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The Role of Central- and Peripheral-Type Benzodiazepine Receptors in Anxiolytic-Agent Augmentation of NaCl Solution Intake: Effects of Clonazepam and Ro 5-4864

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TANG, M., W. R. PLAESSMANN AND J. L. FALK. *The role of central- and peripheral-type benzodiazepine receptors in anxiolytic-agent augmentation of NaCl solution intake: Effects of clonazepam and Ro 5-4864.* PHARMACOL BIOCHEM BEHAV **30**(3) 749–752, 1988.—Two 1,4 benzodiazepines bind preferentially to the central- and peripheral-type benzodiazepine receptor in the brain, clonazepam and Ro 5-4864, respectively. They were administered to rats to determine if the relation between known anxiolytic action and efficacy in augmenting NaCl solution ingestion in rehydrating rats would remain the case for these prototypic agents. Clonazepam (0.062–32.0 mg/kg, PO) was highly potent and efficacious and increased 1.5% NaCl solution intake in a dose-related fashion. Water intake could also be increased, but to a relatively minor degree. Ro 5-4864 (4–8 mg/kg, IP) did not affect 1.5% NaCl solution ingestion, nor did this dose range suppress the augmenting effect of clonazepam (0.5–2.0 mg/kg, PO) on the solution intake. Since clonazepam does, and Ro 5-4864 does not, possess punishment-attenuation properties in other tests, drug augmentation of NaCl solution ingestion by rehydrating rats continues to correlate well with known anxiolytic action.

Clonazepam Ro 5-4864 NaCl intake Anxiolytic Central-type benzodiazepine agonist Peripheral-type benzodiazepine agonist

BENZODIAZEPINES bind with differing affinities to "central" and "peripheral" binding sites. The pharmacological specificities of these sites are such that the more potent anxiolytic and anticonvulsant benzodiazepines possess higher affinities for the "central" receptors than do the less potent derivatives [17], while an agent such as Ro 5-4864, which binds preferentially to "peripheral" sites, displays little affinity for "central" sites. Conversely, the "peripheral" sites exhibit low affinity with respect to agents such as clonazepam which bind well to "central" receptors. While first located in peripheral tissues such as the kidney [4], "peripheral" binding sites were later found in the central nervous system [20]. The functional significance of these "peripheral" sites is unknown, although it is suggestive that Ro 5-4864 is behaviorally active. In contrast to the profiles of most benzodiazepines in clinical use, Ro 5-4864 was reported to possess sedative, convulsant and anxiogenic actions [18]. It had no antipunishment activity in a punished-drinking test [18].

A strong relationship has been noted between the affinity to bind to the central-type receptor and anxiolytic activity for a number of benzodiazepines as measured by both punished-drinking and lever-press-conflict models of antipunishment action in rats [21]. Further, both benzodiazepines and barbiturates that have proven clinical action as anxiolytics in humans produce an increased intake of hypertonic NaCl solution in rehydrating rats [11]. Hypertonic NaCl solution (1.5%) was initially regarded as an aversive stimulus. Thus, the increased solution intake occasioned by benzodiazepines and barbiturates would follow from their punishment-attenuating action in much the same way that behavior suppressed by response-contingent electric shock is increased by these agents [11]. However, antipunishment drugs also increase the intake of highly-acceptable fluids and foods in a pharmacologically selective fashion [7,12]. Hence, while the initial notion that these drug-induced increases are due to release from the suppression produced by an aversive taste may be inadequate, the

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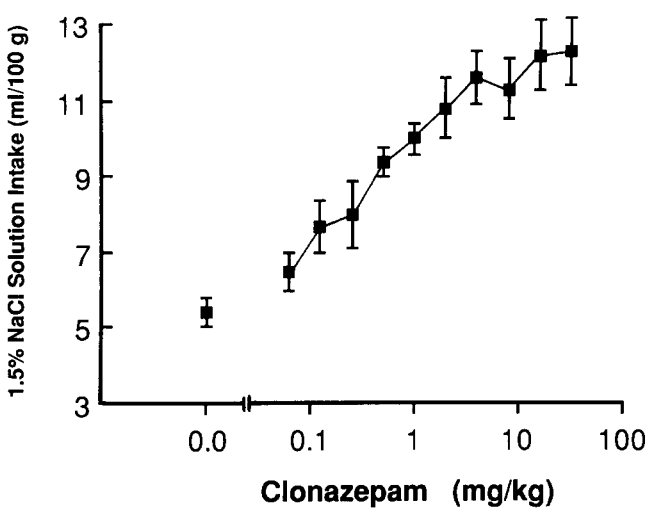

FIG. 1. Mean (±SE) 1-hr ingestion (ml/100 g) of 1.5% NaCl solution by rehydrating rats (N=8) as a function of clonazepam dose (30 min presession, PO).

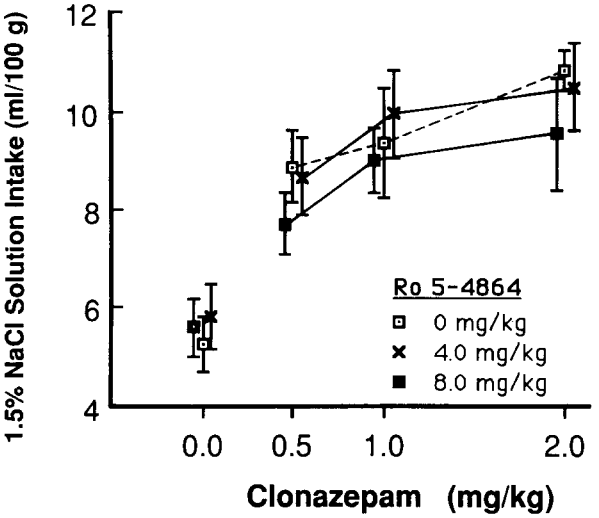

FIG. 2. Mean (±SE) 1-hr ingestion (ml/100 g) of 1.5% NaCl solution by rehydrating rats (N=8) as a function of clonazepam (30 min presession, PO) or Ro 5-4864 (20 min presession, IP) doses given alone or in combination.

correlation between anxiolytic action in humans and increased hypertonic NaCl solution ingestion in rats remains strong. In order to explore this relation specifically in the light of "central" and "peripheral" binding affinities, clonazepam and Ro 5-4864 were employed since they bind selectively and with great affinity to these two sites, respectively. Further, the behavioral interaction of these two agents was explored.

EXPERIMENT 1: CLONAZEPAM AND FLUID INTAKE

METHOD

Animals

Eight male Holtzman rats (Madison, WI) with an initial mean body weight of 304.1 g (range: 282–333 g) were used. Animals were housed individually in standard Acme stainless-steel cages in a temperature-regulated room under a 12-hr on/12-hr off light-dark cycle (lights on 0700–1900 hr).

Drug

Clonazepam was generously supplied by Dr. Peter F. Sorter of Hoffmann-LaRoche, Inc. (Nutley, NJ). The drug was suspended in a 50 mg/100 ml solution of Agent K (BioServ, Inc., Frenchtown, NJ) in distilled water with 1–2 drops of Tween 80 added as necessary for the higher doses. All drug doses were administered orally. This was accomplished by allowing rats to lick the tasteless drug suspension from the blunted end of a 13 ga needle that was attached to a 1-ml syringe. The blunted needle was extended approximately 3 cm into an animal's home cage for drug delivery.

Procedure

Animals were adapted to a 23-hr water-deprivation schedule. This consisted of ad lib access to food (Purina Lab Chow, pelleted) 23 hr per day, with distilled water being presented only during the 1 hr when food was not present. At 1300 hr each day, food was removed from the home cages, animals were weighed and distilled water was made available from individual ball-bearing stainless-steel spouts attached to 100-ml Nalgene graduated cylinder water reservoirs. At the end of the 1-hr drinking period, fluid intakes were recorded, the drinking tubes were removed and food was replaced in the cages.

When the session fluid intakes stablized (13 days), the effect of various doses of clonazepam (0.0, 0.062, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 mg/kg) on session fluid intake was assessed. On drug-testing days, a 1.5% (w/w) NaCl solution substituted for distilled water as the session fluid. Drug doses from 0.0–0.5 mg/kg were assigned to each animal in a random order. On completion of this series of doses, the 1.0–32.0 mg/kg doses were given in an ascending dose order. All doses were given orally 30 min before the drinking session once every 5–7 days. A second vehicle (0.0 mg/kg) treatment was administered to all animals following either the 4.0 or 8.0 mg/kg dose (after the usual 5–7 day interval) in order to redetermine the undrugged level of NaCl solution session intake to ensure that no ingestive baseline shift had occurred. At the end of the dose-effect determination, the 0.0 and 1.0 mg/kg doses were given again, except that water instead of NaCl solution was presented as the session drinking fluid.

RESULTS

Oral administration of acute doses of clonazepam to rats drinking on a 23-hr water-deprivation schedule resulted in a dose-related increase in 1.5% NaCl solution intake. Figure 1 shows that the increase was linearly related to dose magnitude up to 4.0 mg/kg of clonzaepam, with only modest additional increases at the 16.0 and 32.0 mg/kg points. Analysis of variance yielded significance for the dose factor, $F(10,70)=23.532$, $p<0.001$. The vehicle-administration NaCl solution ingestion baseline which was redetermined late in the dose-effect determination series revealed a decrease of only marginal significance, $F(1,7)=5.443$, $p<0.10$, from its determination early in the series (4.00 versus 5.37 ml/100 g). The point plotted for the vehicle in Fig. 1 is the mean of these two values. This nonsignificant decrease in the baseline of

NaCl solution ingestion was small in relation to the magnitude of the effects for the later, larger drug doses and actually would function to yield an estimate of greater drug efficacy if the $p<0.01$ level were to be accepted.

The last two treatments which compared vehicle and 1.0 mg/kg clonazepam administration on session water intake yielded values of 6.15 and 7.25 ml/100 g, respectively, a significant difference, $F(1,7)=12.64$, $p<0.01$.

EXPERIMENT 2: Ro 5-4864 AND ITS INTERACTION WITH CLONAZEPAM

METHOD

Animals

Eight male Holtzman rats (Madison, WI) with an initial mean starting body weight of 359.3 g (range: 347–383 g) were used. Housing conditions were as in Experiment 1.

Drugs

Clonazepam was prepared and administered as in Experiment 1, except that it was suspended in a 1 mg/ml solution of Agent K in distilled water. Ro 5-4864 was generously supplied by Dr. Peter F. Sorter of Hoffmann-LaRoche (Nutley, NJ) and was prepared as was clonazepam.

Procedure

The general procedures were identical to those in Experiment 1 except that there were 4–7 days between drug administrations. Ro 5-4864 was administered IP as 0.0, 4.0, or 8.0 mg/kg doses 20–25 min before the 1-hr 1.5% NaCl solution drinking session. Clonazepam was given orally as 0.0, 0.5, 1.0, or 2.0 mg/kg doses 45 min presession. Doses were given both singly and in all combinations, which comprised 12 treatments. Treatments were given to each animal in random order.

RESULTS

The results are shown in Fig. 2. Ro 5-4864 had no effect on the ingestion level of 1.5% NaCl solution. When given alone, the effect of clonazepam on increasing NaCl solution ingestion was comparable to the data of Experiment 1 as shown in Fig. 1. There is no effect of Ro 5-4864 in combination with clonazepam at any dose that modifies the clonazepam effect. There is a suggestion that 8.0 mg/kg of Ro 5-4864 might have suppressed the clonazepam-induced increase, but it is not a significant effect.

GENERAL DISCUSSION

The present results indicate that clonazepam is the most potent and efficacious of the antipunishment agents yet explored for increasing fluid intake in rehydrating rats. The high potency is borne out by the low value found for its minimally effective oral dose (0.16 mg/kg) in the rat lever-press conflict test [6]. While the potency of clonazepam estimated by these two methods agrees quite well, clonzaepam appeared to have no greater efficacy over other effective 1,4 benzodiazepines in the rat conflict test [6]. This contrasts with the unusually elevated NaCl solution intakes attained in the present experiment and the graded, dose-related response obtained throughout most of the dose range explored. The maintenance of maximum efficacy at the high end of the dose-effect relation shown in Fig. 1 contrasts with marked depression in both punished and unpunished response rates at 2.5 mg/kg clonazepam in the rat conflict test [6]. In general, it is not unusual in rehydration ingestion tests to demonstrate increased intake in spite of signs of frank sedation produced by agents such as the barbiturates [11]. However, no such signs occurred in the present experiment.

A study by Estall and Cooper [10] also explored the effect of clonazepam on rats rehydrating in a 30-min drinking period. While clonazepam produced a marked increase in isotonic NaCl (0.9%) intake at 0.31 and a peak effect at 0.63 mg/kg, larger doses (up to 5.0 mg/kg) produced progressively smaller effects. By comparison, the present study found that effects began at much lower doses and failed to decrease even at the extreme dose of 32.0 mg/kg. While the drinking periods and the NaCl solution concentrations differed in these studies, the major difference was probably the route of drug administration. The present study employed the oral route while Estall and Cooper used intraperitoneal injection. Choice of route may be critical as we found (unpublished study) that clonazepam administered subcutaneously (0.062–0.5 mg/kg) 15 min prior to drinking failed to elevate the intake of 1.5% NaCl solution over vehicle-administration levels. On the other hand, clonazepam administered intraperitoneally (0.078–1.25 mg/kg) yielded graded, dose-related increases in the intake of a palatable food [9], a result that agrees completely with the initial portion of the relation shown in Fig. 1. Larger doses of clonazepam were employed in a later study (0.625–5.0 mg/kg, IP), but the marked increase in palatable-food intake did not change as a function of dose [8]. The 1.5% NaCl solution drinking procedure, which included progressively increasing the doses beyond 1.0 mg/kg, shows that extending the clonazepam dose-effect relation reveals an elevated efficacy not detected by other behavioral techniques.

Ro 5-4864 at the dose levels used did not affect NaCl solution intake nor did it suppress clonazepam-induced solution ingestion. Ro 5-4864 at 20 mg/kg did not increase, but rather reduced, the number of shocks accepted by rats in a punished-drinking situation [18]. Similarly, low doses of Ro 5-4864 did not affect the consumption of a palatable food, but did suppress it at doses of 20 or 40 mg/kg [8]. In an unpublished study, we observed a suppressive effect on NaCl solution intake by a 16.0 mg/kg dose of Ro 5-4864. Current evidence agrees, then, that Ro 5-4864 fails as a punishment attenuator and has only suppressive effects on ingestive tests at rather high doses. Studies that have explored the interaction of other benzodiazepines and Ro 5-4864 used large doses of Ro 5-4864 (approximately 20 mg/kg) to suggest that there may be a specific pharmacologic interaction such that one agent opposes the other [18,27]. However, it is not surprising that suppressed behavior or convulsions can be somewhat ameliorated by low doses of benzodiazepines, since such doses both increase response rates [19] and are known anticonvulsants [16]. The present data do not lend strong support for graded, competitive antagonism between these agents, but rather suggest nonspecific, physiological antagonism.

Drug-enhancement of NaCl solution ingestion by rehydrating rats correlates well with the antipunishment action of those drugs [10–13, 15, 23, 24, 26]. The present data suggests that clonazepam would function as an anxiolytic agent of high potency and efficacy. While clonazepam has been used clinically mainly in the treatment of seizure disorders, it has received increasing attention in the treatment of panic disorders, agoraphobia, mania, and generalized anxiety disorder [1–3, 5, 14, 22, 25].

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