Blockade of 5-HT2A Receptors May Mediate or Modulate Part of the Immobility Produced by Inhaled Anesthetics

Yi Zhang, MD, Michael J. Laster, DVM, Edmond I Eger II, MD, Caroline R. Stabernack, MD, and James M. Sonner, MD

Department of Anesthesia and Perioperative Care, University of California, San Francisco, California

Many inhaled anesthetics block the *in vitro* effect of the excitatory neurotransmitter serotonin on the 5-HT2A receptor, supporting the view that this receptor might mediate the capacity of inhaled anesthetics to produce immobility during noxious stimulation (i.e., would underlie MAC, the minimum alveolar concentration required to suppress movement in response to a noxious stimulus in 50% of subjects). In the present investigation in rats, we found that intrathecal administration of the 5HT-2A blocker, ketanserin, can decrease isoflurane MAC. This effect, presumably mediated by blockade of serotonin transmission in the spinal cord, reaches a maximum of 20%–25%. An additional decrease (to 60%) may be obtained by IV infusion of ketanserin, and presumably this decrease results from ketanserin's actions on supraspinal centers. The IV doses of ketanserin that decreased MAC were approximately 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in rats, compared with usual clinical doses of 1.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in humans. These results indicate that 5HT2A receptors are in the neural circuitry influencing isoflurane MAC. These results, together with the blocking action of isoflurane on expressed 5HT2A receptors, strengthen the case for a role for 5HT2A receptors to isoflurane-induced immobility. However, because MAC for isoflurane is predominantly determined in the spinal cord, this result is consistent at most with a minor contribution of these receptors to the immobilizing action of isoflurane.

(Anesth Analg 2003;97:475–9)

Results from studies from *in vitro* receptor expression systems reveal that inhaled anesthetics can block the effect of serotonin on serotonin receptors, including the G protein coupled 5HT2A receptors (1), doing so at concentrations of approximately 1 MAC (the minimum alveolar concentration of anesthetic that eliminates movement in response to a noxious stimulus in 50% of subjects). 5HT2A receptors may participate in nociceptive processes, as evidenced by results from several studies using a specific blocker of 5HT2A receptors, ketanserin (2–10). Although many of these studies focus on the supraspinal actions of 5-HT and ketanserin, some also suggest a direct spinal effect of ketanserin (6,9,11). This is of interest because the spinal cord mediates most, if not all, of the capacity of inhaled anesthetics to produce immobility (12–15).

Ketanserin effects are potentially of importance to anesthesiologists because ketanserin may be used to control blood pressure during anesthesia (16–18). The decrease in blood pressure may decrease blood loss (19). Ketanserin also may be used to decrease postoperative shivering (20,21), and to reverse the rigidity that can be associated with the administration of opioids (22).

We hypothesized that the administration of ketanserin might influence anesthetic requirement. The present report tests this notion.

Methods

With approval of the Committee on Animal Research of the University of California, San Francisco, we studied male Sprague-Dawley rats (Crl:CD[SD]BR) weighing 300–450 g obtained from Charles River Laboratories (Hollister, CA).

Intrathecal or IV catheters were inserted under anesthesia with isoflurane. An intrathecal 32-gauge polyurethane catheter (Micor Inc., Allison Park, PA) was placed through the atlantooccipital membrane using methods previously described (23). The catheter was threaded caudally 6–8 cm toward the lumbar sac, the length depending on the size of the rat. At the neck,

This work was supported by National Institutes of Health Grant 1P01GM47818.

EIE is a paid consultant to Baxter Healthcare Corp., who donated the isoflurane used in these studies.

Accepted for publication March 20, 2003.

Address correspondence and reprint requests to Dr. James M. Sonner, Department of Anesthesia, S-455i, Box 0464, University of California, San Francisco, CA 94143-0464. Address e-mail to sonnerj@anesthesia.ucsf.edu.

DOI: 10.1213/01.ANE.0000070229.94485.17

0003-2999/03

sutures were used to fix the catheter to adjacent muscle and skin. IV catheters made of PE 10 were placed in the right internal jugular vein of the animals. Rats were allowed to recover from anesthesia and surgery for at least 24 h before study.

In a third group of rats, we placed cannulae into the third ventricle. Each rat was anesthetized with isoflurane, the skull was exposed, and a hole was drilled 1.8 mm posterior to the bregma in the midline. Through this hole we inserted a 24-gauge stainless steel guide cannula to a depth of 4.1 mm from the skull surface. The cannula was secured by cementing to 2 screws that were placed into the skull approximately 5 mm to either side of the cannula. Injections of artificial cerebrospinal fluid (aCSF) without and with ketanserin were made through a needle inserted into the guide cannula.

MAC was determined in two to four rats at a time. Each rat was placed in a gas-tight clear plastic cylinder. A rectal temperature probe was inserted, and the temperature probe and the tail of the rat were separately drawn through holes in the rubber stopper used to seal one end of the cylinder. Ports through the rubber stoppers in each end of the cylinder allowed delivery of gases. The gases entered at the head end of the cylinder and exited at the tail.

To determine MAC, isoflurane was introduced into the oxygen delivered to the cylinders (at a flow that allowed a delivery of at least 1 L/min to each cylinder) from a conventional vaporizer, starting with a partial pressure of approximately 1.0% of an atmosphere. Anesthetic partial pressures were monitored using an infrared analyzer (Datascope, Helsinki, Finland). Animals were equilibrated with the isoflurane partial pressure for 30 min. After 30 min, a tail clamp was applied for 1 min or until the animal moved. The isoflurane partial pressure was then measured by gas chromatography. If the animal moved, the isoflurane partial pressure was increased by 0.1%–0.2% atmospheres. After equilibration for 30 min, the tail clamp was applied again and isoflurane partial pressure measured. This procedure was repeated until a partial pressure at which the animals did not move was achieved.

Each study consisted of two parts. In the first, we infused aCSF alone, and in the second we infused aCSF to which we added ketanserin (Sigma Chemical Co., St. Louis, MO). The second infusion also contained 5%–12.5% dimethyl sulfoxide (needed to ensure solution of the ketanserin). The stock solutions for aCSF were made daily as described previously (23). The final composition of aCSF was 154.7 mM Na^+, 0.82 mM Mg^{2+}, 2.9 mM K^+, 132.49 mM Cl^-, 1.1 mM Ca^{2+}, 5.9 mM glucose, at a pH of 7.4. Intrathecal infusions were at a rate of 1–4 μL/min (ketanserin concentrations from 0.25 to 16 mg/mL). IV infusions were at a rate of 4–80 μL/min (ketanserin concentrations from 4 to 8 mg/mL). Intraventricular infusions were at a rate of 1–4 μL/min (ketanserin concentrations were 16 mg/mL).

We allowed an hour between the first and second parts of each study, during which time the anesthetic partial pressures were maintained at levels that permitted each rat to respond to stimulation. Only one infusion rate was given per rat.

Three controls examined the effect of the aCSF that included dimethyl sulfoxide. We determined the MAC of isoflurane in 4 rats during an intrathecal infusion of 2 μL/min aCSF, and then redetermined MAC during an infusion of aCSF containing 12.5% dimethyl sulfoxide. In a second group of 2 rats, we similarly determined MAC before and during an IV infusion of 80 μL/min aCSF containing 7.5% dimethyl sulfoxide. In a third group of 2 rats, we similarly determined MAC before and during an intraventricular infusion of 4 μL/min aCSF containing 12.5% dimethyl sulfoxide.

MAC was defined as the average of the partial pressures that just prevented and permitted movement in response to clamping the tail. The change in MAC was calculated as the ratio of the MAC for the second part of each study to the first part. We calculated the mean and standard deviation for the change at each dose of each antagonist. We compared the increases in MAC among the three anesthetic groups using a one-way analysis of variance (ANOVA).

We used a Gow-Mac gas chromatograph (Gow-Mac Instrument Corp., Bridgewater, NJ) equipped with a flame ionization detector to measure isoflurane concentration. The 4.6-m-long, 0.22-cm (inside diameter) column was packed with SF-96. The column temperature was 138°–151°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 (volumetric) standards were prepared for each anesthetic, and the linearity of the response of the chromatograph was determined. We usually used secondary (cylinder) standards referenced to primary standards for isoflurane.

The decreases in MAC at a given infusion rate of ketanserin for a given route were compared using ANOVA. Where a significant ($P < 0.05$) difference was found, we applied a Student-Newman-Keuls test to determine which comparisons were significant. Decreases in MAC for a given anesthetic produced by successively increased infusion rates of ketanserin were tested for significance using Student's *t*-test.

Table 1. Effect of Ketanserin by Different Routes on Isoflurane MAC

Route	Drug	Dose	Isoflurane MAC change (%, mean ± SD)	*n*
IT	DMSO	*12.5% at 2 μL/min*	−2.7 ± 0.3	4
IV	DMSO	7.5% at 80 μL/min	−2.3 ± 0.0[a]	2
ICV	DMSO	12.5% at 4 μL/min	−1.0 ± 0.0[a]	2
IV	Ketanserin	16 μg/min	−4.9 ± 5.6	4
IV	Ketanserin	32 μg/min	−18.1 ± 7.7	4
IV	Ketanserin	160 μg/min	−43.5 ± 4.9	4
IV	Ketanserin	320 μg/min	−60.0 ± 3.5	4
IV	Ketanserin	640 μg/min	No recovery	
IT	Ketanserin	0.25 μg/min	−0.6 ± 5.6	4
IT	Ketanserin	1 μg/min	−16.8 ± 1.4	4
IT	Ketanserin	4 μg/min	−21.9 ± 5.3	4
IT	Ketanserin	16 μg/min	−27.2 ± 5.6	4
IT	Ketanserin	32 μg/min	−24.7 ± 7.1	8
IT	Ketanserin	64 μg/min	−31.5 ± 0.0	3
ICV	Ketanserin	16 μg/min	−28.1 ± 7.4	4
ICV	Ketanserin	64 μg/min	−32.7 ± 8.9	2

[a] Both animals in these groups had the same change in MAC.
MAC = the minimum alveolar concentration of anesthetic producing immobility in 50% of individuals, IT = intrathecal, DMSO = dimethyl sulfoxide, ICV = intracerebroventricular.

Results

Results are tabulated in Table 1 and displayed in Figure 1.

No appreciable effect of vehicle was found in the three control studies, in which the DMSO vehicle was infused intrathecally, IV, and intracerebroventricularly.

The slowest IV infusion of ketanserin (16 μg/min) did not significantly decrease MAC but infusion rates of 32–320 μg/min all significantly decreased MAC, doing so in a rectilinear manner. ANOVA confirmed the significance of the progression ($P < 0.001$), and the Student-Newman-Keuls test showed that the results at each succeeding step significantly differed from the results at the preceding step. An infusion rate of 640 μg/min did not permit recovery to occur ($n = 2$).

Similarly, the slowest intrathecal infusion of ketanserin (0.25 μg/min) did not significantly decrease MAC, but infusion rates of 1–64 μg/min all significantly decreased MAC. ANOVA indicated a significant decrease related to dose ($P < 0.001$). The Student-Newman-Keuls test showed that the effect of the smallest dose differed from that of each succeeding dose; the second smallest dose effect differed from that of the effect of the three largest doses; and the effect of the third smallest dose differed from the effect of the largest dose. Although the faster infusion rates seemed to produce a larger decrease in MAC, in part or all, this may be attributed to the systemic effects of these intrathecal doses. That is, if one subtracts the results at a given IV infusion rate from those at the same intrathecal infusion rate, there was no significant decrease less than that found with an intrathecal infusion rate of 1 μg/min.

An intraventricular infusion of ketanserin of 16 μg/min significantly decreased MAC, and an increase in infusion rate to 64 μg/min caused no further decrease. These decreases did not differ from those seen with IV infusion at comparable rates.

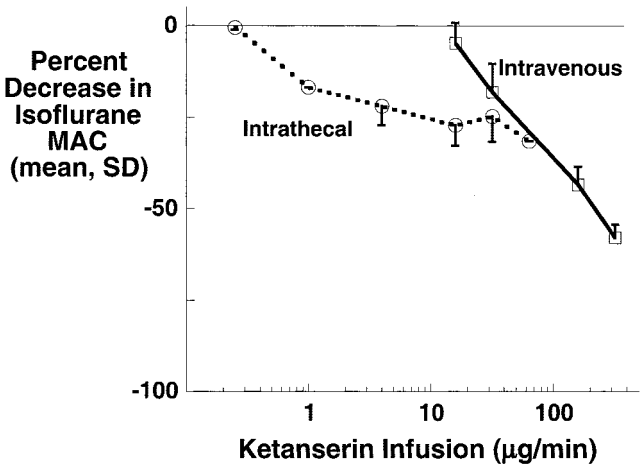

Figure 1. Intrathecal administration of ketanserin in rats produces a 20%–25% decrease in minimum alveolar anesthetic concentration (MAC), independent of effects on supraspinal centers. This effect seems to have a ceiling and is less than the effect on MAC that ketanserin may produce when given IV (i.e., the IV administration affects higher centers in the central nervous system). Values are given as the mean and standard deviation (SD).

Discussion

As predicted by our hypotheses, we found that the administration of ketanserin decreased the MAC of isoflurane (Fig. 1). Up to a 20%–25% decrease could be mediated by the spinal cord, because the intrathecal infusion of ketanserin decreases MAC by 20%–25% at

doses an order of magnitude less than the systemic (IV) doses required to decrease MAC (Fig. 1). The IV administration of increasing doses of ketanserin progressively decrease MAC, survival apparently imposing an upper limit of perhaps a 60% decrease in MAC.

The finding of a greater effect of ketanserin on higher centers (i.e., the greater effect of IV as opposed to intrathecal infusion) parallels the result found by Roberts et al. (24) in sheep. Cervical intrathecal injection of α-methyl-5-HT markedly increased mechanical nociceptive threshold whereas lumbar intrathecal injection did not.

Our results seem to differ from those reported by Dringenberg (25) who found no anesthetic effect consequent to intraperitoneal administration of 700 μg/kg ketanserin. Several differences between his study and ours may explain our failure to confirm Dringenberg's results. First, the anesthetics he used (ketamine, sodium pentobarbital, and chloral hydrate) differed. Second, he applied less vigorous measures of stimulation than did we. Although he used a tail clamp, this was applied to the distal end of the tail, the metal edges of the clamp were padded, the clamp was used for a maximum of 10 seconds, and apparently was not moved during application. Third, intraperitoneal injection may be slightly less effective because of hepatic first-pass removal of ketanserin (26). In addition, in contrast to the sustained or increasing effect of continuous infusion (as in our study), the effect of an intraperitoneal injection would wane over Dringenberg's 200-minute study because of redistribution.

Other investigations suggest that ketanserin may block the analgesia mediated by serotonin receptors in higher centers, such as the midbrain tectum (7) or the inferior colliculus (7). It is difficult to see how such blockade would translate to a decrease in MAC. Similarly, ketanserin seems to decrease κ-mediated analgesia (2) and it is difficult to see how this, too, would decrease MAC.

Regardless of the mechanistic basis, our results suggest that the administration of large doses of ketanserin can affect anesthetic requirement. It should be noted that the doses of ketanserin used clinically in humans (10–20 mg as a single dose, or 10 mg followed by an infusion of 1.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$) are much less than the doses changing MAC in our study in rats (32 μg/min, or approximately 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$). However, given the size of the bolus dose in humans and the greater metabolic rate seen in small versus large animals (in humans, ketanserin has a terminal half-life of 15–30 hours (27,28), but in rats the terminal half-life is 2–5 hours (26)), these doses, particularly immediately after a single IV dose, may be similar for a short span of time. Thus, the use of ketanserin to decrease blood pressure (or prevent increases in blood pressure) (16–19) during anesthesia might transiently decrease anesthetic requirement. Similarly, if ketanserin is used to decrease postoperative shivering (20,21), or to reverse the rigidity that can be associated with the administration of opioids (22), such use might decrease anesthetic requirement.

In summary, we find that ketanserin can decrease anesthetic requirement, but that the effect on the spinal cord is modest. These results do not reveal whether inhaled anesthetics might act directly or indirectly through their effects on 5HT-2A receptors.

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