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Investigation of the Actions of the Benzodiazepine Antagonists Ro 15-1788 and CGS 8216 Using the Schedule-Controlled Behavior of Rats

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SANGER, D. J. *Investigation of the actions of the benzodiazepine antagonists Ro 15-1788 and CGS 8216 using the schedule-controlled behavior of rats.* PHARMACOL BIOCHEM BEHAV **25**(3) 537–541, 1986.—Ro 15-1788 and CGS 8216 antagonise many of the pharmacological effects of benzodiazepines but both of these compounds have also been shown to exert behavioral effects when administered alone. In the present study the effects of Ro 15-1788 and CGS 8216, alone and in combination with diazepam and with the benzodiazepine receptor ligand zolpidem, were investigated. Diazepam and zolpidem produced dose-related decreases in rates of food-reinforced lever-pressing maintained by a fixed-ratio (FR 10) schedule. CGS 8216 also reduced response rates although Ro 15-1788, at several doses, produced small, but statistically significant, increases in responding. When the diazepam and zolpidem dose-response curves were re-established in the presence of a dose of Ro 15-1788 or CGS 8216 the depressant effects of the higher doses were antagonised. However, neither diazepam nor zolpidem blocked the rate reducing effect of CGS 8216 which may not therefore be due to an action at benzodiazepine receptors.

Diazepam Zolpidem Ro 15-1788 CGS 8216 Schedule-controlled behavior Rats

IN recent years several compounds have been described which bind to benzodiazepine receptors but antagonise the pharmacological actions of benzodiazepines. The most intensively investigated of such compounds, flumazepil (Ro 15-1788) has been shown to antagonise the anticonvulsant, muscle relaxant and sedative actions of benzodiazepines in experimental animals [5,23] and also blocks the effects of benzodiazepines in man [12,24]. Among the behavioral actions of benzodiazepines antagonised by flumazepil are anti-punishment effects [3, 15, 27, 29, 32, 41], discriminative stimulus properties [2, 22, 35, 38] and increases in food intake [7, 20, 25]. However, the extent of the antagonism produced in such studies has varied from 100% antagonism (e.g., [3,29]) to a complete lack of effect [11].

One possible explanation for the variability in the benzodiazepine antagonist action of flumazepil is that this compound is itself behaviorally active (see review by File and Pellow [18]). In some instances the behavioral effects of flumazepil resemble those of the benzodiazepines, suggesting partial agonism [14, 21, 30, 40]. However, in other studies the actions of flumazepil have been found to differ from those produced by benzodiazepines [3, 19, 34, 43, 44] and combination of flumazepil with a benzodiazepine may produce effects which differ from those of either drug when administered alone [3,30]. It was also reported recently that flumazepil produced mild stimulant effects in human volunteers [36].

CGS 8216, like flumazepil, displaces benzodiazepines from their binding sites and antagonises many of the pharmacological effects of these drugs [4, 10, 46]. This compound is also known to exert a number of behavioral effects when administered alone. These include reduced social interaction in rats [16], reduced rates of punished drinking [26,31], decreased food intake [7], reduced rates of lever pressing maintained by electrical brain stimulation [30] and increased latencies to switch on such stimulation [21]. Some of these actions are in the opposite direction to the effects produced by benzodiazepines in similar procedures and it has been proposed that CGS 8216 may be a partial inverse agonist at benzodiazepine receptors [6]. It has also been reported that CGS 8216 can antagonise the anticonvulsant action of flumazepil in a kindling procedure [33].

The present study was carried out to investigate in more detail the behavioral effects of flumazepil and CGS 8216 when administered alone or in combination with diazepam or zolpidem. Zolpidem is a novel non-benzodiazepine drug which displaces benzodiazepines from their binding sites and shows several of the pharmacological properties of the benzodiazepines but with preferential sedative actions [1,13]. The behavior used in the present experiment was the operant lever pressing of rats maintained by a fixed-ratio (FR 10) schedule of food reinforcement. This schedule was chosen because it can provide a useful behavioral baseline sensitive to the sedative effects of drugs but also because it does not

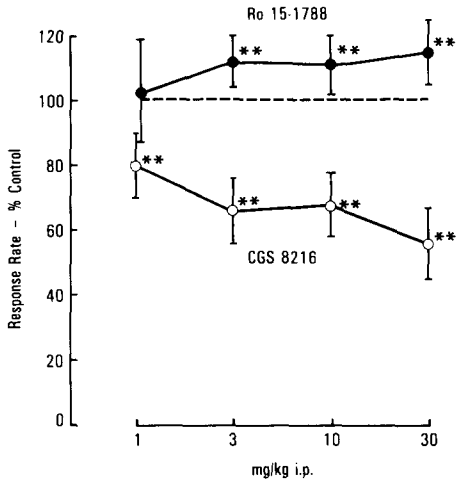

FIG. 1. Dose response curves showing the effects of flumazepil (Ro 15-1788) and CGS 8216 on overall rates of lever pressing maintained by the FR 10 schedule. Each value is the mean±SEM response rate expressed as a percentage of the rate after saline. For flumazepil, n=12 and for CGS 8216 n=8. *$p<0.05$, **$p<0.01$, difference from preceding (saline) day, Wilcoxon's test.

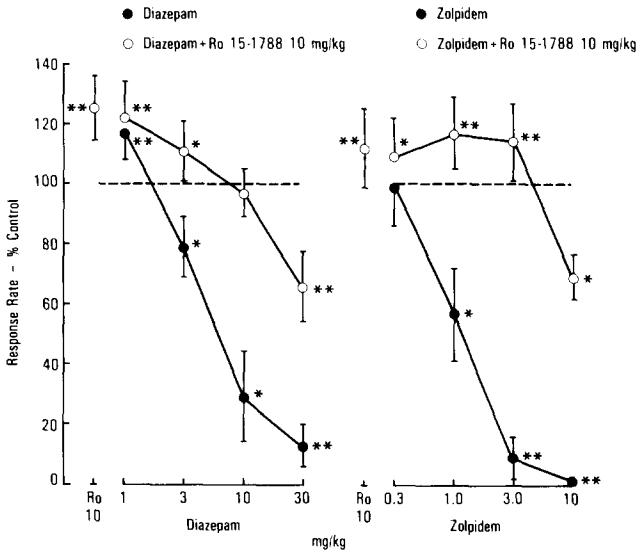

FIG. 2. Effects of diazepam and zolpidem alone and in combination with a dose of flumazepil (Ro 15-1788). Each value is the mean±SEM response rate expressed as a percentage of the rate after saline. Eight rats were tested with diazepam and another group of 8 with zolpidem. *$p<0.05$, **$p<0.01$, difference from preceding (saline) day, Wilcoxon's test.

utilise conditions involving stress or anxiety. Thus drug-induced changes in rates of FR responding are not normally interpreted as increases or decreases in levels of anxiety.

METHOD

Animals

The subjects were 32 male Wistar rats (Charles River, France). They weighed 150–200 g when obtained from the supplier and were allowed to grow during the experiment so that they weighed up to 500 g at the end of the study. All rats were housed individually under standard laboratory conditions. At the start of the experiment they were deprived of food for two days and were subsequently given a standard quantity of chow (15 g/day) each evening and on weekends. This method has been found satisfactory for maintaining a level of motivation which gives rise to a relatively stable behavioral baseline but allows the animals to gain weight. Water was available at all times in the home cages.

Procedure

The experiment was carried out in standard, two lever operant test chambers (Campden Instruments). Rats were trained to press the lever to the right of the food tray. After initial training the number of lever presses required to obtain reinforcement of a 45 mg food pellet (Bioserv) was gradually increased to 10 (fixed-ratio 10: FR 10). This FR 10 schedule was maintained in operation throughout the study. Sessions were 15 min in duration and were given on all weekdays.

When stable day to day rates of lever pressing were obtained rats were injected with several drugs as outlined below. A maximum of two drug administrations were given each week with at least two non-drug days intervening. No carry-over effects were observed the day after drug administration. Injections of the drug vehicle were given on all non-drug days and response rates after drug administration were compared with rates on the immediately preceding day. At least 8 rats were tested with each drug and doses were given in a non-systematic order which was different for each animal. Several rats were used in more than one experiment. When a dose-response curve was established or the effects of a drug were compared with the effects of the same drug in combination with an antagonist the same animals received all doses or dose combinations.

Drugs

The drugs used were diazepam, zolpidem, flumazepil (Ro 15-1788) and CGS 8216. They were prepared as solutions or suspensions in deionized water to which 2 drops of Tween 80/10 ml had been added. Injections were given in a volume of 2 ml/kg 30 min before the start of a session. All doses were calculated as the base and all injections were given IP. Each animal received two injections (vehicle-vehicle, vehicle-drug or drug-drug) before every session.

Statistics

To evaluate the effects of drug administration rates of responding after each injection were compared with response rates on the immediately preceding day when injections of drug vehicle had been administered, using Wilcoxon's matched-pairs signed ranks test. All levels of significance quoted are for two tailed tests.

RESULTS

The FR 10 schedule maintained relatively stable rates of responding in individual rats although these rates varied between 40 and 120 responses/min in different animals. Be-

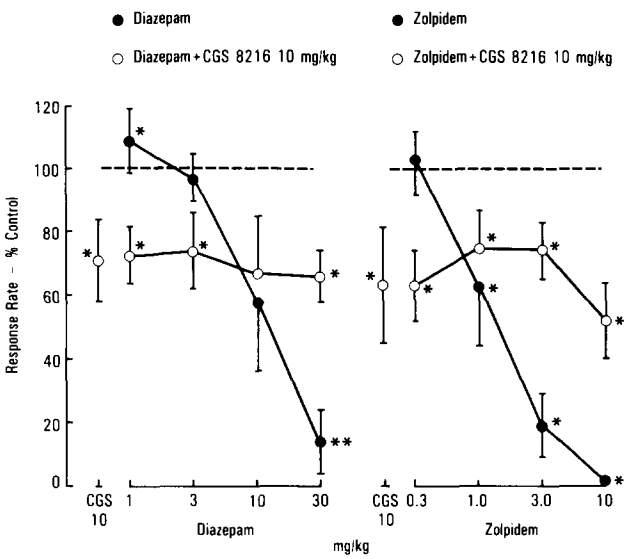

FIG. 3. Effects of diazepam and zolpidem alone and in combination with a dose of CGS 8216. Each value is the mean±SEM response rate expressed as a percentage of the rate after saline. Eight rats were tested with diazepam and 7 with zolpidem. An eighth rat which was used in the zolpidem experiment did not complete the experiment and thus its data were not included. *$p<0.05$, **$p<0.01$, difference from preceding (saline) day, Wilcoxon's test.

cause of this variation the response rates after drug administration were converted to percentages of rates on the immediately preceding sessions for graphical presentation. The figures show the mean±SEM of these response rates as percentages of values on preceding days. It should be noted, however, that as all drug effects were assessed with animals used as their own controls, these SEM values are useful only to provide an indication of the variability between animals and are not useful in indicating differences between different drugs, between different doses or between drug and vehicle conditions.

Figure 1 shows the effects of flumazepil and CGS 8216 on overall response rates expressed as percentages of rates after vehicle administration. It is clear that flumazepil at doses of 3.0, 10 and 30 mg/kg produced small increases in response rates. This effect was unexpected and the number of rats tested with this drug was increased from 8 to 12. Although flumazepil did not increase response rates in all animals the effect was reliable and statistically significant. However, the dose-response curve was flat with all three active doses producing equivalent increases in response rates.

In contrast to flumazepil, CGS 8216 reduced response rates at all doses (1–30 mg/kg). This effect appeared to be dose-related, although, as Fig. 1 shows, the slope of the dose-response curve was very shallow. This decrease in response rates was statistically significant at all doses but there were differences in the effects of CGS 8216 between different individual animals. In one rat this compound had no effect on response rates at any of the doses tested, whereas, in another animal, it almost completely suppressed responding at all doses.

The effects of diazepam and zolpidem administered alone and in combination with a dose of 10 mg/kg of flumazepil are shown in Fig. 2. The doses of diazepam and zolpidem were chosen as those which would reduce response rates, which they did, with zolpidem being approximately 10 times more potent than diazepam. Diazepam also produced a small but statistically significant increase in response rates at the lowest dose (1.0 mg/kg). The decreases in response rates produced by the higher doses of both diazepam and zolpidem appeared to be blocked by the dose of flumazepil. Flumazepil (10 mg/kg) alone produced small increases in rates as did combination of flumazepil with 1.0 and 3.0 mg/kg of diazepam and 0.3, 1.0 and 3.0 mg/kg of zolpidem. It was particularly striking that the combination of 10 mg/kg of flumazepil with 3.0 mg/kg of zolpidem produced response rates significantly above control values.

Figure 3 shows the effects of diazepam and zolpidem alone and in combination with a dose of 10 mg/kg of CGS 8216. Diazepam and zolpidem again produced dose-related decreases in response rates and the effects of the higher doses appeared to be antagonised by CGS 8216. However, response rates after combination of CGS 8216 with either diazepam or zolpidem were always similar to rates after CGS 8216 when given alone, i.e., 60–80% of saline control values. Thus, it appears that although CGS 8216 blocked the depressant effect of higher doses of both diazepam and zolpidem, neither of these drugs antagonised the smaller depressant action of CGS 8216.

DISCUSSION

Diazepam and zolpidem produced dose-related decreases in rates of FR 10 responding in the present experiment. The doses of both drugs used were relatively high and this effect presumably reflects sedative and muscle relaxant actions. Previous laboratory studies have shown that zolpidem is a potent hypnotic drug with less muscle relaxant activity than benzodiazepines [13]. In the present study zolpidem was approximately 10 times more potent than diazepam in decreasing response rates.

The rate-decreasing effects of both diazepam and zolpidem were antagonised by flumazepil and CGS 8216. These results are therefore consistent with many previous studies showing that flumazepil and CGS 8216 are effective benzodiazepine antagonists. However, the present results also showed that these compounds were themselves active at altering response rates. Flumazepil, at doses of 3, 10 and 30 mg/kg, increased response rates while all doses of CGS 8216 (1–30 mg/kg) reduced rates.

The effect of flumazepil was small but nevertheless reliable and statistically significant. Rates of responding maintained by FR schedules are high under control conditions and are only infrequently increased after drug administration. However, previous studies have reported that flumazepil can increase rates of operant responding in rats maintained by variable-interval schedules of food presentation [15] or electrical stimulation of the brain [30]. Increased intake of NaCl solutions has also been observed [14,39]. Such effects may indicate a partial agonist profile of flumazepil which may also account for the increased response rates produced by flumazepil in the present study as the lowest dose of diazepam tested (1.0 mg/kg) gave rise to a similar increase in responding. However, it is interesting to note that response rates were increased by flumazepil from the dose of 3.0 mg/kg whereas previous studies showing effects consistent with partial agonism, anticonvulsant effects for example, have generally observed effects of flumazepil only at much higher doses [42,45].

In contrast to the rate-increasing effect of flumazepil,

CGS 8216 gave rise to a dose-related decrease in response rates. This effect occurred at all doses in the range 1.0–30 mg/kg and was not associated with any obvious sedative, muscle-relaxant or toxic actions of this compound. Pellow *et al.* [30] previously reported that CGS 8216 reduced rates of lever pressing maintained by electrical stimulation of the brain in rats and it has also been found that CGS 8216 reduces food intake [7]. However, other studies reported that similar doses had no effect on the lever pressing of rats maintained by a differential reinforcement of low rate (DRL) schedule [28] or on locomotor activity in mice [9,34]. A number of authors have interpreted the behavioral effects of CGS 8216 as due to increases in fear or anxiety [16, 26, 31]. It is not clear that the reduced rates of FR responding observed in the present study can be interpreted as due to such anxiogenic actions.

Combination of CGS 8216 with either diazepam or zolpidem produced rates of responding similar to those produced by the dose of CGS 8216 given singly. This suggests that the antagonist action of CGS 8216 was non-competitive [37] and that the depressant effect of CGS 8216 was not antagonised by either diazepam or zolpidem. Cooper *et al.* [8] found that the decrease in food intake produced by CGS 8216 could not be antagonised by flumazepil or midazolam and the CGS 8216-induced decrease in social interaction is also not antagonised by benzodiazepines [17]. It is possible, therefore, that these intrinsic behavioral actions of CGS 8216 are not mediated through activity at benzodiazepine binding sites. Further experiments will be necessary to investigate this possibility, including studies of potential interactions between flumazepil and CGS 8216.

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