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BRIEF COMMUNICATION

Withdrawal, Tolerance and Sensitization After a Single Dose of Lorazepam

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FILE, S. E., L. J. WILKS AND P. S. MABBUTT. *Withdrawal, tolerance and sensitization after a single dose of lorazepam.* PHARMACOL BIOCHEM BEHAV 31(4) 937–940, 1988.—Forty-eight hours after a single dose of lorazepam (0.25 mg/kg), there was tolerance to the lorazepam-induced reduction of locomotor activity in the holeboard; but no tolerance to the reductions in exploratory head-dipping or rearing. Mice tested undrugged at this time showed significant hyperactivity and increased rearing, indicating withdrawal responses, but no change in head-dipping. In the elevated plus-maze, no tolerance could be detected to the effects of lorazepam (0.25 mg/kg) when the mice were tested 48 hr after an initial dose; in fact, there was a trend towards enhanced effects in this group. When mice were tested undrugged 24, 48 or 72 hr after a single dose of lorazepam there was an increase in the % time spent on the open arms, compared with controls, that reached significance for the 24 hr group. This indicates a sensitization to the anxiolytic effects of lorazepam, as assessed in the plus-maze. These results demonstrate long-lasting effects of even a single dose of lorazepam.

Benzodiazepine Lorazepam Mouse Tolerance Withdrawal Hyperactivity Anxiety Sensitization

VERY rapid tolerance to the sedative effects of benzodiazepines has been reported in man following overdose, even at times when the plasma concentrations remain high (12), and tolerance to the hypnotic effects of a single high dose of diazepam has been found in mice (21). Tolerance has been found to anticonvulsant effects following a single dose of clobazam in mice (4) and following a single dose of lorazepam in mice and rats (16). In both these studies tolerance occurred at a time when there was still some residual drug acting at the benzodiazepine receptor. A recent in vivo binding study indicated that following an acute dose of oxazepam in mice there was a change in the affinity of the benzodiazepine receptor, such that higher concentrations of oxazepam were needed to produce the same level of receptor occupancy (20). Tolerance developed to the deficits in rotarod performance after two doses of diazepam (13), indicating that some behavioral effects of benzodiazepines do not show tolerance after single doses. Indeed there is good evidence that tolerance develops at different rates to the different behavioral effects of the benzodiazepines (7), and it has been suggested that different mechanisms might underlie the development of tolerance to the various responses (14). The purpose of the present experiment was to determine whether it was possible to detect tolerance to the effects of benzodiazepines in the holeboard and in the elevated plus-maze test of anxiety, following a *single* dose of lorazepam.

A second purpose of the study was to determine whether tolerance and withdrawal responses appeared in parallel following a single injection of lorazepam, as they seem to do following chronic benzodiazepine treatment (11). In order to do this, additional groups of mice were tested undrugged at the same time-points after a single dose of lorazepam. There is less evidence on the occurrence of withdrawal responses following a single dose of benzodiazepine. None could be detected in a drug discrimination paradigm 8 hr after a single extremely high dose of diazepam (3), but it was possible to demonstrate sensitization to the proconvulsant effect of a benzodiazepine inverse agonist 6 hr after a single dose of lorazepam, indicating an 'acute withdrawal' response (16). A spontaneous withdrawal syndrome has been described in the rat, occurring 100–124 hr after an exceptionally high (450 mg/kg) dose of chlordiazepoxide (2).

METHOD

Drugs

Lorazepam (as Ativan injection, Wyeth) was further diluted to a concentration of 0.06 mg/ml and administered

orally at a dose of 0.25 mg/kg. Behavioral testing took place 1 hr after dosing since this is the time of peak effect after oral administration.

Animals

Male albino mice (Bantin & Kingman), weighing 25–30 g, were housed in groups of 5 with food and water freely available.

Apparatus

The holeboard was a wooden box 40×40×27 cm, with four 3.2 cm diameter holes in the floor. It provides independent measures of exploratory head-dipping, rearing and locomotor activity (10,15). All responses were automatically recorded by the interruption of infrared photobeams placed under the holes and in the walls, respectively.

The plus-maze was wooden with two open arms, 17×8 cm, and two enclosed arms with walls 30 cm high. It was raised 50 cm above the floor.

Procedure

Experiment 1. Each mouse was given a 7.5 min trial in the holeboard and the animals were tested in an order randomised for drug treatment, between 0800 and 1300 hr. In a previous study we had found increased locomotor activity 48 hr after a single dose of lorazepam, and therefore this time interval was selected for the holeboard study. Ten mice were randomly allocated to each of the following 4 groups: control group (vehicle 48 hr and 1 hr before test); acute lorazepam (vehicle 48 hr and lorazepam 1 hr before test); acute tolerance to lorazepam (lorazepam 48 hr and 1 hr before test); withdrawal from acute lorazepam (lorazepam 48 hr and vehicle 1 hr before test).

Experiment 2. In this experiment, 14–15 mice were allocated to each of the treatment groups used in Experiment 1, but the mice were tested for 5 min in the elevated plus-maze test of anxiety. Two additional groups were included, tested undrugged 24 and 72 hr after lorazepam, because of the possibility that withdrawal responses in the elevated plus-maze might occur at a different time from the withdrawal responses in the holeboard. Certainly, following chronic lorazepam treatment, we have found that the times at which withdrawal responses can be detected vary for different responses (14). Each mouse was placed in the center of the plus-maze and allowed to enter freely into any of the arms. The time spent on the open arms as a percentage of the total time in arms, and the number of entries onto the open arms, as a percentage of the total arm entries, provide measures of anxiety (10,12). Total arm entries provides a measure of general activity. An arm entry was defined as all four paws in the respective arm and the behavior was scored by an observer, with no knowledge of the drug groups, seated 1 m from the maze. Testing took place in an order randomised for drug treatment, between 0730 and 1200 hr.

RESULTS

Experiment 1

It can be seen from Fig. 1 that 48 hr after an injection of lorazepam (0.25 mg/kg), a second test injection had significantly less sedative effect, as reflected in a decrease in locomotor activity, compared with the group receiving only a single test injection. The group tested undrugged 48 hr after a single injection of lorazepam showed significant hyperactivity, compared with the controls.

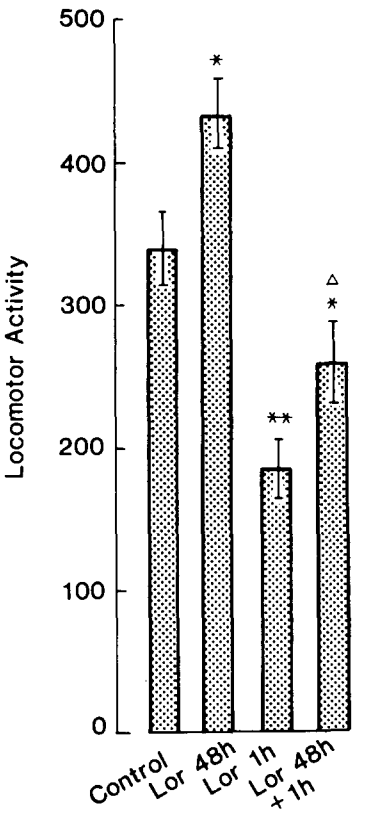

FIG. 1. Mean (±sem) locomotor activity in mice tested undrugged 48 hr after an injection of lorazepam (Lor 48 hr), after an acute dose of lorazepam (Lor 1 hr) or following a probe dose of lorazepam given 48 hr after an earlier dose (Lor 48 hr + 1 hr). $^{**}p<0.01$, $^{*}p<0.05$ compared with controls; $\triangle\ p<0.05$ compared with acute lorazepam group; Dunnett's tests after analysis of variance.

An increase in the number of rears could also be detected 48 hr after a single dose of lorazepam (see Fig. 2). However, there was no evidence for tolerance to the effects of lorazepam to reduce rearing, and the group that received lorazepam both 48 and 1 hr before test had scores no different form those of the group receiving lorazepam only 1 hr before test (see Fig. 2). There was no evidence for either tolerance to, or withdrawal from, an acute dose of lorazepam in the measures of exploratory head-dipping (see Table 1).

Experiment 2

From Fig. 3 it can be seen that lorazepam (0.25 mg/kg 1 hr before test) significantly increased the % time spent on the open arms, and the % number of entries onto open arms, but was without effect on total arm entries. This indicates an anxiolytic effect of acute lorazepam. The increases in % time on open arms and % number of entries onto open arms were as marked in animals that had received lorazepam 48 hr earlier as for the acute treatment group, and in fact the trend was for a greater effect. However, the difference between the two lorazepam groups did not reach significance, partly because of the larger standard error in the 48 hr + 1 hr group.

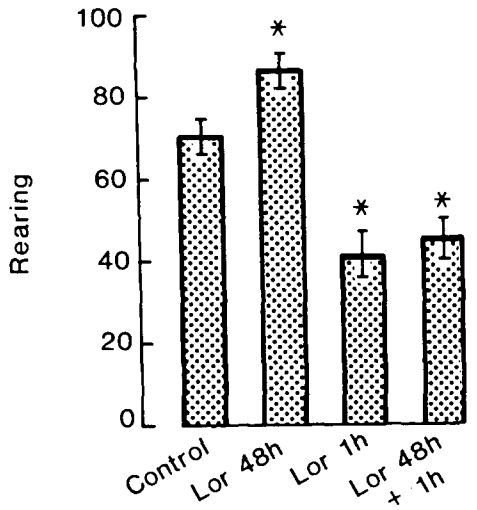

FIG. 2. Mean (±sem) number of rears by mice tested undrugged 48 hr after an injection of lorazepam (Lor 48 hr), after an acute dose of lorazepam (Lor 1 hr) or following a probe dose of lorazepam given 48 hr after an earlier dose (Lor 48 hr + 1 hr). *$p<0.05$ compared with controls; Dunnett's tests after analysis of variance.

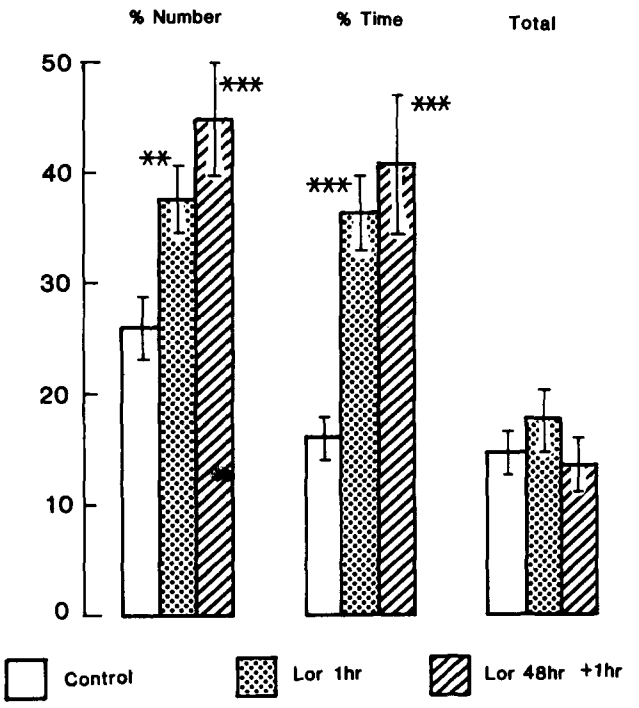

FIG. 3. Mean (±sem) % time spent on open arms, % number of entries onto open arms and total arm entries for groups of mice tested after an acute dose of lorazepam (0.25 mg/kg) (Lor 1 hr) or following a test dose of lorazepam (0.25 mg/kg) given 48 hr after an earlier dose (Lor 48 hr + 1 hr). **$p<0.01$, ***$p<0.001$ compared with controls, analysis of variance.

TABLE 1

MEAN (±sem) NUMBER OF HEAD-DIPS AND TIME SPENT HEAD-DIPPING (sec) BY MICE INJECTED WITH AN ACUTE DOSE OF LORAZEPAM (0.25 mg/kg), OR INJECTED WITH A PROBE DOSE OF LORAZEPAM 48 hr AFTER AN EARLIER DOSE, OR TESTED UNDRUGGED 48 hr AFTER A DOSE OF LORAZEPAM

Drug Treatment		Number of Head-Dips	Time Spent Head-Dipping
48 hr	1 hr		
Con	Con	70.8 ± 8.4	40.2 ± 8.1
Con	Lor	54.0 ± 10.7*	26.9 ± 6.1*
Lor	Lor	44.5 ± 8.1*	20.7 ± 5.5*
Lor	Con	72.8 ± 11.6	39.3 ± 7.9

The control group received vehicle injections both 48 hr and 1 hr before test. *$p<0.01$, compared with controls, Dunnett's test after analysis of variance.

TABLE 2

MEAN (±sem) % TIME SPENT ON OPEN ARMS, % NUMBER OF ENTRIES ONTO OPEN ARMS AND TOTAL ARM ENTRIES FOR GROUPS OF MICE TESTED 24, 48 or 72 HR AFTER A DOSE OF LORAZEPAM (0.25 mg/kg)

	% Number Entries Onto Open Arms	% Time on Open Arms	Total Arm Entries
Control	27.1 ± 2.5	15.6 ± 1.7	13.3 ± 1.6
Lor 24 hr	34.5 ± 3.8	27.6 ± 5.1*	16.6 ± 3.2
Lor 48 hr	33.8 ± 5.0	20.0 ± 2.0	16.8 ± 1.9
Lor 72 hr	24.4 ± 4.4	22.1 ± 5.7	14.5 ± 2.3

*$p<0.05$ compared with controls, Duncan's test after analysis of variance.

This was because some of the animals showed a very marked enhancement. There was no evidence of a decrease in the % time spent on open arms or the % number of entries in the groups tested 24, 48 or 72 hr after the single dose. Thus, there was no evidence of any withdrawal responses. On the contrary, Table 2 shows that the % time spent on the open arms was higher for the groups tested after lorazepam than for the controls, and this was significant for the 24 hr group. This, together with the results of the 48 hr + 1 hr test group, indicates that there is some sensitization to the anxiolytic effects of lorazepam.

DISCUSSION

We have been able to detect tolerance to the lorazepam-induced reductions in locomotor activity 48 hr after only a single injection. Tolerance to the reductions in exploratory head-dipping and rearing could not be detected. However, tolerance does develop relatively rapidly to these effects, and can be observed in mice after 10 days of diazepam treatment (9). Shorter time intervals were not tested in mice, but tolerance to the lorazepam-induced reductions in head-dipping have been observed in rats after 3 days (5). We were also unable to detect any 'acute tolerance' to the anxiolytic effects of lorazepam in the elevated plus-maze. This is in

accord with the results from a drug discrimination experiment where rats tested after a single dose of diazepam did not select the lever previously associated with the anxiogenic drug, pentylenetetrazole (3). It is also in agreement with results from chronic treatment where tolerance has not been found to the anxiolytic effects of benzodiazepines in the plus-maze before 14 days of treatment in mice (18) or 21 days in rats (11).

The different time-course of development of tolerance to the different behavioral effects of benzodiazepines (7) suggests that different mechanisms underlie the development of tolerance to different responses. If, as has recently been proposed (14), the development of tolerance to benzodiazepine effects and the incidence of withdrawal responses are both reflections of a common underlying mechanism, then one would not expect to be able to detect tolerance to a particular behavioral effect, without also observing changes in that response on withdrawal of the drug. Our results following acute treatment are in general agreement, except for the effects of lorazepam on rearing. Tolerance was not detected, but an increase in rearing was observed 48 hr after the single dose of lorazepam. Rearing is a measure partly reflecting exploratory behavior and partly reflecting general motor activity (6) and this may explain the discrepant results with this measure. However, the mechanisms underlying 'acute tolerance and withdrawal' may be distinct from those underlying the tolerance and withdrawal detected after chronic treatment with benzodiazepines. There are insufficient data at present to determine the extent to which the incidence of withdrawal responses following a single dose of benzodiazepine is linked to the development of an acute tolerance to the behavioral effects.

Rather than detecting withdrawal responses in the plus-maze, which would have been indicated by a *decrease* in the % time spent on the open arms, there was an indication of an *increase* in this measure particularly 24 hr after a single dose of lorazepam. This suggests the possibility of a sensitization to the anxiolytic effects of lorazepam occurring at least from the first to the second dose of a benzodiazepine. There is a report of a similar enhancement of diazepam's anticonvulsant effect. Mice tested 14 days after an initial dose of diazepam had a significantly greater anticonvulsant response than mice tested with diazepam for the first time (1).

These experiments have shown that it is possible to detect tolerance to some, but not all, of the behavioral effects of lorazepam after even a single dose. It is also possible to detect behavioral changes on withdrawal from this single dose. There is some evidence from a human volunteer study that tolerance to some of the behavioral effects of lorazepam can be detected after a single dose (8). The possibility that similar effects will occur when the drug is used clinically suggest that even short-term lorazepam should be used with caution. However, the indication of a sensitization to the anxiolytic effects following a single dose does suggest that at least over the first few administrations the anxiolytic efficacy of benzodiazepines might become more pronounced. This would certainly explain the failure to demonstrate tolerance to the anxiolytic effects in less than 2–3 weeks, since any developing tolerance would be offset by the development of sensitization.

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REFERENCES

1. Antelman, S. M.; Kocan, D.; Edwards, D. J.; Knopf, S. A single injection of diazepam induces long-lasting sensitization. Psychopharmacol. Bull. 23:430–434; 1987.
2. Boisse, N. R.; Periana, R. M.; Guarino, J. J.; Kruger, H. S.; Samoriski, G. M. Pharmacologic characterization of acute chlordiazepoxide dependence in the rat. J. Pharmacol. Exp. Ther. 239:775–783; 1986.
3. Emmett-Ogelsby, M. W.; Mathis, D. A.; Lal, H. Diazepam tolerance and withdrawal assessed in an animal model of subjective drug effects. Drug Dev. Res. 11:145–156; 1987.
4. Freely, M.; Gent, J. P.; Haigh, J. R. M.; Peaker, S. Acute clobazam administration induces anticonvulsant tolerance to N-desmethylclobazam in mice. Br. J. Pharmacol. 89:643P; 1986.
5. File, S. E. Rapid development of tolerance to the sedative effects of lorazepam and triazolam in rats. Psychopharmacology (Berlin) 73:240–245; 1981.
6. File, S. E. Variability in behavioral responses to benzodiazepines in the rat. Pharmacol. Biochem. Behav. 18:303–306; 1983.
7. File, S. E. Tolerance to the behavioral effects of benzodiazepines. Neurosci. Biobehav. Rev. 9:113–122; 1985.
8. File, S. E.; Lister, R. G. Does tolerance to lorazepam develop with once weekly dosing? Br. J. Clin. Pharmacol. 16:645–650; 1983.
9. File, S. E.; Pellow, S. No cross-tolerance between the stimulatory and depressant actions of benzodiazepines in mice. Behav. Brain Res. 17:1–7; 1985.
10. File, S. E.; Wardill, A. G. Validity of head-dipping as a measure of exploration in a modified holeboard. Psychopharmacologia 44:53–57; 1975.
11. File, S. E.; Baldwin, H. A.; Aranko, K. Anxiogenic effects from benzodiazepine withdrawal are linked to the development of tolerance. Brain Res. Bull. 19:607–610; 1987.
12. Greenblatt, D. J.; Woo, E.; Allen, M. D.; Orsulak, P. J.; Shader, R. I. Rapid recovery from massive diazepam overdose. JAMA 240:1872–1874; 1978.
13. Henauer, S. A.; Gallaher, E. J.; Hollister, L. E. Long-lasting single-dose tolerance to neurologic deficits induced by diazepam. Psychopharmacology (Berlin) 82:161–163; 1984.
14. Lader, M. H.; File, S. E. The biological basis of benzodiazepine dependence. Psychol. Med. 17:539–547; 1987.
15. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 92:180–185; 1987.
16. Lister, R. G.; Nutt, D. J. Mice and rats are sensitized to the proconvulsant action of a benzodiazepine-receptor inverse agonist (FG 7142) following a single dose of lorazepam. Brain Res. 379:364–366; 1986.
17. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
18. Stephens, D. N.; Schneider, H. H. Tolerance to the benzodiazepine diazepam in an animal model of anxiety. Psychopharmacology (Berlin) 87:322–327; 1985.
19. Wilks, L. J.; File, S. E. Withdrawal from three different anticonvulsants: effects on aggression, exploration and seizure threshold. Soc. Neurosci. Abstr. 12:906; 1986.
20. Wilks, L. J.; File, S. E.; Martin, I. L. Evidence of strain differences in GABA-benzodiazepine coupling. Psychopharmacology (Berlin) 93:127–132; 1987.
21. Yoong, Y. L.; Lee, H. S.; Gwee, M. C. E.; Wong, P. T. H. Acute tolerance to diazepam in mice: pharmacokinetic considerations. Clin. Exp. Pharmacol. Physiol. 13:153–158; 1986.