FLUMAZENIL DOES NOT ANTAGONIZE HALOTHANE, THIAMYLAL OR PROPOFOL ANAESTHESIA IN RATS†

T. MURAYAMA, K. SHINGU, T. OGAWA, K. TOMODA, K. SHINDO, S. TAMAI AND K. MORI

SUMMARY

We have studied the effects of flumazenil on sleep time and EEG in rats anaesthetized with 1.5% halothane, propofol 20 mg kg⁻¹, thiamylal 30 mg kg⁻¹, or combinations of diazepam 5 mg kg⁻¹ and anaesthetic agents. We also studied the effects of flumazenil 0.3, 3 and 30 mg kg⁻¹ on behaviour and EEG. Flumazenil 0.3 and 3 mg kg⁻¹ alone had no effect on behaviour or EEG, but flumazenil 30 mg kg⁻¹ had depressive effects similar to those of diazepam on behaviour and EEG. Flumazenil 0.3, 3 and 30 mg kg⁻¹ i.v. antagonized the effects of diazepam 10 mg kg⁻¹ i.v. on behaviour and EEG. Flumazenil had no antagonistic effect on sleep time induced by anaesthetic agents, but flumazenil 30 mg kg⁻¹ potentiated propofol-induced anaesthesia. Flumazenil did not affect anaesthesia-induced EEG changes. Diazepam 5 mg kg⁻¹ potentiated anaesthesia. Flumazenil antagonized the effects of diazepam potentiating varied with anaesthetic agent: flumazenil 0.3 mg kg⁻¹ potentiated diazepam action in halothane anaesthesia, but 30 mg kg⁻¹ was required in propofol anaesthesia; this large dose was insufficient in thiamylal anaesthesia.

KEY WORDS


Benzodiazepines are used often in association with general anaesthesia. The benzodiazepine receptor is part of the GABAₐ receptor, which includes the GABA-binding sites, barbiturate binding sites and Cl⁻ channels [1,2]. Benzodiazepines and barbiturates increase GABA-induced Cl⁻ conductance.

In this study, we have investigated the role of the benzodiazepine receptor in general anaesthesia by examining the effects of flumazenil on sleep time and EEG in rats anaesthetized with halothane, thiamylal and propofol; the interactions of diazepam and anaesthetics; the effects of flumazenil on these interactions; and any agonistic or inverse agonistic effects of flumazenil on behaviour and EEG.

MATERIALS AND METHODS

The study was approved by our Institutional Animal Care Committee. We studied Wistar male rats (200-280 g); standard rat food and water were provided ad libitum up to the time of the experiment. The animals were kept under standard conditions of temperature (22 ± 2 °C) and relative humidity, in a non-reversed light–darkness cycle of 12–12 h. Each animal was anaesthetized with halothane and a cannula (0.8 mm o.d.) filled with normal saline was placed in the femoral vein for administration of drugs. Rats were subjected to the studies at least 1 h later.

Animals were allocated to nine groups of 40 rats each, according to agent. Each group consisted of four subgroups of 10 rats, according to the dose of flumazenil: control rats (flumazenil only) or flumazenil 0 (solvent), 0.3, 3 and 30 mg kg⁻¹.

Three different concentrations of flumazenil (6, 3 and 0.3 mg ml⁻¹) were prepared by dissolving the powdered drug with equal volumes of propylene glycol and polyethylene glycol 400. The 6-mg ml⁻¹ solution was used for administering 30 mg kg⁻¹ and the other solutions for 3 and 0.3 mg kg⁻¹. The volumes administered were 5 ml kg⁻¹ at a dose of 30 mg kg⁻¹ and 1 ml kg⁻¹ at 3 and 0.3 mg kg⁻¹. The effects of solvent were studied with different volumes (1 and 5 ml kg⁻¹) in five rats each. Thiamylal was dissolved with distilled water to concentrations of 30 and 27 mg ml⁻¹. Concentrations of diazepam and propofol solutions were 5 and 10 mg ml⁻¹, respectively.


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Doses of anaesthetic agents were selected during a pilot study, to produce sleep of a similar duration (10–12 min): propofol 20 mg kg\(^{-1}\) i.v. ; diazepam 10 mg kg\(^{-1}\) i.v.; 3 % halothane in oxygen in a plastic box for the initial 5 min and then 1.5 % with a face mask for the succeeding 15 min. Thiamylal 30 mg kg\(^{-1}\) was used in the initial study, but produced a longer period of sleep compared with other agents; therefore we added a thiamylal 27 mg kg\(^{-1}\) group.

Flumazenil was administered 5 min after administration of the i.v. agents or 5 min before discontinuation of halothane. Effects of flumazenil on sleep time and behaviour were investigated during anaesthesia with anaesthetic agents alone and with combinations of anaesthetic agents and diazepam 5 mg kg\(^{-1}\) (table I). The sleep time was measured as the interval from the time of i.v. administration of drug or discontinuation of halothane exposure to the time when the rat lifted its head. Rectal temperature was monitored and maintained at 36.5—38.5 °C using radiant heat.

Flumazenil-induced EEG changes were studied in two rats in each group. The animals were anaesthetized with halothane and three stainless steel screws, 1 mm diameter, were placed in the skull: one for grounding and the other two for EEG electrodes. The screws were soldered to a small socket, which was fixed to the skull with dental cement. After 3 days recovery, the rates were subjected to the drug study. The EEG was recorded on a polygraph (Sanei, Tokyo) at a paper speed of 10 mm s\(^{-1}\).

### Statistics

The effect of flumazenil on sleep time was analysed using one-way analysis of variance (ANOVA) and the Newman–Keuls test. \(P < 0.05\) was considered significant. Values are expressed as mean (SD).
EFFECT OF FLUMAZENIL DURING ANAESTHESIA

Table II. Effects of flumazenil on mean (SD) sleep time in rats anaesthetized with diazepam or anaesthetic agents and their combinations (n = 10 each value) *P < 0.001 vs solvent

<table>
<thead>
<tr>
<th>Sleep time (s)</th>
<th>Solvent</th>
<th>Flumazenil 0.3 mg kg⁻¹</th>
<th>Flumazenil 3 mg kg⁻¹</th>
<th>Flumazenil 30 mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam 10 mg kg⁻¹</td>
<td>784 (140)</td>
<td>323 (7)*</td>
<td>318 (3)*</td>
<td>316 (3)*</td>
</tr>
<tr>
<td>Halothane + diazepam 5 mg kg⁻¹</td>
<td>604 (242)</td>
<td>746 (331)</td>
<td>625 (198)</td>
<td>647 (164)</td>
</tr>
<tr>
<td>Thiamylal 30 mg kg⁻¹ + diazepam 5 mg kg⁻¹</td>
<td>2748 (316)</td>
<td>839 (316)*</td>
<td>621 (166)*</td>
<td>828 (124)*</td>
</tr>
<tr>
<td>Thiamylal 27 mg kg⁻¹ + diazepam 5 mg kg⁻¹</td>
<td>1522 (449)</td>
<td>1833 (759)</td>
<td>1633 (643)</td>
<td>1868 (220)</td>
</tr>
<tr>
<td>Propofol 20 mg kg⁻¹ + diazepam 5 mg kg⁻¹</td>
<td>6138 (873)</td>
<td>4397 (874)*</td>
<td>2804 (1099)*</td>
<td>2935 (538)*</td>
</tr>
<tr>
<td>Control</td>
<td>691 (53)</td>
<td>651 (55)</td>
<td>660 (51)</td>
<td>1036 (140)*</td>
</tr>
<tr>
<td>Flumazenil 30 mg kg⁻¹</td>
<td>2893 (205)</td>
<td>1405 (452)*</td>
<td>1011 (195)*</td>
<td>782 (58)*</td>
</tr>
</tbody>
</table>

**Control**

![EEG waveform](https://academic.oup.com/bja/article-abstract/69/1/61/243939)

**Flumazenil 30 mg kg⁻¹**

**Fig. 1. Effect of flumazenil 30 mg kg⁻¹ on EEG in the rat. Flumazenil increased amplitude and decreased frequency of EEG activities.**

produced rhythmic 5–7 Hz, 80–100 μV waves during injection, followed by an almost flat EEG with occasional 4–6 Hz, 10–20 μV waves. Successive changes in EEG were: development of sharp waves of 60–120 μV, increased incidence of sharp waves, burst suppression and high amplitude (80–120 μV) 8–12 Hz waves. Flumazenil had no effect on EEG changes induced by propofol. The addition of diazepam 5 mg kg⁻¹ shifted the EEG changes of thiamylal and propofol to those associated with deeper stages of anaesthesia. High amplitude, slow wave EEG changed to burst suppression and occasional sharp waves with thiamylal, and to almost isoelectric waves with diazepam. With propofol, diazepam decreased the incidence of sharp waves. These shifts induced by diazepam were antagonized by flumazenil.

**DISCUSSION**

At a dose of 0.3 mg kg⁻¹, flumazenil antagonized completely diazepam-induced behavioural and EEG changes in rats. In contrast with its action on diazepam-induced changes, flumazenil had no antagonistic effect on the behavioural and EEG changes induced by halothane, thiamylal or propofol.

The interactions of flumazenil and anaesthetics observed in the present study reflect both pharmacodynamic and pharmacokinetic factors. Measurements of blood concentrations of anaesthetics are required to assess pharmacokinetic factors in the antagonistic actions of flumazenil on sleep time and EEG. Flumazenil has a short duration of action, which may explain the failure of flumazenil to antagonize general anaesthesia. However, flumazenil 30 mg kg⁻¹ (100 times greater than that which antagonized the effects of diazepam) did not antagonize general anaesthesia.

Conflicting data have been reported on the interaction of flumazenil with anaesthetics. Acceleration of recovery from halothane anaesthesia has been reported in both mice [13] and humans [14], but no effects were confirmed on the MAC of halothane [13, 15], enflurane [16] or isoflurane [16]. The sleep time of methoxyflurane was not affected by flumazenil [17]. Spinal cord and brain electrical activity studies have demonstrated absence of flumazenil interaction with pentobarbitone [18], Roald, Forsman and Steen [19] reported that flumazenil reversed partially the isoflurane-induced depression of cerebral oxygen consumption and EEG changes, while Schwartz and colleagues [20] reported an opposite view, that flumazenil decreased the MAC of isoflurane in dogs. The present results confirm that flumazenil does not antagonize halothane, thiamylal or propofol anaesthesia. These results indicate that general anaesthesia with these agents is not mediated through benzodiazepine receptors. Barbiturates have been reported to increase the affinity of these receptors for benzodiazepines [1, 21, 22]. However, no endogenous benzodiazepine-like substance has been detected, and an antagonistic effect of flumazenil in thiamylal anaesthesia was absent in this study. These findings suggest that the contribution of the benzodiazepine receptor may be insignificant in barbiturate anaesthesia if benzodiazepines are not co-administered.

Diazepam prolonged the sleep time of thiamylal, propofol and halothane to a greater extent than expected by a simple summation of the sleep time of diazepam and those of the individual anaesthetics. The prolongation of thiamylal sleep time was greater than those of halothane and propofol. The smallest dose of flumazenil, 0.3 ml kg⁻¹, antagonized diazepam during emergence from halothane anaesthesia in a manner similar to that when diazepam was administered alone. This result probably indicates that diazepam was simply additive to the action of halothane.

The flumazenil-induced antagonism of diazepam was, however, modified by thiamylal and propofol, and a greater dose was required to antagonize the drug. In the case of thiamylal, a dose of 30 mg kg⁻¹ (100 times that required to antagonize anaesthetized rats) was not sufficient. The same dose of flumazenil was required in the case of propofol and the antagonism showed a dose-related response manner. Barbiturates bind to barbiturate receptors, which are part of a supramolecular structure that...
include benzodiazepine receptor, GABA-binding sites and Cl⁻ channels [1,2]. Barbiturates increase the affinity of the GABA-binding site for GABA, directly open Cl⁻ channels (at a high dose), and increase the duration of the open state of Cl⁻ channels [23]. Benzodiazepines increase the frequency of opening of Cl⁻ channels by increasing the affinity of GABA-binding sites for GABA [24]. The combination of barbiturates and benzodiazepines increases both the frequency and the duration of open states of Cl⁻ channels, increases Cl⁻ influx and depresses excitabilities of neural membrane. Barbiturates affect the function of the benzodiazepine receptor and facilitate the affinity of benzodiazepines for their receptors [1,21,22]. The present study indicated that thiaymidyl modified the interaction of flumazenil and diazepam. The modification may be exerted, at least partly, through their actions on the GABA–benzodiazepine receptor–Cl⁻ channel complex. The mechanism of modification is beyond the scope of the present study.

An interesting finding of this study was the interaction of flumazenil with propofol. Flumazenil 0.3 and 3 mg kg⁻¹ had no effect on the action of propofol, but 30 mg kg⁻¹ prolonged sleep time. Furthermore, although the prolongation of propofol sleep time by diazepam was similar to that by halothane, it required a greater dose of flumazenil to antagonize diazepam in the case of propofol compared with halothane. All these findings combined together indicate a possibility that a large dose of propofol activates, at least in part, the benzodiazepine receptor.

An agonist-like effect of flumazenil was reported previously in animal behaviour [11] and GABA-induced Cl⁻ conductance [12]. A similar agonist-like action was noted also in the present study: a large dose of flumazenil (30 mg kg⁻¹) prolonged the sleep time of propofol, slowed the EEG activity and increased sleep time by diazepam was similar to that by halothane. All these findings combined together indicate a possibility that a large dose of propofol activates, at least in part, the benzodiazepine receptor.

Frequently, intraoperative supplementation with diazepam prolongs recovery from anaesthesia. The present study has shown that the antagonizing effects of flumazenil on the potentiating action of anaesthetics by diazepam were modified, depending on the anaesthetic agent used. Flumazenil did not always reverse the effects of diazepam.

REFERENCES