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Midazolam-Induced Increase in NaCl Solution Ingestion: Differential Effect of the Benzodiazepine Antagonists Ro 15-1788 and CGS 8216[1]

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FALK, J. L. AND M. TANG. *Midazolam-induced increase in NaCl solution ingestion: Differential effect of the benzodiazepine antagonists Ro 15-1788 and CGS 8216.* PHARMACOL BIOCHEM BEHAV 21(6) 965–968, 1984.—After adaptation to a 23-hr water deprivation regimen, under which rats were allowed a daily 1-hr water rehydration session, they were injected (SC) with 1 or 2 drugs presession and given 1.5% NaCl solution to drink in place of water. Midazolam (0.5–1.0 mg/kg) increased the intake of 1.5% NaCl solution as did Ro 15-1788 (2.5–10.0 mg/kg). This confirmed a previously noted agonist effect of midazolam and partial agonist action of Ro 15-1788. When injected in combination with midazolam, Ro 15-1788 (2.5–10.0 mg/kg) antagonized the effect of midazolam. CGS 8216 (2.5–20.0 mg/kg) revealed no partial agonist action on the NaCl solution ingestion procedure nor did it block the effect of midazolam.

Ro 15-1788 CGS 8216 Anxiolytic agent Midazolam Fluid intake NaCl intake Benzodiazepine

IN previous research, both anxiolytic benzodiazepines and barbiturates were found to produce increases in NaCl solution intake by rehydrating, water-deprived rats [13, 14, 36, 37]. Centrally-active agents that do not act as punishment-attenuators (i.e., that are not anxiolytic) do not produce this general type of increase in the ingestion of various fluids [13, 20, 23, 24, 42]. As part of a series of experiments using the NaCl solution ingestion procedure to evaluate known anxiolytic and putative anxiolytic agents, the effect of the benzodiazepine antagonist Ro 15-1788 was ascertained [37]. The result was somewhat unexpected as it revealed a partial agonist action at a moderate dose range (2.5–10.0 mg/kg).

In the present study, one aim was to explore a second benzodiazepine antagonist for possible agonist action. But the major aim was to ascertain the action of two antagonists (Ro 15-1788 and CGS 8216) on the known agonist effect of midazolam.

METHOD

Animals

A total of 16 adult (mean body weight=367.8 g, range=347–381 g) male, albino Holtzman rats (Madison, WI) were used in the present studies. They were housed individually in stainless-steel cages in a temperature-controlled room with a 12-hr on, 12-hr off light-dark cycle. Experimental procedures and measures were done during the light portion of the cycle.

Drugs

Midazolam maleate (Ro 21-3981) and the benzodiazepine blocking agent Ro 15-1788 were both supplied by Dr. W. E. Scott of Hoffman-La Roche, Inc. (Nutley, NJ). A second benzodiazepine blocking agent CGS 8216 was obtained from Mr. C. A. Brownley, Jr. of Ciba-Geigy (Summit, NJ). Both Ro 15-1788 and CGS 8216 were suspended in a cornstarch vehicle prepared in the following manner: 2 g of cornstarch was added to 98 ml of distilled water and heated to a rolling boil while stirring. After cooling to room temperature, the mixture was combined with approximately 0.1 ml Tween 80, mixed thoroughly and stored under refrigeration for later use. The Ro 15-1788 suspension was always prepared immediately prior to injection by gradually adding 1 ml of vehicle to every 5 mg of drug. The CGS 8216 suspension was prepared in a similar manner except that the concentration was 10 mg of drug to every ml of the vehicle. Midazolam was dissolved in distilled water (1.0 mg/ml). All injections were administered subcutaneously into the loose skin at the back of the neck and were always less than 1 ml in volume.

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Procedure

The procedure for both the first and second experiment was as follows: all animals were adapted to a 23-hr water deprivation schedule for 18 days, i.e., water (distilled) was available for only 1 hr each day. Food (Purina Lab Chow, pelleted) was available at all times except during the 1-hr drinking period. Water was available during the 1-hr drinking period from a stainless-steel drinking spout (Ancare, TD-300) attached to a 100-ml Nalgene calibrated cylinder. At the end of the 1-hr period, fluid intakes were recorded and drinking tubes removed. Food was then replaced. Day 19 and every 6–8 days thereafter were designated as test days and the animals were given a drug or drug combinations before the drinking period. When a blocking drug was administrated it preceded the midazolam injection by 10 min. On injection days a 1.5% NaCl solution was given as the fluid available during the drinking session rather than water.

EXPERIMENT 1: EFFECTS OF Ro 15-1788 ON MIDAZOLAM-INDUCED INCREASE IN NaCl ACCEPTANCE

Eight animals were given drug combinations of 0.0, 2.5, 5.0 or 10.0 mg/kg Ro 15-1788 with either 0.0, 0.5 or 1.0 mg/kg of midazolam. All drug combinations were given in a random order. Fluids were available 15 min after the second injection and fluid intakes were recorded 1 hr later as on non-injection days.

Results

Figure 1 shows the effect of various combinations of Ro 15-1788 and midazolam on 1-hr deprivation-induced NaCl solution intake. The data are presented as percent change in intake from that following vehicle injections (0.0 midazolam and 0.0 Ro 15-1788). Inspection of the figure reveals that NaCl acceptance was increased over vehicle baseline when various doses of Ro 15-1788 (2.5–10.0 mg/kg) were given alone. However, when given in combination with midazolam, Ro 15-1788 produced a dose-related decrease in 1.5% NaCl solution intake. This blocking effect of Ro 15-1788 is still evident at the higher midazolam dose (1.0 mg/kg), although the dose-dependent aspect of the blocker is no longer present. The amount of NaCl solution intake (ml/100 g body weight) at various dose combinations of midazolam and Ro 15-1788 is presented in Table 1. Due to the large between-subject baseline intake differences, an overall analysis of variance was performed on the square-root transformations of the raw data. A significant midazolam effect was obtained, $F(2,77)=3.947$, $p<0.05$. The interaction of midazolam and Ro 15-1788 was also significant, $F(6,77)=3.607$, $p<0.01$. Further analyses on the simple Ro 15-1788 effects at each dose level of midazolam revealed significant Ro 15-1788 blocking effects at both 0.5 and 1.0 mg/kg midazolam, $F(3,21)=4.147$, $p<0.05$ and $F(3,21)=5.812$, $p<0.01$, respectively. In the absence of midazolam (0.0 dose), Ro 15-1788 significantly increased the intake of 1.5% NaCl solution, $F(3,21)=3.261$, $p<0.05$.

EXPERIMENT 2: EFFECTS OF CGS 8216 ON MIDAZOLAM-INDUCED NaCl ACCEPTANCE

A dose-response curve was determined for CGS 8216 in 8 rats. Doses of 2.5, 5.0, 10.0 and 20.0 mg/kg were administered in a random order 15 min pre-session. After the determination of the initial dose-response curve, the animals were given drug combinations of either 0.0, 5.0, 10.0 or 20.0 mg/kg CGS with either 0.5 or 1.0 mg/kg of midazolam. As in Experiment 1, drug combinations were administered in a random order with the blocker being given 10 min before midazolam. The fluid intake test was identical to that of Experiment 1.

TABLE 1

MEAN INTAKE (ml/100 g BODY WEIGHT) OF 1.5% NaCl SOLUTION (N=8 RATS)

Midazolam (mg/kg)	Ro 15-1788 Doses (mg/kg) 0.0	2.5	5.0	10.0
0.0	5.93	6.66	6.67	7.07
0.5	8.54	7.72	7.35	6.56
1.0	8.86	7.54	7.27	7.77

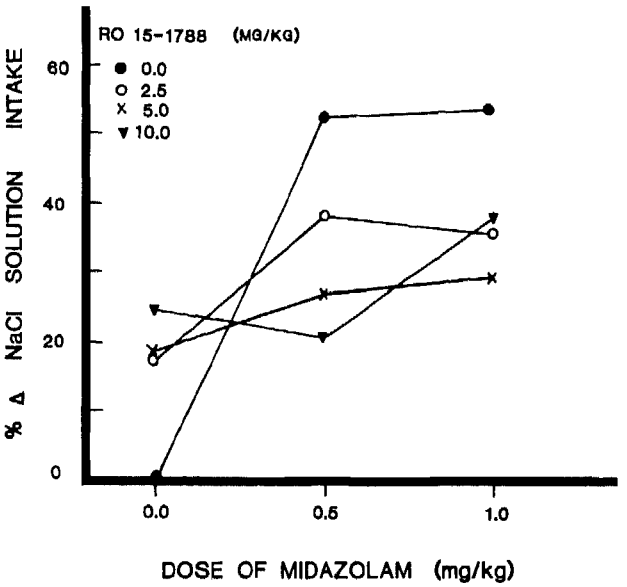

FIG. 1. Mean % increase in 1-hr ingestion of 1.5% NaCl solution by rehydrating rats (N=8) as a function of midazolam or Ro 15-1788 doses given alone or in combination.

Results

The CGS 8216 dose-response function is presented in Fig. 2. Unlike the case of Ro 15-1788, CGS 8216 (2.5–20.0 mg/kg) did not have an intrinsic effect on deprivation-induced 1.5% NaCl solution acceptance. An overall analysis of variance performed on the intake data did not yield a significant treatment effect. Table 2 shows the amount of NaCl solution ingestion (ml/100 g body weight) following various dose combinations of midazolam and CGS 8216. As in Experiment 1, an overall analysis of variance was performed on the square-root transformations of the raw data. Again, a significant midazolam effect was obtained, $F(1,49)=7.114$, $p<0.01$. In contrast to the blocking effect Ro 15-1788 had on the midazolam effect, a mild but statistically significant potentiation was observed with CGS 8216, $F(3,49)=3.858$, $p<0.05$. The interaction between midazolam and CGS 8216 dose levels was not significant.

TABLE 2

MEAN INTAKE (ml/100 g BODY WEIGHT) OF 1.5% NaCl SOLUTION (N=8 RATS)

Midazolam (mg/kg)	CGS 8216 Doses (mg/kg) 0.0	5.0	10.0	20.0
0.5	6.39	7.20	6.84	7.84
1.0	7.25	7.90	7.46	7.73

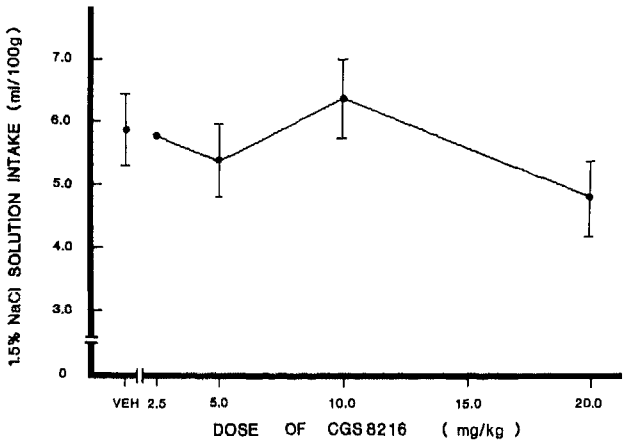

FIG. 2. Mean (±SE) 1-hr ingestion (ml/100 g body weight) of 1.5% NaCl solution by rehydrating rats (N=8) as a function of CGS 8216 dose.

DISCUSSION

Two distinct classes of chemicals, the imidazobenzodiazepines (e.g., Ro 15-1788) and the pyrazoloquinolinones (e.g., CGS 8216) bind with high affinity to benzodiazepine receptors and act as antagonists to the benzodiazepines [5, 6, 7, 10, 19, 32]. In previous research using the NaCl solution intake procedure, we found not only that benzodiazepines and phenobarbital [14, 15, 36, 37] increased intake in the rehydrating rat, but Ro 15-1788 also had a partial agonist action in this regard [37]. The present experimental results further illuminate the interaction of benzodiazepine agonist and antagonist action. We confirmed the results of our previous study again finding that midazolam increased the intake of NaCl solution and that Ro 15-1788 had a partial agonist effect. As well as revealing a partial agonist action at moderate dose levels, Ro 15-1788 was an effective midazolam antagonist at these same dosages. Recently, a number of investigations have reported partial agonist actions for Ro 15-1788. At a high dose level (50 mg/kg) Ro 15-1788 exhibited an anticonvulsive (agonist) action, while at a lower dose (10 mg/kg) it reversed the anticonvulsive actions of diazepam and CL 218,872 [24,40]. However, there is evidence that Ro 15-1788 also yields an anticonvulsive partial agonist action at the lower dose levels of 8 mg/kg [1] and 1 mg/kg [29]. At high dose levels of Ro 15-1788 there is further evidence of partial agonist effects with respect to electrophysiological responses [34], increased escape latencies to aversive stimulation [22], and generalization from training on clorazepate in a drug discrimination procedure [11]. But again at lower doses (5–10 mg/kg) increases in exploratory head-dipping [17], antiaggressive [2] and anticonflict [16] behavior occurred, all of which were interpreted as partial agonist responses. The present study confirmed our previous finding of a partial agonist effect for Ro 15-1788 over the 2.5–10 mg/kg dose range [37].

Ro 15-1788 is most notable for its antagonist properties with respect to agents acting as agonists at central benzodiazepine binding sites such as diazepam. A wide range of the physiological and behavioral effects produced by these agonists are blocked by Ro 15-1788: antipunishment, muscle relaxant, sedative and anticonvulsive actions [6, 7, 19]. The present experiment found that Ro 15-1788 blocked the increased drinking of NaCl solution produced by midazolam over the same dose range that it acted as a partial agonist for this behavior, i.e., 2.5–10 mg/kg (cf. Fig. 1). Similarly, Feldon *et al.* [16] showed that a 10 mg/kg dose of Ro 15-1788 had a partial-agonist effect on punished responding in the Geller-Seifter paradigm, but would antagonize this effect when it was produced by 5 mg/kg of chlordiazepoxide using the same procedure.

A number of punishment-attenuating drugs have been found to increase deprivation-induced fluid intake. These include the anxiolytic benzodiazepines (chlordiazepoxide, midazolam, diazepam, flurazepam, lorazepam), barbiturates, meprobamate and methaqualone [8, 13, 14, 21, 24, 26, 30, 31, 35, 41]. Many of these studies used water as the intake fluid, but in earlier [14] and recent [15, 36, 37] research we found greater and more reliable drug effects when NaCl solutions were employed as the drinking fluid. However, using water Cooper [9] found that Ro 15-1788 antagonized midazolam hyperdipsia, but not that produced by phenobarbital. The present study confirmed the blocking effect of Ro 15-1788 on midazolam-induced hyperdipsia when NaCl solution was the fluid ingested.

The present experiment found no evidence of intrinsic agonist action for CGS 8216 with respect to the enhancement of NaCl solution intake by water-deprived animals (cf. Fig. 2). Further, unlike Ro 15-1788, CGS 8216 did not antagonize the effect of midazolam on NaCl solution intake (cf. Table 2). In fact, a small, but significant increase was observed. The difference between Ro 15-1788 and CGS 8216 with respect to the saline ingestion procedure is of interest in the light of the otherwise similar spectrum of action of these two antagonists. Both agents effectively antagonize the anticonvulsant, muscle relaxant, sedative and anxiolytic actions of diazepam [6]. With respect to drug discrimination, Ro 15-1788 was found to block the discriminative control of diazepam [18], oxazepam [12], and lorazepam [3], while CGS 8216 is likewise effective in blocking diazepam [5,33]. As measured by conflict procedures, Ro 15-1788 was effective in antagonizing the anxiolytic action of chlordiazepoxide [4, 27, 39], U 43,465 F [38], diazepam [6] and lorazepam [28]; CGS 8216 was similarly effective in blocking chlordiazepoxide [27] and diazepam [5,6]. Both Ro 15-1788 and CGS 8216 show comparably high affinity for brain benzodiazepine binding sites [6].

Comparing Ro 15-1788 and CGS 8216 with regard to their effects on the midazolam-induced increase in NaCl solution intake, only Ro 15-1788 revealed a partial agonist action and an antagonist action. In the light of the rather similar effectiveness of these antagonists on a variety of benzodiazepine actions this difference deserves further study.

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