J. Pharm. Pharmacol. 1979, 31: 743–746 Received April 12, 1979

Effects of acute or chronic administration of chlordiazepoxide on feeding parameters using two food textures in the rat

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The effects of acute or chronic administration of chlordiazepoxide on several feeding parameters were studied in the rat. Given acutely, chlordiazepoxide reduced the rate of feeding, at lower dose levels (5·0 or 10·0 mg kg^{-1}) tended to prolong feeding duration, and at a higher dose (15·0 mg kg^{-1}) reduced the amount of food intake. Food texture can influence feeding parameters too; but the effects of the drug did not interact with the effects of food texture. After 9 daily injections of the drug, there were several indications of the development of tolerance to the effects of the drug on the feeding parameters. A possible role of 5-HT mechanisms in the reduction of eating rate produced by the drug is considered.

Benzodiazepines reliably facilitate feeding behaviour in a wide variety of mammalian species (Bainbridge 1968; Brown et al 1976; Fratta et al 1976; Randall et al 1960; Soubrié et al 1975). Little experimental attention however has been paid to the effects of chronic treatment with benzodiazepines on feeding behaviour; long term administration of diazepam or chlordiazepoxide increases feeding in the dog, leading to an increase in body weight (Heilman et al 1974; Randall et al 1960). There is only one report which addresses the possibility that tolerance can develop to the effects of benzodiazepines upon feeding responses. Wise & Dawson (1974) showed that with daily administration of 2·5 mg kg^{-1} diazepam in the rat, the amount of food consumed in a daily 45 min feeding test increased, particularly over the first week of treatment. They interpreted this result in terms of the development of tolerance to the sedative effects of the drug, which acted initially to suppress the amount of food intake. A main purpose of our experiment was to look for the development of tolerance to the effects of chlordiazepoxide, using a range of dose values.

An important development in feeding research has been to apply techniques for observing the microstructure of feeding behaviour (Wiepkema 1971) to the study of the pharmacology of feeding (Blundell & Latham 1978). In order to examine the effects of chlordiazepoxide on feeding in more detail, the measure of the amount of food-intake was subdivided into two components: the duration of feeding and the rate of food-intake whilst feeding is in progress. Drugs which affect the amount of food-intake equally, may, nevertheless, exert quite distinct effects upon these two components (Cooper & Francis 1978, 1979).

In recent work on benzodiazepines and feeding responses in the rat, we have used a short-exposure feeding test, which lasts 10 min, in order to restrict our attention to the factors which govern the initial approach to and consumption of food (Cooper & Crummy 1978). Taste is one factor which can interact with the facilitatory effect of chlordiazepoxide on feeding behaviour (McGuigan 1978). The present experiment looks for possible interactions between chlordiazepoxide treatment and food texture in determining feeding responses. Texture can be conveniently manipulated by presenting the standard laboratory diet in either powdered or pellet form.

METHODS

Animals. The subjects were 96 male Sprague-Dawley rats, supplied by Olac Southern Ltd., which were housed 3 per cage before the start of the experiment. The cages were a metal grill type (Bowman MRC cages). At testing, the rats weighed 260–285 g. The rats were maintained in a 12 h light: 12 h dark cycle, with lights on at 0700 h. Food and tap water were freely available in the home-cage. Room temperature was 21–23 °C and humidity >50%.

Drug. Chlordiazepoxide HCl was dissolved in 0·9% NaCl (saline), and made up to 3 dose levels: 5·0, 10·0 or 15·0 mg kg^{-1} (expressed as base). All injections were administered i.p. in 0·1 ml/100 g.

Procedure. On arrival in our laboratory, the rats were divided into 2 equal groups. Half the cages

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housing the animals were provided with standard diet (Diet 41B) in pellet form, and half with the same food in powdered form. Each animal was handled daily for 7 days as a taming procedure, before any further treatment began. Within each food texture condition, half the animals were allocated to the chronic treatment group. In this group, each rat was injected daily for 9 days at one of 3 dose levels of chlordiazepoxide (5·0, 10·0 or 15·0 mg kg^{-1}) or with a control saline solution. Within each food texture condition, the remaining animals were allocated to the acute treatment group. These rats received the same handling as the animals in the chronic group for 8 days (except that no injections were made). On the 9th day, each rat was injected at one of the same 3 dose levels or with a control saline solution. There were thus 12 rats tested under each injection condition, in both chronic and acute treatment groups (half with pellets, half with powdered food).

At 17.00 h on the 8th day, food was removed, and each rat was run in the feeding test on the 9th day (between 10·00 and 12.30 h). Food tests were restricted to a short period in the morning to avoid any time-of-day effect on the feeding response.

For the feeding test itself, the rat was transferred to a test-cage (of the same type as the home-cage), which contained a standard amount of food (11 g) available in a shallow glass Petri dish. The type of available food (powdered chow or pellets) corresponded to that in the home-cage; water was not available during the test. Two observers were used to record the time actually devoted to eating food during the 10 min test period. It is important to note that rats obtained the 2 types of food from the Petri dish in distinctly different ways. Eating from an open dish, rats usually grasped pellets in the forepaws, detaching small pieces, and masticated these before swallowing. Eating time in this case was taken from the time when chewing and biting the food began, and was recorded as long as the animal exhibited chewing movements. The powdered food was treated differently. Rats licked the powder rapidly, as if it were a liquid, and swallowed with little sign of chewing. Eating time was taken as the time devoted to licking up powder. Time in contact with the food, without evidence of eating, was not recorded.

The food was weighed before and after the test (subtraction giving the amount of food consumed). Feeding rate was later calculated in terms of the amount of food consumed (g) per 100 s devoted to feeding. The duration of the feeding test was limited to 10 min, to restrict observation to within the first meal following the overnight deprivation experience, and to ensure that drug levels remained relatively constant throughout the period of observation. This short exposure test yields highly reliable comparisons amongst drugs in terms of the measures taken: amount of food intake, eating duration and eating rate (Cooper & Francis 1978, 1979). All injections on the test day were given 30 min before the feeding test.

RESULTS

Amount of food intake (g)

Results for the amount of food consumed in the 10 min feeding test are shown in Table 1. Analyses of variance indicated that neither the food texture (pellets vs powdered food) nor the administration of the drug (chronic vs acute) had any significant effect on the amount of food intake. However, there was a significant effect of drug dosage ($F(3,80) = 5{\cdot}6$, $P < 0{\cdot}002$). Over all conditions there was a tendency for food intake to increase after administration of 5·0 mg kg^{-1} chlordiazepoxide, although this effect only reached a significant level for the chronic group fed with powdered food (Table 1). In the acute group, but not in the chronically-treated animals, the highest dose of chlordiazepoxide reduced the amount of food intake (Table 1).

Table 1. Amount of food intake (g) for powdered and pellet food after either chronic or acute chlordiazepoxide administration.

Drug (mg kg^{-1})	Chronic		Acute	
	pellets	powder	pellets	powder
0·0	1·71 ± 0·24	1·50 ± 0·13	1·65 ± 0·19	1·40 ± 0·15
5·0	1·88 ± 0·23	*2·07 ± 0·17	1·76 ± 0·15	1·57 ± 0·13
10·0	1·40 ± 0·30	1·68 ± 0·31	1·90 ± 0·25	1·32 ± 0·23
15·0	1·30 ± 0·43	1·42 ± 0·26	*0·80 ± 0·18	**0·88 ± 0·15

Each value is mean (±s.e.m.). n = 6 per cell.
Significantly different from corresponding control group: *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

Feeding duration (s)

The texture of the food did affect the time devoted to feeding ($F(1,80) = 8{\cdot}10$, $P < 0{\cdot}006$. The rats spent consistently more time eating powdered food (Fig. 1), and the effect was constant over the range of drug dosage, there being no significant interaction term. The effect was also independent of whether rats were allocated to either chronic or acute injections conditions. There was a significant effect of chlordiazepoxide on eating duration ($F(3{\cdot}80) = 3{\cdot}79$, $P < 0{\cdot}01$), in that the drug increased eating duration at certain dose levels (Fig. 1). However, the peak effect of chlordiazepoxide did depend upon whether

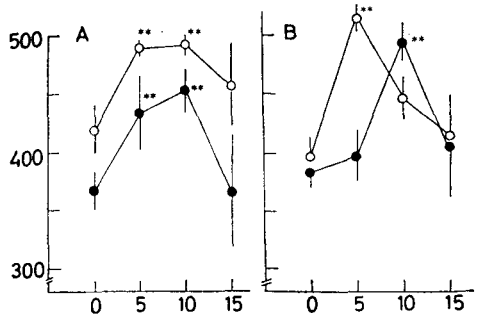

FIG. 1. Feeding duration (s). A: Effects of food presented in either powder (○—○) or pellet (●—●) form on the time (s) devoted to feeding. Chlordiazepoxide dosages indicated on the abscissa. B: Effects of either acute (○—○) or chronic (●—●) treatment with chlordiazepoxide on the time (s) devoted to feeding. Circles indicate the means (N = 12 rats per group), and the vertical lines indicate s.e. mean. Comparisons of drug effect with saline control using a *t*-test: ** $P < 0{\cdot}01$.

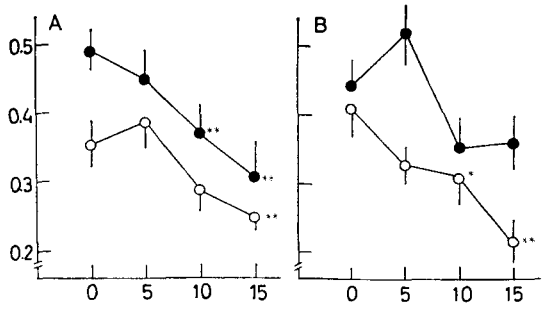

FIG. 2. Feeding rate (g/100 s feeding). A: Effects of food presented in either powder (○—○) or pellet (●—●) form on the rate of feeding calculated as the amount consumed (g) per 100 s devoted to eating. B: Effects of either acute (○—○) or chronic (●—●) treatment with chlordiazepoxide on the rate of feeding. Other conventions as in Fig. 1. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

the drug had been administered chronically or acutely. In the acutely-treated animals, the peak effect of the drug occurred at 5·0 mg kg^{-1}, whereas in the chronically-treated animals, the peak effect occurred at 10·0 mg kg^{-1}. The magnitude of the peak effect did not differ between the acutely-treated and chronically-treated groups ($t = 1{\cdot}30$, n.s.). The overall effect of chronic treatment with drug was to shift the dose effect curve to the right (Fig. 1).

Feeding rate (g/100s feeding)

The texture of the food did affect the feeding rate ($F(1,80) = 12{\cdot}14$, $P < 0{\cdot}001$), since rats consumed powdered food consistently more slowly than the pellet food (Fig. 2). Chlordiazepoxide also affected feeding rate ($F(3,80) = 7{\cdot}51$, $P < 0{\cdot}0004$), and generally acted to reduce feeding rate (Fig. 2). There was not a significant interaction term between food texture and drug dosage, indicating that their effects upon feeding rate were effectively additive. Chronic treatment with chlordiazepoxide did modify the drug's effects on feeding rate ($F(1,80) = 1{\cdot}47$, $P < 0{\cdot}001$, there being less reduction in feeding rate in the chronically-treated animals compared with the acutely-treated animals (Fig. 2). This effect was particularly striking at the lowest dose of chlordiazepoxide, where the feeding rate in the chronically-treated rats actually exceeded the control rate after saline injections (Fig. 2).

Body weights were not affected by the chlordiazepoxide treatment before the feeding test. An analysis of variance on the increase in body weight over the 8 days before the feeding test yielded no significant drug effect. At the time of the test therefore, rats chronically-treated with drug were matched in terms of body weight gain with acutely-treated rats.

DISCUSSION

The outcome of the feeding test, measured in terms of the amount of food ingested, depended upon the effect of chordiazepoxide on the time devoted to feeding, and secondly, on the rate of food ingestion during episodes of feeding. In the present experiment, chlordiazepoxide exerted significant effects upon these two latter feeding levels at doses which did not show any effect on the amount of ingested food. The importance of looking at feeding behaviour in terms of a number of feeding parameters, as emphasized by other authors (Blundell & Latham 1978; Wiepkema 1971), is also borne out by our work with (+)-amphetamine and (±)-fenfluramine. When matched in terms of the reduction in the amount of food intake that they produce, they differ quite clearly in terms of their respective effects on feeding duration and feeding rate (Cooper & Francis 1978, 1979).

Chlordiazepoxide acted to increase feeding duration, although this effect was largely counterbalanced by a reduction in eating rate, so that the effects on the amount of food intake tended to cancel (Table 1). Nevertheless, at 5 mg kg^{-1}, chlordiazepoxide consistently increased food intake, compared with control intake, across all 4 experimental conditions (Table 1). The reduction in feeding rate may involve some effect on a central 5-hydroxytryptaminergic system. Blundell & Latham (1978) have proposed that one effect of 5-HT on food intake is to reduce the rate of eating. Fenfluramine, the effects of which

on feeding behaviour appear to be mediated by 5-HT mechanisms (Blundell 1977), acts to reduce the rate of eating. When chlordiazepoxide and fenfluramine are given in combination, their separate effects on rate of eating summate to produce a gross retardation in feeding rate (Cooper & Francis 1978).

The effects of chlordiazepoxide on feeding duration and feeding rate were independent of the food texture in this experiment. It remains to be seen whether other textural manipulations might interact with the drug's effects on feeding parameters. By contrast, taste does appear to interact with the effect of chlordiazepoxide on feeding, since rats treated with the drug eat more sweet-flavoured food in preference to unadulterated food than do control animals (McGuigan 1978). Evidently the effects of chlordiazepoxide on feeding parameters are also unaffected by the type of feeding action employed by the rat, whether it is chewing or licking. This being so, it would be expected that chlordiazepoxide should decrease the rate of licking liquids, in the same way that it reduces feeding rate. This inference has been confirmed (Knowler & Ukena 1973).

Tolerance to the effects of chlordiazepoxide did develop over the course of the 9 daily injections. This is revealed in 3 ways. Firstly, there was a shift to the right in the dose-effect curve for the feeding duration (Fig. 1) in the chronically-treated animals. Hence, a higher dose was required to produce the peak increase in feeding duration. Secondly, there was less of a reduction in feeding rate in chronically-treated animals (Fig. 2) and thirdly, only in acutely-injected animals did the highest dose of chlordiazepoxide significantly reduce the amount of food intake (Table 1). It has been suggested that tolerance develops to the sedative effects of benzodiazepines over a course of chronic administration (Goldberg et al 1967; Margules & Stein 1968; Wise & Dawson 1974). A reduction in the sedative effects would account for the less pronounced effect of the drug on feeding rate in the chronically-treated animals. Evidence from another type of feeding test indicates that whereas tolerance develops to the sedative effect of benzodizepine treatment, tolerance does not develop to the hyperphagic effect (Wise & Dawson 1974). Our results are consistent with this view, in that the slightly higher level of food intake at 5 mg kg^{-1} chlordiazepoxide was not diminished but was improved by chronic treatment.

The differences observed between chronically-treated and acutely-treated rats were due to the difference in the chlordiazepoxide treatment alone. Saline control animals, under the two procedures of acute and chronic administration, were indistinguishable. Hence acutely-treated animals did not respond differently to the injection procedure itself compared with the chronically-treated animals.

Compared with acute treatment with chlordiazepoxide, chronic administration can affect the turnover of 5-HT in the midbrain, hypothalamus and cerebral cortex (File & Vellucci 1978). A reduction in 5-HT turnover in various brain regions may therefore help to account for the onset of behavioural tolerance to chlordiazepoxide. This mechanism may accord with our proposal that chlordiazepoxide reduces the rate of eating possibly by an action on 5-HT in the brain, that this effect on eating reflects the sedative action of the drug, and that tolerance develops to the reduction in eating rate (Fig. 2) with repeated chlordiazepoxide administration.

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