

Antagonism of the 5-HT₃ Receptor Does Not Alter Isoflurane MAC in Rats

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ONDANSETRON is a potent, selective, competitive antagonist of the serotonin subtype 3 (5-HT₃) receptor^{1,2} that can decrease postoperative nausea.^{3,4} This ionophore is a member of the fast ligand-gated receptor superfamily that includes γ -aminobutyric acid receptor type A, glycine, and neuronal nicotinic acetylcholine receptors. The function of these latter members of the superfamily is known to be sensitive to volatile anesthetics and, in turn, to influence anesthetic potency. For example, in the presence of a volatile anesthetic, charge transfer through the ionophore of γ -aminobutyric acid receptor type A or glycine is increased, and these effects in turn tend to reduce neuronal excitability.^{5,6}

Although ondansetron administration has not been reported to cause significant sedation, some evidence suggests that serotonergic pathways have a role in general anesthesia. For example, Roizen *et al.*⁷ demonstrated that ablation of serotonergic neurons in the nucleus raphe dorsalis decreased halothane minimum alveolar concentration (MAC) in rats by 25%. Volatile anesthetics, including isoflurane, potentiate ion channel currents in cloned, homomeric 5-HT₃ receptors expressed in *Xenopus*.⁸

These observations suggest the possibility that intraoperative administration of ondansetron to minimize postoperative nausea and vomiting might influence anesthetic potency. Accordingly, we examined the effect of ondansetron on the MAC of isoflurane.

Methods

Our study protocol was approved by the University of California, San Francisco, Committee on Animal Research. Sterile ondansetron solutions were prepared in four concentrations (0, 0.05, 0.20, and 0.80 mg/ml; to be administered at infusion rates that would deliver 0, 0.5, 2.0, and 8.0 mg · kg⁻¹ · h⁻¹) and placed in blind coded vials. Seventy-two Sprague-Dawley rats, age-matched at 3 months, with equal gender distribution were prospec-

tively randomized into four gender-balanced groups, with all rats in a given group scheduled to receive the same concentration of ondansetron.

Each animal was placed in a 30-cm-long transparent cylinder with an ID of 6.4 cm. The head-end stopper contained a gas inflow port through which isoflurane was delivered in a fresh gas flow rate of 1 l/min. The tail-end stopper had ports for gas effluent, an intravenous line, the rat's tail, and a temperature probe. Anesthesia was induced with 2.0% isoflurane, and then a 20-gauge femoral vein catheter and a three-lead electrocardiograph were placed. Rectal temperature was controlled to 37.5 ± 0.5°C.

After instrumentation, the inspired isoflurane concentration was set to 1.5%, and the animals were allowed to equilibrate for 40 ± 7 min (mean ± SD). Inspired isoflurane concentration was monitored continuously with an infrared analyzer (model 222; Puritan Bennett, Lenexa, KS). Infusion of the blinded ondansetron solutions were simultaneously initiated at 10 ml · kg⁻¹ · h⁻¹ using independent syringe pumps (model 22; Harvard Apparatus, Holliston, MA) to administer ondansetron at 0, 0.5, 2.0, or 8.0 mg · kg⁻¹ · h⁻¹. The MAC of isoflurane was determined by purposeful movement in response to the stimulus of a modified alligator clip applied to the proximal tail for 60 s and vigorously manually rotated ±45° at approximately 1 Hz. Respiratory movements were excluded. After assessing the first response, the inspired isoflurane concentration was adjusted in 0.15 vol% changes, depending on the animal's response, until each rat had shown two independent-move-no-move cross-overs. The equilibration time after achieving a stable inspired target concentration for each 0.15 vol% step was 27 ± 1 min (mean ± SD). The MAC for each rat was calculated as the mean of the concentrations at the midpoint of its two independent-move-no-move cross-overs.

After determination of MAC, the rats were killed with excessive isoflurane. Concurrently, we stopped infusing ondansetron and obtained a 10-ml blood sample from the inferior vena cava under direct vision. Serum was separated and frozen at -70°C. Ondansetron concentrations in the serum were determined by high-performance liquid chromatography using the method adapted from Colthup *et al.*⁹ The mean MAC values and serum ondansetron concentrations for each group were compared using one-way analysis of variance, and a value of $P \leq 0.05$ was accepted as significant.

Because of unanticipated mortality in the group given 8 mg · kg⁻¹ · h⁻¹ ondansetron, that study arm was

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Table 1. Rat Characteristics by Group (Mean \pm SD)

	Ondansetron Infusion Rates (mg \cdot kg ⁻¹ \cdot h ⁻¹)				P (A,B,C)
	0.0	0.5	2.0	8.0	
n	21	20	20	10	
% Female	52.4	55	50	20	
Age (days)	98 \pm 8	97 \pm 7	99 \pm 8	102 \pm 5	0.351
Weight (g)	358 \pm 100	340 \pm 92	355 \pm 77	418 \pm 71	0.140
MAC (vol%)	1.3 \pm 0.1	1.3 \pm 0.1	1.4 \pm 0.1	—	0.696
Plasma concentration (ng/ml)	0	144 \pm 39	741 \pm 187	7,091 \pm 2,242	0.0001

There were no differences observed in rat characteristics or in the observed minimum alveolar concentration (MAC) values. Rats in the highest infusion rate range died before MAC determination.

abandoned, and two supplemental studies were performed. First, we gave three rats 4.0 mg \cdot ml⁻¹ \cdot h⁻¹ during 1.5% isoflurane. Two of these rats also died. No animals to which lower doses were administered expired, and abnormal respiratory patterns were noted to develop in none. Four additional rats had tail vein intravenous access established and received 8.0 mg \cdot kg⁻¹ \cdot h⁻¹ ondansetron for 4 h while awake. No side effects were observed in these rats.

Results

The mean ages and weights of the rats in each of the first four experimental groups did not differ (table 1). All anesthetized rats to which 8.0 mg \cdot kg⁻¹ \cdot h⁻¹ ondansetron was administered died with abnormal respiration, followed by apnea, bradycardia with atrioventricular block, and then asystole within 60–90 min of starting the infusion, concurrent with administration of 0.7–1.1 MAC (0.9–1.4 vol%) isoflurane. The MAC of rats to which saline placebo was administered did not differ from the MAC of rats to which 0.5 mg \cdot kg⁻¹ \cdot h⁻¹ or 2.0 mg \cdot kg⁻¹ \cdot h⁻¹ was administered (table 1). Rats to which 8.0 mg \cdot kg⁻¹ \cdot h⁻¹ was administered did not survive long enough to allow adequate determination of MAC. Plasma concentrations of ondansetron in the 8.0 mg \cdot kg⁻¹ \cdot h⁻¹ group were within the lethal range described for awake rats (J. F. Pritchard, Ph.D., Glaxo Research Institute, oral communication, October 1991). Rats to which 0.5 and 2.0 mg \cdot kg⁻¹ \cdot h⁻¹ was administered had plasma concentrations within the human therapeutic range.

Discussion

We based our ondansetron infusion rates on pharmacokinetic data from an intravenous bolus in awake rats¹⁰ that indicated a $t_{1/2}$ of 0.2 h and a plasma clearance of 117 ml \cdot min⁻¹ \cdot kg⁻¹. Serum ondansetron concentrations in the range of 1,400 ng/ml have been associated with some behavioral changes in the rat, and the dose lethal to 10% of subjects (LD₁₀) is 4,000–10,000 ng/ml (depending on rat strain; J. F. Pritchard, Ph.D., Glaxo

Research Institute, oral communication, October 1991). We chose a maximal target serum concentration of approximately 80% of the dose causing the aforementioned central nervous system changes in awake animals:

$$0.80 \cdot 1,400 \text{ ng/ml} \cdot 117 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ \approx 7.86 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$$

Given the $t_{1/2}$ of 0.2 h, a constant infusion should produce a serum concentration within 95% of steady state within 0.6 h. Plasma concentrations of ondansetron at the 8.0-mg \cdot kg⁻¹ \cdot h⁻¹ infusion rate were six times higher (7,091 ng/ml *vs.* 1,150 ng/ml) than expected. This result may be caused by the decrease (21%) in total hepatic blood flow reported in spontaneously ventilating (and mildly hypoxic) rats exposed to 1.0 MAC isoflurane¹¹ or by the inhibition of ondansetron metabolism by isoflurane.

Ondansetron is highly selective for the serotonergic subtype 3 receptor with a selectivity ratio of at least 1,000-fold in all systems studied.¹² Subtype 3 receptors exist in the area postrema, the amygdala, the hippocampus, the nucleus tractus solitarius, and other cerebral and peripheral (gut) sites. Roizen *et al.*⁷ previously demonstrated that ablation of the serotonergic neurons in the nucleus raphe dorsalis decreased halothane MAC in rats by 25%. Similar decreases in MAC occur after microdialysis of serotonergic antagonists in the nucleus raphe dorsalis (S. H. Lockhart, Ph.D., M.D., Assistant Professor, University of California, San Francisco, oral communication, 1992). Serotonin_{1A} receptors exist in the nucleus raphe dorsalis,¹³ but no current evidence indicates the existence of subtype 3 at that location.

Rats to which the highest infusion rate of ondansetron with 1.0 MAC isoflurane was administered died, apparently from a drug interaction that led to ventilatory depression and death. Serotonergic neurons modulate the control of breathing in rats.¹⁴ Serotonin_{1A} agonists increase respiratory frequency and amplitude, whereas subtype_{1B} agonists have the opposite effect. However, administered in antiemetic doses (8 or 16 mg intravenously), ondansetron does not alter either the magnitude

of alfentanil-induced respiratory depression or the rate of recovery in volunteers.¹⁵

Several observations are consistent with our finding that ondansetron does not affect MAC. Ondansetron is minimally sedating and does not seem to alter normal awake behavior.¹⁶ In a mouse model of chemically induced pain, antinociceptive effects result from subcutaneous but not intraventricular administration of ondansetron.¹⁷ However, ondansetron has no antinociceptive effect for mechanical or thermal pain, regardless of its mode of administration, nor does it increase the analgesic effect of morphine.¹⁶ Other investigators found that ondansetron did not alter the analgesic potency of alfentanil.¹⁸

In summary, ondansetron is an antiemetic agent that acts by antagonizing 5-HT₃ receptors. Ondansetron plasma concentrations within the human therapeutic range do not alter depth of anesthesia in rats. These data suggest that antiemetic doses of ondansetron administered during anesthesia will affect anesthetic potency little and that 5-HT₃ receptors do not seem to have a significant role in the mechanism of action of isoflurane production of surgical immobility.

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