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A Microgram Dose of Diazepam Produces Specific Inhibition of Ambulation in the Rat

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COOPER, S J *A microgram dose of diazepam produces specific inhibition of ambulation in the rat* PHARMACOL BIOCHEM BEHAV 22(1) 25–30, 1985 —There is considerable consistency in the experimental literature showing that non-sedating doses of benzodiazepines can enhance the consumption of food, water and salt solutions It is of great interest, therefore, that in a previous report low dose treatments with diazepam were found to significantly suppress the level of consumption of a palatable 0 005 M sodium saccharin solution in nondeprived male rats The present study was designed to elucidate the behavioral characteristics of the inhibitory action of low dose diazepam treatments. Food consumption and general activity measures were chosen for analysis to examine the possibilities that low dose diazepam treatments might suppress ingestive behavior in a general way, or that the treatments might affect nonconsummatory responses including components of spontaneous motor activity. The results of two experiments succeeded in locating a highly specific inhibitory effect produced by 100 μg/kg diazepam First, food consumption was not inhibited. Instead, 1 0 mg/kg diazepam produced significant elevations in food intake in both food-deprived and nondeprived animals Second, vertical activity (rearing) and fine body movements were unaffected over the dose-range 0 1–3 0 mg/kg diazepam. Hence, low dose treatments with diazepam did not produce a generalised nonspecific behavioral depression However, 100 μg/mg diazepam significantly inhibited coarse activity (measured automatically) and the corresponding ambulation measure (recorded by direct observation). The effect was present throughout a 1 hr test period and did not interact with the declining baseline level of activity The results therefore confirm the presence of low dose diazepam-induced behavioral inhibition in quite a different context from the saccharin solution consumption study The effects evidently occur at dose levels below those of diazepam which are normally employed in psychopharmacological investigations. They draw attention to an important new dimension in diazepam's effects on behavioral variables, and suggest a novel mechanism of action occurring in the microgram dose range

Diazepam Food intake Locomotion Rearing Behavioral inhibition

BENZODIAZEPINES enhance food consumption in many species (e.g., [1, 3, 4, 8, 9, 14, 16, 20, 22, 23, 27, 35]). They have also been reported to increase water consumption, the consumption of sweetened fluids and the intake of salt solutions (e.g , [10, 11, 13, 14, 19, 21, 24, 25, 30, 31, 32, 33, 36]) Above certain doses, however, side-effects of the drug treatments, including sedation, exert a counter effect and intake is progressively reduced. Impairments in ingestional responses can be detected, for example, by increases in the latency to consume foods and fluids, and by decreases in the rate of feeding and drinking [10,15] However, until recently, there has been no experimental basis for postulating that there may be inhibitory effects of benzodiazepine treatments following low dose treatments.

In the previous study [12], nondeprived male rats were given access to a highly palatable 0.005 M sodium saccharin solution. Within the first hour of access to the solution, control animals had consumed 25 ml, and by the end of six hours, the mean consumption was approximately 50 ml. These data are similar to those reported earlier [28]. Unexpectedly, it was found that diazepam, at dose levels *lower* than those normally reported to increase the intake of food and water, exerted a highly-significant suppression of the saccharin drinking [12]. The effect was present during the first hour of the drinking test, was produced by 0.1 and 0.3 mg/kg diazepam, and persisted undiminished throughout the 6 hour test period. There was no evidence that this inhibitory effect could be attributed to sedation.

The inhibitory effect on saccharin solution drinking produced by diazepam may have been a unique phenomenon, and have no generality outside the particular experimental situation employed in the study. If so, there would still be a need to explain the highly specific nature of the drug effect Alternatively, low dose diazepam treatment may inhibit consummatory responses more generally and thus, for example, inhibit feeding responses as well. A second alternative is that the diazepam treatments may affect one or more components of general activity, and so produce inhibitory effects on behavioral responses not necessarily concerned with ingestion To study the phenomenon in more detail, therefore, the present study was designed to investigate the possible effects of diazepam (0.1–3.0 mg/kg) on food intake in nondeprived and food-deprived animals, and, separately, on several measures of general activity. The aim was

to detect the possible expression of additional behavioral inhibitory effects following administration of low doses of diazepam in the rat

EXPERIMENT 1

The aim of the first experiment was to determine if there was a low dose inhibitory effect of diazepam in relation to food consumption The control level of feeding was manipulated by comparing the effects of diazepam treatments (0 1–3 0 mg/kg) in nondeprived and in 22 hr food-deprived animals

METHOD

Animals

The subjects were 80 experimentally naive male, hooded rats (General strain) bred in our laboratory They were housed in threes in stainless steel cages, with free access to food pellets (modified Diet 41B, Heygate and Sons, U.K) and water. They were maintained under a 12 hr light-12 hr dark cycle (lights on at 7:00 a m.) and room temperature was kept constant at 21°C The animals were thoroughly accustomed to handling before testing, and weighed in the range 280–350 g at the time of the study.

Procedure

The subjects were randomly allocated to two equal groups, the nondeprived and food-deprived groups. In the case of the food-deprived animals, food was removed from the home cage 22 hr before the feeding test. Within each group, the animals were allocated to 5 sub-groups, according to injection conditions: 0, 0.1, 0.3, 1.0 and 3.0 mg/kg diazepam, respectively. These doses were chosen to match those used in a previous study [12] The drug vehicle was propylene glycol and water (48.52% by volume), and the solutions were administered intraperitoneally. Solutions were made up immediately before use.

For the feeding tests, animals were removed from the home-cage and were placed individually in identical cages in a quiet room Food was not initially present in these test cages. Thirty minutes before presentation of food, each animal received an appropriate injection At the end of the 30 min interval, food was presented in a spillproof glass jar for a 1 hr test period The food was a palatable mixture of crushed standard food pellets and corn oil (in a ratio of 3.1, by weight). The food and jar were weighed before and after the test period, and the weight of food consumed was calculated from the difference Weighings were made to an accuracy of 10 mg Any spillage which did occur was collected beneath the cage, weighed and the weight taken into account when calculating the amount consumed In addition to measurement of food intake, the latency to begin feeding (s) was noted in each case Water was not available during the feeding test It was considered important to run the experiment under blind conditions. Therefore solutions were coded by letter, and the experimenter performing the injections and weighing the food did not know the identity of either the drug being tested or the doses of the drug employed

The latency data were first subject to a logarithmic transformation to normalise a markedly skewed distribution of scores The transformed latency scores and the raw food intake data were analysed separately using a 2-way analysis of variance for independent factors [34] Differences between individual dose conditions and the corresponding control group were analysed using Dunnett's t test

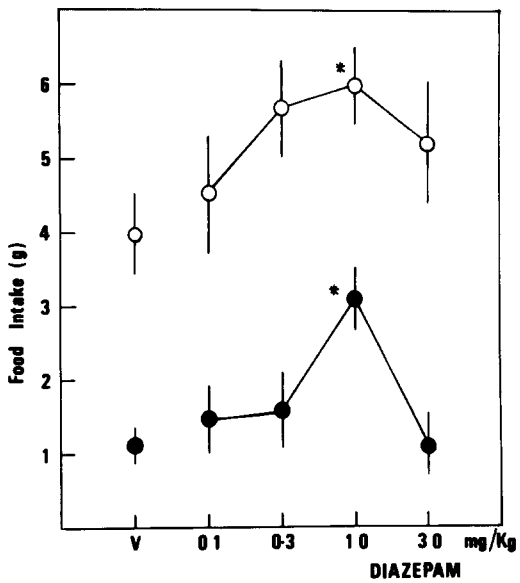

FIG 1 Effects of diazepam treatments (0 1–3 0 mg/kg, IP) on the food consumption (g) of nondeprived (●) and 22 hr food-deprived (○) male rats in a 60 min test period The results are shown as the mean±S E M (n=8 per group) *Indicates group significantly different from controls (V), $p<0$ 05 (Dunnett's t test)

RESULTS

Food Intake (g)

The effects of diazepam (0 1–3 0 mg/kg) on the food consumption of deprived and nondeprived animals are shown in Fig 1 Under control conditions, nondeprived animals consumed a mean of 1.13 g in the 1 hr test period, and this was increased to 3 99 g in the 22 hr food-deprived animals The difference in food consumption between the deprived and nondeprived animals across all conditions of the experiment was highly significant, $F(1,70)=82$ 29, $p<0.001$ There was also a significant drug main effect, $F(4,70)=2$ 76, $p<0$ 05, but the interaction term was not significant, $F<1$ 0 Hence, the effect of diazepam on food consumption was statistically independent of the variation in food intake attributable to the levels of food deprivation (0 and 22 hr)

There was only one condition under which feeding was depressed This occurred in nondeprived animals which had been injected with the highest dose of diazepam, 3 0 mg/kg. Fifty percent of the animals failed to consume any food in the test. The mean intake of the remaining animals in the group was 2 45 g, and of these, two animals consumed only 0 11 g and 0 23 g, respectively All animals in this group exhibited clear signs of drug-induced sedation. In striking contrast, all the food-deprived animals injected with the same dose of diazepam consumed some food (range 1 50–8 39 g). The occurrence of sedation was therefore apparently counteracted by an increased arousal due to the food deprivation

There was no indication of an inhibition of feeding following low dose treatments with diazepam. Instead, significant increases in food consumption occurred following the administration of 1 0 mg/kg diazepam in both food-deprived and nondeprived groups (Fig. 1) In each case, the increase in food consumption was approximately 2 g Hence, the diazepam-induced hyperphagia was a constant effect and not influenced by the deprivation state of the animals

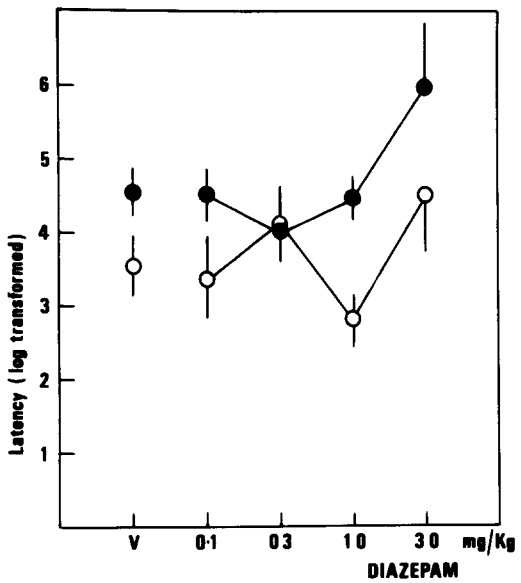

FIG 2 Effects of diazepam treatments (0 1–3 0 mg/kg, IP) on the latency to initiate feeding (log transformed scores raw data in seconds) in nondeprived (●) and 22 hr food-deprived (○) male rats in a 60 min test period The results are shown as the mean±S E M (n=8 per group)

Latency to Feed

The effects of diazepam (0.1–3.0 mg/kg) on latency to feed (log transformed scores) in the test are shown in Fig 2 Food-deprived rats, as expected, were significantly faster to start eating compared with non-deprived animals, $F(1,70)=9\ 27$, $p<0.005$. Overall, there was not a significant drug effect, $F(4,70)=2.33$, N.S., and the interaction term was not significant, $F<1.0$ It should be noted, however, that 4 of the 8 nondeprived animals which were injected with 3 0 mg/kg diazepam failed to eat. They were given the maximum latency score, therefore, which contributed to the noticeably higher mean latency score in this group compared with that of the controls (Fig. 2).

DISCUSSION

The results indicate that low dose treatments with diazepam did not alter food consumption in either nondeprived or deprived animals However, following the administration of 1.0 mg/kg diazepam, food consumption was significantly increased by about 2 g in both nondeprived and deprived animals. The diazepam-induced hyperphagia is consistent with other reports, which have shown similar hyperphagic effects at doses of 1.5 mg/kg [3], 2 0 mg/kg [22] and 2.5 mg/kg [35] In the present study, there was an indication of a hyperphagic effect in deprived animals following injection of 0.3 mg/kg diazepam, but it failed to reach the 5% level of significance. For comparison, it is interesting to note that diazepam has been shown to enhance water consumption in water-deprived rats at a dose of 1.25 mg/kg [10]. Taken together, the results of these studies suggest that 1.0 mg/kg diazepam may be close to a lower limit for obtaining reliable enhancement of ingestional responses.

The present data confirm and extend a previous observation by Cole [8], who showed that the hyperphagic effects of chlordiazepoxide and food deprivation were additive These results are theoretically interesting, in part because the effects of other drugs on food consumption are known to interact strongly with the effects of food deprivation. Such interactions have been reported, for example, with amphetamine [6,7] and morphine [29]. Benzodiazepines clearly do not potentiate the effects of food deprivation, since a similar drug-induced hyperphagia could readily be obtained in hungry and satiated animals We are not in a position to judge, at present, the extent to which the mechanisms which underlie benzodiazepine-induced hyperphagia coincide with those which mediate deprivation-induced hyperphagia.

The suppression of saccharin solution consumption could not therefore have been due to a general inhibition of ingestive responses. This conclusion is supported by a similar failure to observe decreases in water consumption in water-deprived rats following comparable low dose treatments with diazepam (unpublished data). Therefore, the suppression of saccharin solution consumption may depend on factors intrinsic to the particular experimental conditions used. The possibility that low doses of diazepam may inhibit noningestional responses was investigated in the second experiment.

EXPERIMENT 2

The aim of the second experiment was to assess the possibility of a low dose inhibitory effect of diazepam in relation to measures of spontaneous motor activity. Automatic measures of fine and coarse movements were taken, and these were supplemented by direct observations of ambulation and rearing

METHOD

Animals

The subjects were an additional 40 experimentally naive male hooded rats (General strain) from our laboratory. They were within the same weight range as for subjects used in the first experiment, and were housed under the same conditions. The animals were accustomed to handling over a period of 7 days before testing.

Procedure

For the activity test, rats were placed individually in a novel open-field apparatus made of a clear-sided Perspex container (55×55×45 cm). The floor was covered with a fine layer of sawdust, and was marked into quadrants. Automatic measures of activity were made using a Stoelting electronic activity monitor (cat. no. 31404). The two counters for coarse activity and for total activity were adjusted respectively to detect large body movements (ambulation, rearing) and small body movements (head movements, scratching, grooming), in addition to large body movements. The settings for the two monitors were then held constant across all animals of the study. Each animal remained in the apparatus for 60 min. The automatic measures of activity were supplemented by direct observational analysis. A record was kept of the frequency of rears (standing on hind legs with forelimbs raised over half the vertical height), and of ambulation (crossing from one floor quadrant to an adjacent one with all four paws). Measures of total and coarse activity, rearing and ambulation were recorded at 10 min intervals. Animals were tested at the same time of day, 9.00 a.m.–12.00 noon, to minimise circadian variability in activity.

The subjects were assigned at random to 5 injection groups, identical to those used in the first experiment. Again,

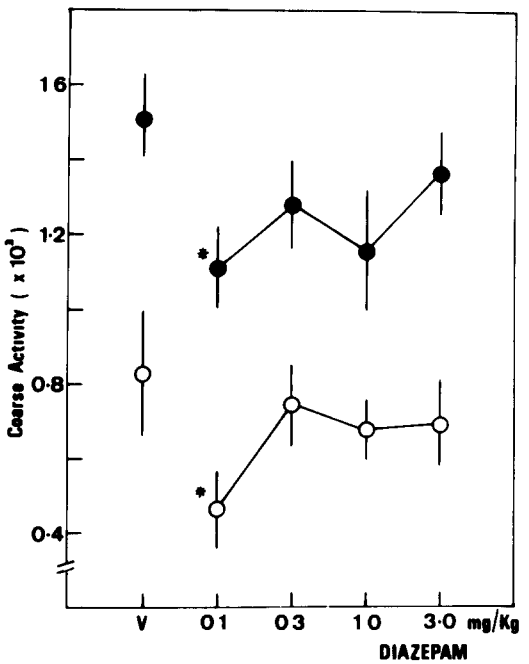

FIG 3 Effects of diazepam treatments (0 1–3 0 mg/kg, IP) on a measure of coarse activity (frequency counts × 10^3) during the first 30 min (●) and the second 30 min (○) of a 60 min test period Diazepam (0 1 mg/kg) significantly inhibited coarse activity over the course of the 1 hr test The results are shown as the mean±S E M (n=8 per group) *Indicates a significant difference from the control condition (V), $p<0$ 05 (Dunnett's t test)

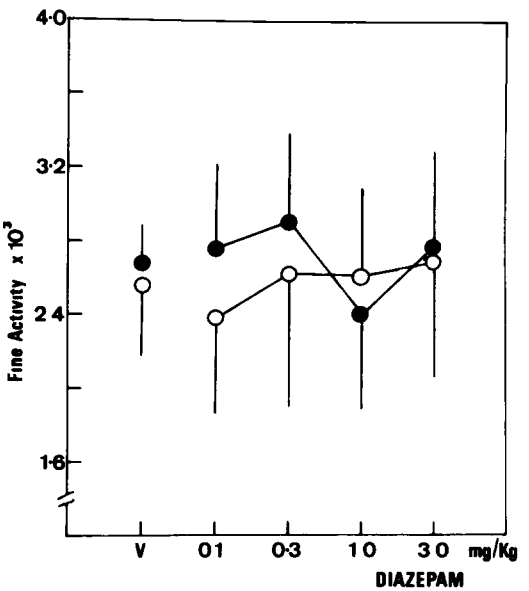

FIG 4 Diazepam treatments (0 1–3 0 mg/kg, IP) had no effect on a measure of fine activity (frequency counts × 10^3) during the first 30 min (●) and the second 30 min (○) of a 60 min test period The results are shown as the mean±S E M (n=8 per group)

the experiment was conducted under blind conditions with a new letter code to designate the treatments. Injections were administered IP, 30 min before the test of activity. Solutions were made up immediately before use The vehicle for the diazepam was 48% propylene glycol 52% water.

Analyses of variance were carried out on the activity scores The data were reduced to first 30 min and second 30 min scores, to give a repeated measures factor with 2 levels To obtain a measure of fine activity, the coarse activity scores for each rat were subtracted from the total activity scores before analysis of the results. Tests of significance between individual dose conditions and the control vehicle condition were made using Dunnett's t test.

RESULTS

The effects of the diazepam treatments on the coarse activity measure for the first and second 30 min periods of the test are shown in Fig 3 Over the 1 hr test, coarse activity declined and this is illustrated by the highly significant difference between the levels of activity in the two halves of the test, $F(1,14)=45$ 18, $p<0$ 001. There was also a significant effect of diazepam treatments, $F(4,56)=3.04$, $p<0.01$ Individual group comparisons confirmed that the lowest dose employed, 100 μg/kg diazepam, significantly reduced the coarse activity measure (Fig 3) Interestingly, the diazepam effect did not interact with the time factor, $F=0.328$ Thus, the effect was present not only in the first 30 min period, but also in the second. Furthermore, the inhibitory effect produced by 100 μg/kg diazepam was similar despite the reduction in control levels of activity from the first half of the test to the second (Fig. 3). Significant effects on coarse activity were not observed at other dose levels

In contrast, diazepam had no effects on the measure of fine activity, $F<1.0$, as shown in Fig 4 Furthermore, the level of fine activity did not alter as a function of time, $F<1$ 0 Levels were equivalent in the two halves of the test

Ambulation scores followed a closely similar pattern to those for coarse activity, and consequently are not shown graphically There was a highly significant difference between ambulation scores in the two halves of the test, $F(1,14)=164$ 57, $p<0.001$, a significant diazepam effect, $F(4,56)=2$ 55, $p<0$ 05, and no interaction between the two, $F(4,56)=1$ 08, N.S. Individual group comparisons showed that 100 μg/kg diazepam significantly depressed ambulation scores in both halves of the test, $p<0$ 05

Finally, rearing activity declined significantly from the first to the second half of the test, $F(1,14)=53$ 38, $p<0.001$, but there was not a significant diazepam effect, $F<1$ 0 The rearing scores are shown in Fig 5

In summary, therefore, over the course of the 1 hr test period, coarse activity, ambulation and rearing showed substantial decrements from the first half of the test to the second Diazepam at a dose of 100 μg/kg significantly inhibited activity measured by ambulation and coarse activity scores This low dose inhibitory effect was behaviorally specific, probably related to locomotion and exploratory activity about the test enclosure, and did not extend either to vertical activity (rearing) or fine activity (including grooming, small body movements)

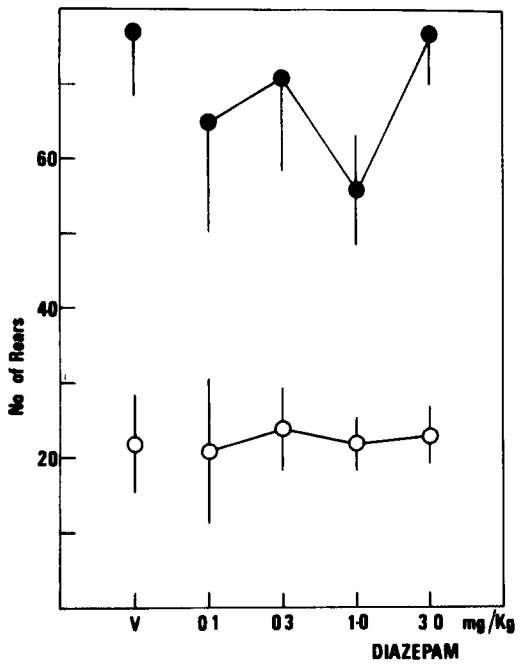

FIG 5 Diazepam (0 1–3 0 mg/kg, IP) did not significantly affect the frequency of rears during the first 30 min (●) and the second 30 min (○) of a 60 min test period The results are shown as the mean± S E M (n=8 per group)

GENERAL DISCUSSION

The results of the present study provide the first evidence of a specific inhibitory effect of diazepam on a major component of spontaneous motor activity, which occurred at a dose level substantially lower than those typically used in behavioral pharmacology. This is not an isolated example, however, since it is now firmly established that low dose treatments with dopamine receptor agonists result in behavioral depression as a consequence of selective action at dopamine autoreceptors [5, 17, 18, 26]. The similarity between the two examples may be only coincidental However, it does emphasize that more attention should be directed to possible anomalous behavioral effects of the benzodiazepines, particularly following low dose treatments It is also worth noting that although amphetamine-induced anorexia is generally taken to be axiomatic, there are instances where low dose treatments with amphetamine have been shown reliably to increase food consumption [2]. Hence, it is very important that the familiar behavioral effects of the benzodiazepines should not be extrapolated uncritically to lower dose ranges without empirical testing.

The results of Experiment 2 indicate that 100 μg/kg diazepam produced a significant inhibition of locomotor activity (indexed by coarse activity and ambulation measures). The effect could not have been due to sedation, and probably did not involve an interaction with habituation processes. The size of the inhibitory effect remained constant from the first to the second half of the test, despite the fall in baseline. Animals did become progressively less active (measured by coarse activity, ambulation and rearing) throughout the 60 min test period. The diazepam effect was independent of this general reduction in behavior. The inhibitory effect of the 100 μg/kg diazepam treatment did not extend to the control of food consumption which supports the conclusion that the effect could not have occurred as a result of general behavioral depression.

Of particular interest is the result that low dose diazepam treatment specifically inhibited ambulatory activity, but did not affect rearing. Although rearing and ambulation scores are usually closely associated, rearing was not sensitive to the inhibitory effect, whereas ambulation was The low dose diazepam treatment appears to have produced at least a partial dissociation between the two forms of motor activity, suggesting some distinction between the events controlling them.

Previously it had been shown that 100 μg/kg diazepam was sufficient to suppress the level of saccharin solution consumption over a 60 min period in nondeprived male rats [12]. It is now clear that the inhibition of saccharin solution drinking was not due to a general suppression of ingestional responses (Experiment 1; unpublished results). At the present time, it seems possible that the inhibitory effect of diazepam on saccharin solution drinking may be closely linked in some way to the inhibitory effect on locomotion.

These behavioral results indicate a novel mechanism of action for diazepam which cannot be accommodated by any current model of benzodiazepine action. Potent and highly selective inhibition of behavior was achieved with a dose of diazepam as low as 100 μg/kg in the rat. The current intense interest in the effects of novel drugs which act at benzodiazepine receptors (including antagonists and reverse agonists) should not lead us to neglect closer scrutiny of the 'classical' benzodiazepine compounds The present data indicate that considerably more attention should be paid, than has been the case, to novel behavioral and biochemical actions of benzodiazepines in the microgram dose range. At the very least, unsuspected mechanisms of action may carry important clinical implications, especially with regard to a class of drugs which are so extensively prescribed

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