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Agonistic Action of a Benzodiazepine Antagonist: Effects of Ro 15-1788 and Midazolam on Hypertonic NaCl Intake[1]

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TANG, M., S. SOROKA AND J. L. FALK. *Agonistic action of a benzodiazepine antagonist: Effects of Ro 15-1788 and midazolam on hypertonic NaCl intake.* PHARMACOL BIOCHEM BEHAV **18**(6) 953–955, 1983.—Rats adapted to a 23-hr water deprivation regimen were allowed a daily 1-hr water rehydration session. On test days the session drinking fluid was 1.5% NaCl solution rather than water. One group was injected (SC) with the benzodiazepine antagonist Ro 15-1788 (2.5–10 mg/kg) and another group with the agonist agent midazolam (0.13–2.0 mg/kg) every 5–6 days at 10 and 15 min before a test session, respectively. Both Ro 15-1788 and midazolam increased 1.5% NaCl solution intake in a dose-related manner. In this study and in previous research, benzodiazepines and barbiturates were shown to increase the intake of hypertonic NaCl solutions. The present results reveal a similar effect for Ro 15-1788, indicating an agonistic dimension to the spectrum of action of this specific receptor antagonist.

Anxiolytic agent Ro 15-1788 Benzodiazepine antagonist Midazolam Conflict Fluid intake

THE selective benzodiazepine antagonist agent Ro 15-1788 specifically blocks centrally mediated effects of benzodiazepines, has no benzodiazepine-like action, and lacks overt central stimulant activity in all tested species [6]. In behavioral studies it does not produce effects characteristic of the benzodiazepines (sedation, muscle relaxation, anticonvulsant, and antipunishment effects); rather, it antagonizes these benzodiazepine-produced effects [1,6]. Further, Ro 15-1788 acts as a competitive antagonist of the discriminative stimulus effects of diazepam in rats [5].

Previous research demonstrated that the punishment-attenuating agents chlordiazepoxide and phenobarbital produced an increased intake of hypertonic saline solution (1.5% NaCl) in 23-hour water-deprived rats [4]. In the interests of using the hypertonic NaCl drinking procedure to develop a screening technique for anxiolytic action, a midazolam [10] dose-effect relation was determined, and we planned to evaluate the effect of Ro 15-1788 on the NaCl intake enhancing effecting of midazolam. Two initial studies, then, planned to explore first the independent dose-effect relations of midazolam and Ro 15-1788 with respect to 1.5% NaCl solution intake. These two studies comprise the present report.

METHOD

Animals

Twelve adult male, albino rats of the Holtzman strain (Madison, WI), with an initial mean body weight of 364 g (range: 341–397 g), were used. They were housed in individual, stainless-steel cages in a temperature-controlled animal room with a 12 hr on, 12 hr off light-dark cycle. Experiments were done during the light portion of the cycle.

Drugs

Two drugs, generously supplied by Dr. W. E. Scott of Hoffman-La Roche, Inc. (Nutley, NJ), were used: midazolam maleate (Ro 21-3981) and the benzodiazepine blocking agent Ro 15-1788. Midazolam was dissolved in distilled water. The vehicle for Ro 15-1788 was a suspension of Agent K (Bio Serv., Inc., Frenchtown, NJ) at a concentration of 40 mg/100 ml distilled water. All injections were administered subcutaneously (SC) into the loose skin at the back of the neck and were less than 1 ml in volume. Solutions were prepared immediately before each injection.

Procedure

The 12 animals were adapted to a 23-hr water-deprivation schedule for at least 15 days, i.e., distilled water was available for only 1 hr daily. Food (Purina Lab Chow, pelleted) was always available except during the 1-hr drinking period. Water was available during the 1-hr daily drinking period from a stainless-steel drinking spout (Ancare, TD-300) attached to a 100-ml calibrated reservoir. At the end of the 1-hr

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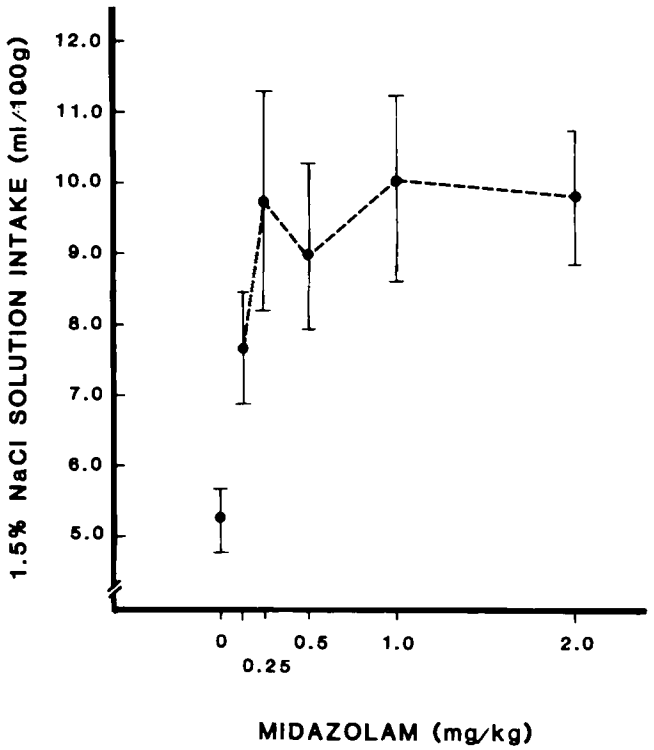

FIG. 1. Mean (±SE) 1-hr ingestion (ml/100 g body weight) of 1.5% NaCl solution by rehydrating rats (N=6) as a function of midazolam dosage (SC injection). 0 mg/kg=vehicle injection.

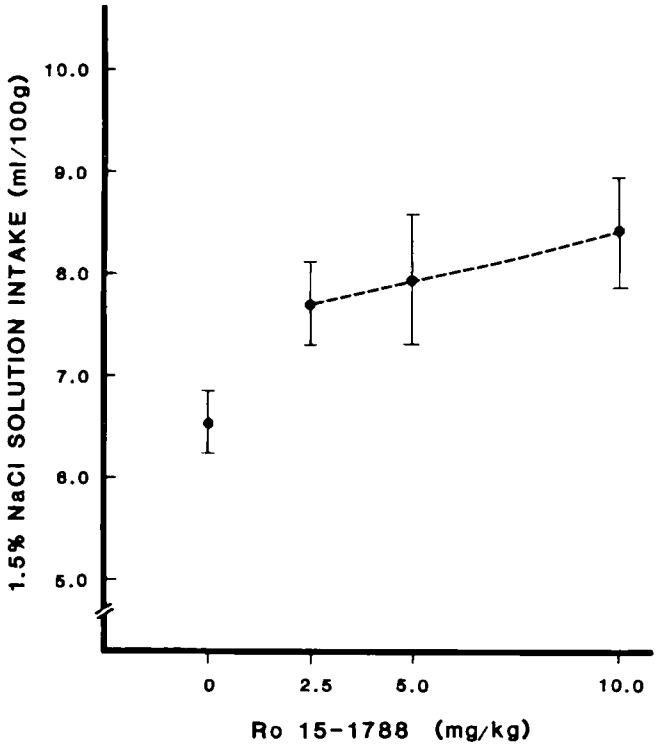

FIG. 2. Mean (±SE) 1-hr ingestion (ml/100 g body weight) of 1.5% NaCl solution by rehydrating rats (N=6) as a function of Ro 15-1788 dosage (SC injection). 0 mg/kg=vehicle injection.

drinking period, fluid intakes were recorded, the drinking tubes were removed, and food was restored.

The animals were divided randomly into two groups (N=6 each) and maintained on the above fluid-availability regimen. However, every 5–6 days 1.5% NaCl solution was the fluid available during the drinking period rather than distilled water. Coincident with this condition either a drug dose or vehicle was injected 15 min before the drinking period. For one group of animals the drug was midazolam given at doses of 0.13, 0.25, 0.5, 1.0 and 2.0 mg/kg. The other group received Ro 15-1788 at 2.5, 5.0 and 10.0 mg/kg doses. Each drug dose level was given 1 or 2 times to each animal and dose order was random.

RESULTS

Figure 1 shows the significant, $F(5,25)=4.41$, $p<0.01$, increase in 1.5% NaCl solution ingestion produced by midazolam. Although the shape of the dose-effect relation suggests that the smallest dose (0.13 mg/kg) had a less pronounced effect than the larger doses, in fact analysis of variance does not yield a significant F-value across doses. The antagonist agent Ro 15-1788 yielded a similar result (Fig. 2). It increased 1.5% NaCl solution ingestion compared to treatment with the vehicle, $F(3,15)=3.63$, $p<0.05$. Again, the effect was not significantly different across doses of the drug.

DISCUSSION

The enhanced 1.5% NaCl intake produced by midazolam confirmed previous studies in which a benzodiazepine (chlordiazepoxide) and a barbiturate (phenobarbital) also yielded this effect [4,12]. Unpublished work from our laboratory using this procedure with nephrectomized and with ureter-tied rats indicated that the benzodiazepine enhancement of NaCl solution ingestion is secondary to neither water nor electrolyte urinary losses. The status of this ingestion test as a screen for anxiolytic action is at present unclear. Ro 15-1788 itself produces no antipunishment effects in doses up to 100 mg/kg [1] and this test correlates well with anxiolytic action. Further, Ro 15-1788 blocks the antipunishment effects of diazepam [1].

Ro 15-1788 selectively blocks a wide variety of the behavioral and neuropharmacological actions of benzodiazepines. It blocks sedative, anticonvulsant, and antipunishment actions as well as drug stimulus discriminative effects [1,5]. It specifically attenuates the cerebellar cyclic GMP reduction induced by diazepam, but not that effected by barbiturates, ethanol, meprobamate, muscimol and neuroleptics [6]. Recently, Ro 15-1788 administration has precipitated an abstinence syndrome in animals given chronic prior exposure to particular benzodiazepines [2, 7, 8, 11].

In agreement with our present findings, not all studies indicate a pure antagonist role for Ro 15-1788; partial agonist properties have been reported. While confirming that Ro 15-1788 blocked the antipunishment effect of clorazepate, Dantzer and Perio [3] found that at high doses of Ro 15-1788 (40 and 80 mg/kg) rats trained to discriminate clorazepate from saline generalized to Ro 15-1788. Using the models of (a) seizure threshold changes to pentylenetetrazol and (b) GABA depolarization of isolated rat cervical sympathetic ganglion, Nutt *et al.* [9] reported that high doses of Ro 15-1788 (50 mg/kg and greater) showed on one hand anticonvulsive activity, and on the other, at high perfusion

concentrations, amplitude-increasing effects similar to those produced by chlordiazepoxide. Both effects could be interpreted as intrinsic, agonist actions.

There are two important aspects to the present findings. First, the increased ingestion of NaCl solution in rehydrating animals occurs not just under phenobarbital or chlordiazepoxide treatment, but for an additional benzodiazepine, midazolam, as well as others (unpublished studies). Second, the similar effect produced by midazolam and Ro 15-1788 indicates a benzodiazepine agonist action for the latter. Furthermore, unlike the evidence heretofore available on Ro 15-1788 agonist action, the present study reveals a marked agonist action occurring in a dose range typically associated with its antagonist effects.

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