

Effect of Differential Delivery of Isoflurane to Head and Torso on Lumbar Dorsal Horn Activity

Joseph F. Antognini, M.D.,* Earl Carstens, Ph.D.,† Etsuo Tabo, M.D.,‡ Viktor Buzin, M.D.§

Background: The spinal cord appears to be the site where anesthetic agents prevent movement in response to noxious stimuli. When isoflurane is differentially delivered to the head and torso (with low torso concentrations), cranial anesthetic requirements increase compared with systemic administration. The aim of the current study was to test the hypothesis that isoflurane action in the brain has descending influences on spinal cord dorsal horn neurons. A secondary aim was to determine the association, if any, of high cranial concentrations of isoflurane (>6%) with dorsal horn activity.

Methods: Ten goats were anesthetized with isoflurane and the carotid arteries and jugular veins isolated and cannulated for cerebral bypass. A laminectomy was performed for recording from single lumbar dorsal horn neurons with hind limb mechanical receptive fields (one cell per goat). A standard noxious mechanical stimulus was applied to the dew claw or hoof bulb during a control period with end-tidal isoflurane at 1.3% and during bypass with the following head/torso isoflurane concentrations: 1.3%/1.3%, 3.2%/1.3%, 9.4%/1.3%, 1.3%/0.2%, 3.0%/0.2% and 8.8%/0.3%.

Results: When torso isoflurane concentration was 1.3%, increasing cranial isoflurane concentration to 3% or 9% had no significant effect on the activity of dorsal horn units. When torso isoflurane was 0.2–0.3%, spontaneous activity increased; however, at these torso concentrations, evoked responses

were significantly decreased (–60%) only when cranial isoflurane concentration was increased to 9%.

Conclusions: Isoflurane action in the brain had an inhibitory effect on dorsal horn activity with the combination of supra-clinical cranial and low torso concentrations. (Key words: Anesthesia mechanisms; cardiopulmonary bypass.)

RECENT studies have demonstrated that the spinal cord is an important site of anesthetic action. Rampil *et al.* found that decerebration and spinal cord transection in rats did not change anesthetic requirements for suppression of movement in response to a noxious stimulus.^{1,2} We have determined that differential delivery of isoflurane to the goat brain (with low concentrations of isoflurane to the torso and spinal cord) produced exaggerated anesthetic requirements in the brain to suppress noxious-induced movement,³ but the mechanism by which this occurs is unknown. An additional intriguing finding is the occurrence of spontaneous movement when isoflurane is differentially delivered to the brain at supraclinical concentrations (>6%).^{4,5} This movement sometimes appears to be purposeful (*e.g.*, coordinated limb movements) and is not due to seizure activity.

The primary aim of this study was to test the hypothesis that isoflurane, when differentially delivered to the brain and spinal cord, influences the spontaneous and evoked activity of spinal cord dorsal horn neurons. Dorsal horn neurons were chosen for study because they represent the first relay in ascending nociceptive and spinal reflex pathways,⁶ and inhibition of their activity is thought to be a mechanism underlying analgesia.^{7,8}

Methods

This study was approved by the local animal care and use committee. Ten adult female goats weighing 51 ± 10 kg (mean \pm SD) were anesthetized with isoflurane by mask and were intubated. Bilateral neck dissections were performed, and the carotid arteries and jugular

* Associate Professor, Department of Anesthesiology.

† Professor, Section of Neurobiology, Physiology and Behavior.

‡ Visiting Assistant Professor, Department of Anesthesiology.

§ Research Assistant, Department of Anesthesiology.

Received from the University of California, Davis, Davis, California. Submitted for publication June 5, 1997. Accepted for publication December 3, 1997. Supported in part by the Foundation for Anesthesia Education Research with a grant from Abbott Laboratories and a research grant from the University of California, Davis. Presented in part at the meeting of the Association of University Anesthesiologists, Washington, D.C., April 18–20, 1997; the 5th International Meeting on Mechanisms of Anaesthesia, Calgary, Alberta, Canada, June 17–20, 1997; and the annual meeting of the American Society of Anesthesiologists, San Diego, California, October 20–22, 1997.

Address reprint requests to Dr. Antognini: TB-170, Department of Anesthesiology, University of California, Davis, Davis, California 95616. Address electronic mail to: jfantognini@ucdavis.edu

veins were isolated and the occipital arteries ligated, as previously described.^{3,4} In the goat, blood normally flows caudally down the basilar artery; however, during cerebral bypass, systemic blood pressure exceeds cranial blood pressure, which prevents normal flow of blood.⁹ A peripheral intravenous catheter was inserted for infusion of lactated Ringer's solution. A catheter was placed into the carotid artery to measure systemic blood pressure and for analysis of arterial blood gases and hematocrit. Pancuronium (0.1–0.2 mg/kg) was administered and repeated every 1–2 h. Torso temperature ($38.4 \pm 0.9^\circ\text{C}$ [mean \pm SD] measured from the rectum) was maintained with a heating lamp, and during bypass head temperature ($37.8 \pm 0.9^\circ\text{C}$ [mean \pm SD] measured from the nasopharynx) was maintained with the heat exchanger of the oxygenator.

A laminectomy exposed the lumbar spinal cord for recording. The spine was suspended in a frame with four vertebral clamps. The dura was slit and retracted to allow a tungsten recording microelectrode (resistance, 5–10 M Ω) to be advanced into the dorsal horn using a hydraulic microdrive (D. Kopf Instruments, Tujunga, CA). Dorsal horn units with mechanical receptive fields on the distal hind limb, including the dew claws or hoof, were searched. Included in our sample are wide-dynamic range (WDR) and nociceptive-specific type neurons. Extracellular action potentials from the unit were amplified and displayed by conventional means and fed to a personal computer programmed to construct peristimulus-time histograms (bin width, 1 s) of activity.¹⁰ We selected for study only units that responded reproducibly to a standard noxious mechanical clamp stimulus applied to the dew claw or a hoof bulb. The stimulus was delivered with a hemostat, the jaws of which spanned the dew claw or hoof bulb and were closed to the first ratchet. This stimulus is noxious and can be considered a supramaximal stimulus akin to surgical incision. Stimuli were delivered to the same site for 10 s. The person applying the stimulus was always the same and was unaware of the anesthetic condition. During the control period, end-tidal isoflurane concentration was maintained at 1.3%, which is the minimum alveolar concentration in goats.^{3,11} Unit activity was recorded for 60 s before and for 60 s after the onset of each stimulus. The stimulus was applied two to four times (usually three), with 5 min between applications; this usually evoked responses that were fairly consistent in amplitude (within $\pm 30\%$ of the

mean). The responses were averaged for each anesthetic condition.

After control dorsal horn unit activity was recorded, 4 mg/kg heparin (repeated 2 mg/kg every 1–2 h) was administered intravenously, a cannula was placed into the carotid artery, and a Y cannula was placed into the jugular vein. Another cannula was placed into the other jugular vein to increase venous return. Blood was drained into a bubble oxygenator (B-10, Bentley; American Edwards, Irvine, CA). Gas flow to the oxygenator was 95% O₂/5% CO₂ at 5–6 l/min. An enflurane vaporizer filled with isoflurane was placed in line with the gas flow. Isoflurane concentration in the arterial blood perfusing the head and brain was estimated from the isoflurane concentration in the exhaust of the oxygenator,^{9,12} and isoflurane concentration in the torso (and spinal cord) was determined from end-tidal samples. Oxygenator exhaust and end-tidal gases were monitored with a calibrated Puritan-Bennett (Wilmington, MA) agent analyzer.^{3,4} Cerebral bypass was initiated by clamping off cranial venous return to the body and diverting cranial venous blood to the oxygenator. Blood flow to the head was initiated at 500–600 ml/min, and complete bypass was achieved by clamping off the other carotid artery.^{3,4} Glucose was infused (10–20 mg/min) into the oxygenator. After ≈ 10 –15 min of stabilization, dorsal horn unit activity was recorded with the cranial and torso isoflurane at 1.3%. The noxious mechanical stimulus was applied in the same manner as described previously. Spontaneous and evoked activities were recorded with cranial isoflurane at 1.3%, 3%, and $\approx 9\%$, with torso isoflurane at 1.3% and at 0.2–0.3%. The order was alternated, and the person performing the noxious stimulation was unaware of the isoflurane concentrations. At least 15 min elapsed at each new isoflurane concentration before the dorsal horn activity was recorded. In four goats, dorsal horn unit activity (spontaneous and evoked) was redetermined in the postbypass condition with end-tidal isoflurane concentration at 1.3%.

At the end of the experiment, an electrolytic lesion was made at the spinal recording site by passing direct current through the recording microelectrode. The goat was killed with potassium chloride and isoflurane. The cord was removed, fixed in formalin, cut in 50- μm frozen sections, and mounted on microscope slides. Electrolytic lesions were observed under the light microscope and plotted onto a camera lucida drawing of the spinal cord section. In two instances, the recording site

CRANIAL ISOFLURANE AND DORSAL HORN ACTIVITY

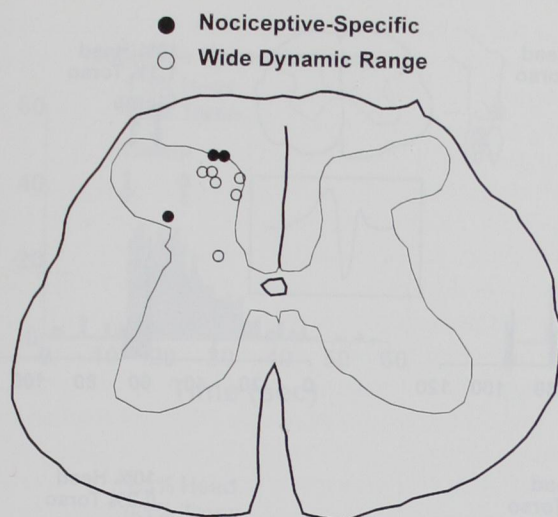


Fig. 1. Spinal recording sites, compiled on representative section through lumbar spinal cord (at the approximate level of L5).

was estimated from the electrode depth, and data for one site were lost.

Statistical Analysis

A log transformation was performed,¹³ and repeated-measures analysis of variance was used to assess statistical significance for comparisons of the evoked response

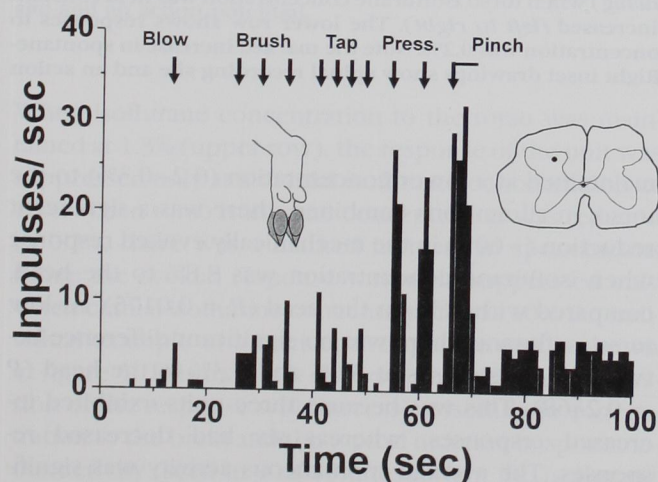


Fig. 2. Example of a wide dynamic range-type unit. This peristimulus-time histogram shows the responses of the unit to innocuous (air blow, brush, tap, pressure) and noxious (pinch) stimuli delivered to the receptive field on the hoof (inset figurine). Right inset drawing shows spinal recording site.

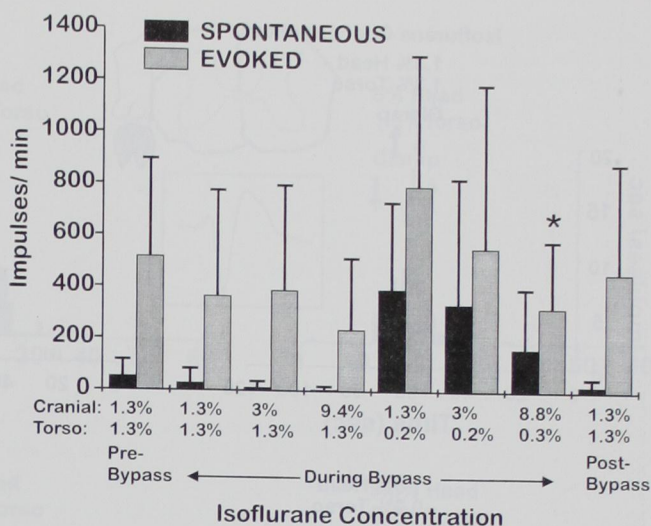


Fig. 3. Population data showing mean number of impulses per minute during each of the experimental conditions. Error bars = SD. With torso isoflurane concentration at 1.3%, evoked responses were not significantly affected by increased cranial isoflurane concentration. When torso isoflurane concentration was 0.3%, 8.8% isoflurane administered to the head decreased the response by 60% (* $P < 0.02$) compared with 1.3%/0.2%. ($n = 7-10$ in each group, except the postbypass group, for which $n = 4$.)

at cranial isoflurane concentrations of 3% and 9% to the evoked response at 1.3%; *post hoc* analysis was performed using a Tukey-Kramer multiple comparisons test. A Student's *t* test was used to compare the change in spontaneous activity when torso isoflurane was decreased from 1.3% to 0.2-0.3% and to compare pre- and postbypass evoked activity. A probability value < 0.05 was considered significant.

Results

Successful recordings were made in 10 goats. The units were localized to the superficial ($n = 2$) or deeper laminae of the dorsal horn (fig. 1). Three units were nociceptive specific, including the two in the superficial dorsal horn (fig. 1), in that they responded only to noxious levels of pressure and pinch stimuli. The remaining were WDR neurons, responding with increasing discharge rate as mechanical stimulus intensity increased from light blowing of hair follicles, brushing, and tapping, to pressure and pinch. Receptive fields ranged in size from part of the hoof to large areas of the distal hind limb. We did not observe any notable

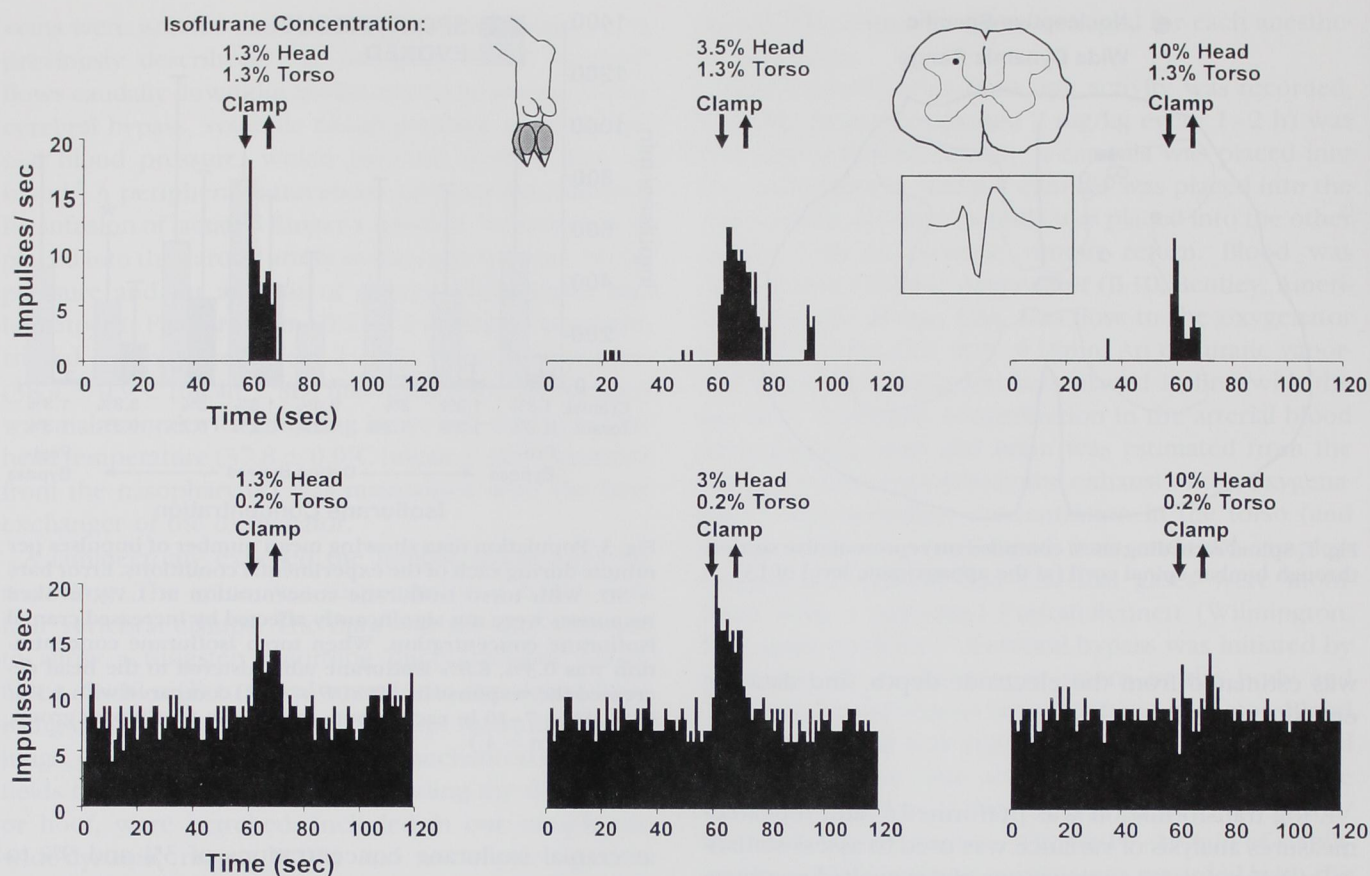


Fig. 4. Example of the effect of different combinations of cranial and torso isoflurane concentrations on response of WDR neuron to noxious stimulation. The upper row of the peristimulus-time histograms shows the responses of the unit to the noxious clamp stimulus delivered to the lateral hoof bulb (*inset drawing*) when torso isoflurane concentration was held constant at 1.3% and cranial isoflurane concentration was systematically increased (*left to right*). The lower row shows responses to increasing cranial isoflurane (*left to right*) when torso isoflurane concentration was 0.2%. Note the marked increase in spontaneous firing at 0.2% torso isoflurane (lower row of the histograms). Right inset drawings show spinal recording site and an action potential.

differences in effects of anesthetic agent on these two unit types and therefore have pooled data from all units. A typical response of a WDR-type unit to graded mechanical stimuli is shown in figure 2.

We were able to record responses of eight units during all combinations of anesthetic delivered independently to the head and torso, whereas incomplete data were recorded in the other two units. All data are presented as means \pm SD. Figure 3 shows averaged spontaneous and mechanically evoked activity for the 10 units at each combination of anesthetic agents to the head and torso. When isoflurane concentration was maintained at 1.3% to the torso, the average response was not significantly affected by increasing cranial isoflurane concentration to 3.0% or 9.4%. When isoflurane was

maintained at a lower concentration (0.2–0.3%) to the torso, in all neurons combined, there was a significant reduction ($\sim 60\%$) in the mechanically evoked response when isoflurane concentration was 8.8% to the head, compared with 1.3% to the head ($P = 0.0175$). At low torso isoflurane, there was no significant difference between the responses at 3.0% and 1.3% to the head ($P = 0.2468$). This was because three units exhibited increased responses, whereas six had decreased responses. The average spontaneous activity was significantly higher at a concentration of 0.3% isoflurane to the torso compared with 1.3% to the torso ($P = 0.0043$). Postbypass-evoked activity was not significantly different from that obtained before bypass ($P = 0.8285$). A typical example of a WDR unit is shown in figure 4.

CRANIAL ISOFLURANE AND DORSAL HORN ACTIVITY

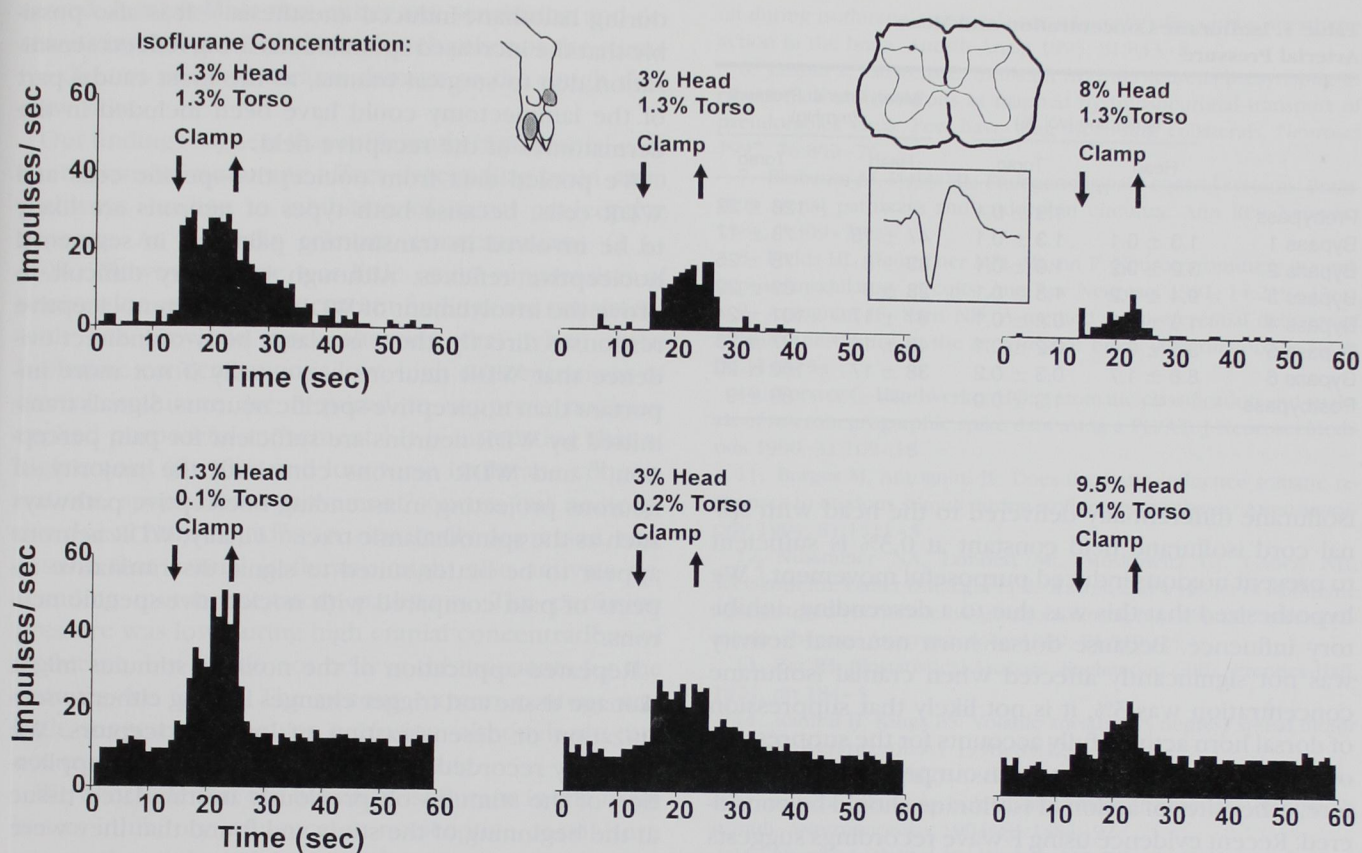


Fig. 5. Example of the effect of different combinations of cranial and torso isoflurane concentrations on a nociceptive-specific neuron. Note the dose-related depressant effect of increasing cranial isoflurane concentration and increased spontaneous firing at low torso isoflurane concentration (lower row of peristimulus-time histograms). Right inset drawings show spinal recording site and an action potential.

When isoflurane concentration to the torso was maintained at 1.3% (upper row), the response of the unit was suppressed only at a cranial isoflurane concentration of 10%. When torso isoflurane concentration was reduced to 0.2%, there was a marked increase in spontaneous firing; the evoked response was again suppressed only when cranial isoflurane concentration was 10%. An individual example of a nociceptive-specific unit is shown in figure 5. This example shows a dose-related suppression of the response with increasing isoflurane concentration to the brain and the typical increase in spontaneous activity (seen in 9 of 10 units) when the isoflurane concentration to the torso was reduced.

Systemic mean arterial pressure was 126 ± 22 mmHg before bypass, 90 ± 25 mmHg during bypass, and 89 ± 9 mmHg after bypass. Mean cranial blood pressure decreased as cranial isoflurane concentration increased,

as would be expected from its vasodilatory effects (table 1). Hematocrit, glucose, and blood gas values are presented in table 2. Cranial blood flow was set at 500–600 ml/min, and cranial venous oxygen content was high, indicating adequate perfusion.

Discussion

We observed that increasing cranial isoflurane concentration to 9% inhibited noxious-evoked responses in dorsal horn neurons, but this effect was statistically significant only when the torso (and presumably spinal cord) isoflurane concentration was 0.3%. The direct spinal effect of isoflurane to suppress nociceptive dorsal horn neuronal activity likely would mask any descending effects from the brain. As previously noted, 3%

Table 1. Isoflurane Concentration and Mean Arterial Pressure

| | Isoflurane (vol %) | | Mean Arterial Pressure (mmHg) | |
|------------|--------------------|-----------|-------------------------------|----------|
| | Head | Torso | Head | Torso |
| Prebypass | — | 1.3 ± 0.1 | — | 126 ± 22 |
| Bypass 1 | 1.3 ± 0.1 | 1.3 ± 0.1 | 47 ± 15 | 78 ± 17 |
| Bypass 2 | 3.2 ± 0.2 | 1.3 ± 0.1 | 39 ± 8 | 78 ± 25 |
| Bypass 3 | 9.4 ± 1.2 | 1.3 ± 0.1 | 28 ± 8 | 89 ± 29 |
| Bypass 4 | 1.3 ± 0.1 | 0.2 ± 0.1 | 61 ± 17 | 101 ± 24 |
| Bypass 5 | 3.0 ± 0.1 | 0.2 ± 0.1 | 45 ± 14 | 97 ± 25 |
| Bypass 6 | 8.8 ± 1.7 | 0.3 ± 0.2 | 38 ± 17 | 100 ± 20 |
| Postbypass | — | 1.3 ± 0.0 | — | 89 ± 9 |

isoflurane differentially delivered to the head with spinal cord isoflurane held constant at 0.3% is sufficient to prevent noxious-induced purposeful movement.³ We hypothesized that this was due to a descending, inhibitory influence. Because dorsal horn neuronal activity was not significantly affected when cranial isoflurane concentration was 3%, it is not likely that suppression of dorsal horn activity fully accounts for the suppression of noxious-evoked movement in our prior study.³ Therefore, other sites of action of isoflurane should be considered. Recent evidence using F-wave recordings suggests that anesthetic agents might suppress movement by direct inhibitory action on spinal motor neurons.^{14,15}

Spontaneous neuronal activity increased significantly with low torso concentrations of isoflurane. In acute decerebrate preparations, spontaneous firing of spinal neurons increases as the anesthetic agent is withdrawn.¹⁶ In chronically instrumented animals, spontaneous activity of spinal (including WDR) neurons is often low¹⁷ but can be as high as 8 spikes/s, with a decrease

during halothane-induced anesthesia.¹⁸ It is also possible that the increased spontaneous firing reflects sensitization due to surgical trauma, as the most caudal part of the laminectomy could have been included in the dermatomes of the receptive field.

We pooled data from nociceptive-specific cells and WDR cells, because both types of neurons are likely to be involved in transmitting pain and in segmental nociceptive reflexes. Although it is very difficult to prove the involvement of WDR neurons in nociceptive responses directly, there is a large body of indirect evidence that WDR neurons are equally if not more important than nociceptive-specific neurons. Signals transmitted by WDR neurons are sufficient for pain perception,¹⁹ and WDR neurons constitute the majority of neurons projecting in ascending nociceptive pathways such as the spinothalamic tract.²⁰ Finally, WDR neurons appear to be better suited to signal discriminative aspects of pain compared with nociceptive-specific neurons.²¹

Repeated application of the noxious stimulus might damage tissue and trigger changes leading either to sensitization or desensitization of local nociceptors. We routinely recorded unit responses to repeated application of the stimulus on previously unstimulated tissue at the beginning of the study and found that they were constant in magnitude. In several animals, we also were able to record responses to the identical stimuli at the conclusion of the study after ending bypass, and responses were again similar in magnitude. This suggests that any sensitizing or desensitizing influences that might have occurred were not sufficient to alter neuronal response magnitude. Had sensitization occurred because of tissue trauma, one would expect an increase in the size of evoked responses, but this was not ob-

Table 2. Hematocrit (Hct), Glucose, and Blood Gas Values

| | Prebypass Arterial | Bypass-Body Arterial | Bypass-Oxygenator-Arterial | Bypass-Oxygenator-Venous | Postbypass Arterial |
|-------------------------|--------------------|----------------------|----------------------------|--------------------------|---------------------|
| Hct | 32 ± 5 | 22 ± 5 | 22 ± 5 | 22 ± 5 | 21 ± 7 |
| Glucose (mg/dL) | 78 ± 34 | 95 ± 35 | 117 ± 45 | 112 ± 42 | 67 ± 23 |
| pH | 7.43 ± 0.06 | 7.40 ± 0.10 | 7.27 ± 0.07 | 7.25 ± 0.07 | 7.37 ± 0.05 |
| pO ₂ (mmHg) | 428 ± 141 | 480 ± 107 | 491 ± 69 | 277 ± 98 | 509 ± 48 |
| pCO ₂ (mmHg) | 31 ± 4 | 32 ± 7 | 41 ± 7 | 45 ± 7 | 35 ± 2 |
| BE (mEq/L) | -2 ± 5 | -3 ± 5 | -7 ± 6 | -8 ± 4 | -4 ± 2 |

Hct = hematocrit; pO₂ = partial pressure of oxygen; pCO₂ = partial pressure of carbon dioxide; BE = base excess.

Values are mean ± SD.

CRANIAL ISOFLURANE AND DORSAL HORN ACTIVITY

served. It is possible that either any sensitizing and desensitizing influences balanced each other or the stimulus did not produce serious damage to the tough dew claw skin.

Our findings suggest that sufficiently high cranial concentrations of isoflurane affect descending brain stem pathways, leading to inhibition of spinal nociceptive neurons. At clinically relevant concentrations (1.3–3.0%), however, there was little or no suppression of neuronal responses. The current findings are consistent with our previous work showing that when torso isoflurane concentration is low, high concentrations of cranial isoflurane are required to suppress noxious-evoked purposeful movement.³ It is interesting that at high cranial (6–10%) and low torso isoflurane concentrations, spontaneous movement⁴ occurs but nociceptive dorsal horn neurons are maximally depressed, suggesting that cranial isoflurane might also activate descending motor excitatory pathways. The perfusion pressure was low during high cranial concentrations of isoflurane, but we do not believe this accounts for the changes we found. The venous oxygen content was high, suggesting adequate perfusion, although we cannot exclude the possibility of shunting.

We conclude that the action of isoflurane in the brain has an inhibitory influence on nociceptive dorsal horn neuronal activity when cranial isoflurane is given at high, supraclinical concentrations and torso (and hence spinal cord) concentrations are low.

References

1. Rampil IJ, Mason P, Singh H: Anesthetic potency (MAC) is independent of forebrain structures in the rat. *ANESTHESIOLOGY* 1993; 78:707–12
2. Rampil IJ: Anesthetic potency is not altered after hypothermic spinal cord transection in rats. *ANESTHESIOLOGY* 1994; 80:606–10
3. Antognini JF, Schwartz K: Exaggerated anesthetic requirements in the preferentially anesthetized brain. *ANESTHESIOLOGY* 1993; 79:1244–9
4. Antognini JF: Movement associated with high cerebral concentrations of isoflurane: No evidence of seizure activity. *Can J Anaesth* 1996; 43:310–4
5. Antognini JF, Berg K: Cardiovascular responses to noxious stimuli during isoflurane anesthesia are minimally affected by anesthetic action in the brain. *Anesth Analg* 1995; 81:843–8
6. Jasmin L, Carstens E, Basbaum AI: Interneurons presynaptic to rat-tail flick motoneurons as mapped by transneuronal transport of pseudorabies virus: Few have long ascending collaterals. *Neurosci* 1997; 76:859–76
7. Basbaum AI, Fields HL: Endogenous pain control systems: Brain-stem spinal pathways and endorphin circuitry. *Ann Rev Neurosci* 1984; 7:309–38
8. Fields HL, Heinricher MM, Mason P: Neurotransmitters in nociceptive modulatory circuits. *Ann Rev Neurosci* 1991; 14:219–45
9. Antognini JF, Kien ND: A method for preferential delivery of volatile anesthetics to the *in situ* goat brain. *ANESTHESIOLOGY* 1994; 80:1148–54
10. Forster C, Handwerker HO: Automatic classification and analysis of microneurographic spike data using a PC/AT. *J Neurosci Methods* 1990; 31:109–18
11. Borges M, Antognini JF: Does the brain influence somatic responses to noxious stimuli during isoflurane anesthesia? *ANESTHESIOLOGY* 1994; 81:1511–5
12. Nussmeier NA, Lambert ML, Moskowitz GJ, Cohen NH, Weiskopf RB, Fisher DM, Eger EI II: Washin and washout of isoflurane administered via bubble oxygenators during hypothermic cardiopulmonary bypass. *ANESTHESIOLOGY* 1989; 71:519–25
13. Zar JH: *Biostatistical Analysis*. Englewood Cliffs, Prentice-Hall, 1974, pp 184–5
14. Rampil IJ, Kings BS: Volatile anesthetics depress spinal motor neurons. *ANESTHESIOLOGY* 1996; 85:129–34
15. King BS, Rampil IJ: Anesthetic depression of spinal motor neurons may contribute to lack of movement in response to noxious stimuli. *ANESTHESIOLOGY* 1994; 81:1484–92
16. Namiki A, Collins JG, Kitahata LM, Kikuchi H, Homma E, Thalhammer JG: Effects of halothane on spinal neuronal responses to graded noxious heat stimulation in the cat. *ANESTHESIOLOGY* 1980; 53:475–80
17. Uchida H, Kishikawa K, Collins JG: Effect of propofol on spinal dorsal horn neurons: Comparison with lack of ketamine effects. *ANESTHESIOLOGY* 1995; 83:1312–22
18. Herrero JF, Headley PM: Cutaneous responsiveness of lumbar spinal neurons in awake and halothane-anesthetized sheep. *J Neurophysiol* 1995; 74:1549–62
19. Mayer DJ, Price DD, Becker DP: Neurophysiological characterization of the anterolateral spinal cord neurons contributing to pain perception in man. *Pain* 1975; 1:51–8
20. Willis WD, Trevino DL, Coulter JD, Maunz RA: Responses of primate spinothalamic tract neurons to natural stimulation of hindlimb. *J Neurophysiol* 1974; 37:358–72
21. Maixner W, Dubner R, Bushnell MC, Kenshalo DR Jr, Oliveras JL: Wide-dynamic-range dorsal horn neurons participate in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. *Brain Res* 1986; 374:385–8