

## LABORATORY INVESTIGATIONS

Anesthesiology  
70:764-766, 1989

# *Flumazenil Decreases the Minimum Alveolar Concentration of Isoflurane in Dogs*

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Flumazenil is a potent-specific benzodiazepine receptor antagonist that has been shown to reverse CNS depressant effects mediated by benzodiazepine agonists. These agonists are known to affect the interaction of gamma aminobutyric acid (GABA) with its receptor. Because the action of volatile anesthetic agents may be mediated by GABA, the authors determined the MAC of isoflurane in 16 dogs before and after one of three doses of intravenous flumazenil (0.15, 0.3, and 0.45 mg/kg) or the drug vehicle. The flumazenil produced a reduction in MAC from  $1.39 \pm 0.15\%$  (mean  $\pm$  SD) to  $1.23 \pm 0.11\%$  after 0.15 mg/kg ( $P < 0.05$ ), from  $1.50 \pm 0.35\%$  to  $1.08 \pm 0.20\%$  after 0.3 mg/kg ( $P < 0.01$ ), and from  $1.45 \pm 0.14\%$  to  $1.09 \pm 0.08\%$  after 0.45 mg/kg ( $P < 0.01$ ). Administration of drug vehicle produced no change in MAC. This reduction in isoflurane requirement by flumazenil may be due to its benzodiazepine receptor agonist action or its analgesic effect. (Key words: Anesthetics, volatile: isoflurane. Antagonists, benzodiazepine: flumazenil. Potency, anesthetic: MAC.)

BENZODIAZEPINES are often used clinically to enhance the efficacy of inhalational anesthetics. Intravenous diazepam at a dose of 0.2 mg/kg reduces the MAC of halothane in humans by approximately 35%.<sup>1</sup> A similar effect has been reported for midazolam.<sup>2</sup> This reduction in MAC is probably due to benzodiazepine facilitation of gamma aminobutyric acid (GABA) inhibition of synaptic nerve conduction.<sup>3</sup>

The enhancing effect on GABAergic synaptic inhibition is initiated by the interaction of benzodiazepines with membrane proteins to which benzodiazepines bind with high affinity and specificity. This benzodiazepine receptor is thought to be part of a supramolecular assembly that includes the GABA receptor and chloride ionophore.<sup>4</sup> Flumazenil (Ro 15-1788), an imidazobenzodiazepine, is

a potent, selective benzodiazepine receptor antagonist, with limited agonistic activity.<sup>5</sup> To clarify the interaction of the benzodiazepine system and inhalational anesthetics, the effect of flumazenil on the anesthetic action of isoflurane was investigated.

### Methods

This study was approved by the Institutional Committee on Research Involving Animal Subjects. General anesthesia was induced by inhalation of isoflurane and oxygen *via* mask in 16 mongrel dogs (nine male, seven female) weighing 15–19 kg. Tracheal intubation was accomplished with a cuffed 8.0-mm endotracheal tube without the use of muscle relaxants. The dogs' lungs were mechanically ventilated at a rate of 8 breaths/min at a tidal volume adjusted to produce an end-tidal carbon dioxide tension between 30 and 40 mmHg as measured by infrared analysis (Siemens®). Central venous and femoral arterial catheters were inserted and a continuous infusion of lactated Ringer's solution was begun. A heating pad was used to maintain nasal temperature at  $37.0 \pm 0.5^\circ$  C. Temperature, ECG, and arterial blood pressure were continuously recorded (Siemens® 4-channel). Isoflurane concentration was measured by infrared analysis with a Puritan Bennett Anesthetic Agent Monitor 222 (Wilmington, Massachusetts). The analyzer was calibrated for each study according to the manufacturer's specifications. Exhaled gas was obtained from a 5.25-inch 16-g Teflon catheter (Angiocath®) inserted through the endotracheal tube. A 1-m length of narrow-bore nylon tubing connected the catheter to the analyzer.

The MAC of isoflurane necessary to prevent purposeful movement in response to tail clamping was determined according to the method of Eger *et al.*<sup>6</sup> The base of the tail was shaved. After a stable end-tidal isoflurane concentration was maintained for at least 15 min, a large Kocher clamp was applied to the base of the tail and closed to full ratchet lock for 1 min or until purposeful movement was elicited from the dog. Following the determination of the response to the tail-clamp stimulus, the end-tidal isoflurane concentration was adjusted either up or down as appropriate, and the response to stimulation was again determined. Purposeful movement was defined as gross

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Received from the Department of Anesthesiology, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York, and the Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, New York. Accepted for publication December 6, 1988.

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movement of the head or extremities and did not include coughing, chewing, swallowing, or increased respiratory effort. MAC was determined to be that concentration midway between the stable end-tidal concentrations of isoflurane (to the nearest 0.1%) at which point the animal did or did not move in response to the applied stimulus.

Following determination of the control isoflurane MAC, the animals were divided into three groups. Group 1 (n = 5) received flumazenil at a dose of 0.15 mg/kg, group 2 (n = 6) received flumazenil 0.3 mg/kg, and group 3 (n = 5) received the drug vehicle, 4.5 ml/kg, and later flumazenil, 0.45 mg/kg. The flumazenil was injected 5–10 min after completion of the determination of MAC and 15 min prior to the application of the tail clamp for the next determination of MAC. Flumazenil (Hoffmann-La Roche) was given as the aqueous formulation in a 0.1 mg/ml solution. In addition, each milliliter contained 0.1 mg edetate disodium, 0.1 mg glacial acetic acid, and 9.3 mg sodium chloride. The pH was adjusted to 4.0 with either hydrochloric acid or sodium hydroxide. Values for MAC before and after flumazenil and vehicle injection were compared by the two-tailed *t* test for paired data. Comparisons of percent reductions in MAC among groups were made by analysis of variance with Bonferroni correction. *P* < 0.05 was considered statistically significant.

### Results

As shown in Table 1, the MAC of isoflurane decreased from  $1.39 \pm 0.15$  volume percent (mean  $\pm$  SD) to  $1.23 \pm 0.11\%$  after 0.15 mg/kg flumazenil administration (*P* < 0.05). Flumazenil at a dose of 0.30 mg/kg reduced MAC from  $1.50 \pm 0.35\%$  to  $1.08 \pm 0.20\%$  (*P* < 0.01), and the 0.45 mg/kg dose reduced MAC from  $1.45 \pm 0.14\%$  to  $1.09 \pm 0.08\%$  (*P* < 0.01). Administration of the drug vehicle produced no change in the MAC of isoflurane.

The times between determinations of MAC before and after vehicle and flumazenil administration of 0.15, 0.30, and 0.45 mg/kg were  $64 \pm 12$ ,  $74 \pm 25$ ,  $81 \pm 23$ , and  $74 \pm 14$  min, respectively. The percent reductions in MAC after flumazenil 0.15, 0.30, and 0.45 mg/kg were  $11.2 \pm 7.3\%$ ,  $27.2 \pm 10.1\%$ , and  $24.8 \pm 10\%$ , respectively. The percent reduction in MAC after the 0.15 mg/kg dose of flumazenil was less than the percent reduction following the 0.30 mg dose (*P* < 0.05).

### Discussion

Benzodiazepines produce their effect on the CNS by facilitating the interaction of GABA with its receptor.<sup>7,8</sup> In both behavioral and biochemical studies, flumazenil has been shown to antagonize the CNS effects of benzodiazepines. Hunkeler *et al.* demonstrated antagonism of diazepam-induced impairment of mice to climb a wire

TABLE 1. MAC of Isoflurane Before and After Flumazenil

N	Flumazenil (mg/kg)	MAC Before (vol %)	MAC After (vol %)	Time (min)
5	0.15	$1.39 \pm 0.15$	$1.23 \pm 0.11^*$	$74 \pm 25$
6	0.30	$1.50 \pm 0.35$	$1.08 \pm 0.20^\dagger$	$81 \pm 23$
5	0.45	$1.45 \pm 0.14$	$1.09 \pm 0.08^\dagger$	$74 \pm 14$
	Vehicle	$1.45 \pm 0.14$	$1.45 \pm 0.14$	$64 \pm 12$

\* *P* < 0.05 compared with MAC before flumazenil.

† *P* < 0.01 compared with MAC before flumazenil.

following orally administered flumazenil.<sup>4</sup> In addition, flumazenil reversed the anxiolytic effect of diazepam in rats given simultaneously both food and an electric shock in response to pressing a lever. In humans a 2.5 mg iv bolus of flumazenil completely reversed the hypnotic effects of a midazolam infusion.<sup>9</sup>

There is also evidence that flumazenil has some benzodiazepine agonist activity. Robertson and Riives showed that intraperitoneal (ip) flumazenil reduced seizure activity in rats, the brains of which were electrically stimulated.<sup>10</sup> Skerrett and Macdonald demonstrated moderate enhancement of GABA responses in mouse spinal cord treated with flumazenil.<sup>11</sup> In humans flumazenil has been reported to have an intrinsic anticonvulsant effect.<sup>11</sup>

Flumazenil may also produce inverse agonist effects. Corda *et al.* induced seizure activity in rats by administration of subcutaneous isoniazid.<sup>12</sup> The subsequent administration of iv flumazenil increased the number, duration, and severity of convulsant episodes. In social interaction tests, flumazenil has been reported to produce anxiogenic behavior in rats.<sup>13</sup>

Volatile anesthetics, like benzodiazepines, are known to alter CNS activities mediated by GABA. Halothane has been shown to prolong the inhibition of mitral cells in the olfactory cortex induced by GABA.<sup>14</sup> Furthermore, in rat cerebral cortex slices, halothane has been reported to inhibit the metabolism of GABA.<sup>15</sup> Alterations in GABA-modulated effects have also been attributed to chloroform, enflurane, ether, and isoflurane.<sup>16–18</sup>

In addition, flumazenil has been shown to produce analgesia in rats. Walsh *et al.* reported an increased hot plate latency in rats after treatment with ip flumazenil (10–60 mg/kg).<sup>19</sup> Morgan *et al.* showed increased tail-flick latency in flumazenil-treated rats.<sup>20</sup> The analgesic effect was time-dependent and dose-dependent.

Our results contrast with those of Schwiager *et al.*,<sup>21</sup> who reported that flumazenil at the larger doses of 0.5, 1.0, and 1.5 mg/kg did not affect isoflurane MAC.<sup>21</sup> Similarly, Greiner *et al.* reported no effect of intraarterially

¶ Scollo-Lavizzari G: The anticonvulsant effect of the benzodiazepine antagonist, Ro 15-1788: An EEG study in 4 cases. *Eur Neurol* 23:1–6, 1984.

administered flumazenil on the MAC of halothane in rats.<sup>22</sup> Their doses of 0.1 and 1.0 mg/kg would also be expected to produce higher brain concentrations of flumazenil than those achieved in our study. The difference is not surprising in light of evidence that the action of flumazenil as an agonist or inverse agonist is dose-dependent.<sup>23</sup> In humans flumazenil 100 mg po produced benzodiazepine agonist effects, whereas a lower dose (30 mg) produced behavioral effects opposite to those normally associated with benzodiazepine action.<sup>24</sup> Furthermore, in the hot plate test in rats, low doses of flumazenil caused analgesia, whereas high doses did not.<sup>19</sup> Also, Schwieger *et al.*,<sup>21</sup> administered iv succinylcholine to awake dogs prior to induction of anesthesia. Paralysis in conscious dogs would be predictably anxiogenic and may have confounded their results because stress is well known to modulate benzodiazepine receptor function.<sup>25</sup>

Our data indicate that flumazenil potentiates the analgesic effect of isoflurane. The reduction in isoflurane MAC may be due to the analgesic effect of flumazenil or may be due to its partial agonist activity.

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