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Differential Effects of Benzodiazepine Receptor Ligands on Isotonic Saline and Water Consumption in Water-Deprived Rats

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ESTALL, L. B. AND S. J. COOPER. *Differential effects of benzodiazepine receptor ligands on isotonic saline and water consumption in water-deprived rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 247–252, 1987.—Water-deprived male rats were adapted to a 30 min test of water or saline drinking in a single-bottle acceptance test. The potent benzodiazepine agonist, clonazepam, produced significant increases in both water and saline consumption. Increases in the consumption of both were also obtained with the non-benzodiazepine agonist, zopiclone (a cyclopyrrolone), but not with the pyrazoloquinoline agonist, CGS 9896. Hence, some, but not all, benzodiazepine receptor agonists enhance drinking responses. The benzodiazepine receptor antagonists, Ro15-1788 and CGS 8216, had no significant effect on the intake of either isotonic saline or water. In contrast, the β-carboline FG 7142, which has been described as an inverse agonist acting at benzodiazepine receptors, reduced both saline and water drinking at 10 and 20 mg/kg. Although the baseline level of saline drinking was considerably higher than that of water, there was no general indication that any drug effect on consumption interacted with the type of fluid in the drinking test. However, in the case of agonist-induced increases in consumption, peak effects occurred at different doses; they were lower for saline- than for water-drinking.

Clonazepam CGS 8216 CGS 9896 FG 7142 Saline intake Ro15-1788 Thirst Water intake Zopiclone

DRUGS which act as agonists at central benzodiazepine receptors increase the level of water consumption in water-deprived rats, e.g., [7–9, 13, 36–39, 43]. There is also evidence that they enhance the ingestion of NaCl solutions. Falk and Burnidge [22] first reported that chlordiazepoxide increased the ingestion of a hypertonic NaCl solution, and their result was subsequently extended to include diazepam and the imidazobenzodiazepine, midazolam [23, 45, 46]. The effect is not limited to hypertonic saline, however, and it was shown that chlordiazepoxide increased consumption not only of water, but also of isotonic saline [47]. More generally, chlordiazepoxide raised the complete saline acceptance-rejection function for concentrations of NaCl solution ranging from 0.5–3.0% [23].

Following our earlier study [47], the aim of the present experiments was to investigate the effects of a number of relatively novel benzodiazepine receptor ligands on the consumption of water and of 0.9% NaCl solution in rehydrating rats. The potent compound, clonazepam, was chosen as a reference benzodiazepine receptor agonist. It binds selectively to 'central-type' benzodiazepine recognition sites, and has little affinity for 'peripheral-type' sites [30, 31, 40]. Two nonbenzodiazepine anxiolytics were selected for comparison to determine if they had effects similar to those of clonazepam, in the tests of saline and water drinking. Both show high-affinity binding to benzodiazepine sites [29,30]. They were the cyclopyrrolone, zopiclone [35], and the pyrazoloquinoline, CGS 9896 [49]. In behavioural tests, zopiclone produces effects which are very similar to those of the benzodiazepines [35, 41, 42]. CGS 9896 acts more selectively, and shares anxiolytic, anticonvulsant, and discriminative stimulus properties with the benzodiazepines, but lacks sedation and ataxia as side-effects [1–3, 41, 42]. Neither compound has been tested previously for effects on saline and water consumption, and we were interested to determine whether the ingestion measures would differentiate between them.

Two other benzodiazepine receptor ligands, the imidazobenzodiazepine derivative Ro15-1788, and the pyrazoloquinoline CGS 8216, have both been intensively investigated as selective benzodiazepine antagonists [18, 30, 31, 33]. Neither compound, however, is completely devoid of intrinsic activity. The data on this point has recently been reviewed for Ro15-1788 [28], and other evidence indicates that CGS 8216 can act as a weak benzodiazepine receptor 'inverse agonist' [25, 27, 34]. The detection of intrinsic activity for these drugs is likely to be test-dependent [26]. For example, Ro15-1788 exhibited partial agonist activity when water-deprived rats consumed a hypertonic NaCl solution

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[24,46], but not when they consumed water [7]. There was a failure to detect an effect of CGS 8216 on hypertonic saline consumption [24]. For purposes of comparison, we also tested the β-carboline FG 7142 [5,44], which has previously been shown to have antidipsogenic effects [10].

Using single-bottle (acceptance) tests [21], it is well-established that rehydrating rats consume far more isotonic saline than water, as a consequence of drinking for a longer duration before satiety [19,32]. Hence, the present study was designed to test for possible interactions between drug effects and the type of fluid being consumed. In addition, we determined dose-response relationships, and time-courses for drinking responses. The overall aim of the present series of experiments was to indicate ways in which benzodiazepine receptor ligands may be differentiated, according to measures of water and isotonic saline consumption.

METHOD

Animals

The subjects were 60 adult, male rats (blackhooded General strain) which were bred in the Animal Laboratory of the Psychology Department. They were housed in pairs in stainless steel cages with ad lib access to food pellets (modified Diet 41B, Heygate & Sons, U.K.). They were maintained under a 12 hr light-12 hr dark cycle (lights on at 7 a.m.) and the room temperature was kept constant at 21–22°C. The animals were accustomed to being handled, and were in the weight range 300–470 g at the start of testing.

Drugs

The following drugs were tested: zopiclone (generously donated by Rhone-Poulenc Sante) in doses of 1.25–20 mg/kg; CGS 9896 (2-(p-chlorophenyl) pyrazolo [4,3-c] quinolin-3(5H)-one) in doses of 1.25–20 mg/kg, and CGS 8216 (2-phenylpyrazolo [4,3-c] quinolin-3(5H)-one) in doses of 1.25–20 mg/kg (both generously donated by CIBA-Geigy Corp., Summit, NJ); clonazepam (0.3125–5.0 mg/kg) and Ro15-1788 (1.25–20 mg/kg) (generously provided by Hoffman-La Roche, Basel, Switzerland); FG 7142 (N'-methyl-β-carboline-3-carboxyamide) in doses of 1.25–20 mg/kg (supplied by courtesy of Dr. E. N. Petersen, Ferrosan, Soeborg, Denmark). All drugs were ultrasonically dispersed in distilled water to which Tween 80 had been added (2 drops per 10 ml). They were all administered by IP injection in a volume of 1 ml/kg. In addition, CGS 9896 was also tested following administration by oral route, cf. [14]. Injections were given 30 min prior to tests of fluid consumption.

Procedure

First, animals were adapted to a 23 hr water-deprivation schedule and to obtaining fluid from a 50 ml calibrated cylinder clipped to the front of test cages (which were identical to home-cages) in 30 min daily sessions. Following drinking sessions, the animals were returned to their home-cages for a further 30 min access to water with food. Food was not made available in test cages. The procedure was adopted of giving each rat access to a 0.9% saline solution on days which alternated with access to water. Adaptation took place over 8 consecutive days, and then drug tests were initiated.

On each day of drug testing, animals were allocated at random to 6 injection conditions (N=10 for all drug treatments, except for CGS 8216 and Ro15-1788 when N=9 per group). Each group was subdivided, and on a given day, half were given access to 0.9% saline and half to water. Doses of a particular compound would be tested, and on the following test day, the procedure was repeated with the fluid conditions reversed. Thus, for each compound tested, each animal was tested (for a given dose condition) on both saline and water drinking, with 48 hr between consecutive injections. Two- to four-day intervals separated tests with different compounds. Throughout the series of tests, baseline (control) levels of fluid consumption remained virtually constant. During drug tests, fluid intake (to nearest 0.5 ml) was measured at 6 min intervals over the 30 min test.

Statistical Analysis

Intake data were analysed using a three-way analysis of variance, with one independent factor (drug dose), and two repeated-measures factor: type of fluid (0.9% NaCl solution and water) and time periods (five 6-min intervals). To assess significant differences between individual dose conditions and the corresponding vehicle condition, Dunnett's t-test was used, following one-way ANOVAs on 30 min intake data for a particular drug and fluid condition [48].

RESULTS

Benzodiazepine Receptor Agonists

Clonazepam (0.3125–5.0 mg/kg) had a significant effect on fluid intake, $F(5,54)=3.13$, $p<0.05$. As expected, there was a marked difference between isotonic saline- and water-consumption, $F(1,54)=373.5$, $p<0.001$. Following vehicle administration, the rats consumed on average 28–29 ml of isotonic saline and 12–13 ml of water in the 30 min test (Figs. 1 and 2). The drug dose × fluid interaction term was not significant, $F(5,54)=1.87$, N.S., and clonazepam stimulated fluid consumption in the case of isotonic saline (Fig. 1) and water (Fig. 2). For saline drinking, all doses tested produced a significant elevation in the level of consumption compared with the vehicle condition ($p<0.05$, Dunnett's t-test). The peak effect occurred at a dose of 0.625 mg/kg, at which dose animals consumed an additional 10.7 ml in excess of the control level of intake (Fig. 1). This represents a maximum 38.1% increase in intake. Larger doses of clonazepam produced progressively less increases in saline consumption (Fig. 1). In the case of water drinking, doses of 0.625 mg/kg and above produced significant increases in consumption ($p<0.05$). Interestingly, clonazepam's effect was dose-related, and the peak effect occurred at 5.0 mg/kg (Fig. 2). At this dose, water intake was increased by 8.5 ml above baseline, representing a maximum 70.2% increase in consumption. Hence, although there was no overall interaction effect between drug treatments and fluid condition, it is important to note that peak effects occurred at different doses (an eight-fold difference). In absolute terms, the maximal increase in saline-drinking was slightly greater than that for water. In percentage terms, due to the lower baseline of water-drinking, the increase in water consumption greatly exceeded that for isotonic saline consumption.

Zopiclone (1.25–20 mg/kg) also increased fluid consumption, $F(5,54)=2.90$, $p<0.05$. There was also no significant drug dose × fluid interaction term, $F(5,54)=1.37$, N.S. For saline drinking, all doses tested produced a significant elevation of consumption ($p<0.05$). A peak occurred at 2.5 mg/kg, with progressively less of an effect with increasing dose

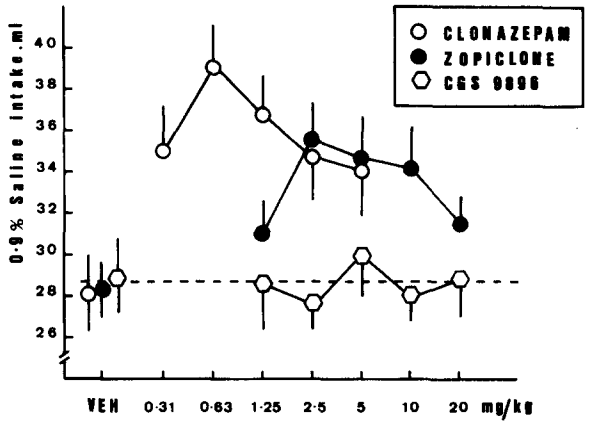

FIG. 1. Consumption of 0.9% saline (ml) in a 30 min test in rehydrating rats, following IP administration of clonazepam, zopiclone and CGS 9896. Results are shown as mean intake (±S.E.M.). N=10 per group. The horizontal dashed line represents the average baseline intake (VEH) across the three drug conditions. All doses of clonazepam and zopiclone produced significant increases in saline consumption ($p<0.5$, at least, Dunnett's t-test). CGS 9896 had no effect on saline intake.

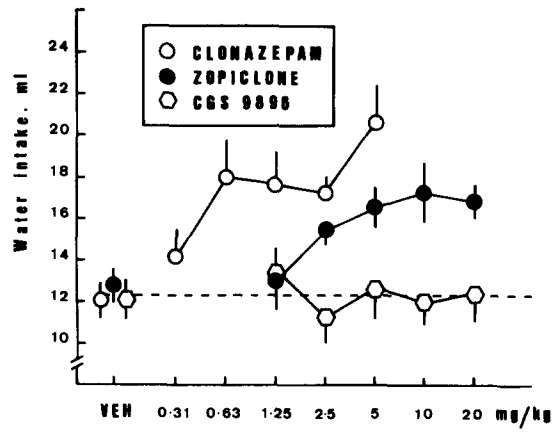

FIG. 2. Consumption of water (ml) in a 30 min test in rehydrating rats, following IP administration of clonazepam, zopiclone and CGS 9896. Results are shown as mean intake (±S.E.M.). N=10 per group. The horizontal dashed line represents the average baseline intake (VEH) across the three drug conditions. Doses of 0.625 mg/kg and above of clonazepam, and 2.5 mg/kg and above of zopiclone, produced significant increases in water intake ($p<0.05$, at least, Dunnett's t-test). CGS 9896 had no effect on water consumption.

levels (Fig. 1). The maximal elevation of consumption was 7.3 ml, representing an increase of 25.7%. Again, the dose-response relationship was somewhat different for the water-consumption data (Fig. 2). Doses of 2.5 mg/kg and above produced significant increases in intake ($p<0.05$), with a peak effect occurring at 10 mg/kg. At this dose, water intake was increased by 4.4 ml above baseline, an increase of 34.5%.

The pyrazoloquinoline CGS 9896 (1.25–20 mg/kg, IP) had no effect on either saline (Fig. 1) or water (Fig. 2) consumption at any dose tested. Furthermore, when the experiment was repeated using the oral route of administration, there was also no significant effect of CGS 9896 at any dose on fluid consumption (data not shown). In water-deprived animals, therefore, CGS 9896 lacked hyperdipsic activity.

Figure 3 illustrated examples of the time-course of drinking following clonazepam administration. Peak drug effects are shown for comparison. When drinking water, the animals showed rapid satiation and most of their drinking took place within the first 6 min period (panel B). When drinking isotonic saline, satiation was retarded, and there was still a substantial amount of drinking in the second 6 min period (panel A). Despite the baseline difference, the effect of clonazepam was similar in the two cases. For saline drinking, clonazepam (0.625 mg/kg) produced significant increases in consumption during the third and fourth 6-min periods, at which time the control level of consumption was falling (panel A). The same effect occurred earlier in the case of water drinking following clonazepam at 5.0 mg/kg, during the second and third 6-min period, when the level of consumption was also falling (panel B). The intervals at which significant clonazepam effects occurred were dependent on the temporal pattern of drinking. Similar effects, but not as pronounced, occurred with zopiclone (not illustrated).

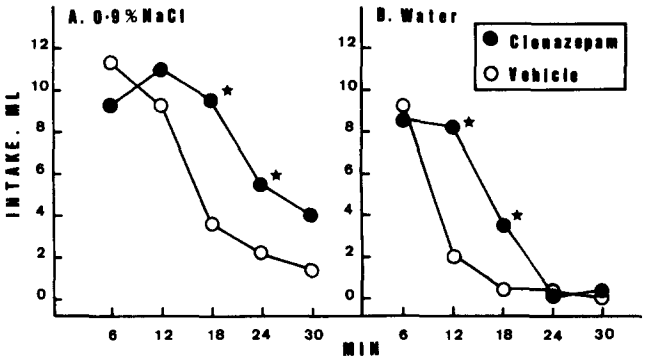

FIG. 3. Time-course for 0.9% NaCl consumption following clonazepam (0.625 mg/kg, IP) or vehicle injection (panel A); time-course for water consumption following clonazepam (5.0 mg/kg, IP) or vehicle injection (panel B). Results are shown in terms of mean intake (ml) for consecutive 6 min intervals over a 30 min test. N=10 per group. Significant increases in consumption produced by clonazepam in comparison with baseline drinking: ☆$p<0.05$ (Dunnett's t-test).

Ro15-1788 and CGS 8216

There was no significant overall main effect of Ro15-1788 treatments on fluid consumption, $F(5,48)=0.33$, N.S., and no drug dose × fluid condition interaction, $F(5,48)=1.43$, N.S. Intake data are shown in Table 1. Likewise, for CGS 8216, there was no overall main effect of drug treatments on fluid consumption, $F(5,48)=0.37$, N.S., and no interaction with fluid condition, $F(5,48)=1.24$, N.S. Intake data are shown in Table 1.

FG 7142

There was a significant effect of FG 7142 to reduce fluid consumption, $F(5,54)=2.54$, $p<0.05$, but no interaction with the type of fluid consumed, $F(5,54)=1.23$, N.S. Much of the effect of FG 7142 on consumption occurred in the first 6 min interval, and this is reflected in a significant drug dose × time

TABLE 1

CONSUMPTION OF 0.9% SALINE AND WATER (ml) FOLLOWING ADMINISTRATION OF Ro15-1788, CGS 8216 AND FG 7142

	Dose (mg/kg)					
	0	1.25	2.5	5.0	10.0	20.0
			Ro15-1788			
Water intake	13.6 ±0.7	14.6 ±1.7	15.5 ±1.2	14.4 ±0.9	15.6 ±1.0	13.2 ±1.2
Saline intake	28.0 ±1.1	31.2 ±2.3	29.8 ±1.9	29.0 ±3.3	27.0 ±2.0	31.7 ±2.9
			CGS 8216			
Water intake	13.2 ±1.2	13.4 ±1.3	13.1 ±1.0	13.7 ±1.5	13.0 ±1.3	11.4 ±1.4
Saline intake	25.4 ±1.3	25.8 ±3.4	30.4 ±3.1	26.6 ±1.7	26.3 ±2.7	25.8 ±1.9
			FG 7142			
Water intake	12.1 ±1.3	12.9 ±0.8	10.8 ±1.0	12.1 ±1.2	9.0* ±1.3	9.2* ±1.9
Saline intake	28.0 ±2.6	27.5 ±1.2	27.4 ±1.7	24.9 ±2.7	18.2* ±2.9	19.1* ±2.9

Results are shown as mean ± S.E.M. intake in a 30 min period. N=9 per group for Ro15-1788 and CGS 8216; n=10 per group for FG 7142.

Level of significance: *$p<0.05$ (Dunnett's t-test).

interval interaction, $F(20,216)=2.63, p<0.001$. The results of the first 6 min interval are shown in Fig. 4. At 10 and 20 mg/kg, FG 7142 produced significant reductions ($p<0.05$) in drinking, and the effects were very similar for the two conditions of saline and water drinking. Smaller doses were ineffective. Thirty-minute intake data are shown in Table 1.

DISCUSSION

The potent benzodiazepine agonist, clonazepam, stimulated the consumption of water and isotonic saline in rehydrating rats, confirming earlier observations on benzodiazepine-induced hyperdipsia [9,12], and indicating that this effect was due to actions at central-type receptors. Zopiclone and CGS 9896, two nonbenzodiazepines which act as benzodiazepine agonists, were completely dissociable in terms of drinking responses. Zopiclone, like clonazepam, increased the consumption of both isotonic saline and water, although it was less potent and its effects less marked. In contrast, CGS 9896 administered by either oral or intraperitoneal route, had no effect on drinking responses.

Both zopiclone and CGS 9896 share anxiolytic and anticonvulsant properties of classical benzodiazepine agonists [1–3, 29, 30, 35, 41, 42, 50]. Nevertheless, they were clearly differentiated in terms of isotonic saline and water consumption by thirsty rats. There is a parallel, here, with tests of food consumption. Zopiclone, like benzodiazepines, increased consumption of food by deprived [42] and nondeprived [16] rats. On the other hand, CGS 9896 had little or no effect on feeding in deprived or nondeprived rats [4, 14, 42].

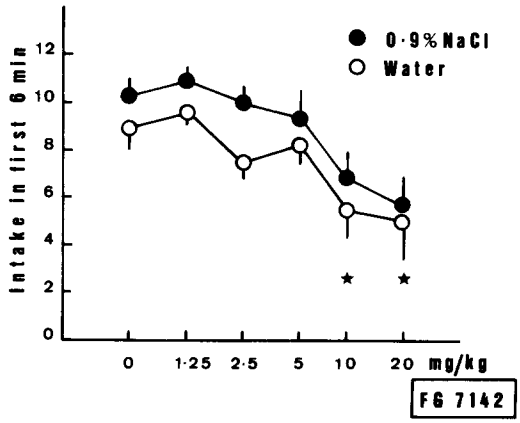

FIG. 4. Reduction in consumption of isotonic (0.9%) saline and water by water-deprived rats following administration of the β-carboline FG 7142. Results are shown as mean intake (±S.E.M.) for the first 6 min interval of a 30 min test. Significant reductions occurred at 10 and 20 mg/kg (IP): ☆$p<0.05$ (Dunnett's t-test).

This suggests that CGS 9896 may differ from benzodiazepines and zopiclone, and behave as an agonist in tests of anxiolytic and anticonvulsant activity, but to be virtually inactive as an agonist in tests of ingestional responses.

Neither Ro15-1788 or CGS 8216, benzodiazepine receptor antagonists, had effects on either saline or water drinking. Interestingly, Ro15-1788 has exhibited partial agonist activity in a test of hypertonic saline ingestion [24,46]. Hence, using water-deprived rats, the type of available fluid in the acceptance test appears to be an important determinant of partial agonist activity. This provides a pharmacological indication that somewhat different mechanisms may be involved in determining effects of benzodiazepine receptor ligands on hypertonic saline drinking. Unlike Ro15-1788, CGS 8216 did not affect hypertonic saline consumption [24], and present studies show that it did not affect consumption of water or isotonic saline, either.

Comparisons with feeding behaviour for these two compounds are particularly interesting. Ro15-1788 lacked effect on food intake on food-deprived animals [4], and in nondeprived animals eating a palatable diet [11,14]. However, in comparable tests, CGS 8216 produced significant reductions in food consumption [4,11], and its anorectic effect appears to be benzodiazepine-receptor mediated [20]. Thus, CGS 8216 appears to act selectively; in drinking tests, it was devoid of activity, but in feeding tests, it reduced food intake. The difference cannot be accounted for in terms of differences in motor requirements for ingestion, since licking for nutritive milk and sucrose solutions was also reduced by CGS 8216 (Estall and Cooper, in preparation). Consumption of water or of salt solutions were unaffected by CGS 8216. The β-carboline FG 7142 appears to act differently from CGS 8216. It not only reduced food consumption [11,17] but it also reduced the ingestion of isotonic saline and water (present results). The basis for effects of FG 7142 remain uncertain, but our data connot exclude a non-specific depression or interference with feeding and drinking.

Until the advent of novel benzodiazepine receptor ligands, the pharmacological picture concerning benzodiazepines and their effects on feeding and drinking responses was relatively straightforward. The consumptions of

water, solutions and food were increased [6, 9, 12]. The present set of results, taken in conjunction with previously-published work, reveals major qualitative and quantitative distinctions between effects of a variety of benzodiazepine receptor ligands. In general, the distinctions that can be drawn in relation to drinking responses apply also to feeding behaviour. The contrast between CGS 8216 and FG 7142 remains as an apparent exception, since our results did not show a decrease in either saline or water consumption with the former compound. When ingestional responses are compared with data from tests of anxiolytic/anxiogenic activity, the pyrazoloquinolines, CGS 9896 and CGS 8216, are particularly remarkable. CGS 9896 has anxiolytic effects [1–3, 42, 43], but did not affect drinking responses (present results) or food intake [4,14]. CGS 8216 is active in some tests of anxiogenic activity [27,44] but did not depress consumption of saline or water. Whether such distinctions will be explicable in terms of benzodiazepine receptor subtypes, or partial agonism at a single type of receptor, remains to be determined [15].

There was no general evidence for interactions between effects of benzodiazepine receptor ligands, and the type of fluid consumed, despite the appreciable difference between water and saline drinking conditions. Hence, there appeared to be no major dependency of drug effect on either taste or baseline level of consumption. Nevertheless, there were differences in detail. Thus, peak effects for clonazepam and zopiclone occurred at different doses for the two fluid conditions.

Finally, the time course data indicated that effects of the benzodiazepine receptor agonists became evident when baseline levels of drinking were beginning to fall. Consequently, the times at which effects occurred differed between the saline- and water-drinking conditions. The overall effect of agonists was to prolong the initial period of avid drinking, cf. [8, 13, 43, 47]. The antidipsogenic effect of FG 7142 was observed at an early stage in the drinking tests.

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