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Clonazepam-Induced Hyperphagia in Nondeprived Rats: Tests of Pharmacological Specificity With Ro5-4864, Ro5-3663, Ro15-1788 and CGS 9896

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COOPER, S. J. AND D. B. GILBERT. *Clonazepam-induced hyperphagia in nondeprived rats: Tests of pharmacological specificity with Ro5-4864, Ro5-3663, Ro15-1788 and CGS 9896.* PHARMACOL BIOCHEM BEHAV 22(5) 753–760, 1985.—Nondeprived male rats were familiarised with daily 60 min access to a highly palatable diet, consisting of powdered rat diet, sweetened condensed milk and water. Clonazepam (0.625–5.0 mg/kg, IP) produced a substantial increase in food consumption within the first 30 min of access. The increase was similar across all dose conditions, suggesting that a maximal effect may have been achieved with a dose as small as 0.625 mg/kg. The hyperphagia induced by clonazepam was reversed by the benzodiazepine receptor antagonist, Ro15-1788 (5.0–20.0 mg/kg), indicating that the effect was benzodiazepine receptor-mediated. Treatments with the peripheral-type benzodiazepine agonist, Ro5-4864, did not induce a hyperphagic response. Instead, food consumption was significantly depressed following the administration of Ro5-4864 at 20 and 40 mg/kg, IP. A comparison of the clonazepam and Ro5-4864 data suggests that benzodiazepine-induced hyperphagia is mediated by central-type benzodiazepine binding sites. The pyrazoloquinoline, CGS 9896, binds with high affinity to benzodiazepine sites and has recently been described as a nonsedating anxiolytic. CGS 9896 (2.5–20.0 mg/kg, administered either IP or PO) did not affect consumption of the highly palatable diet. In consequence, anxiolytic and hyperphagic effects of drug actions at benzodiazepine receptors may be dissociated in the case of this compound. The atypical 1,4-benzodiazepine, Ro5-3663, a GABA antagonist which may act at the picrotoxinin site, produced a dose-related reduction in food consumption. Comparison with the results for Ro5-4864 rules out an interpretation for the anorexia in terms of anxiogenic effects. Ro5-3663 (1.25–5.0 mg/kg, IP) did not diminish the hyperphagia induced by clonazepam (1.25 mg/kg), indicating that it was ineffective as an anorexic agent in the presence of clonazepam. Taken together, these results define more clearly the nature of drug-receptor interactions which may underlie benzodiazepine-induced hyperphagia.

Clonazepam CGS 9896 Food intake Ro5-4864 Ro5-3663 Ro15-1788 Palatability Rats Benzodiazepines Hyperphagia

IN recent experiments on feeding responses in the laboratory rat, we have obtained stable, high baseline levels of food consumption in nondeprived animals provided with limited daily access to a highly palatable diet [16,17]. This allows us to investigate the effects of drug treatments on feeding behaviour which is aroused in response to incentive qualities of food, as distinct from deprivation-induced feeding. Our results show that feeding which is sustained by the availability of a highly palatable diet is very sensitive to treatments which either increase or decrease consumption [16,17]. This is an important consideration for a rigorous analysis of drug effects and drug interactions since problems of ceiling or floor effects can be avoided. More detailed and less ambiguous interpretations of drug effects can be made.

Our concern is to understand the actions of drugs at benzodiazepine and at functionally-related sites in terms of their effects on feeding responses. It is well known that benzodiazepines stimulate increased food consumption, and this finding has been confirmed in a wide variety of mammalian species [14,16]. Typically, however, the increase in the amount of food consumed following benzodiazepine treatments is comparatively modest (e.g., [12, 13, 15, 33, 42, 45]). We have recently found that in nondeprived rats given access to a familiar, highly palatable diet, the maximum hyperphagic effect of a benzodiazepine treatment can be quite substantial. Thus, following intraperitoneal administration of the imidazobenzodiazepine, midazolam, food consumption increased from a baseline of 14.0 g to 25.7 g, within a 30 min test period [16]. In comparisons between drug treatments, it was found that the benzodiazepine receptor antagonist, Ro15-1788 [6, 7, 25, 28, 40], when administered alone (1.25–10.0 mg/kg, IP), had no effect on food consumption. As contrasting examples, the compounds CGS 8216 [6, 9, 18, 29] and FG 7142 [9, 10, 29], each which binds with high affinity to benzodiazepine sites, exerted potent, dose-related *anorectic* effects in nondeprived animals with access to the highly palatable diet [16].

The present series of experiments were designed to continue these initial observations. The first aim was to compare the possible effects of clonazepam and Ro5-4864 on food

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consumption. This comparison may provide a means to distinguish between so-called "central-type" and "peripheral-type" benzodiazepine binding sites in relation to benzodiazepine-induced hyperphagia. Clonazepam is a weak inhibitor of binding of labelled ligand to peripheral-type sites, but is very potent in displacing [^{3}H] diazepam from high-affinity central-type sites [1, 3, 31, 32]. Conversely, Ro5-4864 has been used as a ligand which binds selectively to peripheral-type sites. Using [^{3}H] Ro5-4864 labelling, it has been shown that sites occur in a variety of peripheral tissues, on astrocytes, cultured neuronal cells lines, and also in brain tissue [1, 2, 3, 11, 27, 31, 32, 35, 41, 46, 48, 49]. Although it is established that peripheral-type receptors are present within the central nervous system, their regional distribution within the brain and their subcellular localization differs quite strikingly from those of central-type benzodiazepine sites.

The second question to be considered is the reversibility of benzodiazepine-induced hyperphagia. Therefore, data are reported for the outcome of combined treatments with clonazepam and the benzodiazepine receptor antagonist, Ro15-1788. Thirdly, we were interested in the pyrazoloquinoline, CGS 9896. This nonbenzodiazepine compound exhibits a high affinity for rat brain benzodiazepine binding sites, but, interestingly, does not displace [^{3}H] Ro5-4864 binding from kidney or brain membranes [5, 22, 23, 53]. In several animal procedures, it has been shown that CGS 9896 has anxiolytic and anticonvulsant effects, apparently in the absence of muscle relaxation, ataxia, or other signs of neurotoxicity [4, 5, 24, 38, 47]. A question of immediate concern, therefore, was whether this compound would also induce hyperphagia in rats.

Finally, we examined the effect of Ro5-3663, an atypical 1,4-benzodiazepine, which is thought to bind to the picrotoxinin site of the chloride channel-GABA receptor-benzodiazepine complex [30, 36, 37, 50, 51]. On the basis of electrophysiological evidence, Ro5-3663 acts to antagonise inhibitory GABAergic neurotransmission [26,43], and may do so by reducing chloride ion movement into the post-junctional cell. Theoretically, therefore, Ro5-3663 should have an effect on feeding which is opposite to the effect of typical benzodiazepines, i.e., produce *hypophagia*.

Taken together, the present series of experiments were designed to provide more information of relevance to an understanding of benzodiazepine receptor mechanisms in the context of the control of feeding responses.

METHOD

Animals

The subjects were 50 adult male rats (hooded General strain) which were bred in the animal laboratory of the Psychology department. They were housed individually in stainless steel cages with continuous access to standard laboratory 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 21–22°C. The animals were weighed regularly before drug testing, to accustom them to being handled. They were in the weight range 250–350 g at the start of testing.

Drugs

In addition to the benzodiazepine agonist, clonazepam, the following drugs were tested: Ro15-1788 (ethyl 8-fluoro-5,6-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate), a benzodiazepine receptor antagonist [7, 28, 40]; Ro5-4864 (7-chloro-5-(4-chlorophenyl-1,3-dihydro-1-methyl-2H-1,4-benzodiazepine-2-one) a ligand for peripheral-type and for micromolar benzodiazepine binding sites [1, 8, 35, 46]; CGS 9896 (2-(4-chlorophenyl)-2,5-dihydropyrazolo [4,3-c] quinoline-3 (3H)-one), a nonbenzodiazepine ligand for brain benzodiazepine receptors, which has anxiolytic and anticonvulsant efficacy [4, 5, 47]; Ro5-3663 (1,3-dihydro-5- methyl-2H-1,4-benzodiazepine-2-one), an atypical benzodiazepine, with reported anxiogenic and convulsant effects [21,39]. All drugs were prepared by suspension in distilled water to which Tween 80 was added (2 drops in 10 ml), and the suspensions were made up immediately before use. With one exception, injections were always given intraperitoneally, in a volume of 1 ml/kg, 25–30 min prior to the feeding test. In the exceptional case, CGS 9896 was administered orally, 60 min before the test. The vehicle (distilled water and Tween 80) was used in the control injections.

Procedure

The animals were first familiarized with the highly palatable diet. Each day, animals were transferred to individual test cages, identical to the home cages for 60 min during the morning light period. About 30 g of freshly prepared diet placed in a clean perspex petri dish was positioned inside each test cage. The diet was made up to the following formula: 50 ml Nestles brand sweetened condensed milk, 150 ml ground rat maintenance diet No.1 (Special Diet Services Ltd, Essex, U.K.), and 200 ml distilled water. Within 10 min of thorough mixing, this food sets to a relatively firm consistency, and tastes very sweet to human tasters. The animals were adapted to this procedure over a period of two weeks, by which time latency to begin eating the diet in the test cage was at an absolute minimum in all cases, and food consumption had stabilised at an asymptotic high level. Most food was consumed within the first 30 min of the test period. The water supply and the rats' standard diet were not available during the palatable food test.

Consumption of the diet was measured by successive weighings, and intake was calculated after 30 and 60 min of the test. Weighings were made on a sensitive electronic top-loading balance (Sartorius 1203 MP) and were recorded to an accuracy of 0.1 g. Care was taken to collect any food spillage, and to make appropriate corrections to the weighings.

(1) For the first experiment, animals were allocated at random to 5 equal groups (n=10 per group), and were injected with 0, 0.625, 1.25, 2.5 and 5.0 mg/kg clonazepam, respectively. On each subsequent test, animals were reallocated at random on each occasion to 5 groups. At least three days separated consecutive tests in the animals. The experiments were then conducted in the following sequence. (2) Each animal received two injections, one given immediately after the first. One group was administered two vehicle injections, and the remaining four were administered 1.25 mg/kg clonazepam in combination with 0, 5.0, 10.0, 20.0 mg/kg Ro15-1788, respectively. (3) Five groups were administered 0, 1.25, 2.5 5.0 and 10.0 mg/kg Ro5-4864, respectively. (4) Five groups were administered 0, 2.5, 5.0, 10.0 and 20.0 mg/kg CGS 9896, respectively. (5) A week later the CGS 9896 experiment was repeated, but on this occasion the drug was administered orally, 60 min prior to the feeding test. There is evidence that it is more potent when given orally [5]. (6) Five groups were administered 0, 0.625, 1.25, 2.5 and 5.0

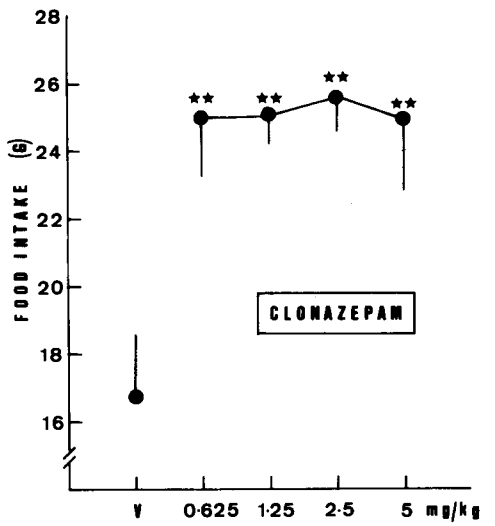

FIG. 1. Clonazepam (0.625–5.0 mg/kg, IP) produced a significant elevation in the consumption of highly palatable food in the non-deprived rat, $F(4,45)=5.09$, $p<0.005$. The results are shown as mean food intake (g) in the first 30 min period of access to the palatable diet. Vertical lines represent S.E.M.'s. N=10 per group. Level of significance for individual dose conditions compared with vehicle (V) group: $^{**}p<0.01$ (Dunnett's t test).

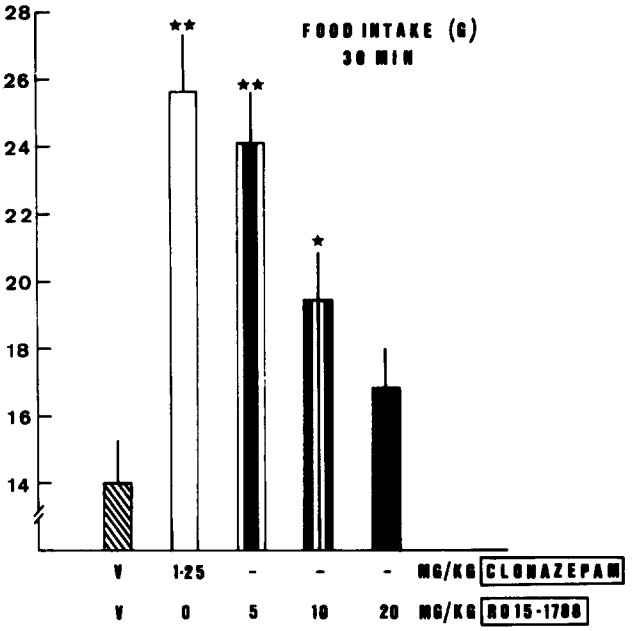

FIG. 2. Hyperphagia induced by clonazepam (1.25 mg/kg, IP) was dose-dependently reversed by the benzodiazepine receptor antagonist, Ro15-1788 (5.0–20.0 mg/kg, IP). The results are shown (left to right) for the control group intake following two vehicle (V,V) injections; 1.25 mg/kg clonazepam administered with the antagonist vehicle (unfilled histogram bar); 1.25 mg/kg clonazepam given in combination with three ascending doses of Ro15-1788, respectively (histogram bars with shading). Each histogram shows mean food intake(g) in the first 30 min period of access to the palatable diet. Vertical lines represent S.E.M.'s. N=10 per group. Levels of significance for individual dose conditions compared with control (V,V) group: $^{*}p<0.05$; $^{**}p<0.01$ (Dunnett's t-test).

mg/kg Ro5-3663, respectively. (7) Each animal received two injections, one given immediately after the first. One group was administered two vehicle injections, and remaining four were administered 1.25 mg/kg clonazepam in combination with 0, 1.25, 2.5 and 5.0 mg/kg Ro5-3663, respectively. (8) A further supply of Ro5-4864 was obtained from Hoffmann-La Roche, and three groups were injected with 0, 20.0 and 40.0 mg/kg, respectively. On days between drug testing, which continued over the course of several weeks, animals were repeatedly tested with the highly palatable diet in the absence of any injection procedure, as a check on the continuing stability of baseline food consumption.

The data for 30 and 60 min food intake were analysed using an analysis of variance procedure for independent drug groups, and comparisons between individual drug dose conditions and the control group were made using Dunnett's t-test.

RESULTS

Clonazepam

Clonazepam (0.625–5.0 mg/kg, IP) produced a very potent enhancement of feeding in the palatable food test (Fig. 1). Following administration of the drug vehicle, food intake was 16.7 g (mean value) in the first 30 min period of the feeding test, rising to 18.6 g by the end of the 60 min period. Clonazepam in doses of 0.625 mg/kg and above appeared to produce a maximal effect, when the treated rats consumed 24.9 g (mean value) in the first 30 min period of the feeding test. This increase of 8.2 g is a little less than the maximal effect observed following midazolam treatment in a previous study [16]. In a separate study, clonazepam (0.08–1.25 mg/kg) produced clear dose-related increases in food intake (Cooper and Moores, unpublished data). Clonazepam did not elevate food intake within the second 30 min period of the feeding test.

Clonazepam-Ro15-1788 Interaction

We have shown elsewhere that Ro15-1788, administered alone over a dose range of 1.25–10.0 mg/kg, had no effect on food consumption in the palatable food test [16]. As Fig. 2 shows, however, Ro15-1788 (5.0–20.0 mg/kg) antagonized the hyperphagia induced by 1.25 mg/kg clonazepam, in a dose-dependent manner. Food consumption was not significantly in excess of the control value when 1.25 mg/kg clonazepam was given in combination with 20.0 mg/kg Ro15-1788.

Ro5-4864

In the first stage of the experiment with the 'peripheral-type' benzodiazepine receptor agonist, Ro5-4864, there was no effect on the consumption of the highly palatable diet over the 60 min test period (Table 1). Later, however, when Ro5-4864 was administered in the larger doses of 20.0 and 40.0 mg/kg, significant reductions in food consumption occurred. Clearly, over the range of doses which were examined, the effects of Ro5-4864 did not mimic the hyperphagia induced by the clonazepam treatments.

CGS 9896

The data obtained in the palatable food test indicated that CGS 9896 (2.5–20.0 mg/kg), administered intraperitoneally, had no effect on food consumption (Table 2). Since CGS

TABLE 1

EFFECTS OF THE 'PERIPHERAL-TYPE' BENZODIAZEPINE RECEPTOR AGONIST, Ro5-4864 (1.25–40.0 mg/kg), ON THE CONSUMPTION OF A HIGHLY PALATABLE DIET IN NONDEPRIVED MALE RATS

	Ro5-4864 (mg/kg)				
(a)	0	1.25	2.5	5.0	10.0
30 min	13.9 ±1.1	16.1 ±1.0	12.4 ±1.3	13.0 ±1.4	16.0 ±1.7
60 min	15.6 ±1.8	17.4 ±1.2	14.5 ±1.3	14.5 ±1.7	17.9 ±1.7

	Ro5-4864 (mg/kg)		
(b)	0	20.0	40.0
30 min	17.1 ±2.0	12.6* ±1.0	10.3* ±1.2
60 min	18.5 ±1.9	14.8 ±1.2	11.7† ±1.0

Results are shown as food intake (mean ± S.E.M., n=10 per group) in grams. Ro5-4864 was injected IP 25–30 min before the feeding test. 30 min and 60 min cumulative intakes are shown. Experiment was carried out in two stages, (a) and (b). Food intake significantly less than control values: *$p<0.05$; †$p<0.01$ (Dunnett's t-test).

TABLE 2

LACK OF EFFECT OF BENZODIAZEPINE RECEPTOR AGONIST, CGS 9896 (2.5–20.0 mg/kg), ON THE CONSUMPTION OF A HIGHLY PALATABLE DIET IN NONDEPRIVED MALE RATS, FOLLOWING INTRAPERITONEAL OR ORAL ADMINISTRATION

(a) Intraperitoneal. 30 min before feeding test

	CGS 9896 (mg/kg)				
	0	2.5	5.0	10.0	20.0
30 min	15.1 ±1.1	17.0 ±1.7	13.0 ±1.6	16.1 ±0.9	15.2 ±1.7
60 min	17.4 ±1.0	19.3 ±2.1	14.7 ±1.7	17.4 ±1.1	17.3 ±1.6

(b) Oral administration. 60 min before feeding test

	CGS 9896 (mg/kg)				
	0	2.5	5.0	10.0	20.0
30 min	19.8 ±1.4	19.4 ±1.1	18.7 ±1.3	16.8 ±1.3	19.3 ±1.1
60 min	21.0 ±1.5	19.8 ±1.1	19.7 ±1.2	17.5 ±1.2	20.5 ±0.9

Results are shown as food intake (mean ± S.E.M., n=10 per group) in grams.

9896 may be considerably more potent when administered orally [5], the experiment was repeated using the oral route of administration. Howere, it remained without effect on food consumption (Table 2).

Ro5-3663

Figure 3 indicates that Ro5-3663 dose-dependently reduced the consumption of the highly palatable diet in nondeprived animals, during the first 30 min access. The anorectic effect was significant following doses of 2.5 and 5.0 mg/kg, which were subconvulsant in the strain of rats used in these experiments. There was some recovery in feeding during the second 30 min access in animals injected with 2.5 and 5.0 mg/kg (data not shown). Ro5-3663 may therefore have a relatively short duration of action.

Ro5-3663-Clonazepam Interaction

The anorectic effect of Ro5-3663 was completely reversed by 1.25 mg/kg clonazepam (Fig. 4). When given alone clonazepam 1.25 mg/kg produced a highly significant increase in the level of food consumption, confirming the finding shown in Fig. 1. This hyperphagic effect of clonazepam was not significantly affected by the concurrent administration of Ro5-3663, in doses, which when given alone were sufficient to depress the consumption of food. Ro5-3663 was ineffective in clonazepam-treated rats.

DISCUSSION

These studies first of all confirm that the consumption of a highly palatable diet by nondeprived rats provides a test system which should prove particularly useful in the pharmacological analysis of the hyperphagic effects of the benzodiazepines. Clonazepam, a potent benzodiazepine agonist, produced a striking increase in the amount of food consumed, within a 30 min test period, at dose levels of 0.625 mg/kg and greater (Fig. 1). The substantial increase in consumption which followed clonazepam treatments occurred despite the high control level of feeding. This result, taken with our similar observations obtained with midazolam [16], indicates that benzodiazepine treatments can profoundly stimulate additional food consumption in nondeprived animals given access to a familiar, highly palatable diet.

The hyperphagia induced by clonazepam was reversed dose-dependently by the benzodiazepine receptor antagonist, Ro15-1788 (Fig. 2). We have determined previously that Ro15-1788 (1.25–10.0 mg/kg, IP), when administered alone, had no effect on food consumption under the same conditions of testing [16]. Therefore, it is possible to conclude that the clonazepam-induced hyperphagia was mediated by action at benzodiazepine receptors. It should be noted that complete antagonism of the clonazepam effect was not achieved even with a 20 mg/kg dose of Ro15-1788 (Fig. 2). However, Ro15-1788 appears to have a relatively short duration of action [6], and this may have led to some underestimate of its potency in antagonizing the effect of clonazepam in the present study. There is a recent report that in rabbits, the hyperphagia which followed diazepam administration was reversed by Ro15-1788 [34]. In two species, therefore, there is now evidence that hyperphagia can result from agonist action at benzodiazepine receptors.

Benzodiazepines bind with high affinity not only to sites within the central nervous system, but also to sites in peripheral tissue, including lung, heart, adrenals and kidneys [41]. In principle therefore, behavioural effects of benzodiazepine treatments may depend on actions at centrally-located, or at peripherally-located sites, or both. Fortunately, it is possible to distinguish amongst these alternatives by using drugs which show markedly different affinities for two types of

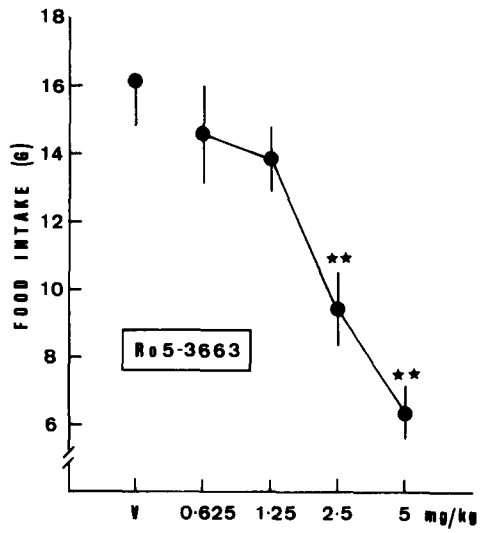

FIG. 3. The consumption of the palatable diet was dose-dependently suppressed in nondeprived rats by the atypical 1,4-benzodiazepine, Ro5-3663, $F(4,45)=8.67$, $p<0.001$. The data are shown as mean food intake (g) in the first 30 min period of access to the palatable diet. Vertical lines represent S.E.M.'s. N=10 per group. Significance level (see legend to Fig. 1).

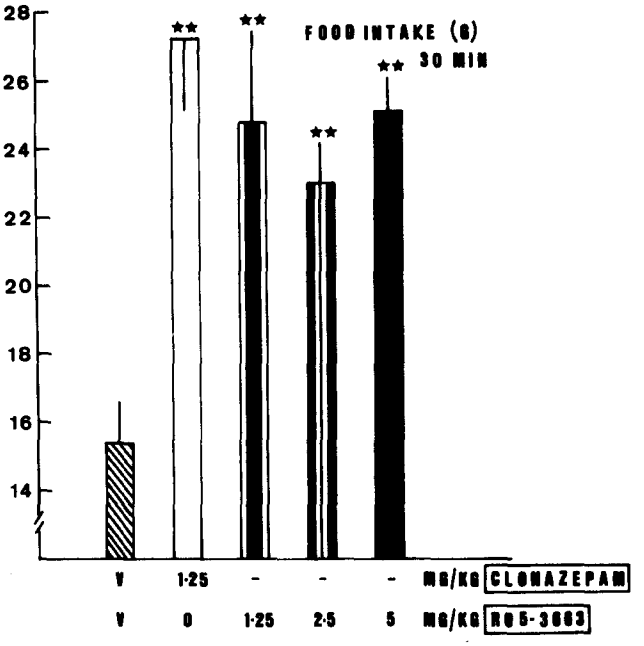

FIG. 4. The hyperphagia induced by clonazepam (1.25 mg/kg, IP) was undiminished in the presence of Ro5-3663 (1.25–5.0 mg/kg, IP). The results are shown (left to right) for the control group intake (diagonal shading); 1.25 mg/kg clonazepam administered with Ro5-3663 vehicle (unfilled histogram bar); 1.25 mg/kg clonazepam given in combination with three ascending doses of Ro5-3663, respectively (histogram bars with shading). For further explanation, see legend to Fig. 2.

receptor. Both labelled Ro5-4864 and PK11195 have been used as very potent ligands for the "peripheral type" benzodiazepine receptor [1, 2, 3, 29, 32]. Potency relationships for displacing labelled ligands from "peripheral type" receptors are reported to be Ro5-4864 > diazepam > clonazepam, but are in the reverse direction, clonazepam > diazepam > Ro5-4864, for "central type" receptors. Hence, the comparison between clonazepam and Ro5-4864, each selective for one of the two classes of benzodiazepine binding site, is an important one in understanding the receptor characteristics which underlie benzodiazepine-induced food consumption. The so-called "peripheral type" benzodiazepine binding sites have also been detected in the central nervous system [2, 3, 5, 41, 46].

It is of considerable interest, therefore, that Ro5-4864 failed to stimulate additional food consumption in our present study (Table 1). This, in contrast to the positive result obtained with clonazepam, suggests to us that "peripheral type" receptors are irrelevant to the hyperphagic effect of benzodiazepines, at least under the particular conditions used in the present study. The corollary of this conclusion is that benzodiazepine-induced hyperphagia depends on actions of drugs at "central type" receptors within the central nervous system.

Nevertheless, Ro5-4864 did have some effect on food consumption. At 20 and 40 mg/kg, Ro5-4864 reduced the level of food consumption (Table 1). However, there is evidence that at these doses, Ro5-4864 may have produced a relatively non-specific depression of behavior. In a social interaction test using rats, 20 mg/kg Ro5-4864 reduced motor activity [19], and in a holeboard test, the same dose produced a significant reduction in locomotor activity and in the number of rears, and also reduced the frequency of head-dipping [20]. More interestingly, however, Ro5-4864 dose-dependently reduced the time spent in active social interaction between pairs of male rats, over the dose range, 5–20 mg/kg [19]. Our data suggest that these effects observed with the smaller doses could not have been due to non-specific decrements in behavior. File and Lister [19], in fact, interpret their results in terms of a specific anxiogenic action of Ro5-4864, which they infer from the reduction in social interaction. If their interpretation is the correct one, then clearly under the conditions employed in our experiments, which were designed to minimize any possible anxiety- or stress-provoking environmental stimuli, the *potential* anxiogenic effect of Ro5-4864 did not affect food consumption. In larger doses Ro5-4864 is a convulsant [39]. This effect has been tentatively linked to an action of Ro5-4864 at the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex, an action it may share with other convulsants which are GABA antagonists [52]. We did not observe any convulsions induced in our rats by our dose range of Ro5-4864.

CGS 9896 is a relatively novel compound, which is structurally related to the putative benzodiazepine receptor antagonist CGS 8216 [5]. CGS 9896 binds with high affinity to benzodiazepine sites in rat brain [5, 22, 23]; it exhibits anticonvulsant activity [5] and also shows potent anxiolytic activity in several animal models [45,47]. In the present study, CGS 9896, whether administered orally or by the intraperitoneal route, did not have any effect on food consumption (Table 2). This was an interesting, but unexpected finding. First, it represents an important example of a compound which has anxiolytic effects, but fails to induce hyperphagia. This provides evidence for a dissociation between

the two effects, and thus offers further support for an earlier hypothesis concerning the benzodiazepines [14]. Second, it suggests some distinction between CGS 9896 and benzodiazepines in terms of their actions at benzodiazepine receptors.

However, the present results do not provide a unique example of a dissociation between the behavioral effects of CGS 9896 and those of benzodiazepine agonists. For example, chronic treatments with CGS 9896 apparently did not cause benzodiazepine-like dependence in baboons (data of Griffiths, cited in [5]). Further, animals trained to discriminate diazepam from vehicle did not show generalisation from the diazepam cue when tested with CGS 9896 [44]. Preliminary results of this kind require further investigation because they imply that anxiolytic and /or anticonvulsant properties of drugs may not be the best predictors of certain other behavioral effects which are closely associated with the actions of benzodiazepines. One can speculate that CGS 9896 may bind more specifically to receptors which have a particular role in mediating anxiolytic and anticonvulsant effects of the benzodiazepines. Another possibility, which has been raised elsewhere [5], is that CGS 9896 may act as a full agonist at some brain benzodiazepine receptors, but exhibit antagonist action at other benzodiazepine receptors. Certainly there are important distinctions between the binding characteristics of CGS 9896, compared with diazepam and other typical benzodiazepine agonists [5]. It will be interesting to discover the critical binding characteristics which are necessary in order to elicit the benzodiazepine hyperphagic effect. It can be noted, at this point, that the structurally-related compound, CGS 8216, is a potent anorectic agent [16].

Ro5-3663 (0.625–5.0 mg/kg, IP) produced a dose-dependent reduction in food consumption (Fig. 3). There is evidence that Ro5-3663 binds to the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex [36, 37, 50, 51], and that it antagonizes GABAergic neurotransmission in a manner which is similar to the effect of picrotoxin [26,43]. From what we understand of the mechanisms by which benzodiazepines affect GABAergic neurotransmission [9, 25, 36, 37, 50], it could be predicted that Ro5-3663 produces an anorectic effect. Although Ro5-3663 is thought to be anxiogenic in some circumstances, it is unlikely that this action explains the reduction in food intake. Although Ro5-3663 and Ro5-4864 produce a similar anxiogenic effect measured in the rat social interaction test [19,21], their effects on food consumption were different. Ro5-3663 reduced food consumption in doses which decreased social interaction, but Ro5-4864 did not. Furthermore, under the completely familiar conditions of the feeding test we employed, we never observed any sign of induced anxiety in animals treated with Ro5-3663 (i.e., no defaecation, urination, vocalisation, struggling, etc. on handling, and no behavioral signs of stress in the test situation). Therefore, the effect of Ro5-3663 in the feeding test was more likely related to an effect on the feeding response, itself.

When Ro5-3663 and clonazepam were given in combination, the hyperphagic effect of clonazepam remained unaffected (Fig. 4). This complete reversal of the anorectic effect of Ro5-3663 by clonazepam was unexpected. Further work will be needed to explain this interesting interaction, but it should be noted that it was shown recently that picrotoxin, which also antagonises GABAergic neurotransmission, failed to block chlordiazepoxide's hyperphagic effect in rats [42]. Either GABAergic neurotransmission is not really relevant to benzodiazepine-induced hyperphagia, which at present has to be considered unlikely; or occupancy of benzodiazepine receptors by agonists in some way blocks the effects of picrotoxin and Ro5-3663 at the picrotoxinin site which is involved in the control of chloride conductance. The second possibility suggests a functional interaction of some importance between benzodiazepine and picrotoxinin sites.

In summary, therefore, the present set of results provides additional pharmacological data regarding benzodiazepine-induced hyperphagia. A comparison of the results for clonazepam and Ro5-4864 indicates that the hyperphagia is most probably due to action at central-type benzodiazepine sites. In addition, the increased consumption of palatable diet produced by clonazepam was reversed by Ro15-1788, but not by Ro5-3663. These data imply that the action of clonazepam was at a benzodiazepine site, and argue against an effect at the picrotoxinin site. Finally, the putative non-sedating anxiolytic, CGS 9896, did not stimulate increased food consumption in our feeding model. This result provides pharmacological confirmation of a dissociation between anxiolytic/anticonvulsant effects, on the one hand, and hyperphagic effects, on the other, for drug actions at benzodiazepine sites.

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