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Intrathecal Veratridine Administration Increases MAC in Rats

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Abstract

Background—Results from several studies point to sodium channels as potential mediators of the immobility produced by inhaled anesthetics. We hypothesized that the intrathecal administration of veratridine, a drug that enhances the activity or effect of sodium channels, should increase MAC.

Methods—We measured the change in isoflurane MAC caused by intrathecal infusion of various concentrations of veratridine into the lumbothoracic subarachnoid space of rats. We compared these result to those obtained from intracerebroventricular infusion.

Results—As predicted, intrathecal infusion of veratridine increased MAC. The greatest infused concentration (25 μ M) also produced neuronal injury in the hind limbs of two rats and decreased the peak effect on MAC. A concentration of 1.6 μ M produced the greatest (21%) increase in MAC. Intraventricular infusion of 1.6 and 6.4 μ M veratridine did not alter MAC. Rats given 25 μ M died.

Conclusion—Intrathecal administration of veratridine increases MAC of isoflurane, a finding consistent with a role for sodium channels as potential mediators of the immobility produced by inhaled anesthetics.

Implications—Intrathecal administration of veratridine can increase MAC, presumably by an effect on sodium channels.

Keywords

Anesthetics; inhaled; isoflurane; Anesthetic potency; MAC; EC₅₀; Catecholamines; Mechanisms of anesthetic action; Sodium channels; Veratridine

Introduction

Inhaled anesthetics may act by blocking the passage of excitatory impulses through the central nervous system. In vitro studies indicate that inhaled anesthetics can block sodium channels at anesthetizing concentrations, and that such blockade can, in turn, block the release of excitatory neurotransmitters such as glutamate.¹ To be relevant to the capacity of inhaled anesthetics to produce immobility, such an action must occur in the spinal cord.² Consistent

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with this requirement and a role for sodium channels, an increase in intrathecal, but not intracerebroventricular, sodium concentration increases MAC.³

If inhaled anesthetics act by blocking sodium channels, then intravenous administration of a blocker of sodium channels, lidocaine, should and does decrease MAC (the minimum alveolar concentration of anesthetic that eliminates movement in response to noxious stimulation in 50% of subjects) in a dose-related manner.⁴ Conversely, administration of veratridine, a lipid-soluble drug that enhances the activity or effect of sodium channels (by decreasing the rate of inactivation of such channels),⁵ should increase MAC and should do so much more if given intrathecally than if given systemically or into the cerebral ventricles. Present evidence indicates that veratridine activates all voltage-gated sodium channels without affecting other ion channels.⁶ Although its effects on each of the cloned Na_v isoforms have not been determined, veratridine activates isolated rat brain, cardiac and skeletal muscle type Na channels.⁷

The present study tested the prediction that intrathecal but not intracerebroventricular administration of veratridine would increase MAC.

Methods and Materials

Materials

Isoflurane was obtained from Baxter Healthcare Corp. (New Providence, NJ). Veratridine was obtained from Sigma (St. Louis, MO). With approval of the Committee on Animal Research of the University of California, San Francisco, we studied 66 male, 12–15 week-old, Long-Evans rats weighing 300–450 g obtained from Charles River Laboratories (Hollister, California). Each animal was caged with up to as many as 2 additional rats before surgical preparation, and singly after preparation. All had continuous access to standard rat chow and tap water before study.

Studies of isoflurane MAC in rats given veratridine intrathecally (46 rats)

Fig. 1 provides the outline of the experimental plan. On day 1, rats were anesthetized with isoflurane, and a 32 gauge polyurethane catheter (Micor Inc., Allison Park, PA) was placed through the atlantooccipital membrane according to the method described by Yaksh and Rudy.⁸ The catheter was threaded caudally 6 to 8 cm towards the lumbar sac, the length depending on the size of the rat. The catheter then was tunneled through to the external auditory meatus where it exited and could be accessed and sutured in place. The skin over the posterior wound was sutured closed, and the wound was infiltrated with 0.25% bupivacaine. Rats recovered from anesthesia and surgery for at least 24 hours before study.

Groups of up to 8 rats were placed in individual clear plastic cylinders. Each cylinder received approximately 1L/min oxygen containing approximately 2% isoflurane. An infusion of artificial cerebrospinal fluid (aCSF) was begun immediately after induction of anesthesia at 4 μ L/min via the previously placed intrathecal catheters. The aCSF contained (in mM): NaCl 126.6; NaHCO₃ 27.4; KCl 2.4; KH₂PO₄ 0.50; Na₂SO₄ 0.49; CaCl₂ 1.10; MgCl₂ 0.83; and glucose 5.49. The pH was adjusted to 7.4 by bubbling the mixture with carbon dioxide. A rectal temperature probe was inserted. The isoflurane concentration was decreased to 1.0%–1.2% and sustained at this concentration for 50 minutes, after which the tail was clamped and moved by rolling the clamp at 1–2 Hz for up to 1 min (less if the rat moved). After certifying that movement had occurred, the isoflurane concentration was increased by approximately 0.15% to 0.2%, and after a 30 min period of equilibration the tail clamp was again applied and movement or lack of movement determined. Isoflurane partial pressures were monitored using an infrared analyzer (Datascope, Helsinki, Finland), and immediately after determination of

the response to tail clamp, a sample of gas was obtained from one of the chambers and analyzed for isoflurane by gas chromatography. This process continued until all rats failed to move in response to application of the tail clamp. MAC was calculated as the average of the greatest concentration that permitted movement and the smallest concentration that suppressed movement. This value was designated MAC0. Anesthetic administration then was discontinued, and the rats recovered.

The next day, the rats again were anesthetized with isoflurane and MAC redetermined (MAC1). However, on this day, one or two of the rats (the control group rats) received a 4 $\mu\text{L}/\text{min}$ intrathecal infusion of aCSF containing 4% dimethyl sulfoxide (DMSO). The other 6 rats received infusions of a solution containing either 0.025, 0.1, 0.4, 1.6, 6.4, or 25 μM veratridine (only one dose for a given experiment) in aCSF plus 4% DMSO (the experimental groups). Anesthetic administration then was discontinued, and the rats recovered. The investigator making the determination of MAC was blinded to the contents of the infusions.

On the third day, the rats again were anesthetized with isoflurane and the process of MAC determination repeated (MAC2). On this day, all rats received only an infusion of aCSF. The rats again were allowed to recover and were examined for gross abnormalities in hind-limb function.

Thus, these measurements supplied two control assessments. The change in MAC with treatment (MAC1) could be compared with the MAC in the same rat when given aCSF (MAC0). And the change in MAC with treatment could be compared with the MAC in a comparable group of rats given aCSF. Injury from treatment could be assessed by noting whether a gross abnormality in motor function, particularly of the hind limbs, was evident after the third anesthesia, and whether MAC2 differed from MAC0 in the experimental group more than in the control group.

Studies of isoflurane MAC in rats given veratridine intraventricularly (20 rats)

The preceding studies were repeated in rats in which the veratridine was delivered via a cannula placed in the left lateral cerebral ventricle rather than an intrathecal catheter. Under anesthesia with isoflurane, the skull was exposed and a hole drilled 0.5 mm posterior to the bregma and 1.5 mm lateral from the midline. A 25G stainless steel guide cannula was placed through the hole to a depth of 4.2 mm from the surface of the skull. Each cannula was secured by wiring to two screws that were placed into the skull approximately 5 mm to either side of the cannula. The skin around the wound was sutured closed, and the wound was infiltrated with 0.25% bupivacaine. Rats recovered from anesthesia and surgery for at least 24 hours before study. MAC0, MAC1 and MAC2 were determined as described above, except that an infusion of aCSF was begun immediately after induction of anesthesia at 4 $\mu\text{L}/\text{min}$ via the previously placed intraventricular cannula. In this study, we tested 4 control rats, 4 infused with 1.6 μM veratridine, 6 with 6.4 μM , and 6 with 25 μM .

Analysis of inhaled anesthetics

We used a Gow-Mac gas chromatograph (Gow-Mac Instrument Corp., Bridgewater, NJ) equipped with a flame ionization detector to measure concentrations of isoflurane. The 4.6 meter-long, 0.22 cm (ID) column was packed with SF-96. The column temperature was 100°C. The detector was maintained at temperatures approximately 50°C warmer than the column. The carrier gas flow was nitrogen at a flow of 15–20 mL/min. The detector received 35–38 mL/min hydrogen and 240–320 mL/min air. Primary standards were prepared, and the linearity of the response of the chromatograph was determined. We also commonly used secondary (cylinder) standards referenced to primary standards.

Statistical analyses

For each concentration of veratridine infused we determined the ratios MAC 1/MAC0 and MAC2/MAC0. A one-way analysis of variance with Fisher's protected least significant difference (PLSD) was done to determine whether infusion of veratridine significantly affected MAC and whether one or more groups differed significantly. We accepted a value of $P < 0.05$ as significant.

Results

Intrathecal infusion of veratridine significantly ($P < 0.0001$) increased MAC. Relative to MAC in control rats, concentrations of 0.4 μM or more significantly increased MAC1/MAC0 (Fig. 2). A concentration of 1.6 μM caused the greatest increase ($21 \pm 8\%$; mean $\pm$ SD). Greater concentrations (6.4 μM and 25 μM) produced increases of $15 \pm 6\%$ and $13 \pm 6\%$ respectively. The lesser increase at 25 μM differed significantly ($P < 0.05$) from that at 1.6 μM . Recovery values (MAC2/MAC0) did not indicate residual damage overall ($P = 0.095$). However, Fisher's PLSD indicated that the 6.4 ($P < 0.05$) and 25 ($P < 0.01$) μM infusions decreased the MAC for recovery from that found in control rats (Fig. 2), indicating injury at these greatest concentrations. Indeed, two rats given 25 μM displayed weakness of one hind limb 24 hours after infusion of veratridine.

Intraventricular infusions of 1.6 μM and 6.4 μM did not significantly alter MAC (Fig. 3). All rats given 25 μM intraventricularly died during infusion of veratridine.

Discussion

As hypothesized, intrathecal, but not intraventricular, infusions of veratridine increased the MAC of isoflurane, an infusion of 1.6 μM producing the greatest (21%) increase with a dose of 25 μM causing a relative decline. The absence of an increase in MAC from intraventricular infusion is consistent with a spinal site of action for inhaled anesthetics. One might ask why a maximal increase is seen with the intrathecal infusion. Does this represent a limited capacity of sodium channels to influence MAC? At least in part it appears that higher concentrations of veratridine produce a counterbalancing effect of injury. Regardless, the results are consistent with, but do not prove, the notion that spinal voltage-gated sodium channels mediate the immobility produced by inhaled anesthetics. The limited increases in MAC might suggest that the mediation explains only a limited portion of the immobility. Alternatively, the increases seen might simply reflect a non-specific increase (i.e., a modulation) in excitability rather than mediation, a reduction but not elimination by veratridine in the sensitivity of sodium channels to isoflurane, and/or the engagement of a less sensitive immobilizing mechanism/target by isoflurane in the absence of effective sodium channel antagonism.

The isoflurane concentration measured was that in the inspired gas mixture. The definition of MAC refers to expired concentrations. Thus, MAC was not measured. However, the ratio of expired to inspired concentrations was probably constant throughout so the conclusions are not altered by this systematic error, and the error has been shown to be small (perhaps 10%), given the duration of the determination of MAC.⁹ Finally, the experiment was designed to compare MAC values (MAC0, MAC1, MAC2) at the same times after initiation of anesthesia; thus an inspired-to-end-tidal difference likely is the same and does not compromise the comparisons.

Veratridine is a lipid-soluble toxin that prolongs the opening of fast voltage-gated sodium channels, allowing greater sodium influx, albeit at a diminished rate.⁵ Intravenous general anesthetics (propofol, etomidate, alfaxalone, methohexital, thiopental, ketamine) inhibit the veratridine-induced influx of ^{14}C -guanidinium.¹⁰ That is, these anesthetics block the fast

sodium channel, doing so with a potency indicated by the above listing (i.e., propofol most potent).¹⁰ The capacity to block the sodium channel correlates with the lipophilicity of these compounds,¹⁰ a finding consistent with the Meyer-Overton hypothesis.^{11,12} Halothane at 1–2 MAC causes a 50% inhibition of sodium influx into synaptosomes and veratridine-evoked glutamate release from synaptosomes.¹ Isoflurane at a similar MAC-multiple (IC₅₀ 0.41–0.50 mM) also decreases veratridine-evoked glutamate release from synaptosomes.¹³ Similarly, ethanol, diethyl ether, halothane and enflurane all inhibit the veratridine-induced increased uptake of sodium by synaptosomes from rodent brain.¹⁴ Collectively, such results plus the present findings point to anesthetic inhibition of the sodium channel as a plausible candidate for the mediation of immobility produced by inhaled anesthetics.

Acknowledgements

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Intrathecal or Intraventricular Catheter Inserted	MAC0 Determined	MAC1 Determined	MAC2 Determined
Day 1	Day 2 (Infusion of aCSF)	Day 3 (Infusion of test veratridine concentration)	Day 4 (Infusion of aCSF)

Fig. 1.

A schematic representation of the experimental design

Fig. 2.

Intrathecal veratridine infusion increases isoflurane MAC (MAC1/MAC0) with subsequent recovery of MAC (MAC2/MAC0) after cessation of veratridine infusion except for the two highest concentrations infused (6.4 and 25 μM) where recovery was incomplete. The numbers assigned each point indicate the number of rats tested.

Fig. 3. Intrathecal, but not intraventricular, veratridine infusion increases isoflurane MAC (MAC1/MAC0). The numbers assigned each point indicate the number of rats tested.