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Effects of Two Benzodiazepine Inverse Agonists, RO 15-4513 and FG 7142, on Recovery From Pentobarbital and Halothane Anesthesia in the Rat

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WEINGER, M. B., J. F. SCHREIBER AND G. F. KOOB. *Effects of two benzodiazepine inverse agonists, RO 15-4513 and FG 7142, on recovery from pentobarbital and halothane anesthesia in the rat.* PHARMACOL BIOCHEM BEHAV **35**(4) 889–895, 1990.—A new class of drugs, the benzodiazepine inverse agonists, have recently been shown to antagonize some of the behavioral and sedative effects of benzodiazepines, barbiturates, and alcohol. Preliminary studies suggested that at least one of these drugs, RO 15-4513, may also be able to reverse the general anesthetic properties of volatile halogenated agents. Another inverse agonist, FG 7142, exhibits a similar ability to antagonize alcohol or benzodiazepines. However, FG 7142 is less potent than RO 15-4513 and has less affinity for the benzodiazepine receptor (BZR). The present studies were therefore undertaken to compare the analeptic effects and relative potencies of RO 15-4513 and FG 7142 on the anesthetic properties of pentobarbital compared with the general anesthetic agent halothane as measured by the time for recovery of the righting reflex in the rat. Three basic experimental paradigms were employed. Drug (FG or RO) or carrier was administered 5 minutes prior to the induction of pentobarbital anesthesia. Drug or carrier was administered to anesthetized animals 60 minutes after pentobarbital injection. Lastly, drug or carrier was administered 5 minutes prior to 15 minutes of halothane anesthesia. In addition, the selective benzodiazepine antagonist, flumazenil (RO 15-1788), was used to determine if the effects of the benzodiazepine inverse agonists on recovery from barbiturate or halothane anesthesia were due to activity at the BZR. The results revealed that RO was both more potent and more effective than FG at speeding recovery from barbiturate anesthesia in the rat. RO's effects appeared to be primarily due to BZR inverse agonist activity since it could be reversed by the BZR antagonist, flumazenil. In contrast, FG appeared to be less potent but much more effective than RO with respect to reversal of halothane anesthesia. FG's effect could not be antagonized with flumazenil, suggesting a non-BZR-mediated analeptic effect. The results of the present study suggest that compounds such as RO 15-4513 or FG 7142 might prove to be clinically useful for antagonizing barbiturate or volatile anesthetic overdosage.

Benzodiazepine inverse agonist RO 15-4513 FG 7142 Flumazenil Barbiturate Pentobarbital Halothane Volatile anesthesia Righting reflex

A new class of drugs, the benzodiazepine inverse agonists, has recently been the subject of intense interest because of their apparent ability to antagonize some of the behavioral and sedative effects of alcohol (1, 6, 8, 11, 24, 27, 33, 38). These inverse agonists bind to the benzodiazepine receptor (BZR) but produce the physiological opposite effects of benzodiazepine agonists like diazepam (7). These drugs have also been shown in some experimental paradigms to reverse the CNS depressant effects of benzodiazepine agonists (8, 16, 23, 32) and barbiturates (6, 8, 19, 25).

Preliminary studies using rats (40) and recent reports of work with mice (28) and tadpoles (12) suggest that, at least one of these inverse agonist drugs, the imidazobenzodiazepine RO 15-4513, may be able to antagonize the general anesthetic properties of volatile halogenated agents. Another benzodiazepine inverse agonist, FG 7142, appears to exhibit a similar ability to antagonize alcohol or benzodiazepine agonists, but may be less potent than RO 15-4513 (18, 21, 23, 24). This is consistent with the finding that FG 7142 has approximately 1/100th the affinity of RO 15-4513 for the benzodiazepine receptor in vitro (36).

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There may also be functional differences between the two drugs independent of their different binding affinity for the BZR (13,38). In one study, FG 7142, like RO 15-4513, reversed ethanol-induced depression of locomotor activity in rats, but only RO 15-4513 antagonized ethanol-mediated depression of gamma motor neuron activity in cats (31). These apparent differences in pharmacological function may be partially due to differences in species or behavioral/experimental paradigms. Pharmacokinetic or pharmacodynamic differences between the drugs in individual species could also play a role. These factors have suggested that more work is necessary to assess the relative effectiveness of RO 15-4513 and FG 7142 in antagonizing clinically-relevant sedative or anesthetic properties of selected CNS depressants.

It is hypothesized that benzodiazepines and barbiturates produce their sedative and hypnotic effects by acting on adjacent components of the same membrane ionophore complex (34). Benzodiazepines are thought to bind to a receptor linked to this complex. Current theories of the mechanism of action of volatile anesthetic agents are based on a generalized membrane disordering effect rather than a receptor-mediated process (22). However, more recent evidence suggests that general anesthetics, at clinically relevant concentrations, may act specifically in the region of the GABA receptor-chloride channel complex to produce some of their CNS depressant effects (20). Therefore, if the effects of different benzodiazepine inverse agonists on volatile anesthetic action are solely due to receptor-mediated alterations of the GABA receptor-chloride channel complex, some support would be provided to this new model of the mechanism of general anesthesia. In addition, differences in effectiveness of different benzodiazepine inverse agonists independent of benzodiazepine receptor affinity could have important implications for the development of a new class of clinically useful general anesthesia antagonists.

The present study was undertaken to compare the analeptic effects and relative potencies of RO 15-4513 and FG 7142 on the anesthetic properties of pentobarbital compared with the general anesthetic agent halothane in one behavioral test (recovery of the righting reflex) in the rat.

METHOD

Animals

In the present experiments, 339 male Wistar rats (200–360 g, Charles River Laboratories, Wilmington, DE) were studied. All work was performed with the approval of our institutional animal care committee and in compliance with the guidelines established by the National Institutes of Health. In order to conserve animal resources, each rat was used in two independent experiments 7–10 days apart. Animals were either rerandomized to new treatment groups between the two independent studies or, in cases where the same animals were used for the same experiment on successive weeks, a cross-over design was employed.

Drugs

RO 15-4513 (RO) (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5a][1,4] benzodiazepine-3-carboxylate, Hoffmann-La Roche, Basel, Switzerland), flumazenil (RO 15-1788, FLU) (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5a][1,4] benzodiazepine-3-carboxylate), Hoffmann-La Roche Laboratories, Nutley, NJ and FG 7142 (FG) (N-methyl-beta-carboline-3-carboxamide, RBI, Natick, MA) were emulsified and partially dissolved by sonication in a carrier solution of 95% saline, 2.5% alcohol and 2.5% emulphor (23). Both RO and FG were administered intraperitoneally in a dose range (0–15 mg/kg) previously shown to reverse the behavioral and sedative effects of benzodiazepines, barbiturates, and ethanol (1, 5, 8, 11, 16, 19, 23, 25, 27, 32, 37). Because of limited solubility of both benzodiazepine inverse agonists, the concentration of the injected drug solution was kept constant (3 mg/ml) for doses greater than 3 mg/kg, resulting in a larger volume of drug injected intraperitoneally (up to 5 ml/kg maximum). However, since total intraperitoneal injection volume rarely exceeded 1 ml, it is unlikely that the difference in injected volumes resulted in altered absorption of the drug at the higher doses. Because it is significantly less hydrophilic, the highest dose of FG 7142 (15 mg/kg) had to be administered as a suspension in a 5 ml/kg volume of a modified carrier of 85% saline, 5% alcohol, and 5% emulphor.

In all experiments, animals were randomly assigned to receive either drug (RO or FG) or carrier (1–3 ml/kg) treatment as described below. Within each experiment, rats receiving drug treatments were always paired with control rats that had come from the same vendor shipment and had therefore been exposed to precisely the same environmental conditions prior to and throughout the study. Experiments were performed sequentially and drug pretreatment doses were based on optimal responses from previous experiments.

Anesthesia

Halothane (Halocarbon Laboratories, Hackensack, NJ) was administered in 100% medical grade oxygen using an 800 ml flow through a temperature- and flow-compensated Fluotec™ vaporizer delivered to a snug-fitting nose cone. The nose cone was held loosely on the animal's face as necessary until anesthesia was induced. A low-flow vacuum connected to the nose cone insured continuous fresh gas flow. In other experiments, rats breathing room air were injected with pentobarbital (Abbott Laboratories, North Chicago, IL) intraperitoneally (20–40 mg/kg of a 50 mg/ml premixed solution) either before or after the study drug injection.

Righting Reflex

The righting reflex was assessed by placing the animal on his back and measuring the time required to regain a fully upright posture (all four limbs in contact with the ground). Observers timing the righting reflex were always blinded as to treatment group. The time to the recovery of the righting reflex was measured in minutes. In experiments where time to the loss of the righting reflex was assessed, the animal was repeatly placed on his back every 10–15 seconds until loss of righting occurred. A righting reflex was considered absent if the animal did not right itself within 10 sec. Upon initial evidence of lightening of the anesthesia, the experimenter briskly clapped to assess the level of arousal of the animal and stimulate the animal to right itself.

Experimental Design—Phase I

In the first phase of the study, 3 experiments were performed: drug (FG or RO) or carrier administered 5 minutes prior to the induction of pentobarbital anesthesia (Experiments 1 and 3); drug or carrier administered to anesthetized animals 60 minutes after pentobarbital injection (Experiment 2); and drug or carrier administered 5 minutes prior to 15 minutes of halothane anesthesia (Experiment 4).

In the first experiment, animals ($n = 134$) were randomly assigned to receive single intraperitoneal pretreatment doses of either carrier (1 or 3 ml/kg) or drug (either RO (0–9 mg/kg) or FG 7142 (0–15 mg/kg). Five minutes later all animals were given an injection of pentobarbital (40 mg/kg IP). The occurrence of the loss of the righting reflex, the time to the loss of the righting

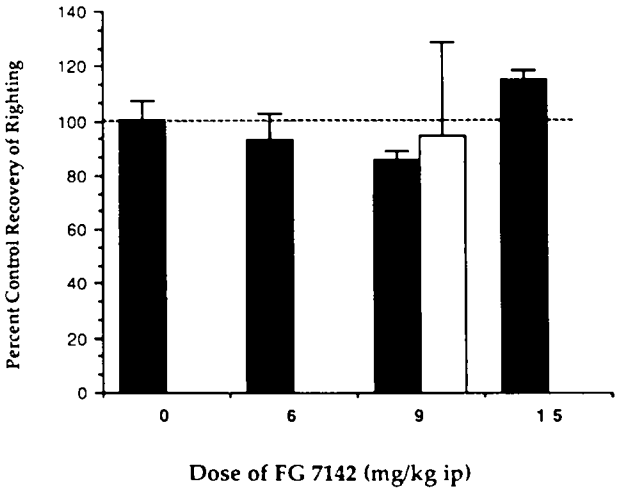

FIG. 1. This figure shows that pretreatment with FG 7142 (0–15 mg/kg IP) 5 minutes prior to the administration of a moderate dose of pentobarbital (40 mg/kg) had no significant effect on time to recovery of the righting reflex compared with carrier-treated controls. Similarly, administration of FG (9 mg/kg) to animals given pentobarbital (40 mg/kg) 60 minutes earlier had no effect on time to recovery compared with animals "posttreated" with carrier. Note that the animals receiving the "zero" dose of FG recovered from pentobarbital in 107.3 ± 7.8 minutes, while the matched placebo controls recovered in 107.1 ± 6.1 minutes. The x-axis gives the dose of FG administered, either as a pretreatment (solid bars) or as a posttreatment (open bars). The y-axis gives the mean (± SEM) recovery values of the FG-treated animals expressed as a percentage of the mean values for recovery of the righting reflex of the paired carrier-treated control animals. The horizontal dashed line represents baseline values (100% of control).

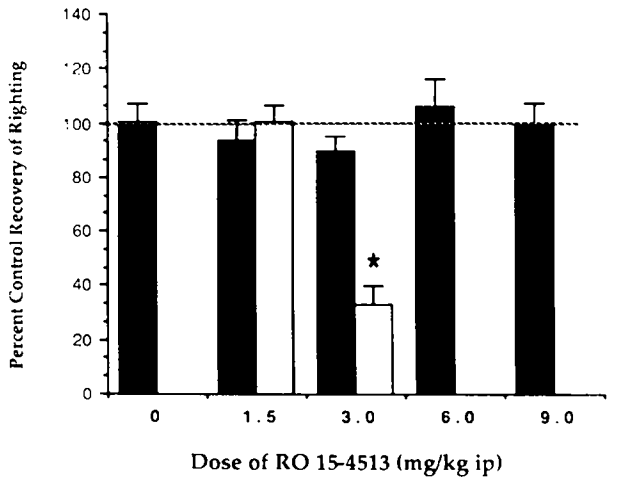

FIG. 2. Like FG 7142 (see Fig. 1), *pretreatment* with RO 15-4513 (0–9 mg/kg IP) also failed to have a significant effect on the recovery of the righting reflex after pentobarbital (40 mg/kg IP) anesthesia. On the other hand, when RO (3.0 but not 1.5 mg/kg IP) was administered 1 hour after pentobarbital injection, a markedly significant decrease ($*p<0.05$) in the time for the recovery of the righting reflex occurred (15.5 ± 3.0 min for the 3 mg/kg dose vs. 47.1 ± 7.9 min for control). Note that the "zero" dose control group was the same as in Fig. 1. The x-axis gives the dose of RO administered, either as a pretreatment (solid bars) or as a postreatment (open bars). The y-axis gives the mean (± SEM) recovery values of the RO-treated animals expressed as a percentage of the mean values for recovery of the righting reflex of the paired carrier-treated control animals. The horizontal dashed line represents baseline values (100% of control).

reflex, as well as the time to the return of the righting reflex were then measured.

In Experiment 2, all animals (n = 53) were first given pentobarbital (40 mg/kg IP). Animals which lost their righting reflex and who still failed to exhibit righting 60 minutes later were then randomized to receive either carrier (1 ml/kg) or drug [either RO (1.5-3 mg/kg IP) or FG (6 mg/kg IP)]. The times for the recovery of the righting reflex were then recorded.

In Experiment 3, rats given a fixed pretreatment dose of RO (3 mg/kg IP) were compared with rats given carrier (1 ml/kg) with respect to the incidence of loss of the righting reflex and duration of anesthesia over a range of pentobarbital doses (20–40 mg/kg IP, n = 47). The pentobarbital was administered five minutes after the animals received either RO or carrier.

In Experiment 4, animals (n = 270) were pretreated with either the carrier solution (1 or 3 ml/kg) or an injection of either RO (0–9.0 mg/kg IP) or FG (0–15 mg/kg IP). Five minutes later, they were anesthetized with halothane using a vaporizer setting of 4% for 2 minutes followed by 2.5% for 13 minutes. The nose cone was immediately removed after the 15 minutes of halothane anesthesia and the animals were then placed on their backs, and the times required to recover their righting reflex were measured.

Experimental Design—Phase II

In the second phase of the study, the benzodiazepine antagonist flumazenil (RO 15-1788) was used to determine if the effects of the benzodiazepine inverse agonists on recovery from barbiturate or halothane anesthesia were due to activity at the BZR.

In experiment 5, animals (n = 16) were given pentobarbital (40 mg/kg IP) and the depth of anesthesia was assessed 1 hour later. Animals that had lost their righting reflexes (n = 15) then received either RO (3 mg/kg IP) or the combination of RO (3 mg/kg IP) and a low dose of flumazenil (FLU) (1 mg/kg IP). The times to the recovery of the righting reflex were then measured.

In Experiment 6, animals (n = 32) were randomized to one of 4 separate treatment groups. Rats that were still anesthetized one hour after pentobarbital injection (40 mg/kg IP, n = 25) received either carrier, RO (3 mg/kg), a high dose of FLU (10 mg/kg), or the combination of RO (3 mg/kg) and FLU (10 mg/kg). The time required to recover the righting reflex was determined.

In the final series of experiments (Experiment 7), the effects of flumazenil on recovery from halothane anesthesia was assessed in the presence or absence of the benzodiazepine inverse agonist FG 7142. Initially, animals (n = 46) were pretreated with either 6 or 9 mg/kg (IP) of FG alone or in combination with FLU (10 mg/kg IP). The recovery of the righting reflex after 15 minutes of halothane anesthesia was then compared with animals given carrier alone. In the last experiment, animals (n = 26) were randomized to one of 4 separate treatment groups. Five minutes before being anesthetized with halothane, rats were injected with either carrier, FG (15 mg/kg), a high dose of FLU (10 mg/kg), or the combination of FG (15 mg/kg) and FLU (10 mg/kg). The time required to recover the righting reflex after termination of the halothane was then determined.

Data Analysis

Differences between groups in the incidence of loss of righting reflex were analyzed using contingency table analysis. Differences in mean animal weights between treatment groups were compared using one-way analysis of variance (ANOVA). Drug-treated

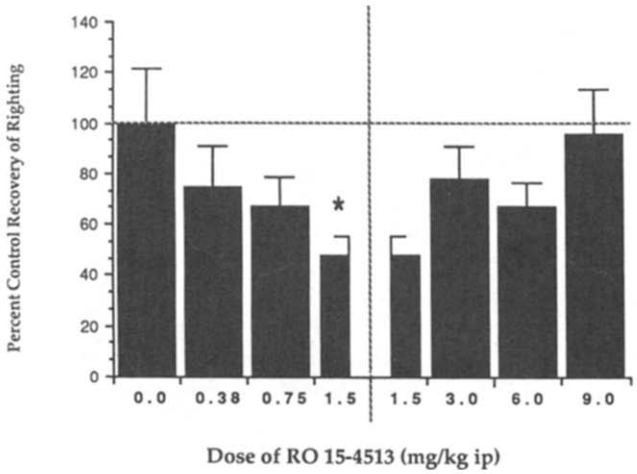

FIG. 3. Overall, pretreatment with RO (0–9.0 mg/kg) had a significant effect on the recovery of the righting reflex after halothane anesthesia. Because a U-shaped dose-response function was obtained, the data were separated into 2 separate (descending and ascending) dose-response curves (vertical dashed line) and then were normalized and transformed. The speed of recovery from halothane anesthesia was significantly ($*p<0.05$) increased after RO (1.5 mg/kg; 2.5 ± 0.4 min) than after carrier (5.0 ± 1.4 min). In this figure, the dose of RO administered is depicted on the x-axis and the mean ($\pm$SEM) recovery values of the RO-treated animals are expressed as a percentage (of mean recovery of righting of matched carrier-treated controls) on the y-axis. The horizontal dashed line represents baseline values (100% of control). Note that the animals receiving the "zero" dose of RO recovered from the halothane anesthetic in 4.96 ± 1.10 minutes, while the matched controls recovered in 4.97 ± 0.66 minutes.

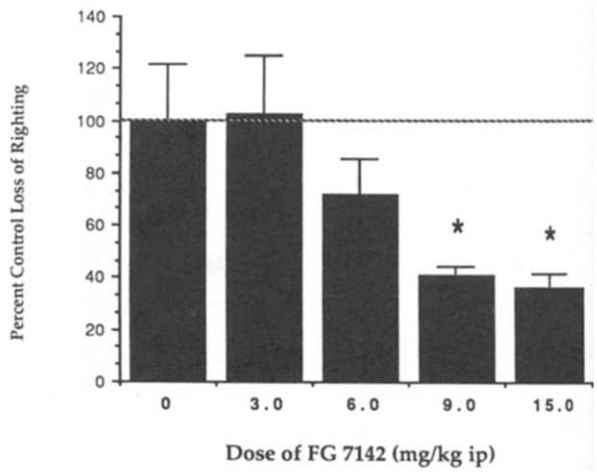

FIG. 4. FG 7142-treated animals recovered significantly faster from halothane anesthesia compared with matched carrier-treated animals ($*p<0.01$) The reduction in sleeping time from control after the 9 mg/kg dose (1.6 ± 0.1 min vs. control of 4.0 ± 0.5 min) and also the 15 mg/kg dose (2.1 ± 0.4 min vs. 5.9 ± 1.8 min) were appreciably greater than the maximal reduction seen in RO-treated animals (see Fig. 3). These data suggest that FG is less potent but much more effective than RO at antagonizing halothane anesthesia. The x-axis shows the dose of FG administered while on the y-axis is displayed the mean ($\pm$SEM) recovery values of the FG-treated animals expressed as a percentage of the recovery of the righting reflex of paired carrier-treated controls. The horizontal dashed line represents baseline values. Note that the "zero" dose control group was the same as in Fig. 3.

animals were always matched with carrier-treated (control) rats from the same vendor shipment because of the potential variability in response to anesthetics among rats exposed to different environmental conditions. Differences in the time to onset and to recovery of righting between groups were evaluated using two-way ANOVA with anesthetic dose and drug treatment group as the dependent variables. Only in cases where an overall drug treatment effect occurred was further statistical analysis performed. Differences in effectiveness between drug doses were then compared relative to matched controls. The data from individual animals in the drug dose groups were divided by the average values from all of the animals in their respective matched control groups. Thus, each drug treatment animal's recovery time was reexpressed as a proportion of the mean control value for that experiment. Because there is a skewed distribution associated with proportions, the normalized percentile data were subjected to an arcsin transformation to stabilize variance (39). A one-way ANOVA (with dose as the dependent variable) followed by Scheffe F-tests were then performed to assess significance. All data were expressed as mean $\pm$ S.E.M. and a $p<0.05$ was considered statistically significant.

RESULTS

There were no significant differences in mean animal weight between treatment and control groups in any experiment. There were no significant differences in the time to *loss* of righting reflex after pentobarbital anesthesia (40 mg/kg) regardless of drug pretreatment. In the RO group, drug-treated animals lost their righting reflex in 5.4 ± 0.7 minutes while the carrier-treated animals lost their righting reflex in 6.2 ± 1.0 minutes. The values in the FG group were similar (4.5 ± 0.6 vs. 5.8 ± 1.0 min).

Pentobarbital

Pretreatment with FG 7142 (0–15 mg/kg IP) five minutes prior to the administration of a moderate dose of pentobarbital (40 mg/kg) had no significant effect on time to recovery of the righting reflex compared with carrier-treated controls (Fig. 1). Similarly, posttreatment with FG (9 mg/kg IP), administered 1 hour *after* pentobarbital (40 mg/kg) to an already well-anesthetized animal, also failed to affect the recovery of the righting reflex compared with the carrier-treated group. However, when RO (3.0 but not 1.5 mg/kg IP) was administered 1 hour after pentobarbital injection, a markedly significant decrease, $F(2,22) = 15.77$, $p<0.05$, in the time for the recovery of righting occurred (Fig. 2). Like FG, *pretreatment* with RO 15-4513 (0–9 mg/kg IP) also failed to have a significant effect on the recovery of the righting reflex after pentobarbital (40 mg/kg IP) anesthesia.

Experiment 3 examined the effects of pretreatment with RO on the incidence of loss of the righting reflex. Of the 8 animals given carrier 5 minutes prior to a low dose of pentobarbital (20 mg/kg IP), 7 lost their righting reflex. In contrast, only 3 out of 8 of the animals pretreated with RO (3 mg/kg) animals lost their righting reflex ($\chi^2 = 3.50$, $p<0.07$).

Halothane

Overall, pretreatment with RO (0–9.0 mg/kg) had a significant but modest effect on the recovery of the righting reflex after 15 minutes of halothane anesthesia, $F(2,7) = 4.76$, $p<0.05$ (Fig. 3). Because the results generated a U-shaped dose-response function, the data were separated into two separate (descending and ascending) dose-response curves and then were normalized and trans-

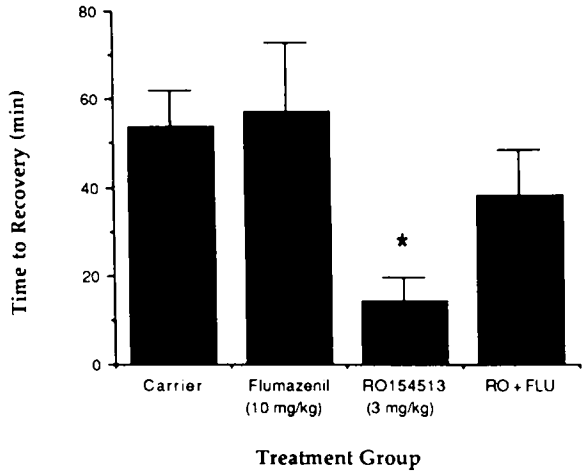

FIG. 5. This figure demonstrates that the benzodiazepine inverse agonist RO 15-4513 (RO) enhances the recovery from pentobarbital anesthesia and that the benzodiazepine antagonist, flumazenil (RO 15-1788), blocks this effect. Each drug treatment combination (depicted on the x-axis) was injected intraperitoneally 1 hour after administration of pentobarbital (40 mg/kg). Recovery of the righting reflex (mean ± SEM) is displayed in minutes on the y-axis. RO shortened the time to recovery of the righting reflex compared with carrier (*$p<0.05$). However, coadministration of RO (3 mg/kg) and flumazenil (10 mg/kg), like flumazenil alone, resulted in a mean recovery time which was not significantly different from that of the carrier control group.

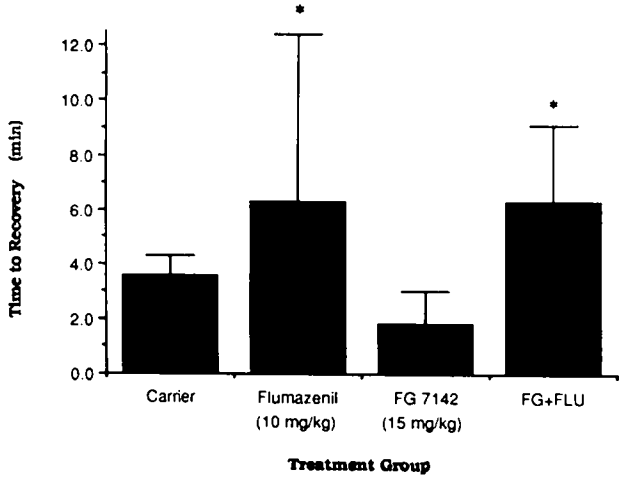

FIG. 6. The benzodiazepine antagonist, flumazenil (FLU) (RO 15-1788) appears to prolong recovery from halothane anesthesia independent of the effects of the benzodiazepine inverse agonist FG 7142 (FG). Each drug treatment combination (depicted on the x-axis) was injected intraperitoneally 5 minutes before a 15-minute halothane anesthetic. Recovery of the righting reflex (mean ± SEM) is displayed in minutes on the y-axis. The decrease in the time to recovery of the righting reflex after FG (15 mg/kg) administration did not attain statistical significance, probably because of the small group of animals studied. However, administration of FLU (10 mg/kg) resulted in a significant increase in recovery time (*$p<0.05$) independent of whether FG or carrier was coadministered.

formed. The speed of recovery from halothane anesthesia was significantly increased after RO (1.5 mg/kg) than after carrier, $F(4) = 3.32$, $p<0.05$. The U-shaped dose-response curve portrayed in Fig. 4 suggests that RO may exhibit some agonist properties at higher doses, although the animals in the three highest RO doses studied were not statistically different in their time to recover their righting reflex.

Consistent with RO's relatively weak ability to antagonize halothane anesthesia, in another experiment a 3.0 mg/kg pretreatment dose failed to significantly hasten recovery from a shorter duration (10 minutes) halothane anesthetic when compared with matched carrier controls (2.0 ± 0.3 vs. 2.6 ± 0.5 min respectively, $p>0.05$, $n = 20$, data not shown). Similarly, when RO (1.5 mg/kg) was administered 5 minutes into 15 minutes of halothane anesthesia ($n = 16$), there was an overall, but nonsignificant reduction in time to the recovery of the righting reflex compared to carrier-treated animals (3.9 ± 0.9 vs. 5.2 ± 0.9 min, $p>0.05$).

On the other hand, FG 7142-treated animals recovered significantly faster from halothane anesthesia compared with matched carrier-treated animals, $F(2,5) = 7.00$, $p<0.01$) (Fig. 4). Transformation of the data to control for differences between experimental groups revealed that the both the 9 and 15 mg/kg doses significantly, $F(5) = 4.81$, $p<0.01$, enhanced the speed of recovery from halothane anesthesia compared with carrier. The percentage reduction in sleeping time from controls (59% and 64%, respectively) at these doses was appreciably greater than the maximal reduction seen in the RO-treated animals (49% at the 1.5 mg/kg RO dose). These data suggest that FG is less potent but much more effective than RO at antagonizing halothane anesthesia in the rat.

Effects of Flumazenil (RO 15-1788)

Treatment with a low dose of the benzodiazepine antagonist flumazenil (1 mg/kg) reversed RO's (3 mg/kg) shortening of the recovery of the righting reflex when both were administered one hour after pentobarbital (40 mg/kg) anesthesia (Experiment 5, $n = 15$). While animals treated with RO alone recovered in 38.4 ± 6.3 minutes ($n = 7$), those treated with both RO and FLU ($n = 8$) recovered in 53.1 ± 3.4 minutes, $F(1,13) = 4.52$, $p<0.06$.

The results of a balanced two-way treatment design (Experiment 6, Fig. 5) confirmed that RO enhances the recovery from pentobarbital anesthesia and also indicates that higher doses of FLU antagonizes this effect. Although the administration of RO (3 mg/kg) 1 hour after pentobarbital (40 mg/kg) shortened recovery of the righting reflex by 73 ± 10% compared with carrier ($p<0.05$), coadministration of RO (3 mg/kg) and FLU (10 mg/kg) resulted in a significant reversal of RO's effects. The mean time to the recovery of the righting reflex in animals in the RO + FLU group was not significantly different from that of either the FLU-only or the carrier groups. The administration of FLU (10 mg/kg) alone had no apparent effect.

In contrast, there was no statistical difference in the time for the recovery of the righting reflex after halothane anesthesia in animals treated with FG (6 mg/kg) alone (4.9 ± 0.9 min, $n = 8$) compared with those given both FG (6 mg/kg) and FLU (10 mg/kg) (5.1 ± 1.2 min, $n = 8$). When the same experiment was repeated using 9 mg/kg of FG, a slight but nonsignificant reduction in the time for the recovery of the righting reflex was noted in the FG alone group (2.9 ± 0.3 min, $n = 16$) compared with the FG plus FLU group (4.1 ± 0.6 min, $n = 14$).

In the final experiment, the administration of flumazenil (10 mg/kg) to rats receiving halothane anesthesia significantly increased the recovery time, $F(1,22) = 5.13$, $p<0.05$, independent of whether FG or carrier was coadministered (Fig. 6). In this single cross-over study, the decrease in the time to recovery of the righting reflex after FG (15 mg/kg) administration did not attain statistical significance, probably because of the small number of animals.

DISCUSSION

The data presented are consistent with previous work showing that RO 15-4513 is able to partially antagonize the anesthetic and sedative properties of pentobarbital. RO appears to be most effective at speeding recovery from a given dose of pentobarbital, if an appropriate dose is given at a time which takes maximal advantage of RO's peak analeptic effects [which previous work suggests occurs within 15 minutes of drug administration (27)]. On the other hand, FG 7142, at doses up to 15 mg/kg, had no apparent effect on recovery from pentobarbital anesthesia. RO's weak ability to speed arousal from halothane anesthesia contrasts with the potent analeptic effects of FG on recovery from halothane anesthesia in the rat. The higher doses of FG required to affect recovery and the U-shaped dose-response curve for the RO groups strongly suggest that FG is less potent, but more effective, at antagonizing halothane anesthesia than RO. Also, RO's effects on barbiturate anesthesia could be reversed with the benzodiazepine antagonist flumazenil, while FG's effects on halothane anesthesia were *not* reversible with the antagonist. This implies that the analeptic effects of these two drugs on the two different types of anesthetic agents are due, at least partially, to different central mechanisms of action.

The results of these experiments suggest that although both RO 15-4513 and FG 7142 are benzodiazepine inverse agonists, RO may have more of a direct effect on the receptor-protein ionophore complex which contains the benzodiazepine, barbiturate, and GABA binding sites. In contrast, FG's analeptic properties appear to either be more indirect or to occur, at least partially, at a site other than the BZR.

There is disagreement in the current literature regarding the relative effectiveness and potencies of the different benzodiazepine inverse agonists. For example, in contrast to the results of the present study, Polc (31) was unable to demonstrate in rats any reversal of barbiturate sedation with RO (3–30 mg/kg PO). Similarly, RO (0.01–20 mg/kg IP) did not antagonize barbiturate-induced depression of discriminitive stimuli in mice (32). Another investigator (4) found RO to be quite effective at reversing or preventing the effects of barbiturates in some tests, e.g., suppression of pentylenetetrazol-induced seizures (ED_{50} 3.5 mg/kg PO) or recovery of balance in the horizontal wire test in mice (ED_{50} 0.3 mg/kg IP) but largely ineffective in other paradigms, e.g., reversal of hexobarbitone-induced loss of the righting reflex in mice (RO 100 mg/kg PO) or depression of locomotor activity in rats (RO 30 mg/kg PO).

The effects of RO 15-4513 on ethanol-induced behavior also appear to depend on the behavioral paradigm being tested (26,29). Both RO and FG reversed ethanol-induced depression of locomotor activity in rats, but only RO antagonized ethanol-mediated depression of gamma motor neuron activity in cats (31). In contrast to the studies cited above which found positive effects, others failed to show any effect with either pre- or posttreatment with RO (10 mg/kg IP) on the duration of ethanol-induced sleep in mice (15) or were unable to demonstrate any influence of RO (2.5 mg/kg IP) on ethanol-induced suppression of wheel-running in the rat (3). Another group of investigators failed to find antagonism by RO (2.5 mg/kg IP) of the effects of moderate ethanol doses on several different tests of motor impairment in several different rat strains (17). These apparent differences in pharmacological function may be partially due to differences in species or behavioral/experimental paradigms. However, there appear to be functional differences between the RO and FG which can not solely be accounted for on the basis of differences in binding affinity for the BZR (13,38).

RO 15-4513 fulfills all of the criteria for a partial inverse agonist at the benzodiazepine receptor (4) including high affinity binding to central benzodiazepine receptor sites, one half as potent as flumazenil at displacing tritiated diazepam binding (35). However, results from multiple studies, such as work on RO's effects on gamma motor neurons and Renshaw cells, are consistent with the hypothesis that RO also acts at non-BZR sites (4,6). In addition, central benzodiazepine receptors are not pharmacologically homogeneous. Tritiated RO 15-4513 apparently binds specifically to different brain sites in the mouse than does [^{3}H]-RO 15-1788 (35).

FG 7142 has approximately $^1/_{10}$th the binding affinity of RO for the benzodiazepine receptor in vitro (36). FG has been shown to be anxiogenic (9,23) and proconvulsant (10,33), consistent with its activity as an benzodiazepine inverse agonist. The present study confirms the analeptic properties of FG and supports the recent findings of others (14,30) that FG is less potent but more efficacious as a benzodiazepine inverse agonist.

Other studies have demonstrated that the benzodiazepine inverse agonists can antagonize the effects of anesthetic drugs. It has been previously shown that RO 15-4513 shifted the dose-response curve to the right for the loss of the righting reflex in tadpoles exposed to a variety of general anesthetic agents (including thiopental, ethanol, and halothane) (12). This effect was present at low RO doses (10 nM) and was reversible with the BZR antagonist flumazenil. In agreement with our data, Bishop and Laverty showed that low doses of RO (5 mg/kg IP) significantly shortened sleeping time after pentobarbitone in mice and that this effect was partially antagonized by FLU (1 mg/kg) (2). As in the present study, these investigators also were unable to show a significant effect of RO on recovery from halothane anesthesia.

Other investigators demonstrated an antagonism of methoxyflurane-induced loss of the righting reflex in rats by the full benzodiazepine receptor inverse agonist ethyl-β-carboline-3-carboxylate (β-CCE) but not by the benzodiazepine antagonist flumazenil (39). The fact that FLU blocked the ability of β-CCE to decrease in half the time for recovery of the righting reflex after methyoxyflurane anesthesia seems to support a role for the benzodiazepine-GABA-chloride channel complex in the β-CCE action on volatile anesthetic agents.

One potential criticism of the present study is the possibility that pretreatment with the BZR inverse agonist could have affected the pharmacokinetics of the subsequently administered anesthetic agents. In earlier work in mice, both RO and FG were able to reverse ethanol-induced behavioral effects without altering ethanol pharmacokinetics (24). In the present study, pretreatment with RO or FG probably had no effect on the depth of the subsequent anesthetic state since there were no significant differences in time of onset of loss of the righting reflex between drug- and carrier-treated groups. Also, if pretreatment with RO or FG had produced nonspecific arousal during the subsequent anesthetic then the resultant increase in hemodynamics or depth of respiration would, in fact, have augmented the depth of barbiturate or halothane anesthesia, thereby slowing awakening.

In summary, RO was both more potent and more effective than FG at speeding recovery from barbiturate anesthesia in the rat. RO's effects appeared to be primarily due to BZR inverse agonist activity since it could be reversed by the BZR antagonist, flumazenil. In contrast, FG appeared to be less potent but much more effective than RO at reversing the anesthetic effects of halothane. FG's effect could not be reliably antagonized with flumazenil, suggesting a non-BZR-mediated analeptic effect. The results of the present study suggest that less toxic structural homologues of these benzodiazepine inverse agonists deserve further study for potential clinical applications.

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