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Effects of a Chronic Administration of Two Benzodiazepines on Food Intake in Rats Given a Highly Palatable Diet

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SEYRIG, J. A., R. FALCOU, D. BETOULLE AND M. APFELBAUM. *Effects of a chronic administration of two benzodiazepines on food intake in rats given a highly palatable diet.* PHARMACOL BIOCHEM BEHAV **25**(4) 913–918, 1986.—Chronic administration of benzodiazepines is known to increase food intake in numerous species. But this effect has been studied only after a unique daily injection and over a short part of the 24 hr cycle. In the present study, during 28 days, drugs were administered to rats receiving ordinary chow or a highly palatable diet (cafeteria diet): diazepam (DZ) (2.5 mg/kg IP) twice a day, or brotizolam (BR) (1 mg/kg IP), a longer acting compound, once a day. In the chow fed rats, DZ and BR provoked a post injection hyperphagia throughout the study, followed by a compensatory hypophagia resulting in 24 hr food intakes not different from those of controls; conversely neither body weight nor weight of fat pads were increased. The cafeteria diet provoked hyperphagia and overweight. DZ did not induce any supplementary hyperphagia. BR provoked a post injection hyperphagia, also compensated in time, resulting again in 24 hr food intakes, body weight gains and weight of fat pads not increased compared to those of cafeteria controls. Thus in the rat, benzodiazepine treatment increases food intake, but only acutely, and does not provoke any trend toward obesity.

Benzodiazepines Food intake Diazepam Brotizolam Cafeteria diet Food preference Food neophobia Brown adipose tissue Rat

THE action of benzodiazepines on eating behavior has been extensively documented with a wide range of drugs: a hyperphagic effect was observed in all species of mammals in which benzodiazepines have been tested, e.g., in rats, hamsters, mice, and also, dogs, cats, and horses [5, 6, 12, 23, 25]. The phenomenon might even be more general since it was reported in pigeons [8]. As far as rodents are concerned, two chronological arguments have suggested that this hyperphagia is a specific response: it is unlikely to be a mere side effect of the sedative action since tolerance to sedation occurs rapidly while it does not (or at least occurs much later) for hyperphagia [27]; it is also unlikely that it is a consequence of stereotyped behaviors of chewing and gnawing since when a choice between wood and food is offered, rats prefer food and also since the chewing response is rapidly extinguishable [20]. Thus it is clear cut that benzodiazepines increase food intake. However, the meaning in terms of energy balance is not documented: in the course of chronic studies, the drug has been injected every day once a day and the eating response has been observed during the time when the drug is supposed to act, i.e., only a short part of the 24 hr cycle [7, 10, 11, 15, 19, 22–24]. Moreover, the relevance of such protocols as models for nutritional effects of medication or self medication in humans suffering from anxiety is not satisfactory since these patients usually take benzodiazepines at least twice a day, and contrary to laboratory animals, have a free access to a large choice of palatable food. Therefore the aim of the present study is to take into account both these conditions by offering rats a cafeteria diet [2] and by treating them chronically, during 28 days, either with the most studied benzodiazepine, diazepam (DZ) or with a more recent and longer acting compound, brotizolam (BR) which has been reported to particularly increase food intake (P. Skolnick, personal communication).

METHOD

Animals

Forty-two male Wistar rats (Iffa Credo) weighing 160–180 g were housed in individual cages at 24°C with lights on between 9 p.m. and 9 a.m. Food and water were available ad lib.

Drugs

DZ and BR were suspended with arabic gum in deionised water at respective concentrations of 2.5 and 1 mg/kg. Both these doses were reported to be border-line for sedative ef-

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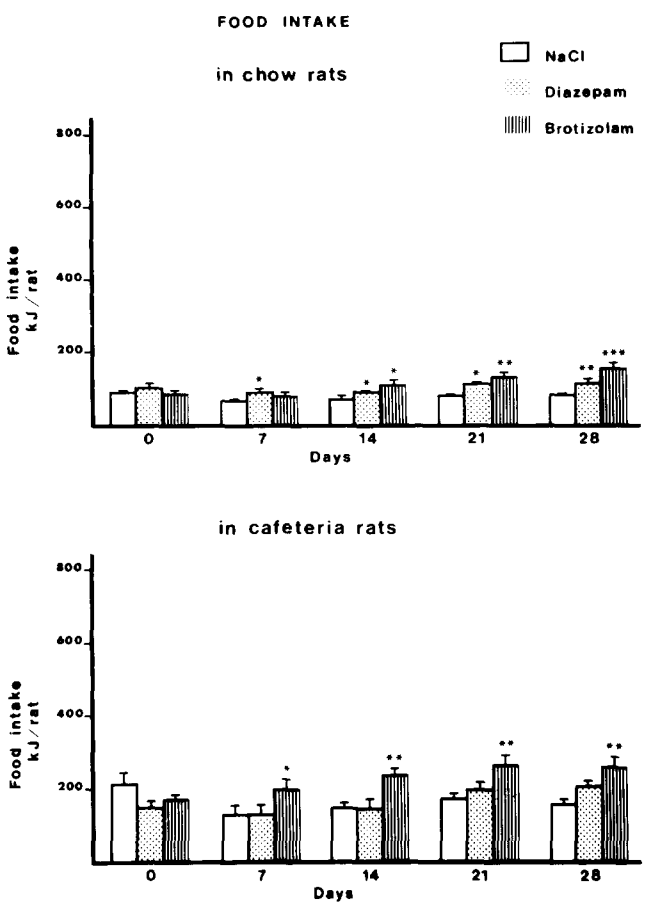

FIG. 1. Effects of brotizolam (1 mg/kg) or diazepam (2.5 mg/kg) versus control injections (NaCl) on food intake (in kJ/rat) during a 3 hour period (9 a.m. to noon) = free access to laboratory chow diet (chow rats) or cafeteria diet (cafeteria rats). Means ± (vertical lines) S.E.M.; n=7. *$p<0.05$; **$p<0.01$; ***$p<0.001$ (vs. NaCl).

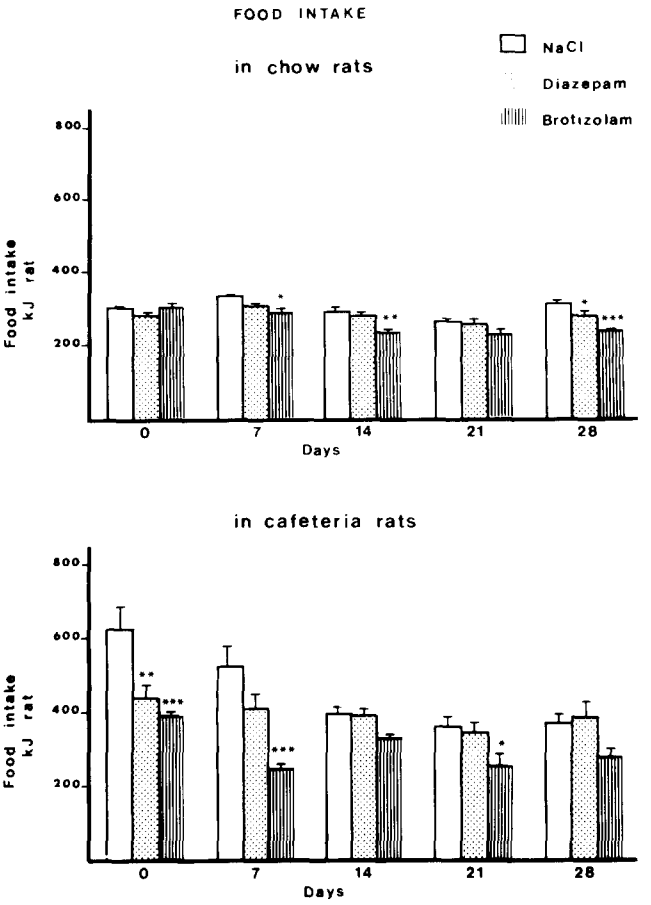

FIG. 2. Effects of brotizolam (1 mg/kg) or diazepam (2.5 mg/kg) versus control injections (NaCl) on food intake (in kJ/rat) during a 21 hour period (noon to 9 a.m.) = chow rats and cafeteria rats. Means±S.E.M.; n=7. *$p<0.05$; **$p<0.01$; ***$p<0.001$ (vs. NaCl).

fects [3, 24, 27] (see also activity test). Control rats received isotonic saline (NaCl). All drugs were administered IP at 9 a.m. and 5 p.m.: two injections of DZ (Produits Roche S.A.) for the DZ groups, one injection of BR (Boehringer Ingelheim) at 9 a.m. and one injection of NaCl at 5 p.m. for the BR groups, two injections of NaCl for the control groups. Since the half-life of BR in rats ranges from 14 to 17 hours [3], we used only one injection per day of BR. The half-life of DZ is about 2 hours in acute injection [17] and more in chronic injection [1,18].

Diet

Half the rats received ordinary chow (Extralabo M 25). The other half were given a cafeteria diet made of four palatable foods in addition to the chow [2]. The four supplementary items were changed each day on a weekly rotation basis.

Food Intake

Food intake tests were conducted for each rat over a period of 3 hours (9 a.m. to noon) and 21 hours (noon to 9 a.m.) the same day. Taking in account the half-life of drugs, the 3 hour period was chosen to correspond well to periods of activity. The food intake tests were performed once a week, with the same "menu" (lard, noodles, cakes, chocolate, and chow). Fresh food was weighed and offered at the beginning of the test. At the end, leftover food was carefully removed and weighed. The energy content and the composition of each item consumed were calculated according to tables [26]. The energy intake was expressed in kJ/rat/3 or 21 hours. Food intake for 24 hours was calculated from the 3 and 21 hour measures.

Procedure

After 14 days of adaptation to the light/dark cycle, with a chow diet, rats were randomly allocated in 6 groups of 7 rats: chow diet-NaCl, chow diet-BR, chow diet-DZ, cafeteria diet-NaCl, cafeteria diet-BR, cafeteria diet-DZ. Daily injections of drugs, and cafeteria diet were started on day zero. Weight and food intake (3 and 21 hours) of each rat were measured on day 0, 7, 14, 21 and 28. To assess a possible accumulation of benzodiazepines inducing a sedative effect,

TABLE 1

FOOD CHOICE: PART OF CHOW IN THE CAFETERIA GROUPS (PERCENTAGE OF TOTAL ENERGY INTAKE) DURING 24 HOURS

Drugs \ Days	1	7	14	21	28
NaCl	4.90	0.21	1.04	0.39	2.41
DZ	15.48*	1.67	0.92	0	0.77
BR	15.45*	9.82*	2.71	0	1.23

n=7.
*$p<0.001$ vs. NaCl.

activity was estimated on day 19 and 20, between 2 and 4 p.m., by an activity score [13].

After 29 days of drug administration, rats were sacrificed and the white adipose tissue (periepididymis, and perikidney) and the brown adipose tissue (interscapular region) were weighed.

Statistical Tests

Data are expressed as means±SEM. The experiment has a three factor design: drugs (three levels: NaCl, BR, DZ), diets (two levels: chow, cafeteria), days (five levels: 0, 7, 14, 21, 28). There were seven rats in each cell of the design. All measures were analysed using a three and two-way analysis of variance (ANOVA), which allowed a comparison between drugs and days, or drugs and diets, and allowed an assessment of any interaction which may occur between them when variance analysis resulted in significant differences or significant interaction. Select comparisons between groups were performed with *t*-test.

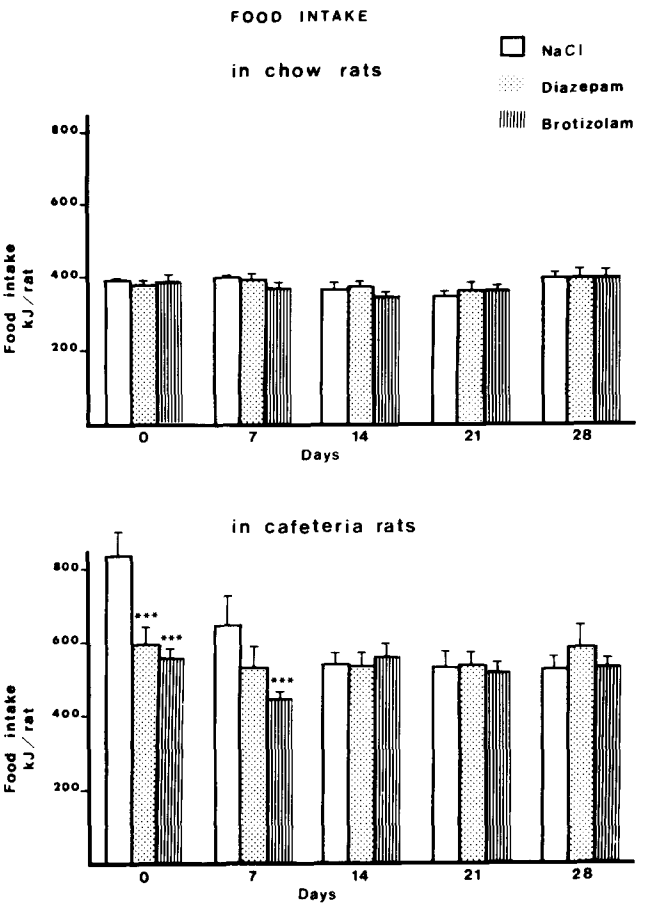

FIG. 3. Effects of brotizolam (1 mg/kg) or diazepam (2.5 mg/kg) versus control injections (NaCl) on 24 hour food intake (in kJ/rat) = chow rats and cafeteria rats. Means±S.E.M.; n=7. ***$p<0.001$ (vs. NaCl).

RESULTS

Food Intake

Three hour food intakes (Fig. 1). In the chow consuming groups, DZ provoked a food intake increase during the 3 hours following each injection (main drug effect: $F(1,60)=17.61$, $p<0.001$); BR provoked a similar increase of food intake, $F(1,60)=28.81$, $p<0.001$.

The cafeteria control group consumed more (about twice) than the chow controls, $F(1,60)=75.94$, $p<0.001$, and the cafeteria DZ and cafeteria BR groups consumed more than the chow DZ, $F(1,60)=29.94$, $p<0.001$, and chow BR, $F(1,60)=88.54$, $p<0.001$. DZ did not induce any significant change in intake of the cafeteria rats; but BR still induced a hyperphagia during the 3 hours following each injection, $F(1,60)=18.52$, $p<0.001$.

Twenty-one hour food intakes (Fig. 2). The 21 hour food intake was bigger in the cafeteria groups, than in the 3 corresponding chow groups (main diet effect for NaCl: $F(1,60)=47.34$, $p<0.001$, for DZ: $F(1,60)=33.02$, $p<0.001$, for BR: $F(1,60)=6.04$, $p<0.02$); when one compared benzodiazepine treated rats to their controls, there was no longer an increase in food intake. On the contrary, in groups (chow diet DZ, chow diet-BR, cafeteria diet-BR) in which DZ or BR had resulted in an initial hyperphagia when measured during 3 hr, there was a subsequent compensatory hypophagia (chow diet-DZ: $F(1,60)=7.86$, $p<0.006$; chow diet-BR: $F(1,60)=28.41$, $p<0.001$; cafeteria diet-BR: $F(1,60)=39.93$, $p<0.001$).

Twenty-four hour food intakes (Fig. 3). As expected, cafeteria diet provokes an increase (two way ANOVA on day zero shows a significant main diet effect, $F(1,36)=87.06$, $p<0.001$, and a significant interaction between drugs and diets, $F(2,36)=8.28$, $p<0.001$). As for the benzodiazepines, the sum of the increase during 3 hours and the decrease during 21 hours results in a total energy intake over 24 hours which is not different from that of controls, either in the chow or the cafeteria groups.

Food Choice

The cafeteria diet as composed by the free choice of the rats is particularly rich in lipids and glucids. This preference for lipids and glucids was not modified by the benzodiazepines. However, while in the cafeteria control group the part of chow was, as is usual with such a diet, very small from the 1st day and negligible thereafter, in the benzodiazepine treated groups it was significantly greater on the first day, and with BR still on the second week (Table 1).

TABLE 2

WEIGHT OF WHITE (EPIDIDYMAL AND PERIRENAL) AND BROWN (INTERSCAPULAR) ADIPOSE TISSUES

	Epididymal (g)		Perirenal (g)		Interscapular (g)	
	Chow	Cafeteria	Chow	Cafeteria	Chow	Cafeteria
NaCl	6.27 ± 0.21	9.74 ± 0.92‡	5.37 ± 0.46	9.24 ± 0.94†	0.42 ± 0.04	0.73 ± 0.06‡
DZ	5.79 ± 0.42	8.06 ± 0.60†	5.05 ± 0.77	8.48 ± 1.11*	0.40 ± 0.03	0.64 ± 0.04‡
BR	5.45 ± 0.52	6.84 ± 0.50§	4.71 ± 0.68	7.34 ± 0.65†	0.38 ± 0.02	0.68 ± 0.04‡

n=7; Means ± SEM.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$ vs. chow group. §$p<0.001$ vs. NaCl cafeteria group

TABLE 3

SCORES OF ACTIVITY

Chow Diet			Cafeteria Diet		
NaCl	DZ	BR	NaCl	DZ	BR
41.36 ± 4.87	39.47 ± 7.53	46.36 ± 4.60	37.86 ± 3.60	33.36 ± 4.91	56.57 ± 3.48*

n=7; Means ± SEM.
*$p<0.001$ vs. NaCl cafeteria group.

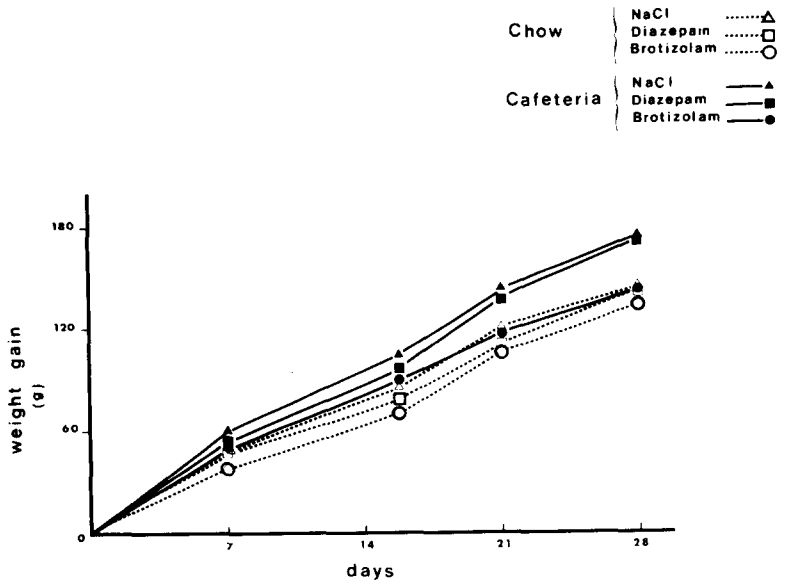

FIG. 4. Effects of brotizolam (1 mg/kg) or diazepam (2.5 mg/kg) versus control injections (NaCl) on weight gain during 28 days=chow rats and cafeteria rats. Values significantly different from control (▲) for cafeteria brotizolam (●) at 21 days $p<0.01$ and 28 days $p<0.001$.

Weight Gain (Fig. 4)

In the chow groups, neither of the benzodiazepines induced overweight: DZ did not modify the body weight curve; the chow BR group had a smaller weight gain than chow NaCl, $F(1,48)=9.93$, $p<0.003$; individual comparisons were not significant. In the cafeteria groups, when no active drug was given, the cafeteria diet provoked, as expected, a significantly higher weight gain than chow, $F(1,48)=20.65$, $p<0.001$. When treated, during the first two weeks, the rats gained no more weight than their cafeteria controls with either of the benzodiazepines; during the third week the weight of the DZ rats followed that of controls, (main diet effect: $F(1,48)=17.26$, $p<0.001$), but paradoxically enough, the weight of BR rats was lower than that of controls (main drug effect: $F(1,48)=19.99$, $p<0.001$).

Adipose Tissue—Table 2

Brown adipose tissue. Intrascapular brown adipose tissue was obviously increased by the cafeteria diet (main diet effect: $F(1,36)=71.88$, $p<0.001$) and the benzodiazepines did not further modify this effect.

White adipose tissue. The cafeteria control rats had heavier white epididymal, $F(1,36)=25.99$, $p<0.001$, and renal pads, $F(1,36)=25.98$, $p<0.001$, and while DZ did not modify this effect, BR inhibited this diet-induced increase in the epididymal pad, $F(1,24)=9.72$, $p<0.004$, which is consistent with BR effect on weight gain.

Activity

Non quantified everyday observation led us to conclude that throughout the study the rats treated with benzodiazepines seemed no sleepier but rather more active and at times aggressive with BR. Scores of activity (Table 3) confirm the absence of any sedative effect.

DISCUSSION

Benzodiazepines and Chow Fed Rats

Both the benzodiazepines tested provoke a post injection hyperphagia throughout the study (except on day 0). This hyperphagia is perfectly consistent with all the previous data [7, 10, 11, 15, 19, 22–24, 27]. It does not disappear over the 4 weeks of the experiment. Such an absence of tolerance is itself consistent with the previous data [10,27]. More striking is the finding that this hyperphagia is followed by a long compensatory hypophagia, in such a way that the mean 24 hour food intakes are not different in groups treated with drugs from those of controls. The efficiency of this compensatory hypophagia is confirmed by the weight evolution: neither the body weight nor the weight of fat pads are increased by the chronic treatment with drugs.

For the body weight effects, the data available in the literature are somewhat confusing: in rodents it has beeen reported that weight may decrease, increase or not be affected according to the doses and the protocols [7, 11, 15]. We found no references concerning the food intake over the 24 hour cycle, and then no references on compensatory hypophagia. Dogs [14], treated chronically, gain weight. Our clear cut results could seem contradictory with this but one possible explanation is that this species has a one meal a day pattern leaving no time for compensation.

Benzodiazepine and Cafeteria Fed Rats

In the cafeteria control group the classical effects of cafeteria diet were confirmed [2,4]: chronic hyperphagia, preference for fat foods, increase in body weight and in weight of fat pads. The only unusual feature was the dramatic hyperphagia on day 0, which we interpret as an effect of the stress due to NaCl injection in naïve rats (we have reported previously [4] that non specific stressor induced hyperphagia is particularly strong in animals offered a choice of foods).

At day 0, in rats treated with benzodiazepines, cafeteria induced hyperphagia occurs, as compared to chow fed rats, but is smaller than in the cafeteria non treated rats. After day 0 this hyperphagia remains within the usual range, with and without treatment. We have no explanation for the decrease in hyperphagia induced by benzodiazepines on the first day, which could appear to be contradictory with the literature. It has been reported that benzodiazepines facilitate the feeding of unfamiliar food [21], and obviously on day 0, the cafeteria food was unfamiliar. There is however an internal consistency in our results: with BR and DZ since at day 0, not only is there a decrease in cafeteria food hyperphagia, but also there is a relatively larger intake of the familiar chow.

For the 3 hr food tests, DZ does not significantly induce any extra hyperphagia (which is consistent with previous results reported with this kind of diet [9,16]), while BR does. This BR hyperphagia is compensated over the 24 hour cycle in such a way that in both treated groups the 24 hr intakes and weight gains are not different from those of the cafeteria control group.

In conclusion, chronic treatment by benzodiazepines does provoke a short term hyperphagia when monotonous food is presented, but this hyperphagia is entirely compensated over the 24 hour period; in cafeteria conditions, the hyperphagia still occurs after the injection of BR, not after DZ; when the hyperphagia occurs (with BR) it is also entirely compensated. This model, which is relevant to the human situation of patients taking benzodiazepines and evidently offered cafeteria diets, could explain the apparent discrepancy between the increased feeding action of benzodiazepines and the rarity of benzodiazepine induced obesity.

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