Anesthesiology
70:644–648, 1989

LABORATORY INVESTIGATIONS

The Effect of Benzodiazepine Receptor Antagonism by Flumazenil on the MAC of Halothane in the Rat

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The effects of a benzodiazepine receptor agonist and an antagonist on the MAC of halothane required to achieve anesthesia were evaluated to explore the possible functional interaction between halothane and the benzodiazepine receptor. Rats were anesthetized with halothane and then administered midazolam (a benzodiazepine agonist) and/or flumazenil (a benzodiazepine antagonist). Flumazenil in doses of 0.1 mg/kg and 1.0 mg/kg was found to have no effect on the MAC of halothane. Midazolam (1.0 mg/kg) lowered the MAC of halothane by 37%. This decrease in MAC was inhibited by coadministration of flumazenil. The absence of an increase in the MAC of halothane in the presence of flumazenil suggests that halothane does not interact with the benzodiazepine receptor, directly or indirectly, to produce its anesthetic action. (Key words: Anesthetics, potency: MAC. Anesthetics, volatile: halothane. Antagonists: benzodiazepine; flumazenil. Hypnotics: benzodiazepine, midazolam.)

BENZODIAZEPINES when used for induction of general anesthesia decrease the MAC of halogenated inhalational anesthetic agents in humans.[1] The site of action of general anesthetics at the molecular level is unknown, although general anesthetics have been shown to interact with water, lipid bilayers, protein, and carbohydrates.[2] It is possible that halothane could cause activation of brain benzodiazepine receptors, either directly by binding to the benzodiazepine receptor, or by indirect mechanisms such as altering the binding or release of endogenous benzodiazepine-like substances.

Benzodiazepine antagonists have recently been developed that reverse the amnestic, sedative, antianxiety, and antiseizure effects of benzodiazepines.[3–9] These antagonist drugs, such as flumazenil, are thought to act competitively at the benzodiazepine receptor. We are unaware of published investigations of the effects of benzodiazepine receptor antagonism on the potency of halogenated inhalational anesthetic agents. The aim of this study was to examine the possibility of reversal of inhalational general anesthesia by benzodiazepine antagonism and to study the interactions among halothane, flumazenil, and the benzodiazepine receptor agonist midazolam.

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Received from the Department of Anesthesia, Pennsylvania State University College of Medicine, Hershey, Pennsylvania. Accepted for publication November 8, 1988. Supported by the Department of Anesthesia, Pennsylvania State University College of Medicine, Hershey, Pennsylvania.

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Materials and Methods

METHODS

Forty-three adult, male Sprague-Dawley rats (Charles River Laboratories, Wellington Massachusetts, weight 245–450 g at time of study) were utilized with approval by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine. All rats received water and rat chow *ad libitum* and were housed in an automatically controlled environment consisting of light from 0700 to 1900 h followed by 12 hours of darkness daily. All rats were anesthetized in a plexiglass induction chamber with halothane 2% in 100% oxygen. Following orotracheal intubation, their lungs were mechanically ventilated with halothane in oxygen using a small animal respirator (Analytical Specialties, Co., St. Louis, Missouri) to maintain an end-tidal CO_2 concentration (FET_{CO_2}) of 5.0 ± 0.5% atmospheric. A mass spectrometric technique was used to monitor FET_{CO_2} and the end-tidal concentration of halothane (FET_H).[10] A carotid artery was cannulated to monitor arterial blood pressure and heart rate *via* a pressure transducer and a Gould® 2800 recorder, and to allow for the measurement of arterial blood gases. Rectal temperature was monitored and servocontrolled with a heating blanket to 37.5 ± 0.5° C. Each change in inspired halothane concentration (FI_H) was followed by an equilibration period of no less than 8 min to ensure less than 0.1% difference between FI_H and FET_H, to approximate a steady-state condition in well-perfused areas such as the brain. Arterial blood gases were obtained to correlate FET_{CO_2} with Pa_{CO_2} and to ensure that no acid–base or oxygenation abnormalities were present. *p*H ranged from 7.34 to 7.50 (mean of 7.44 ± 0.01), and Pa_{O_2} values were in all instances greater than 100 mmHg (mean Pa_{O_2} of 456 ± 13 mmHg).

MAC was determined by measuring the FET_H at the time of application of a standardized rubber shod tubing clamp to the base of the tail.[10] A positive response was the movement of head, leg, or foot during a 60-s period of clamping. The FI_H was varied to produce changes in

FET_H of 0.1% or less, and the highest FET_H at which the rat responded was determined. The lowest FET_H at which the rat did not respond to tail clamp was also determined, and the MAC of halothane for that rat was taken as the average of those two values.[11]

Study 1 Protocol

This portion of the study was designed to determine the flumazenil dose that would be tolerated in the rats. A group of ten rats was anesthetized and the MAC of halothane was determined for each rat. Each rat was given flumazenil 0.1 mg/kg intraarterially into the aortic arch *via* the carotid artery cannula. Intraarterial injection was selected to minimize the possible cardiac effects of a bolus of drug and to decrease any possible first-pass effect through the lungs, although in preliminary experiments (unpublished data) we found no difference between use of iv *versus* intraarterial flumazenil or midazolam on MAC of halothane or cardiovascular variables. Thirty minutes later, the MAC of halothane was determined. Each rat then received flumazenil 1.0 mg/kg intraarterially followed by a MAC determination 30 min later.

Study 2 Protocol

This study involved the administration of both benzodiazepine agonist and benzodiazepine antagonist in varying orders. Four groups of rats were utilized:

Halothane only control group (group I). Six rats were anesthetized with halothane only, and the MAC of halothane was determined at 0, 30, and 60 min to determine whether the MAC of halothane changed over the study period.

Halothane–midazolam control group (group II). Six rats were anesthetized, and after the MAC of halothane was determined, 1.0 mg/kg midazolam was given intraarterially. MAC was determined at 30 and 60 min after midazolam administration to determine the duration of midazolam's effect on halothane MAC.

Flumazenil–midazolam group (group III). Eleven rats were anesthetized, and after the initial MAC of halothane was determined, 1.0 mg/kg midazolam was given intraarterially. MAC was measured again at 30 min. Next, 1.0 mg/kg flumazenil was administered intraarterially, and a final MAC measurement was made 30 min later to examine the ability of flumazenil to antagonize the midazolam effect.

Flumazenil–midazolam group (group IV). Ten rats were anesthetized, and after measuring the MAC of halothane, they received 1.0 mg/kg flumazenil followed by a MAC determination 30 minutes later to evaluate any possible flumazenil–halothane interaction. Next, 1.0 mg/kg of midazolam was administered and a final MAC determination was made 30 min later to document the efficacy of flumazenil in blocking benzodiazepine receptors.

TABLE 1. Effect of Flumazenil on the Anesthetic Potency of Halothane: Study 1 (n = 10)

Time (min)	Drug	Halothane MAC* (%)
0	Halothane	0.98 ± 0.03
30	Flumazenil 0.1 mg/kg	0.98 ± 0.04
60	Flumazenil 1.0 mg/kg	0.96 ± 0.04

All data are presented as mean ± SEM.
* MAC (% end-tidal halothane concentration).

Materials

Midazolam maleate and flumazenil carboxylate (RO15-1788, ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-A] [1,4] benzodiazepine-3-carboxylate) were kindly provided as a gift by Hoffman-La Roche, Nutley, New Jersey. All drugs were diluted to a total volume of 1.0 ml using 0.9% NaCl, and were administered intraarterially *via* the carotid artery catheter into the aortic arch over 30 s.

Statistics

All data are presented as mean ± SEM. Statistical analysis included one- and two-sided paired *t* test within groups and one-way analysis of variance among groups (for initial halothane MAC). Statistical significance was defined at $P < 0.05$. The statistical power of these tests was calculated and found in all cases to be greater than 90% based on a minimum detectable difference of 0.1% MAC halothane.

Results

The mean MAC of halothane for the entire group of 43 rats studied was 0.91 ± 0.02%. Study 1 (table 1) shows in rats receiving halothane followed by flumazenil that neither 0.1 mg/kg nor 1.0 mg/kg flumazenil produced a statistically significant difference in halothane MAC from baseline ($P > 0.99$ and 0.39, respectively). Study 2 (table 2) involved examination of the MAC interaction among halothane, flumazenil, and midazolam.

Group I

Halothane MAC did not change significantly during the study period ($P = 0.084$ for 0 and 30 min, $P = 0.44$ for 0 and 60 min).

Group II

Rats receiving only midazolam during halothane anesthesia showed a 37% decrease in halothane MAC at 30 min and a 33% reduction, which persisted at 60 min post-midazolam administration. This difference between MAC of halothane and MAC of halothane plus midazolam was statistically significant ($P < 0.001$). There was no statistical

TABLE 2. Effects of Flumazenil and Midazolam, Alone and in Combination, on Anesthetic Potency of Halothane: Study 2

Group	Time (min)	Drug	Halothane MAC* (%)
Group I (n = 6)	0	Halothane	0.91 ± 0.03
	30	Halothane	0.93 ± 0.03
	60	Halothane	0.92 ± 0.03
Group II (n = 6)	0	Halothane	0.92 ± 0.03
	30	Midazolam 1 mg/kg	0.58 ± 0.03†
	60	No additional drug	0.62 ± 0.04†
Group III (n = 11)	0	Halothane	0.90 ± 0.02
	30	Midazolam 1 mg/kg	0.55 ± 0.02†
	60	Flumazenil 1 mg/kg	0.87 ± 0.02
Group IV (n = 9)	0	Halothane	0.89 ± 0.04
	30	Flumazenil 1 mg/kg	0.90 ± 0.05
	60	Midazolam 1 mg/kg	0.88 ± 0.04

All data are presented as mean ± SEM.
* MAC (% end-tidal halothane concentration).
† $P < 0.001$ compared with time zero.

difference between MAC values at 30 and 60 min post-midazolam ($P = 0.16$).

GROUP III

Midazolam caused a statistically significant 39% decrease in halothane MAC ($P < 0.001$). The addition of flumazenil reversed the effects of midazolam, causing an increase in MAC toward baseline that was statistically significant ($P < 0.001$). Although the postflumazenil MAC was statistically different from the initial halothane measurement (initial MAC 0.90 ± 0.02%, MAC after midazolam and flumazenil 0.87 ± 0.02%; $P = 0.022$), this difference (0.03%) was below the resolution of our measurement technique.

GROUP IV

We were unable to detect an effect of flumazenil on the MAC of halothane. Rats that received flumazenil 30 min prior to midazolam administration showed no statistically significant differences in MAC, either comparing halothane alone with halothane plus flumazenil ($P = 0.87$) or comparing halothane alone to the addition of midazolam in the presence of flumazenil ($P = 0.17$). One rat in this group was eliminated from statistical analysis due to a marked sensitivity to halothane anesthesia at all times as compared with the other rats studied. The halothane MAC values of the rat were 0.48%, 0.53%, and 0.48% at 0, 30, and 60 min, respectively. Cardiovascular and metabolic variables did not vary significantly from the remainder of the group. One-way analysis of variance performed on the initial MAC determinations of halothane for the four groups of rats showed no statistical difference among groups ($\alpha = 0.05$). Midazolam or flumazenil, alone or in combination, did not significantly affect either mean arterial pressure (MAP) or heart rate (HR) for any group ($P > 0.05$, table 3).

Discussion

This study demonstrates that the benzodiazepine antagonist flumazenil in doses of 0.1 mg/kg and 1.0 mg/kg has no effect on the MAC of halothane in the rat. Flumazenil can completely block the MAC-lowering effect of midazolam. Thus, flumazenil was still exerting a pharmacologic antagonism at benzodiazepine receptors at 30 and 60 min. Our inability to detect an effect of flumazenil to increase halothane MAC was not due to inadequate flumazenil concentration at the receptors.

We observed a 37% decrease in MAC halothane after the administration of 1 mg/kg midazolam. Melvin *et al.* found that midazolam decreases halothane MAC in a dose-dependent manner over the studied dose range of 0–0.60 mg/kg in healthy patients. Their maximum decrease in halothane MAC was 30% at the highest midazolam dose administered.[1] Hall *et al.* have also shown that midazolam produces a dose-dependent decrease in enflurane MAC in the dog.[12]

The benzodiazepine receptor in the CNS is thought to be part of a protein complex containing receptor sites for the inhibitory neurotransmitter gamma-amino butyric acid (GABA), barbiturates, and benzodiazepines, and an associated chloride ion channel. GABA acts at this complex to increase chloride conductance.[13,14] Both barbiturates and benzodiazepine agonists act to potentiate this

TABLE 3. Hemodynamic Variables during Administration of Halothane, Flumazenil, and Midazolam

Group	Initial		30 min		60 Min	
	MAP (mmHg)	HR (beats/min)	MAP (mmHg)	HR (beats/min)	MAP (mmHg)	HR (beats/min)
I	133 ± 15	350 ± 20	119 ± 16	342 ± 35	123 ± 16	390 ± 33
II	135 ± 27	327 ± 26	140 ± 26	348 ± 24	147 ± 31	350 ± 19
III	124 ± 10	336 ± 18	139 ± 12	376 ± 21	118 ± 10	353 ± 20
IV	128 ± 16	330 ± 20	128 ± 18	352 ± 22	131 ± 22	328 ± 33

All data are presented as mean ± SEM. Measurements were recorded during the MAC determinations corresponding to times specified. No within group statistical comparison reached statistical significance ($P > 0.15$ in all cases).

GABA-induced increase in chloride conductance. Kissin *et al.*[15] and Tverskoy *et al.*[16] have shown that barbiturates may affect the benzodiazepine receptor by increasing the binding of benzodiazepines to the benzodiazepine receptor. This results in a supraadditive effect when barbiturates and benzodiazepines are combined as anesthetics. In our studies, flumazenil had no effect on the MAC of halothane. Although the site and mechanism of action of the inhalational general anesthetics in the CNS are not known, it has been suggested by Cheng and Brunner[17,18] that halothane anesthesia may be associated with an inhibition of GABA catabolism leading to increased GABA levels.

If flumazenil had caused an increase in the MAC of halothane (indicating partial reversal of anesthesia), it might have suggested that halothane directly or indirectly activated the benzodiazepine receptor complex. The results of our study suggest that halothane does not bind at the benzodiazepine receptor, nor does it alter the release or binding of an endogenous benzodiazepine receptor ligand. The possibility that halothane may increase chloride conductance by other mechanisms at a postreceptor level has not been explored by our studies.

We are aware of only one study examining the interaction between halothane and flumazenil. Geller *et al.*[19] published in summary form a study of patients who received flumazenil and halothane anesthesia. They found that patients receiving flumazenil had a significant improvement in memory during the first postoperative hour, a faster decline in the incidence of deep tendon clonus, and faster neurologic recovery compared with that of controls. It was not stated what time course or dose of flumazenil was utilized or whether additional drugs, such as benzodiazepines or narcotics, had been administered.

We observed no hemodynamic instability when administering either midazolam or flumazenil (table 3). Geller *et al.* did not observe significant hemodynamic changes by flumazenil in patients receiving or not receiving benzodiazepines.[6,20] Fleischer *et al.* found a decrease in MAP requiring iv fluids and a low-dose phenylephrine infusion after administering iv flumazenil to dogs receiving midazolam during spinal anesthesia.[21] Our rats' lack of hemodynamic instability could be due to the flumazenil being given intraarterially, which would avoid a large bolus to the heart such as could occur with an iv injection. We also did not utilize spinal anesthesia, which may blunt compensatory sympathetic responses. Reves *et al.*[22] and Samuelson *et al.*[23] studied the hemodynamic effects of midazolam as an induction anesthetic of patients with ischemic heart disease and found only modest effects on hemodynamic variables. Our data in healthy rats concur with their findings.

This study was limited by the fact that all animals were anesthetized with halothane prior to receiving flumazenil. It is possible, although not likely, that administering flumazenil prior to halothane may have produced an inhibitory interaction. Also, our tail clamp method, although reproducible, may not be able to detect small changes in MAC (<0.1%). However, it is questionable whether MAC changes smaller than 0.1% would have clinical significance. It is unlikely that our use of one carotid artery for drug administration and monitoring affected our results for several reasons. The rat brain has excellent collateral blood supply, making ischemia difficult to produce; other authors have determined similar halothane MAC of rats without carotid artery cannulation.[10,11,24]

In conclusion, we found no effect of flumazenil in doses of up to 1 mg/kg on the MAC of halothane, suggesting that halothane's anesthetic activity does not involve the benzodiazepine receptor. Flumazenil, in the dose used, could completely block the MAC-lowering effect of midazolam. This effect occurred whether flumazenil was given prior to or after midazolam.

The authors thank Anne M. Semanchick, B.S., for technical assistance, H. Gregg Schuler, B.A., for research assistance, and Hoffman-LaRoche, Nutley, New Jersey, for supplying midazolam and flumazenil.

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