Anesthetic Potency Is Not Altered after Hypothermic Spinal Cord Transection in Rats

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Background: In essence, the clinical goal of general anesthesia is to produce a state of unresponsiveness and amnesia. These endpoints are commonly achieved with drugs like isoflurane, but the sites and mechanisms by which these specific endpoints are achieved remain unknown. Blocking the somatic motor response to painful stimuli is widely used as an indicator of anesthetic adequacy, and the concentration of anesthetic agent (minimum alveolar concentration [MAC]) required to achieve this unresponsiveness is the benchmark of anesthetic potency. Recent work has demonstrated that precollicular decerebration does not alter MAC in rats, suggesting that the forebrain is not a major site of action of isoflurane in blocking motor responses. The brain stem contains systems that modulate pain processing in the spinal cord. The current study was undertaken to assess the relative roles of the brain stem and spinal cord as sites of anesthetic action in blocking somatic responsiveness.

Methods: In seven rats, anesthesia was induced and maintained with isoflurane in oxygen. MAC was determined by observing the response to tail clamp and fore- and hind limb toe pinch at three times: after intubation, after cervical laminectomy, and after staged hypothermic spinal cord transection.

Results: MAC determined by tail clamp did not change during the protocol (1.28 ± 0.08% [mean ± standard deviation] baseline vs. 1.25 ± 0.18% postlaminctomy vs. 1.03 ± 0.40% posttransection). In one animal, the MAC value decreased from a prelesion value of 1.2% to 0.25%, accounting for most of the variance in the postlesion mean; the MAC value as determined by withdrawal to rear paw pinch was unchanged from its prelesion value in this animal. The MAC values as determined by toe pinch in all animals remained unchanged after spinal transection of the lesion both rostrally and caudally.

Conclusions: Somatic motor responsiveness and its sensitivity to isoflurane appeared to be unaltered despite acute loss of descending cortical and bulbar controls. This observation suggests that the site of anesthetic inhibition of motor response may be in the spinal cord. (Key words: Anesthesia; mechanisms. Anesthetics, volatile: isoflurane. Potency: anesthetic; minimum alveolar concentration. Spinal cord.)

GENERAL anesthesia is used clinically to induce a state of unresponsiveness (somatic or motor, hemodynamic, and endocrine) and a lack of awareness and memory. Potent, inhaled anesthetic agents have effects that are widely distributed throughout the central nervous system, and indeed the entire body, but the specific molecular or macroscopic sites of action that mediate the desired effects of these agents are not known. Of particular interest, the potency of general anesthetic agents to inhibit the ability of a patient to respond with movement to painful stimuli has long been used as a test of anesthetic action. This potency, characterized by its ED₅₀, is widely known as the minimum alveolar concentration (MAC). Although this somatic unresponsiveness is a central feature of the general anesthetic state, the mechanism and site(s) at which general anesthetic agents modulate this effect remain unknown.

A recent study reported that precollicular decerebration did not alter the potency of general anesthetics to block somatic response, demonstrating that the neurorheological structures of the forebrain are not required to mediate this change in somatic responsiveness. This observation also suggests that the removed structures do not exert a tonic descending modulatory influence during general anesthesia. Confirming evidence that the principal site of control of somatic responsiveness lies below the forebrain comes from Antognini and Schwartz, who preferentially anesthetized the brains of goats with isoflurane, maintaining the central nervous system from the brain stem downward and the rest of the body with a very low concentration. When isoflurane was delivered only to the brain, the cerebral concentration required to block movement increased to 240% of control, suggesting that depression of the upper central nervous system is a relatively impotent means of depressing somatic responsiveness.

In the past decade, evidence has supported the existence of a neural system within the brain stem that

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provides descending modulation of the transmission of nociceptive information within the spinal cord.\textsuperscript{4,5} In rats, isoflurane causes complex changes in the spontaneous electric activity of cells in a component of the pain modulation system, the nucleus raphe magnus.\textsuperscript{†} The role of this system in mediating anesthetic-induced unresponsiveness is unknown and cannot be ascertained because of the anatomic limits in the studies by Rampil \textit{et al.}\textsuperscript{2} and Antognini and Schwartz.\textsuperscript{5}

The goal of the current study was to isolate further and identify the neural structures that mediate the effect of inhaled anesthetics on the somatic response to painful stimuli by functionally separating the brain stem from the spinal cord.

**Materials and Methods**

With the approval of the Committee on Animal Research, seven mature, male Sprague-Dawley rats (weight 401 ± 59 g [mean ± standard deviation]) were studied. Animals were allowed food and water \textit{ad libitum} until the day of surgery. Anesthesia was induced by inhalation of isoflurane in air, and tracheas were intubated with a 16-G intravenous catheter. Animals were then secured in prone position in a Kopf stereotactic frame (D. Kopf Instruments, Tujunga, CA). Anesthesia was maintained by spontaneous ventilation of isoflurane in oxygen. Inspired and end-tidal airway gas concentrations were continuously measured with a Datex CapnoMac Ultima (Datex Instrumentarium, Helsinki, Finland) using a breathing circuit and sampling system described previously.\textsuperscript{2} Rectal temperature was thermostatically controlled to 37.5 ± 0.5°C using an infrared lamp. Lactated Ringer’s solution was administered subcutaneously, as required, to maintain hydration.

MAC for each rat was determined using a modification of Dixon’s up-and-down method, as described previously.\textsuperscript{6} Administration of isoflurane was adjusted in steps of 0.2%, and 30 min was allowed for equilibration once a stable end-tidal concentration had been obtained at each step. Noxious stimulation was applied by means of an alligator clip clamped to the proximal third of the tail and oscillated ± 45° at approximately 1 Hz for 60 s. Positive movement was defined as purposeful movement of any of the four extremities, including, for the purposes of this study, withdrawal of a single rear limb. If the animal showed a positive response, the isoflurane concentration was increased; if there was no response, the isoflurane concentration was decreased until movement was observed. MAC was calculated as the midpoint between the lowest isoflurane concentration blocking motor response and the highest concentration permitting movement. In addition to tail clamping, at each stage of the protocol, the movement response to separate pinches of the hind and foretoes applied for 15 s by 20-cm fixation forceps (Stille, Sweden) was noted. The toe pinch test, relying on presumably simpler reflex circuitry than tail pinch, was intended as a control measurement for detecting spinal shock.

After baseline MAC determination, a dorsal midline incision and dissection exposed the spinal processes and lamina of C6–T1.\textsuperscript{7} Using a high-speed drill, bilateral laminectomies were performed at C7, and the posterior spinal arch wasatraumatically removed to expose the dura. A thin layer of warmed mineral oil was applied to the tissue and the wound loosely approximated to preserve normothermia. MAC was then remeasured.

Next, the wound was reopened, and a custom-machined probe was lightly applied to the exposed dura at C7. The probe consisted of a partially hollowed 12-mm aluminum rod that tapered to a 5-mm-diameter dual contact surface. The contact surface was instrumented with a subminiature thermocouple. The internal chamber of the probe was connected to a controllable supply of liquid nitrogen. The probe was slowly cooled to a dorsal temperature of \(\approx 10^\circ\text{C}\), held stable for about 2 min to allow the spinal cord to equilibrate, and then rapidly cooled to \(\approx -10^\circ\text{C}\) and maintained at that temperature for about 90 s to produce a complete freeze lesion at that level of the spinal cord. Warm mineral oil was again applied and the wound again approximated. The tissue surrounding the freeze lesion was allowed time to rewarmed to 37.5°C, and then MAC was retested.

After determination of the posttransection MAC, the isoflurane concentration was reduced to 0.3% to confirm the presence of vigorous reflex responses, and the functional extent of the spinal cord lesion was assessed. Animals were assumed to have a functionally (sensormotor) complete transection if, when painfully stimulated at the tail or hind limb, the hind limbs moved vigorously and bilaterally but the forelimbs and neck did not, conversely, if painful pinching of a forelimb elicited vigorous reaction proximally, but not distally, to C7. The rats were then killed with high-
dose isoflurane; in three rats, the spinal cords were excised, fixed with paraformaldehyde, cryosectioned, and stained with cresyl violet to assess histologic damage from the freeze lesion. Changes in MAC were assessed by analysis of variance with repeated measures. A P value of ≤ 0.05 was assumed to be significant.

Results

The baseline MAC value for each rat was within the range expected for this strain and weight (n = 7, 1.28 ± 0.08% isoflurane [mean ± standard deviation]). The postsurgical, prelesion MAC was unchanged at 1.25 ± 0.18, and tail clamping produced movement of all extremities (including forelimbs and occasionally the neck) at this point. The MAC as elicited by hind toe pinch decreased between baseline and postlaminectomy (1.53 vs. 1.28, P = 0.035). After a segment of the spinal cord was frozen, the mean MAC value was not statistically different (1.03 ± 0.40, P = 0.133) (fig. 1). In one animal, the MAC decreased from a prelesion value of 1.2% to 0.25%, accounting for most of the variance in the postlesion mean; the MAC as determined by withdrawal to rear paw pinch was unchanged from its prelesion value in this animal, although even with this stimulus, this animal presented only the simplest motor responses (unilateral withdrawal), suggesting that a degree of spinal shock was present. The MAC values as determined by toe pinch remained unchanged after spinal transection of the lesion both rostrally and caudally. The time required to measure MAC after transection ranged from 45 to 135 min.

All animals demonstrated complete, functional spinal cord transection as defined in Materials and Methods. The posttransection motor responses tended to be slightly less vigorous than those preceding transection, but were of the same types. There was no evidence for sensory or motor communications across the lesion.

With occasional tracheal suctioning of secretions, all animals maintained vigorous respiration throughout the study period.

Histologic examination revealed complete transection of the cord, with necrosis and loss of structural integrity in a zone extending several millimeters along the longitudinal axis of the cord, centered on the site of the cooling probe.

Discussion

The blockade of movement is a central feature of the general anesthetic state. The nocifensive (escape-from-pain) movements triggered by tail or toe pinch or by surgical incision and blocked by general anesthetics appear to be organized as reflexes within the spinal cord. In rodents and carnivores, the isolated spinal cord is capable of complex coordinated actions; in primates and humans, this ability is less commonly observed, but it has been reported. Electrically stimulated and recorded monosynaptic and polysynaptic spinal reflexes are known to be sensitive to inhaled anesthetic agents, and the current study extends these observations to clinically relevant stimuli and responses in vivo. Validation of these findings would permit a neurologically simpler model to be used in uncovering the underlying mechanisms of some aspects of the general anesthetic state. These data also support a distributed, heterogeneous model of anesthetic drug effect, in which the neural structures involved in different components of general anesthesia (i.e., responsiveness vs. awareness or memory formation) may have different anatomic sites with potentially different pharmacologic sensitivities.

There are potential limitations in a study of this type. Most relevant are the physiologic changes due to spinal
transsection, which could be expected to obscure anesthetic-mediated effects. Generally, transection of the spinal cord is associated with a temporal sequence of changes in reflex sensitivity and responsiveness. Initially, in primates, there is a brief (≈ 2-h) period of diminishing responsiveness,\textsuperscript{16} progressing to the state of unresponsiveness known as "spinal shock."\textsuperscript{4} Another common finding in severe spinal injury is gross jerking or fasciculation at the moment of injury, although this was never observed in this series. After a period of time, which depends on the species, the spinal cord below the lesion exhibits hyperreflexia. In Sprague-Dawley rats comparable in size to those used in this study, Sercmin et al. described the sequence of reflex activity in animals whose spinal cords were transected by aspiration during halothane anesthesia.\textsuperscript{9} Most animals, in the absence of anesthesia, were areflexic for more than 1 day postlesion. The mean time for development of weak unilateral withdrawal to toe pinch was 1 day, whereas good unilateral withdrawal required 3 days; similarly, weak contralateral withdrawal was observed at 2 days, and good contralateral withdrawal required more than 4 days. The response to tail clasp was observed in fewer than 10% of animals on the 1st post-surgical day and in most animals by 4–5 days, and consisted initially of bilateral hind limb extension. By 2 weeks, bilateral extension was replaced by alternating extension and flexion or stepping in 66% of animals. Data regarding the sensitivity of these chronic spinal reflexes to anesthetic agents appear to be limited. One early study reported that these reflex responses became resistant to anesthetics after the period of spinal shock.\textsuperscript{17} Both spinal shock or central nervous system plasticity and up-regulation of synaptic receptors would render MAC testing meaningless as a probe of anesthetic mechanism.

Hypothermia has been used in the past for selective, reversible block of synaptic or axonal activity.\textsuperscript{18,19} In the current study, hypothermia was used to "anesthetize" the cord before freezing, to avoid triggering spinal shock, and to allow testing of anesthetic effect in the spinal cord before the changes of plasticity and reorganization became large. That spinal shock was avoided after transection is evidenced by the continued presence of bilateral and complex reflex behaviors that very closely resembled the responses observed during MAC testing while animals were intact. Although any type of complete spinal transection lesion might produce similar results if measurements were obtained in the immediate postlesion period, in a pilot series of transections accomplished with a scalpel blade, gross jerking followed by areflexia occurred immediately.

In summary, in six of seven animals without spinal shock, somatic motor responsiveness and its sensitivity to isoflurane appeared to be unaltered despite acute loss of descending cortical and bulbary controls. This observation suggests that, for lower mammals like rats that have complex autonomous spinal motor pattern generators, the site of anesthetic inhibition of motor response may be in the spinal cord. In addition, that all of the sites identified with the conscious perception of pain were absent in the present model suggests that the somatic motor response could be independent of the perception of pain.

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