cell had become maximally sensitized and did not demonstrate any further sensitization. In addition, isoflurane itself diminishes sensitization.[18] Finally, our use of pancuronium did not likely affect our results, because neuromuscular blocking drugs do not affect sensitization[19] or MAC.[20]

If anesthetic action on dorsal horn cells has a minor role in the suppression of the movement response, then what other sites are likely? Rampil and King have shown that anesthetic agents depress the F wave, which is thought to reflect motor neuron excitability.[21] Prior work reporting anesthetic-induced hyperpolarization of the motoneuron suggested that it might be an important site for suppression of the movement response.[22]

Although our findings indicate that the dorsal horn might not be important to action of isoflurane, we can not extrapolate these findings to other anesthetic agents and anesthetic adjuvants. For example, opiate agents have a profound effect on dorsal horn cells[23] and also decrease MAC,[24] an action that might be mediated solely at the dorsal horn. The finding that opiate agents have a ceiling effect on MAC[24] (increased concentrations of opiate do not further decrease MAC), however, suggests that anesthetic action on other components of sensorimotor integration (*e.g.,* the motor component) is involved in the movement response to noxious stimulation.

We found that isoflurane has a minimal and variable effect on dorsal horn neuronal responses to a supramaximal noxious stimulus in the concentration range that just permitted and just prevented movement. These data suggest that action of isoflurane at the dorsal horn might not be a critical part of the suppression of the movement response that occurs as the result of noxious stimulation.

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