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Novel Benzodiazepine Receptor Ligands Stimulate Intake of Hypertonic NaCl Solution in Rehydrating Rats

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COOPER, S J *Novel benzodiazepine receptor ligands stimulate intake of hypertonic NaCl solution in rehydrating rats* PHARMACOL BIOCHEM BEHAV 27(3) 425–430, 1987 —Experiments were conducted to determine the degree of generality of previous findings that anxiolytics increased the ingestion of hypertonic saline in rehydrating rats Further, potential differential effects amongst recently described benzodiazepine receptor partial agonists were explored Finally, the hypothesis that benzodiazepine receptor partial inverse agonists would decrease the ingestion of hypertonic NaCl solution was tested Results indicated that full agonists (midazolam, ZK 93423, zopiclone) produced substantial dose-related increases in hypertonic saline consumption The putative 5-HT_{1A} agonist, buspirone, produced only a dose-dependent decrease in saline intake Partial agonists fell into two distinct categories ZK 91296, CL 218,872 and two novel benzodiazepines, Ro16-6028 and Ro17-1812, also increased saline ingestion In contrast, two pyrazoloquinolines, CGS 9896 and CGS 9895, had no significant effect on intake Two compounds, CGS 8216 and FG 7142, described as benzodiazepine partial inverse agonists, did not significantly affect consumption of the hypertonic saline

Benzodiazepines Buspirone CGS 8216 CGS 9896 CGS 9895 CL 218,872 FG 7142 Midazolam Ro16-6028 Ro17-1812 ZK 93423 ZK 91296 Zopiclone Hypertonic saline Rats Water-deprivation

ANXIOLYTICS, including the benzodiazepines chlordiazepoxide, diazepam and midazolam, and the barbiturate phenobarbital, increase the acceptance of hypertonic saline in rehydrating rats [11, 12, 34, 38, 39] The effect is independent of renal function, since bilateral nephrectomy did not interfere with diazepam-induced NaCl solution intake [38] In recent years, a number of nonbenzodiazepine compounds have been synthesised, which display high specific affinities for benzodiazepine receptors in the brain [16, 18, 40] They include full agonists, e g , the pyrrolopyrazine derivative, zopiclone [21,22], and the β-carboline ZK 93423 [35,37] The first aim of the present series of experiments was to determine if these anxiolytics would also increase acceptance of a hypertonic NaCl solution To take this question further, a nonbenzodiazepine anxiolytic was also tested, whose effect is not mediated by benzodiazepine receptors Buspirone was chosen as a representative of a class of putative 5-HT_{1A} agonists [28] Comparisons between these compounds and the established benzodiazepines may indicate to what extent hypertonic saline ingestion is generally indicative of anxiolytic activity

In benzodiazepine receptor pharmacology, there is growing interest in *partial* agonists, which retain anxiolytic effects but which show a reduction in, or absence of, behaviourally-depressant side-effects (sedation, ataxia, muscle relaxation) The forerunner of these interesting compounds is the triazolopyridazine derivative CL 218,872, which shares anticonflict and anticonvulsant effects with benzodiazepines, but was initially reported to produce few side-effects [25] Later reports disputed the selectivity of CL 218,872, however, [15,26] Nevertheless, several partial agonists have since been developed, and these compounds do display anxiolytic properties together with a reduced potency to produce side-effects They include the β-carboline ZK 91296 [27,31], the two pyrazoloquinolines CGS 9896 and CGS 9895 [1–4], and novel benzodiazepine derivatives, Ro16-6028 and Ro17-1812 [17] Few data have been published on the latter two compounds, although in rats Ro16-6028 appears to be more potent than diazepam in a conflict test and as an anticonvulsant, but is devoid of sedation and ataxia as side-effects, Ro17-1812 has also anxiolytic and anticonvulsant effects, and gives shallow dose-response curves for reduction of activity in an open field test [17] Since these compounds are likely to be unfamiliar, Fig 1 shows their chemical structures, which are related to that of the benzodiazepine receptor antagonist Ro15-1788 [18]

Nothing so far has been published concerning the possible effects of these partial agonists on the ingestion of hypertonic saline Hence, the second principal aim of these experiments was to establish whether some, all or none of these compounds would prove effective in increasing hypertonic NaCl soltuion intake This is an important issue, since each of these compounds exhibits some anxiolytic activity in one or more animal models of anxiety A particular point of interest

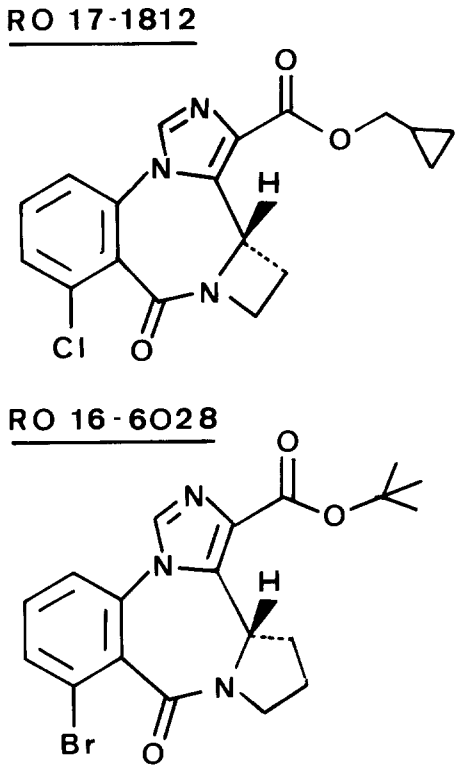

FIG 1 Chemical structure of Ro17-1812 and Ro16-6028

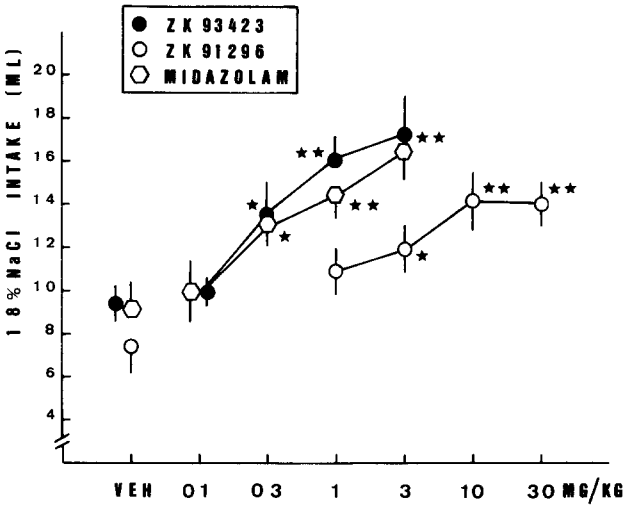

FIG 2 Increased intake of 1 8% NaCl solution produced by midazolam, the β-carboline full agonist ZK 93423 and the β-carboline partial agonist ZK 91296, in rehydrating rats Results are shown in terms of mean intake (ml) ±S E M (N=9 per group) Levels of significance in comparison with control (VEH) group *$p<0$ 05, **$p<0$ 01 (Dunnett's t-test)

was to determine if the test of hypertonic saline ingestion would differentiate between different classes of partial agonists

Finally, a number of compounds active at benzodiazepine receptors have been described as *inverse agonists* [5] Amongst the partial inverse agonists are the pyrazoloquinoline CGS 8216 [42,43] and the β-carboline FG 7142 [19,20] Both appear to have anxiogenic properties in several animal models [13, 14, 29], and therefore the hypothesis was tested that these compounds would decrease hypertonic saline ingestion

Taken together, the data of these experiments should extend our understanding of benzodiazepine receptor-related effects on the control of hypertonic saline ingestion in rehydrating rats

METHOD

Animals

The subjects were 45 adult, male rats (blackhooded General strain) which were bred in the Psychology Department, University of Birmingham They were housed individually in stainless steel cages with ad lib access to food pellets (modified Diet 41B, Heygate and 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 20–21°C The animals were accustomed to being handled, and weighed 300–360 g at the start of the studies

Drugs

The following drugs were used midazolam bimaleate (supplied by Roche Products LTD , U K), the β-carbolines ZK 93423 and ZK 91296 (6-benzyloxy- and 5-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylic acid ethyl ester, respectively), which were supplied through the courtesy of Dr D N Stephens, Schering, West Berlin, the β-carboline FG 7142 (β-carboline-3-carboxylic acid methylamide), which was generously donated by Ferrosan, Denmark through the courtesy of Dr E N Petersen, the benzodiazepines Ro16-6028 (tert-butyl(S)-8-bromo-11,12,13,13a-tetra-hydro-9-oxo-9H-imidazo[1,5-a]pyrrolo-[2,1-c][1,4]benzodiazepine-1-carboxylate) and Ro17-1812 (cyclopropylmethyl(S)-8-chloro-12,12a-dihydro-9-oxo-9H, 11H-aceto[2,1-c]-imidazo-[1,5-a][1,4]benzodiazepine-1-carboxylate), which were kindly supplied through the courtesy of Professor W Haefely, Hoffman-La Roche, Basel, the pyrazoloquinolines CGS 9896 (2-(p-chlorophenyl) pyrazolo [4,3-c] quinolin-3(5H)-one), CGS 9895 (2-(p-methoxyphenyl) pyrazolo[4,3-c]quinolin-3(5H)-one), and CGS 8216 (2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one), which were donated by Ciba-Geigy Corp , Summit, NJ, buspirone hydrochloride, which was generously supplied by Bristol-Myers, Evansville, IN, zopiclone (6-(5-chloro-2 Pyridyl)-6-7-dihydro-7-oxo-5H-pyrrolo [3,4-6]pyrazintyl] 4-methyl-1-piperazine carboxylate), which was obtained from Rhone-Poulenc Sante, France

Midazolam bimaleate and buspirone hydrochloride were dissolved in isotonic saline, and their doses are expressed in terms of salts The other drugs were prepared by ultrasonic dispersion in distilled water to which Tween 80 was added (2 drops in 10 ml) The suspensions were made up immediately before use Injections were administered by intraperitoneal route 20–25 min before the test of hypertonic NaCl intake, with the exception of FG 7142 which was injected by the same route 5–10 min before the test session Drug doses were chosen on the basis of pilot work, or on previously published data

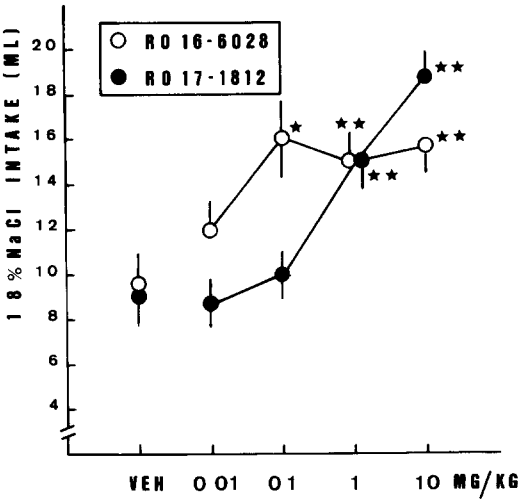

FIG 3 The benzodiazepines, Ro16-6028 and Ro17-1812, described as partial agonists [17], produced marked increases in hypertonic saline intake Results are shown in terms of mean intake (ml) ±S E M (N=9 per group) Levels of significance in comparison with control (VEH) group *$p<0$ 05, **$p<0$ 01 (Dunnett's t-test)

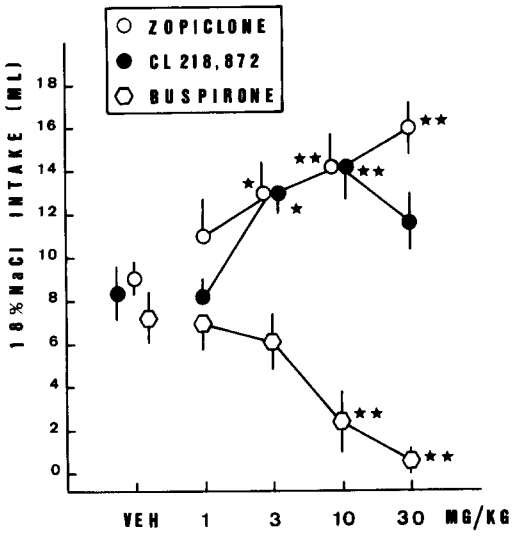

FIG 4 Increases in intake of 1 8% NaCl produced by zopiclone and CL218,872 In contrast, the putative 5-HT_{1A} agonist, buspirone, suppressed hypertonic saline consumption Results are shown in terms of mean intake (ml) ±S E M (N=9 per group) Levels of significance in comparison with control (VEH) group *$p<0$ 05, **$p<0$ 01 (Dunnett's t-test)

Procedure

The animals were first adapted to a 22 hr water-deprivation schedule and to obtaining a 1 8% NaCl solution from a 25 ml calibrated cylinder clipped to the front of the test-cage (which was identical to the home-cage) in daily 30 min sessions Following the drinking sessions, the animals were returned to their home-cages for a further 90 min access to water with food Food was not available in the test-cage This procedure was continued over a 10-day period, and then drug tests were initiated

On each day of drug testing, animals were allocated at random to 5 injection conditions for a chosen compound (N=9 per group), including one control injection group The hypertonic NaCl solution was available in the drinking tube, and intake (to the nearest 0 5 ml) was measured over the 30 min test period Two compounds were tested each week, with two- or three-day intervals between tests There was no systematic order for the series of drug tests, except to note that the first drug to be examined was midazolam (which has been reported previously to enhance hypertonic saline intake [12]) and the final one was FG 7142 In the intervals between drug-testing, the animals continued to receive 30 min daily sessions in test-cages, and on half the occasions water was available in the drinking tube, and on the other half, the hypertonic saline was available It was possible to check, therefore, that the level of consumption of hypertonic NaCl solution remained below that of water consumption over the same 30 min period Throughout the series of drug tests, baseline (control) levels of 1 8% NaCl consumption remained very stable As far as could be judged, there was no drug-order effect on intake, although this was not investigated systematically

The saline intake data were analysed using a one-way analysis of variance for independent groups, followed by Dunnett's t-test for comparisons between individual dose conditions and the corresponding vehicle condition [41]

RESULTS

In agreement with earlier work, e g [11], the level of consumption of the 1 8% NaCl solution remained below that of water For example, half-way through the sequence of drug testing, on two consecutive non-drug days, the intake of water in 30 min following 22 hr water-deprivation was 12 1±0 5 ml (mean±S E M) The corresponding intake of 1 8% saline was 8 7±0 6 (N=45 in each case) The difference is highly significant ($p<0$ 001, correlated t-test), and was typical of the differential levels maintained throughout the experiments

Figure 2 shows the intake of hypertonic NaCl following the administration of midazolam and two β-carbolines, ZK 93423 (a full agonist) and ZK 91296 (a partial agonist) Confirming earlier work, midazolam significantly enhanced hypertonic NaCl consumption, $F(4,40)=6$ 12, $p<0$ 001 The effect was a monotonic function of the dose, with a minimum effective dose of 0 3 mg/kg Intake was increased from the control level of 9 3 ml up to 16 5 ml (77 4% increase) The β-carboline ZK 93423 had an effect which was very similar to that of midazolam, producing a significant dose-related increase in intake, $F(4,40)=8$ 97, $p<0$ 001, with a minimum effective dose of 0 3 mg/kg The partial agonist ZK 91296 also increased hypertonic saline intake significantly, $F(4,40)=5$ 75, $p<0$ 001 However, it was slightly less potent with a minimum effective dose of 3 0 mg/kg Increasing the dose to 10 mg/kg, produced an additional increase in the salt intake, but a further increase in dose to 30 mg/kg had no greater effect on intake No side-effects were noticeable with ZK 91296 at any dose tested, although there were signs of some sedation with the highest dose of midazolam and ZK 93423 tested

Figure 3 illustrates the results obtained with the two novel benzodiazepine partial agonists, Ro16-6028 and Ro17-1812 The data indicate that Ro16-6028 is a potent compound, significantly increasing hypertonic NaCl intake, $F(4,40)=3$ 57,

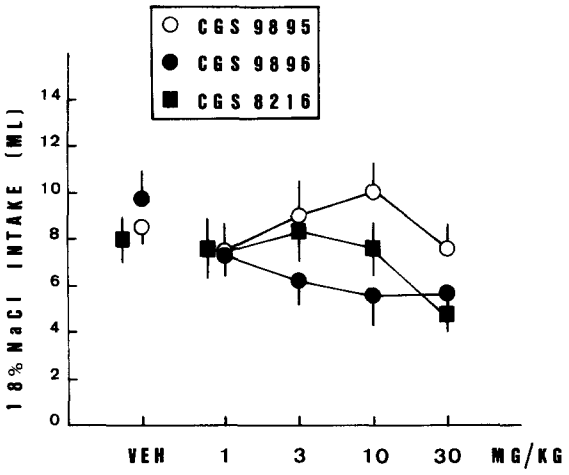

FIG 5 The pyrazoloquinoline partial agonists CGS 9896 and CGS 9895, and the partial inverse agonist CGS 8216, had no significant effects on hypertonic NaCl intake in rehydrating rats

$p<0$ 05, with a minimum effective dose of 0 1 mg/kg However, its effects on salt consumption was not a monotonic function of its dose At 0 1 mg/kg, intake increased from a control level of 9 7 ml up to 15 9 ml (a 63 9% increase) Multiplying the dose a 100-fold had no additional effect on intake The log dose-response relationship for Ro17-1812 was somewhat different It significantly increased salt intake, $F(4,40)=15$ 54, $p<0$ 001, with an effect that was linearly-related to log dose The maximum effect obtained with Ro17-1812 was to increase consumption up to 18 7 ml (an 107 7% increase)

Figure 4 shows that zopiclone increased hypertonic NaCl intake, $F(4,40)=3$ 92, $p<0$ 01, with a minimum effective dose of 3 0 mg/kg Its effect was linearly related to log dose CL 218,872 also increased salt intake, $F(4,40)=4$ 64, $p<0$ 005, with significant effects at 3 0 and 10 mg/kg However, at the higher dose of 30 mg/kg, the significant effect was lost In complete contrast to all the compounds so far considered, buspirone (1–30 mg/kg) produced a dose-dependent *decrease* in hypertonic NaCl intake, $F(4,40)=7$ 35, $p<0$ 001 At the highest dose tested, consumption of the salt solution was almost completely blocked

Analyses of variance indicated that none of the pyrazoloquinolines had a significant overall main effect on hypertonic saline intake (Fig 5) The two agonists, CGS 9896 and CGS 9895, were ineffective, although there was a tendency for intake to rise at 10 mg/kg of CGS 9895, and for it to fall after CGS 9896 treatments The partial inverse agonist, CGS 8216, was also relatively ineffective, although there was a tendency for intake to fall at 30 mg/kg

Finally, over the dose-range 0 1–3 0 mg/kg, FG 7142 also had no significant effect on hypertonic NaCl intake (Table 1)

TABLE 1

CONSUMPTION OF 1 8% SALINE FOLLOWING ADMINISTRATION OF FG 7142 (0 1–3 0 mg/kg, IP) IN A 30 MIN TEST

Dose (mg/kg)	0	0 1	0 3	1 0	3 0
Intake (ml)	9 8	10 2	9 7	10 5	8 8
	±1 0	±1 4	±1 3	±0 9	±1 2

Results are shown as mean intake ±S E M (N=9 per group)

DISCUSSION

The present data extend earlier evidence that full agonists acting at benzodiazepine receptors increase the ingestion of hypertonic saline in rehydrating rats [11, 12, 38, 39] ZK 93423 and zopiclone, like midazolam and other benzodiazepines, produced large dose-related increases in salt intake Nevertheless, this result cannot be generalised to all anxiolytics, since buspirone (a putative 5-HT_{1A} agonist) did not increase ingestion, and at high doses produced a marked suppression of NaCl solution intake (Fig 3)

The most interesting new information derives from the series of tests with the partial agonists The test of hypertonic saline intake distinguishes between them Several compounds (CL 218,872, ZK 91296, Ro16-6028, Ro17-1812) were effective, and produced significant increases in NaCl solution intake This result shows that the increased ingestion of hypertonic saline can be dissociated from the production of typical benzodiazepine side-effects There is considerable evidence, for example, that ZK 91296 (at doses used in the present work) does not produce behavioral depression [27,31] Also, we failed to find any depressant effect of either Ro16-6028 or Ro17-1812 on activity of rats in an open-field test, at doses which increase hypertonic NaCl ingestion (Yerbury and Cooper, unpublished data) Hence, the effect on hypertonic saline intake cannot be due to a reduction of competing behavioural responses

However, the pyrazoloquinolines CGS 9896 and CGS 9895 failed to have a significant effect on saline consumption Yet, these compounds are effective anxiolytics in several animal models (e g , rat conflict test, mouse fear-conditioning test) [1, 3, 32, 33] This points to the conclusion that within the class of anxiolytics acting through benzodiazepine receptors, some but not all are effective in increasing the consumption of hypertonic saline

The present data provide a strong empirical link between the hypertonic saline drinking test and results obtained in a palatable food consumption test with partial agonists CL 218,872 [9], ZK 91296 [6], Ro16-6028 and Ro17-1812 (Yerbury and Cooper, unpublished results), stimulated ingestion of palatable food CGS 9896 and CGS 9895, in contrast, did not [8,10] It is a question for future research to determine the basis for the strong degree of concordance between results obtained in hypertonic saline and palatable food ingestion tests There may be an association, or overlap, between subpopulations of brain benzodiazepine receptors involved in the two types of ingestional response

The reason for the differences between the pyrazoloquinolines and other benzodiazepine partial agonists in terms of ingestion remains to be determined Recently, Petersen *et al* [30] have argued for a distinction to be drawn between partial agonists and selective agonists acting at benzodiazepine receptors Assuming only one type of benzodiazepine receptor, compounds which retain some effects of the benzodiazepine agonists but lack others, can properly be described as *partial agonists* However, if there are benzodiazepine receptor subtypes, compounds may retain efficacy at one subtype, but have little or no efficacy at another In that case, they are more appropriately described as *selective agonists* Applied to pyrazoloquinolines, CGS 9896 and CGS 9895 may be anxiolytic because of action at one type of benzodiazepine receptor, but lack efficacy at another mediating effects on ingestion Only further research

will resolve this question, but it is worth noting that, at this stage, functionally-distinct benzodiazepine receptors deserve serious consideration

Finally, it is of considerable interest that CGS 8216 (1 0–3 0 mg/kg) did not significantly affect hypertonic saline consumption, in confirmation of earlier work [12] We have recently demonstrated that, in a dose as small as 2 5 mg/kg, CGS 8216 significantly reduced ingestion of a mixed saccharin-glucose solution [23], and significantly reduced sham-feeding a sucrose solution [24] in rats Thus in some, but not all, tests of ingestion involving drinking responses, CGS 8216 reduces intake In a similar vein, FG 7142 (0 1–3 0 mg/kg) did not reduce hypertonic saline ingestion, although at comparable doses, it significantly reduced saccharin intake [7] and sham-feeding a sucrose solution in rats (Cooper *et al* , unpublished results) As far as hypertonic saline ingestion is concerned, the description of partial inverse agonist cannot appropriately be applied to either CGS 8216 or FG 7142 Hence, we have to conclude that this type of description may be test situation-dependent Future work with these compounds will be required, to identify those factors which are important in determining which ingestional responses will be affected

In summary several compounds which have been described as partial agonists at benzodiazepine receptors increase the ingestion of hypertonic NaCl solution in water-deprived rats However, two pyrazoloquinolines, CGS 9896 and CGS 9895, failed to do so CGS 8216 and FG 7142, which have been referred to as partial inverse agonists, did not reduce ingestion of hypertonic saline Hence, descriptions of drugs in terms of partial agonists or partial inverse agonists are clearly test-dependent It seems possible that for some behavioral tests, a number of agonists or inverse agonists lack efficacy at certain (as yet undefined) benzodiazepine receptors

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