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Benzodiazepine-Induced Hyperphagia in the Nondeprived Rat: Comparisons with CL 218,872, Zopiclone, Tracazolate and Phenobarbital

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COOPER, S. J. AND W. R. MOORES. *Benzodiazepine-induced hyperphagia in the nondeprived rat: Comparisons with CL 218,872, zopiclone, tracazolate and phenobarbital.* PHARMACOL BIOCHEM BEHAV **23**(2) 169–172, 1985.— Nondeprived male rats were familiarized with 30 min daily access to a highly palatable diet. Clonazepam, midazolam and chlordiazepoxide each produced significant dose-dependent increases in food consumption. Clonazepam was the most potent, and a significant hyperphagic effect was detected following 0.078 mg/kg (IP). Amongst novel non-benzodiazepine anxiolytics, zopiclone and CL 218,872 also produced significant increases in food intake. The smallest doses to produce significant hyperphagia for these two drugs were 10.0 and 2.5 mg/kg (IP) respectively. In contrast, tracazolate caused only a reduction in feeding, evident at 20 and 40 mg/kg (IP). Previous reports indicate that although benzodiazepines, zopiclone and CL 218,872 displace [^{3}H] flunitrazepam binding in rat cerebral cortex preparations, tracazolate enhances the binding. Our results are consistent with the drug-induced hyperphagia depending upon agonist actions at high-affinity benzodiazepine sites. They also provide pharmacological evidence for a dissociation between hyperphagic and anxiolytic drug effects. Phenobarbital (2.5–40.0 mg/kg), like the benzodiazepines, produced a strong stimulation of food intake, indicating that drug action at an alternative site in the benzodiazepine receptor–GABA receptor-chloride channel complex can also lead to hyperphagia.

Chlordiazepoxide Clonazepam CL 218,872 Food intake Hyperphagia Phenobarbital Rats Tracazolate Zopiclone

IT is firmly established that benzodiazepines cause increased food consumption in many mammalian species, including primates and stock animals (for reviews of the evidence, consult [1, 3, 4]). Barbiturates, which share many of the pharmacological characteristics of the benzodiazepines, also stimulate food consumption in rats [9,22]. However, there are several novel non-benzodiazepine anxiolytics for which there is little or no available evidence for possible effects they may have on food consumption. The general aim of this study was to compare the effects of several benzodiazepines with those of CL 218,872, zopiclone and tracazolate in a feeding situation which has proved very sensitive to hyperphagic and anorectic drug effects [4,5]. The effect of phenobarbital was also compared with that of the benzodiazepines, as a test for hyperphagia produced by an action at a receptor distinct from the benzodiazepine site.

The triazolopyridazine derivative, CL 218,872, shares properties with benzodiazepines, including anticonflict and anticonvulsant effects [8, 11, 14, 17]. It was suggested that this compound may be devoid of significant sedation as a side effect [11], although this suggestion has to be revised in the light of more recent evidence [14]. In a drug discrimination study, rats were trained to discriminate a chlordiazepoxide cue from saline, and showed generalization from CL 218,872 to the standard benzodiazepine treatment [12]. The same study also provided evidence for cross tolerance between chlordiazepoxide and CL 218,872 for a sedative effect. Binding studies have shown that CL 218,872 inhibits specific [^{3}H] flunitrazepam or [^{3}H] diazepam binding [8, 20, 23] and that [^{3}H] CL 218,872 labels benzodiazepine receptors in membrane preparations of rat cerebral cortex [25].

Zopiclone is a pyrrolopyrazine derivative, which also shares many properties with benzodiazepines, and is effective in tests of anticonflict and anticonvulsant activity [8, 17, 23]. Similarly, it inhibits the binding of [^{3}H] flunitrazepam or [^{3}H] diazepam to membrane preparations from rat cerebral cortex [2,8]. Tracazolate is a pyrazolopyridine derivative, which has anxiolytic activity in mice, rats and monkeys [8, 16, 17]. Unlike chlordiazepoxide, it has been reported to produce motor incoordination at doses higher than its minimally effective anticonflict doses [8,16]. Unlike benzodiazepines, CL 218,872 or zopiclone, tracazolate causes a significant enhancement of [^{3}H] flunitrazepam binding in rat synaptic membrane fragments [8,13]. Its effect on benzodiazepine binding is therefore clearly different from the other compounds.

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METHOD

Animals

The subjects were 60 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°C. The animals were weighed regularly before drug testing to accustom them to being handled. They were in the weight range 180–220 g at the start of testing.

Drugs

In addition to the benzodiazepine agonists, clonazepam, midazolam, and chlordiazepoxide, the following drugs were tested: CL 218,872 (3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4triazolo[4,3-b]pyridazine) [11,20]; zopiclone(6-(5-chloro-2-pyridyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-b]pyrazin-t-yl]4-methyl-1-piperazine carboxylate) [2]; tracazolate (4-butyl-amino-1-ethyl-6-methyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylic acid ethyl ester) [16,17]; sodium phenobarbital. Midazolam bimaleate, chlordiazepoxide hydrochloride and sodium phenobarbital were dissolved in isotonic saline at room temperature. The solutions were made immediately before use, and were protected from light. Doses are expressed in terms of salts. For these drugs, isotonic saline was used in control injections. 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, and doses are expressed in terms of bases. The suspension medium was used in control injections. All injections were given by the intraperitoneal route, in a volume of 1 ml/kg, 25–30 min prior to the feeding test.

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 30 min during the morning light period. About 35–40 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. 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 of the diet was consumed within the first 30 min of availability. The water supply and the rats' standard diet were not available during the palatable food test. Consumption of the diet was measured after 30 min of the test, and weighings were made on an 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 correction to the weighings.

For each drug tested, the animals were allocated at random to six groups of ten animals each, and each group was assigned to a separate dose condition, including vehicle as 0 mg/kg. Previous experience has shown that in rats which are highly familiarised with the test procedure, baseline levels of food intake remain stable over repeated testing [5]. One drug was tested at a time, and a non-benzodiazepine was alternated with a benzodiazepine compound to balance order effects. For each animal, at least three days separated consecutive drug trials, to avoid drug carry-over effects. The food intake data for each compound were analysed using a one-way analysis of variance (ANOVA) procedure for independent drug groups, and comparisons between individual drug dose conditions and the control vehicle (0 mg/kg) were made using Dunnett's *t*-test [24]. Tests for linear, quadratic and cubic trends were also carried out on the dose-response data for each drug. Only significant ($p<0.05$) trends are described in the text.

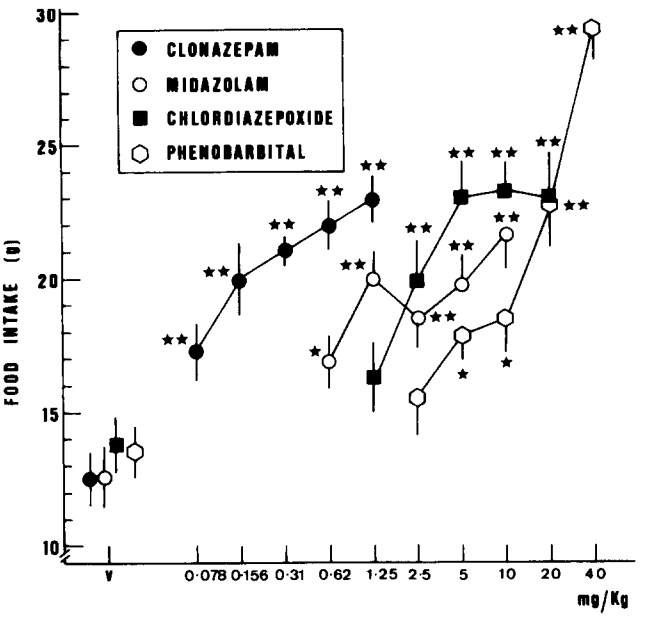

FIG. 1. Hyperphagia produced by clonazepam, midazolam, chlordiazepoxide and phenobarbital in nondeprived rats in a 30 min feeding test. Results are shown in terms of mean (±SEM) food intake (grams). N=10 per group. Levels of significance for comparisons between individual dose conditions and corresponding vehicle (V) treatments: *$p<0.05$; **$p<0.01$ (Dunnett's *t*-test).

RESULTS

Benzodiazepines

Clonazepam (0.078–1.25 mg/kg) produced a highly-significant, dose-related increase in food consumption, $F(5,54)=13.55$, $p<0.001$. Trend analysis confirmed a significant linear trend, relating drug doses to the increase in level of food consumption, $F(1,54)=60.60$, $p<0.001$. Figure 1 shows that following the administration of the smallest dose, 0.78 mg/kg, clonazepam had a significant hyperphagic effect.

Midazolam (0.625–10.0 mg/kg) had a highly-significant dose-related effect on food intake, $F(5,54)=5.82$, $p<0.001$. Linear and cubic trends were significant, $F(1,54)=22.30$, $p<0.001$ and $F(1,54)=5.83$, $p<0.05$, respectively. Each dose of midazolam produced a significant increase in food intake (Fig. 1).

Chlordiazepoxide (1.25–20.0 mg/kg) also exerted a highly-significant hyperphagic effect, $F(5,54)=8.25$, $p<0.001$. There was a highly-significant linear trend in the data, $F(1,54)=38.71$, $p<0.001$. As Fig. 1 indicates, a significant effect of chlordiazepoxide occurred at 2.5 mg/kg, and a

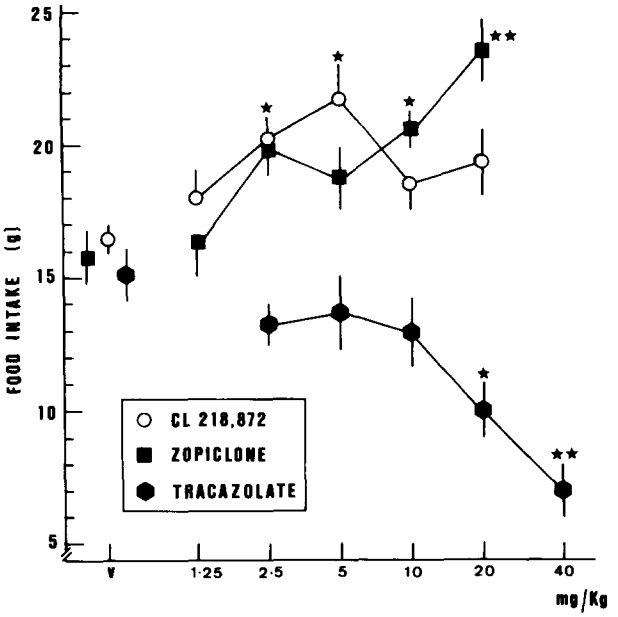

FIG. 2. Hyperphagia effects of CL 218,872 and zopiclone contrast with the feeding suppressant effects of tracazolate. Results are shown as the mean (±SEM) food intake (grams) in a 30 min test. N=10 per group. Levels of significance for comparisons between individual dose conditions and corresponding vehicle (V) treatments: *$p<0.05$; **$p<0.01$ (Dunnett's t-test).

peak effect at 5.0 mg/kg. Increasing the dose to 10.0 or 20.0 mg/kg produced no additional change in chlordiazepoxide's hyperphagic effect.

Phenobarbital

Like the benzodiazepines, phenobarbital (2.5–40.0 mg/kg) produced a highly significant hyperphagic effect, $F(5,54)=19.51$, $p<0.001$. The effect was strongly dose-related, and there were significant linear and quadratic trends in the data, $F(1,54)=90.74$, $p<0.001$ and $F(1,54)=7.95$, $p<0.01$, respectively. As Fig. 1 illustrates, phenobarbital had a significant hyperphagic effect at 5.0 mg/kg. The peak effect, at 40.0 mg/kg, resulted in a mean intake of 29.6 (±1.36) g in a 30 min test. Three rats, at this dose, consumed more than 34.0 g in this time period. The mean intake represents a very substantial increase in feeding over the control level of 13.8 (±1.05) g, i.e., an elevation of 114.5%.

Zopiclone

Zopiclone (1.25–20.0 mg/kg) produced significant increase in food intake, $F(5,54)=4.74$, $p<0.005$, with a strong linear trend in the data, $F(1,54)=21.44$, $p<0.001$. As Fig. 2 shows, the hyperphagic effect was very similar to that associated with the benzodiazepine treatments (cf. Fig. 1), and was significant at 10 and 20 mg/kg.

CL 218,872

Results for CL 218,872 (1.25–20.0 mg/kg) were somewhat untypical. A significant hyperphagic effect was produced, $F(5,54)=2.87$, $p<0.05$, but the effect was not linearly-related to dose. Instead, significant quadratic and cubic trends were found in the data, $F(1,54)=6.58$, $p<0.05$ and $F(1,54)=4.42$, $p<0.05$, respectively. Figure 2 indicates that CL 218,872 at 2.5 and 5.0 mg/kg produced significant increases in food consumption, but did not when larger doses were administered. Hence, the relationship between food intake and dose level was non-monotonic.

Tracazolate

Tracazolate (2.5–40.0 mg/kg) did not increase food intake (Fig. 2). There was a reliable effect on food consumption, $F(1,54)=7.19$, $p<0.01$, which was due to significant reductions in food intake occurring at 20.0 and 40.0 mg/kg.

DISCUSSION

The results extend the previous observation that clonazepam caused a strong stimulation of food consumption in nondeprived rats given access to a high palatability diet [5], to include midazolam and chlordiazepoxide. Clonazepam was the most potent, and produced a significant increase in intake following a dose as small as 0.078 mg/kg (Fig. 1). In a previous study [5], clonazepam treatments (0.625–5.0 mg/kg) produced no further increase in food intake compared with its maximum effect in this experiment. In the present investigation the maximum observed effects of benzodiazepine treatments were exceeded only by phenobarbital (Fig. 1). Hence the limit to the benzodiazepine-induced hyperphagia did not appear to be set by the animals' physical capacity to consume food.

The feeding situation discriminated between the effects of three novel non-benzodiazepine anxiolytics. Zopiclone, like the benzodiazepines, produced a significant dose-related increase in the level of food intake. CL 218,872 also produced an elevation in food consumption, at 2.5 and 5.0 mg/kg (Fig. 2). However, the effect of CL 218,872 was non-monotonically related to dose, and in larger doses did not produce significant increases in food intake. Hyperphagia was never observed following tracazolate treatments, and, instead, significant suppression of feeding was observed following doses of 20 and 40 mg/kg. Compared with animal models which are used to assess anxiolytic activity of drugs, the feeding test used in the present study was more selective in producing positive (i.e., hyperphagic) results. Tracazolate is an example of an effective anxiolytic [8, 16, 17, 23] which did not stimulate additional food consumption.

In a drug discrimination study, CL 218,872 at 5.0 mg/kg (IP) showed approximately 90% generalization in rats trained to discriminate 3.0 mg/kg chlordiazepoxide from saline [12]. At that dose, CL 218,872 produced no effect on response level in drug experienced animals. In the present experiment, the peak effect of CL 218,872 on feeding occurred at 5.0 mg/kg. Some degree of motor impairment may explain, at least in part, the loss of the hyperphagic effect when CL 218,872 was administered in larger doses. Thus, in the drug discrimination study [12], CL 218,872 at 10.0 mg/kg produced a strong suppression of lever responses. Although initial experiments emphasised lack of motor impairment with CL 218,872 [11], recent data demonstrate motor incoordination and reductions in locomotor activity in rats and mice following oral administration of CL 218,872 at doses only slightly larger than those required to inhibit conflict and shock-induced fighting [14]. At doses of tracazolate which produced suppression of feeding (20 and 40 mg/kg), we observed signs of ataxia and sedation. In confirmation, there is a recent report [6] that tracazolate produced significant reductions in spontaneous activities in a rat holeboard test [7], when administered at 25.0 mg/kg. The fall in food consump-

tion may therefore reflect some motor impairment produced by the larger doses. It is worth noting that an anxiolytic effect of tracazolate has been found at 5.0 mg/kg in a rat social interaction test [6], and that it was effective at 10 and 20 mg/kg (IP) in a shock-induced suppression of drinking test [16].

At present, it seems likely that benzodiazepine-induced hyperphagia is mediated by actions at 'central' benzodiazepine recognition sites, since the hyperphagia is reversed by the receptor antagonist, Ro15-1788, and cannot be elicited using the selective 'peripheral-type' ligand, Ro5-4864 [5]. Both zopiclone and CL 218,872 may induce hyperphagia in rats by acting at the same recognition sites, and this would be consistent with observations that like benzodiazepines, zopiclone and CL 218,872 displace [^{3}H] benzodiazepine binding in rat cerebral cortex [8,23]. In a study examining the effects of benzodiazepine receptor ligands using mouse spinal cord neurons in dissociated cell culture [19], clonazepam was most potent in enhancing GABA responses at low nanomolar concentrations. CL 218,872 and zopiclone were weak enhancers of GABA responses at high nanomolar concentrations [19]. In the present experiment, CL 218,872 and zopiclone produced significant hyperphagia but required doses which were 32 times and 128 times greater, respectively, than the smallest dose of clonazepam which produced a significant effect.

Tracazolate is different in that it causes a significant enhancement of [^{3}H] flunitrazepam binding in rat cerebral cortex [8, 13, 23], and it has been suggested that it may affect benzodiazepine binding by acting at the picrotoxin site in the GABA receptor-chloride channel-benzodiazepine receptor complex [18, 13, 23]. Its site of action for producing suppression of feeding is not known, although it should be noted that the benzodiazepine, Ro5-3663, which binds with high affinity to dihydropicrotoxinin sites [10,21], also reduced food consumption [5]. The particular problem which arises, however, is that barbiturates also enhance the binding of benzodiazepine to high-affinity benzodiazepine sites [21], and, as we have seen, strongly stimulate feeding responses (Fig. 1). Understanding the actions of drugs at the GABA receptor–chloride channel-benzodiazepine receptor complex is undoubtedly problematic [10, 15, 18, 21, 23]. Further attention to those actions mediated at the picrotoxin site may help to explain the relationships between the effects of barbiturates, Ro5-3663, tracazolate and associated drugs on feeding responses. More detailed knowledge of drug actions at this site could then be integrated with what has been established concerning the role of the benzodiazepine recognition sites in the control of feeding behavior.

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REFERENCES

1. Baile, C. A. and C. L. McLaughlin. A review of the behavioral and physiological responses to elfazepam, a chemical feed intake stimulant. *J Anim Sci* **49:** 1371–1395, 1979.
2. Blanchard, J. C., A. Boireau, C. Garrett and L. Julou. *In vitro* and *in vivo* inhibition by zopiclone of benzodiazepine binding to rodent brain receptors. *Life Sci* **24:** 2417–2420, 1979.
3. Cooper, S. J. Benzodiazepines as appetite-enhancing compounds. *Appetite* **1:** 7–19, 1980.
4. Cooper, S. J. and L. B. Estall. Behavioural pharmacology of food, water and salt intake in relation to drug actions at benzodiazepine receptors. *Neurosci Biobehav Rev* **9:** 5–19, 1985.
5. Cooper, S. J. and D. B. Gilbert. Clonazepam-induced hyperphagia: Tests of pharmacological specificity with Ro-4864, Ro-3663, Ro15-1788 and CGS 9896. *Pharmacol Biochem Behav* **22:** 753–760, 1985.
6. File, S. E. Behavioural effects of drugs acting at the picrotoxin site. *Neuropharmacology* **23:** 823–824, 1984.
7. File, S. E. and A. G. Wardill. Validity of head-dipping as a measure of exploration in a modified holeboard. *Psychopharmacologia* **44:** 53–59, 1975.
8. Goldberg, M. E., A. I. Salama, J. B. Patel and J. B. Malick. Novel non-benzodiazepine anxiolytics. *Neuropharmacology* **22:** 1499–1504, 1983.
9. Jacobs, B. L. and P. B. Farel. Motivated behaviors produced by increased arousal in the presence of goal objects. *Physiol Behav* **6:** 473–476, 1971.
10. Leeb-Lundberg, F., C. Napias and R. W. Olsen. Dihydropicrotoxinin binding sites in mammalian brain: Interaction with convulsant and depressant benzodiazepines. *Brain Res* **216:** 399–408, 1981.
11. Lippa, A. S., J. Coupet, E. N. Greenblatt, C. A. Klepner and B. Beer. A synthetic non-benzodiazepine ligand for benzodiazepine receptors: A probe for investigating neuronal substrates of anxiety. *Pharmacol Biochem Behav* **11:** 99–106, 1979.
12. McElroy, J. F. and R. S. Feldman. Generalization between benzodiazepine- and triazolopyridazine-elicited discriminative cues. *Pharmacol Biochem Behav* **17:** 709–713, 1982.
13. Meiners, B. A. and A. I. Salama. Enhancement of benzodiazepine and GABA binding by the novel anxiolytic, tracazolate. *Eur J Pharmacol* **78:** 315–322, 1982.
14. Oakley, N. R., B. J. Jones and D. W. Straughan. The benzodiazepine receptor ligand CL 218,872 has both anxiolytic and sedative properties in rodents. *Neuropharmacology* **23:** 797–802, 1984.
15. Olsen, R. W. Drug interactions at the GABA receptor-ionophore complex. *Annu Rev Pharmacol Toxicol* **22:** 245–277, 1982.
16. Patel, J. B. and J. B. Malick. Pharmacological properties of tracazolate: a new non-benzodiazepine anxiolytic agent. *Eur J Pharmacol* **78:** 323–333, 1982.
17. Patel, J. B., C. Martin and J. B. Malick. Differential antagonism of the anticonflict effect of typical and atypical anxiolytics. *Eur J Pharmacol* **86:** 295–298, 1983.
18. Paul, S. M., P. J. Marangos and P. Skolnick. The benzodiazepine-GABA-chloride ionophore receptor complex: common site of minor tranquilizer action. *Biol Psychiatry* **16:** 213–229, 1981.
19. Skerrit, J. H. and R. L. Macdonald. Benzodiazepine receptor ligand actions on GABA responses. Benzodiazepines, CL 218872, zopiclone. *Eur J Pharmacol* **101:** 127–134, 1984.
20. Squires, R. F., D. I. Benson, C. Braestrup, J. Coupet, C. A. Klepner, V. Myers and B. Beer. Some properties of brain specific benzodiazepine receptors: New evidence for multiple receptors. *Pharmacol Biochem Behav* **10:** 825–830, 1979.
21. Ticku, M. K. Benzodiazepine-GABA receptor-ionophore complex. Current concepts. *Neuropharmacology* **22:** 1459–1470, 1983.
22. Watson, P. J. and V. C. Cox. An analysis of barbiturate-induced eating and drinking in the rat. *Physiol Behav* **4:** 325–332, 1976.
23. Williams, M. Molecular aspects of the action of benzodiazepine and non-benzodiazepine anxiolytics: A hypothetical allosteric model of the benzodiazepine receptor complex. *Prog Neuro-Psychopharmacol Biol Psychiatry* **8:** 209–247, 1984.
24. Winer, B. J. *Statistical Principles in Experimental Design, 2nd edition*. New York: McGraw-Hill, 1971.
25. Yamamura, H. I., T. Mimaki, S. H. Yamamura, W. D. Horst, M. Morelli, G. Bautz and R. A. O'Brien. [^{3}H] CL 218,872, a novel triazolopyridazine which labels the benzodiazepine receptor in rat brain. *Eur J Pharmacol* **77:** 351–354, 1982.