Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 499-503, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00020-8

Food Palatability and Hunger Modulated Effects of CGS 9896 and CGS 8216 on Food Intake

SHU-WEN CHEN,[1] M. FRANCES DAVIES AND GILDA H. LOEW

Molecular Research Institute, 845 Page Mill Road, Palo Alto, CA 94304

Received 13 May 1994

CHEN, S.-W., M. F. DAVIES AND G. H. LOEW. *Food palatability and hunger modulated effects of CGS 9896 and CGS 8216 on food intake.* PHARMACOL BIOCHEM BEHAV 51(2/3) 499-503, 1995.—The effect of food palatability and duration of food deprivation on the modulation of food intake by two benzodiazepine receptor (BDZR) ligands, CGS 9896 and CGS 8216, were investigated. Three diets differing in palatability (high, medium, or standard) and three different periods of food deprivation (0, 16, or 24 h) were used in all combinations to compare the effect of these variations on the observed modulation of food consumption by both BDZR ligands. Increasing diet palatability and/or food deprivation increased the baseline food consumption and reduced the sensitivity of the test to the detection of the hyperphagic effect of CGS 9896 but increased the sensitivity to detect the anorexic effect of CGS 8216. Only for the intermediate conditions of food deprivation (16 h) and for a standard or medium palatable diet were both significant hyperphagic effect of CGS 9896 and anorexic effect of CGS 8216 detected. Neither increased palatability nor hunger enhanced the modulation of feeding, indicating that neither "taste preference" nor "hunger" is the key factor in the mechanism of BDZR ligand-induced feeding response.

Benzodiazepines CGS 8216 CGS 9896 Food palatability Food deprivation Food intake Feeding test

IT IS KNOWN that benzodiazepine receptor (BDZR) agonists such as flunitrazepam (16), chlordiaxepoxide (19), clonazepam (4), and midazolam (7) increase food intake. By contrast, other BDZR ligands such as CGS 8216 (17) and β-carbolines (8) are anorexic and are inverse agonists at this endpoint. Ro 15-1788 is a BDZR antagonist that has no intrinsic effect on food intake but blocks the effects of both agonists and inverse agonists (2,14,16). Because BDZR agonists are clinically useful as anxiolytics, it has been proposed that the hyperphagic effect is a consequence of anxiolysis and anorexia is a consequence of the anxiogenic or proconvulsant properties of BDZR inverse agonists (21,25). The effect of CGS 9896, a high-affinity BDZR ligand, on food consumption has become a pivotal and controversial component in evidence for against this hypothesis. Although most anxiolytic BDZR ligands also enhanced food intake, CGS 9896, a known nonsedative, potent anxiolytic, was shown in some studies to be an exception, causing no effect on the ingestion of a highly palatable diet in nondeprived rats (11,14) and dose-dependently reversing the hyperphagic effect of clonazepam (14). Therefore, CGS 9896 was cited as providing evidence of the separation of the anxiolytic and hyperphagic effects of BDZR ligands (5,6,14). In contradiction to this result, in a study using different protocols in which rats were subjected to 16-h food deprivation and fed a standard diet sweetened with 15% sucrose, we have found that CGS 9896 increased food consumption and that this effect was reversed by Ro 15-1788 (16). This hyperphagic effect of CGS 9896 in rats has also been demonstrated in other laboratories using trained and food-deprived rats (22). The origin of the striking qualitative differences in these results, finding of antagonist activity in one and agonist activity in the others, could be the use of different protocols.

In this study, we have systematically investigated the effect of different protocols on modulation of food intake by CGS 9896 and CGS 8216. Specifically, we investigated the effect of two factors, changes in food palatability and duration of food deprivation, on food consumption. CGS 8216 was included in this study as a comparison because it has been reported to be both anxiogenic and to decrease food consumption (14).

Although the effects of food deprivation, diet texture, and palatability have been investigated in previous studies of the mechanism of BDZR ligand-induced feeding response, we be-

[1] To whom requests for reprints should be addressed. E-mail: shuwen@montara.molres.org

lieved that the conclusions reached in these studies (3,26) might not be universally applicable, because in those studies, only "potent" BDZR agonists such as diazepam, chlordiazepoxide, or flunitrazepam were examined. For example, neither hungry nor food-satiated condition was found to affect the hyperphagic effect of diazepam (26) or chlordiazepoxide (3) in rats. Moreover, using chlordiazepoxide, the effect of BDZR ligand was also found not to be sensitive to variation in food texture (10). There is no evidence that these same conclusions would be reached when a less potent or behaviorally selective agonist, such as CGS 9896 (16), or an inverse agonist, such as CGS 8216, is tested under these varied experimental conditions.

In summary, the result of this study should extend the understanding of the mechanism of BDZ-induced feeding response. Another motivation for the work presented here was to identify the origin of the disparity in the results for CGS 9896 reported by different investigators by determining to what extent the results obtained are dependent on the protocols used. The result of present study should help to define a protocol that is able to detect both the anorexic and hyperphagic effects of BDZs regardless of the potency of the drug effect on this endpoint.

METHOD

Animals

Long Evans hooded male rats (Charles River, Wilmington, MA) weighing 300–500 g, housed in groups of two, and maintained on a 12L : 12D reverse cycle (lights off at 1100 h) were used in this study. The housing condition was maintained at 22°C and humidity was 50–70%. All the animals used were adapted to the housing conditions and reversed light/dark cycle for at least 3 weeks prior to the experiment. Water and standard rat chow were available at all times.

Drugs

CGS 9896 and CGS 8216 were provided by Ciba-Geigy (Summit, NJ). Drugs were stirred overnight in 40% w/v Encapsin HPB (Amaizo, Hammond, IN) and deionized water, and then administered IP as a suspension.

Diets

Three test diets were used in this study. The standard rat chow on which rats were maintained was purchased from Purina Mill (diet #5012) and considered to have the lowest palatability. The diet used in Experiment 1, and considered to be of medium palatability, was made from the standard diet sweetened with 15% sucrose (Purina Mill, special mix 5729-D). The highly palatable diet was made according to previously reported specifications (11,14) and contained 300 ml of sweetened condensed milk, 900 g of ground standard rat diet (Purina diet #5012), and 1200 ml of distilled water. Each diet was freshly prepared the day before the experiment and stored at 4°C until used.

Experimental Protocol

Rats with similar weights were used and randomly assigned to one of the groups. All experiments were started between 1100 and 1200 h. Animals were trained to acclimatize to the experimental procedures in a sham experiment conducted on the day before the test day. In both the sham and actual experiments the same procedures were used, including the fasting protocol and testing diet. Before the administration of the test drugs, animals were transferred to individual test cages in a dark testing room illuminated with a 40-W red light bulb and allowed to acclimatize for 1 h. Thirty minutes after the injection of the drug, a preweighed plastic dish containing about 40 g of test diet was placed inside the test cages. In the sham experiment, food was introduced right after the acclimatization period, excluding the drug injection procedure. The duration of the test feeding period was 60 min, and during this time, only the test diet was available. At the termination of the test, animals were returned to their home cage and the cup with the remaining food was weighed. The amount of food consumed was determined as the difference between the weight of the content and cup before and after test. This weight difference was determined to the nearest 0.1 g, with correction for spillage.

The dose-response curve for the CGS 9896 and CGS 8216 effect on food consumption was determined using a previously described protocol (16) in which the experiment was conducted after 16 h of food deprivation with the medium palatable diet described above. Eight to 10 rats in each group were given CGS 9896 at doses of 0, 0.1, 1, 5, or 10 mg/kg. CGS 8216 was given at doses of 0, 0.1, 0.5, 1, 5, 10, or 20 mg/kg.

In the second experiment, a single dose, 5 mg/kg of CGS 9896 or 20 mg/kg of CGS 8216, was administered using three different diets and periods of food deprivation. Rats were randomly given vehicle, CGS 8216, or CGS 9896, fasted for 0, 16, or 24 h, and each of the three test diets was given one at a time. Between tests, animals were returned to the maintenance diet for 1 week, and then the test was conducted using the next diet with the same rats.

Statistical Analysis

The food consumption data were analyzed by factorial ANOVA using the STATVIEW® program. Dunnett's *t*-test was used to assess the effects of different treatment by comparison of individual treatments with corresponding vehicle control group.

RESULTS

As illustrated in Fig. 1, food consumption was dose dependently increased by CGS 9896, $F(4, 52) = 10.565$, $p < 0.001$, and decreased by CGS 8216, $F(6, 55) = 6.534$, $p < 0.0001$, in rats deprived food for 16 h and fed 15% sucrose-added diet. CGS 9896 at 5 and 10 mg/kg caused an increase ($p < 0.001$) of about 50% over the amount of food consumed by control animals. CGS 8216 at 5, 10, and 20 mg/kg ($p < 0.001$) significantly reduced food intake. No significant behavioral change was observed in rats treated with CGS 8216 or CGS 9896, although some rats treated with > 10 mg/kg of CGS 8216 produced soft stool.

Results of the three-factor ANOVA on food consumption from Experiment 2 showed that both diet, $F(4, 135) = 10.631$, $p < 0.001$, and food deprivation, $F(4, 135) = 3.994$, $p < 0.05$, interacted with the effect of the drug. However, there was no three-way interaction among diet, food deprivation, or drug treatment, $F(8, 135) = 1.603$, $p = 0.1294$.

Results separated by different diet are shown in Fig. 2. The amount of food consumption using standard rat diet for the three different intervals of food deprivation are shown in Fig. 2a. We see from Fig. 2a that the effect of both CGS 9896 and CGS 8216 on food intake depended on the fasting interval.

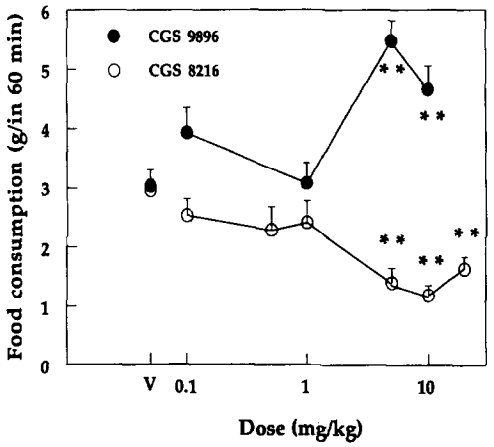

FIG. 1. Average amount of medium palatable diet consumed in 60 min by rats deprived food for 16 h prior to administration of CGS 8216 (open circle) and CGS 9896 (closed circle). Data expressed as mean ± SEM. Statistical comparisons were between vehicle-treated and drug-treated groups. $^{**}p < 0.001$ (Dunnett's t-test).

CGS 9896 increased food consumption in nondeprived and 16-h deprived rats but not in those experiencing 24-h deprivation. CGS 8216 reduced food consumption in 16- and 24-h deprived rats but not in nondeprived rats. The same pattern of modulation was observed when the medium palatable diet was tested (Fig. 2b), although rats tended to eat more when compared to the standard diet group. However, in the third set of results shown in Fig. 2c, in rats fed the highly palatable diet, CGS 9896 increased food consumption only in the nondeprived group whereas the anorectic effect of CGS 8216 was only observed in the 16- and 24-h food deprivation groups.

DISCUSSION

The present results fail to support the hypothesis that CGS 9896 can separate the anxiolytic and hyperphagic effect of BDZs because CGS 9896 clearly increased low and medium palatable diet intake in rats that were not deprived or deprived for 16 h. Because CGS 9896 has been reported to somewhat reduce locomotion activity (16), the hyperphagic effect was not as robust at the highest dose (10 mg/kg) used in the dose-response study. The same inconsistent result at high doses was also observed in a previous study (22). This reduced efficacy at high doses of CGS 9896 could explain why it might act as an apparent antagonist at these doses of the hyperphagic effect of clonazepam (14,15).

The second experiment in this study demonstrated that food deprivation and palatability affected the hyperphagic effect of CGS 9896. CGS 9896 increased consumption of all three diets in nondeprived rats. After a 16-h food deprivation, the hyperphagic effect of CGS 9896 was only observed in rats given standard or medium palatable diets, but not in rats fed the highly palatable diet. After 24 h of food deprivation, CGS 9896-induced hyperphagia completely disappeared, regardless of the palatability of the test diets.

Although our results clearly indicate that CGS 9896 increases consumption of all diets in nondeprived rats, other studies (7,11,14,27) have concluded that CGS 9896 is an antagonist using a similar highly palatable diet and nondeprived rats. The reason for this disparity lies in an important differ-

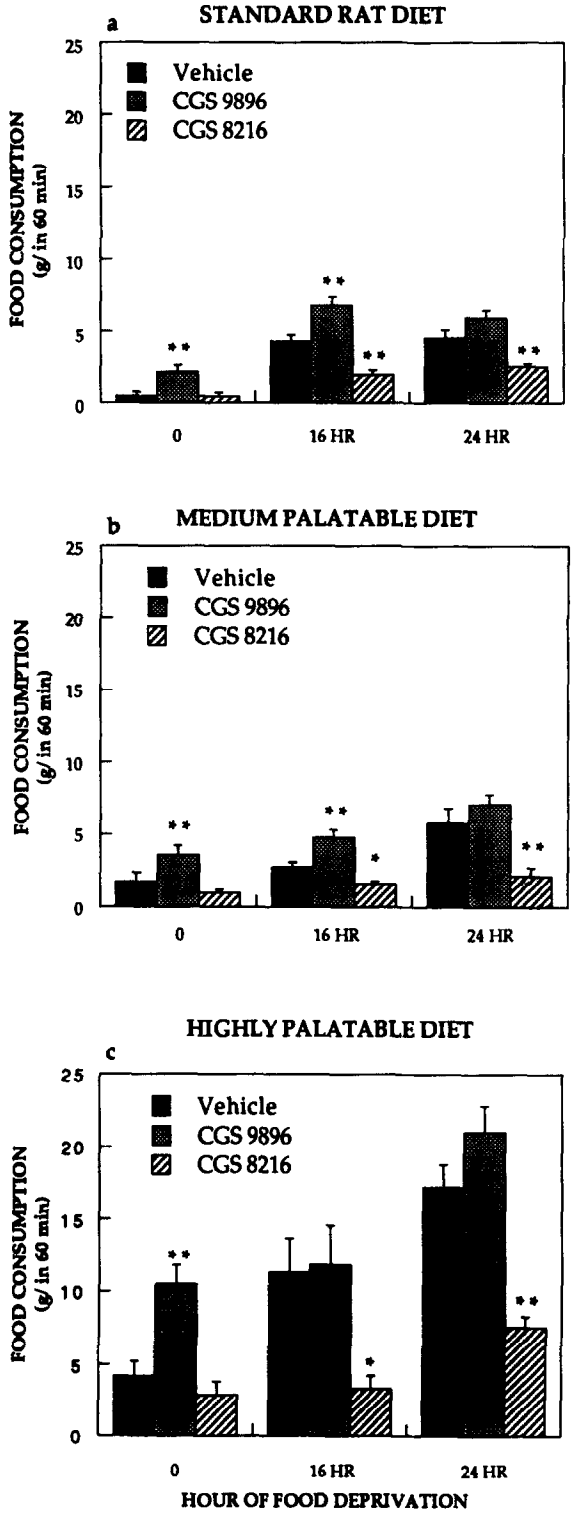

FIG. 2. Average amount of food consumed in 60 min by rats deprived of food for 0, 16, or 24 h prior to administration of vehicle, 5 mg/kg of CGS 9896, or 20 mg/kg of CGS 8216. (a) Result of rats fed the standard rat chow; (b) rats fed the medium palatable diet; (c) rats fed the highly palatable diet. Data expressed as mean ± SEM. Statistical comparisons were between vehicle-treated and drug-treated groups. $^{*}p < 0.05$, $^{**}p < 0.001$ (Dunnett's t-test).

ence in the treatment of the nondeprived rats in the previous studies and in the one used here. In the previous studies, the nondeprived rats were trained for at least 10 days to the daily 30-min presentation of the highly palatable diet. The drug trials were begun only after the daily consumption of highly palatable diet was stable (7,11,14,27). This complicating feature of acclimatization to the highly palatable diet before the actual experiment is not included in our study. We believe that due to this extensive training, these rats might deprive themselves of "plain" regular rat chow with the expectation of the more palatable diet before the experiment. Further evidence that these animals are hungry at the beginning of the experiment is that the baseline food consumption in the vehicle-treated rats was about 20 g in 30 min in those studies, approximately 400% higher than the nondeprived, vehicle-treated rats given a similar diet for 1 h in the present study. As we have mentioned previously, the hyperphagic effect of CGS 9896 in hungry rats was not as robust as in nondeprived rats. Thus, if indeed the rats in the previous experiment were actually hungry, the results found in the two experiments are consistent (5,6,14). The results obtained here clearly indicate that using nondeprived or 16-h deprived rats and standard or medium palatable diet, protocols resulting in a low to medium baseline food consumption, enabled the unambiguous detection of the hyperphagic effect of CGS 9896.

Regardless of diet palatability, the anorexic effect of CGS 8216 could only be found under food-deprived conditions. These results indicate that the effect of BDZR ligands on food intake can be modulated by food deprivation. We proved here that using an inverse agonist such as CGS 8216, the conclusion can be different from using only "potent" BDZR agonists (3,26) that showed no effect of food deprivation. However, without food deprivation, the control animals consumed only a small amount of food and produced a low baseline, which might account for the difficulty in detecting a significant decrease.

Several hypotheses have been advanced to explain the mechanism of BDZR ligand-induced hyperphagia. One idea is that the hyperphagic effect of BDZR agonists is a secondary effect of their anxiolytic action. Consistent with this idea is the fact that no anxiogenic BDZs have been found to be hyperphagic. In addition, BDZR ligand-treated animals also have a higher frequency of visiting food cups (5) and spend more time eating (26) than animals treated with vehicle. Some studies have shown a good correlation between antianxiety and hyperphagia of BDZR ligands (5,6,24). There are, however, counter indications to this hypothesis (23,25). For example, the feeding response to BDZR ligands was found to be similar in animals exposed either to novel food or environments or to familiar food or environment (12). Because novelty usually induces anxiety, these results (12) indicate that the hyperphagic effect of BDZR ligands cannot be solely related to their antianxiety action. A second hypothesis is that, because BDZR ligands increases food intake in satiated animals (5,6,26), BDZR ligands might act to reduce the meal-terminating signals or increase the hunger perceived by these animals. Using rats with gastric fistula, a condition that eliminated the meal-terminating cues, midazolam (13) increased food intake whereas CGS 8216 (20) and FG 7142 (13) decreased it. Therefore, modulation of hunger signals does not change the effect of BDZR ligands, indicating that they might not work by modulating this signal (6,13). The third hypothesized mechanism of the effect of BDZR ligands on food intake is that BDZR ligands modify taste-related palatability; that is, the "taste preference" hypothesis (5,6). Using a multiple-food (15) or multiple-bottle type presentation (8,9,17), BDZ agonists have been shown to increase the proportion of palatable diet intake but not to affect the intake of standard and aversive diets. However, some studies have shown that BDZ increased the reaction to the aversive taste, and the results were opposite to "taste preference" hypothesis (18). In this study, we have shown that the hyperphagic effect of CGS 9896 was more robust in nondeprived rats given less palatable diet and was less consistent in food-deprived rats given a highly palatable diet. These results indicate that neither increase in hunger or in food palatability increases the sensitivity of the feeding response to BDZ. Therefore, food palatability and hunger do not appear to be key factors in the BDZR ligand-induced feeding response.

In conclusion, we found that when rats are subjected to a highly palatable diet and extended periods of food deprivation, hyperphagic effects of BDZR ligands are not easily observable. A less palatable diet with no periods of food deprivation, on the other hand, made detection of the anorexic effect difficult. Therefore, using intermediate palatable diet and food deprivation periods to maintain moderate basal consumption may be an efficient way to detect both hyperphagic and anorexic effects of BDZs in the same test. The results of this study also indicated that neither "taste preference" nor "hunger" is a key factor for the mechanism of BDZ-induced hyperphagia and anorexia because further increase in the food deprivation period and the palatability of the diet did not increase the sensitivity of the feeding response to BDZ. Benzodiazepines appear to affect food intake simply by modifying the amount of food consumed during the testing period.

ACKNOWLEDGEMENTS

This work was supported by NIDA grant DA 06304-05. The authors thank Dr. Mervyn Maze for the helpful discussions and Ms. Lizel Rodriguez for her technical assistance.

REFERENCES

1. Bernard, P. S.; Pastor, G.; Liebman, J. M. CGS 8216, a benzodiazepine antagonist, reduces food intake in food-deprived rats. Pharmacol. Biochem. Behav. 24:1703–1706; 1986.
2. Boireau, A.; Martel, M.; Farges, G.; Dubedat, P.; Laduron, P. M.; Blanchard, J. C. In vivo determination of the profile of benzodiazepine ligands by comparing the inhibition of ^{3}H-Ro 15-1788 binding to the modulation of cGMP levels in mouse cerebellum. Biochem. Pharmacol. 37:3765–3769; 1988.
3. Cole, S. O. Combined effects of chlordiazepoxide treatment and food deprivation on concurrent measures of feeding and activity. Pharmacol. Biochem. Behav. 18:369–372; 1983.
4. Cooper, S. J. The anorectic effect of FG7142, a partial inverse agonist at benzodiazepine recognition sites, is reversed by CGS 8216 and clonazepam but not by food deprivation. Brain Res. 346:190–194; 1985.
5. Cooper, S. J. Benzodiazepine receptor-mediated enhancement and inhibition of taste reactivity, food choice, and intake. Ann. NY Acad. Sci. 575:321–336; 1989.
6. Cooper, S. J. Benzodiazepines and appetite: Recent preclinical advances and their clinical implications. Hum. Psychopharmcol. 4:81–89; 1989.
7. Cooper, S. J.; Barber, D. J.; Gilbert, D. B.; Moores, W. R.

Benzodiazepine receptor ligands and the consumption of a highly palatable diet in non-deprived male rats. Psychopharmacology (Berlin) 86:348–355; 1985.
8. Cooper, S. J.; Bowyer, D. M.; van der Hoek, G. Effects of imidazobenzodiazepine Ro15-4513 on saccharin choice and acceptance, and on food intake in the rat. Brain Res. 494:172–176; 1989.
9. Cooper, S. J.; Estall, L. B. Behavioural pharmacology of food, water and salt intake in relation to drug actions at benzodiazepine receptors. Neuorsci. Biobehav. Rev. 9:5–19; 1985.
10. Cooper, S. J.; Francis, R. L. Effects of acute or chronic administration of chlordiazepoxide on feeding parameters using two food textures in the rat. J. Pharm. Pharmacol. 31:743–746; 1979.
11. Cooper, S. J.; Gilbert, D. B. Clonazepam-induced hyperphagia in nondeprived rats: Tests of pharmacological specificity with Ro5-4864, Ro5-3663, Ro 15-1788 and CGS 9896. Pharmacol. Biochem. Behav. 22:753–60; 1985.
12. Cooper, S. J.; McClelland, A. Effects of chlordiazepoxide, food familiarization, and prior shock experience on food choice in rats. Pharmacol. Biochem. Behav. 12:23–28; 1980.
13. Cooper, S. J.; van der Hoek, G.; Kirkham, T. C. Bidirectional changes in sham feeding in the rat produced by benzodiazepine receptor ligands. Physiol. Behav. 42:211–216; 1988.
14. Cooper, S. J.; Yerbury, R. E. Benzodiazepine-induced hyperphagia: Stereospecificity and antagonism by pyrazoloquinolines, CGS 9895 and CGS 9896. Psychopharmacology (Berlin) 89:462–466; 1986.
15. Cooper, S. J.; Yerbury, R. E.; Neill, J. C.; Desa, A. Partial agonists acting at benzodiazepine receptors can be differentiated in tests of ingestional behaviour. Physiol. Behav. 41:247–255; 1987.
16. Davies, M. F.: Onaivi, E. S.; Chen, S. -W.; Maguire, P. A.; Tsai, N. F.; Loew, G. H. In vivo evidences for functional benzodiazepine receptor heterogeneity. Pharmacol. Biochem. Behav. 49:47–56; 1994.
17. Estall, L. B.; Cooper, S. J. Benzodiazepine receptor-mediated effect of CGS 8216 on milk consumption in nondeprived rat. Psychopharmcology (Berlin) 89:477–479; 1986.
18. Hunt, T.; Poulos, C. X.; Cappell, H. Benzodiazepine-induced hyperphagia: A test of the hunger-mimetic model. Pharmacol. Biochem. Behav. 30:515–518; 1988.
19. Iwahara, S.; Iwasaki, T. Effect of chlordiazepoxide upon food intake and spontaneous motor activity of the rat as a function of hours of food deprivation. Jpn. Psychol. Res. 11:117–128; 1969.
20. Kirkham, T. C.; Cooper, S. J. CGS 8216, a novel anorectic agent, selectively reduces saccharin solution consumption in the rat. Pharmacol. Biochem. Behav. 25:341–345; 1986.
21. Leidenheimer, N. J.; Schechter, M. D. Inverse agonist properties of the FG 7142 discriminative stimulus. Pharmacol. Biochem. Behav. 38:99–104; 1991.
22. Sanger, D. J.; Joly, D.; Zivkovic, B. Behavioral effects of non-benzodiazepine anxiolytic drugs: A comparison of CGS 9896 and zopiclone with chlordiazepoxide. J. Pharmacol. Exp. Ther. 232: 831–837; 1985.
23. Seyrig, J. A.; Falcou, R.; Betoulle, D.; Apfelbaum, M. Effects of a chronic administration of two benzodiazepines on food intake in rats given a highly palatable diet. Pharmacol. Biochem. Behav. 25:913–918; 1986.
24. Soubrie, P.; Kulkarni, S.; Simon, P.; Boissier J. R. Effects of antianxiety drugs on the food intake in trained and untrained rats and mice. Psychopharmacologia 45:203–210; 1975.
25. Tang, M.; Plaessmann, W. R.; Falk, J. L. The role of central- and peripheral-type benzodiazepine receptors in anxiolytic-agent augmentation of NaCl solution intake: Effects of clonazepam and Ro 5-4864. Pharmacol. Biochem. Behav. 30:749–752; 1988.
26. Wise, R. A.; Dawson, V. Diazepam-induced eating and lever pressing for food in the sated rats. J. Comp. Physiol. Psychol. 86:930–941; 1974.
27. Yerbury, R. E.; Cooper, S. J. Novel benzodiazepine receptor ligands: Palatable food intake following zolpidem, CGS 17867A, or Ro23-0364, in the rat. Pharmacol. Biochem. Behav. 33:303–307; 1989.