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Effects of Sodium Phenobarbital on Motor Activity and Exploration in the Mouse: Development of Tolerance and Incidence of Withdrawal Responses

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FILE, S. E. AND L. J. WILKS. *Effects of sodium phenobarbital on motor activity and exploration in the mouse: Development of tolerance and incidence of withdrawal responses.* PHARMACOL BIOCHEM BEHAV **35**(2) 317–320, 1990.—Sodium phenobarbital (20 mg/kg) was without significant effect on exploratory head-dipping, but a higher dose (70 mg/kg) significantly reduced the number of head-dips and the time spent head-dipping 0.5 and 8 hr after acute administration. Tolerance developed rapidly, i.e., by day 7 of treatment, to these reductions. Both doses of phenobarbital significantly increased locomotor activity, the increase was more marked for the 70 mg/kg dose and for this dose was greater 8 hr after administration than 0.5 hr. No tolerance developed over 21 days of treatment to the increase in locomotor activity 0.5 hr after drug administration; some tolerance did develop by day 14 to the increase detected 8 hr after drug administration, but this is likely to be metabolic. After 21 days of treatment, sodium phenobarbital was withdrawn. Twenty-four hours after the last dose of 70 mg/kg there was a significant reduction in the time spent head-dipping; there was also significant reduction in locomotor activity, which peaked 48 hr after the last dose.

Barbiturate Mouse Exploration Motor activity Tolerance Withdrawal

IN low doses the benzodiazepines increased locomotor activity and exploratory head-dipping in a holeboard and, even after 20 days of treatment, tolerance did not develop to these effects (4). This is in contrast to the rapid development of tolerance to the decreases in locomotor activity and exploration caused by higher doses of benzodiazepines [see (3) for review]. There was no cross-tolerance between the stimulant and depressant actions of the benzodiazepines, suggesting that the two are mediated by independent mechanisms (4). Other drugs that act at sites on the GABA-benzodiazepine receptor complex seemed to produce similar effects. Thus, low doses of the triazolopyridazine CL 218,872 and the pyrazolopyridine tracazolate increased exploratory head-dipping and no tolerance developed to these effects after 10 days of treatment; high doses of thee drugs reduced locomotor activity and head-dipping and tolerance developed to these effects within 10 days; once again, there was no cross-tolerance between the stimulant and sedative effects (12).

Sodium phenobarbital has also been reported to have biphasic effects on locomotor activity in mice, with low doses having a stimulant, and high doses a depressant, effect (11). The purpose of the present study was to determine whether tolerance would develop to the effects in the holeboard of a low and a high dose of phenobarbital; the doses (20 and 70 mg/kg) were chosen on the basis of earlier studies (11,15). A second purpose was to determine whether any behavioral changes could be detected on withdrawal of phenobarbital after 21 days of treatment. We have previously found that whereas tolerance did not develop to the anticonvulsant effects or to the enhancement of social behavior seen 0.5 hr after sodium phenobarbital (70 mg/kg), there were significant decreases in seizure threshold 24–48 hr, and a significant reduction in social behavior 48 hr, after drug withdrawal (6).

METHOD

Apparatus

The holeboard was a wooden box, 40 × 40 × 27 cm, with four holes 3.2 cm in diameter, equally spaced in the floor. Head-dipping was measured by the interruption of infrared photobeams under each hole. Interruption of infrared photocells in the walls of the box 1.5 and 5.5 cm from the floor, provided automatic measures of locomotor activity and rearing, respectively. The output from the photocells was amplified and entered directly into an IBM personal computer.

Animals

Male albino mice (Bantin & Kingman TO strain) 35–40 g were housed in a room with lights on from 0600 to 1600 hr, with food and water freely available.

Drug

Sodium phenobarbital (Sigma) was dissolved in distilled water

to give an injection volume of 4 ml/kg body weight. All injections were intraperitoneal. Mice received one injection per day, of sodium phenobarbital or water, as appropriate.

Procedure

Experiment 1: Tolerance. Mice were randomly allocated among the following drug treatment groups: control (distilled water); sodium phenobarbital (20 or 70 mg/kg acutely or after 7, 14 or 21 days of treatment). Half (n = 8–10 mice) of each group was allocated to be tested 0.5 hr after injection, the other half was tested 8 hr after. In order to equate injection experience, all mice received 21 days of once daily injections, water injections being given prior to the appropriate drug treatment.

In order to equate the time of day at which the mice were tested, the injection times were varied so that all mice were tested between 0800 and 1300 hr. Testing took place over four days and on each day at least two mice from each group were tested and the test order was randomised for drug treatment and time since injection. Thus, in this experiment, there was only one control group with respect to the days of treatment (all control mice received 21 days of water injections), but different mice were tested 0.5 and 8 hr after injection.

Mice were placed singly in the holeboard for a 7.5-min trial and the order of testing was randomised for drug treatment. At the end of each trial the holeboard was cleaned and any fecal boluses removed.

Experiment 2: Withdrawal. Mice were randomly allocated among the following groups (n = 8–10 per group): control (21 days water injections) tested 24, 48 or 72 hr after the last injection; sodium phenobarbital (20 or 70 mg/kg for 21 days) tested 24, 48 or 72 hr after the last injection. In this experiment, the different withdrawal groups were tested on different days and, hence, each day of testing had its own control group.

The mice were tested in an order randomised for drug group between 1200 and 1600 hr.

Statistics

The data were analysed with analyses of variance in which phenobarbital treatment was one factor, time of testing (0.5 or 8 hr) was a second factor and days of treatment, or hours of drug withdrawal was the third. Comparisons between individual drug groups were made after analysis of variance with Duncan's multiple range tests.

RESULTS

The scores for the control mice tested 0.5 and 8 hr after injection differed by less than 5%, and, therefore, for illustrative purposes, have been combined in Figs. 1, 2 and 3 and Tables 1 and 2.

Sodium phenobarbital (20 mg/kg) was without significant effect on head-dipping (see Table 1). However, the 70 mg/kg dose significantly reduced both the number of head-dips and the time spent head-dipping, $F(1,136) = 22.8$, $p<0.0001$ and $F = 9.8$, $p<0.005$, respectively. The reductions occurred at both test times, but were significantly greater at 0.5 hr than at 8 hr, $F(1,136) = 11.5$, $p<0.005$ (see Figs. 1 and 2). Tolerance rapidly developed to these reductions [days factor, $F(3,136) = 7.6$ and 5.9 for number of head-dips and time spent head-dipping, respectively, $p<0.005$] and by day 7 the scores were no longer significantly different from the controls (see Figs. 1 and 2).

Both doses of phenobarbital significantly increased locomotor activity at both 0.5 and 8 hr after injection (see Table 1 and Fig. 3). The 70 mg/kg dose had a significantly greater effect than the 20 mg/kg dose ($p<0.01$). There was no evidence of the development of tolerance to the locomotor stimulant effect 0.5 hr after injection (see Table 1 and Fig. 3). However, 8 hr after injection there was evidence of tolerance and by day 21 the effect of the 20 mg/kg dose was significantly less than the effect of that dose acutely (see Table 1); at both days 14 and 21 the effect of the 70 mg/kg dose was significantly less than that seen after acute administration (see Fig. 3).

TABLE 1

MEAN (± sem) NUMBER OF HEAD-DIPS, TIME (SEC) SPENT HEAD-DIPPING AND LOCOMOTOR ACTIVITY 0.5 AND 8 HR AFTER ACUTE AND CHRONIC TREATMENT WITH SODIUM PHENOBARBITAL (20 mg/kg)

Days of Treatment	Time	No. Head-Dips	Time Head-Dipping	Locomotor Activity
1	0.5 hr	113.0 ± 10.3	58.9 ± 7.5	744.3 ± 49.7
7		134.0 ± 10.9	52.4 ± 5.6	791.1 ± 46.6
14		133.3 ± 11.3	84.1 ± 12.0*	750.5 ± 51.6
21		129.7 ± 11.4	60.2 ± 4.9	724.4 ± 18.9
1	8 hr	107.2 ± 10.6	56.1 ± 7.3	794.3 ± 40.4
7		92.4 ± 5.2	47.7 ± 6.7	737.4 ± 37.1
14		121.3 ± 11.0	70.5 ± 7.5	786.9 ± 34.2
21		113.8 ± 14.1	58.5 ± 7.5	633.7 ± 40.8*
Control Scores		118.9 ± 10.0	65.7 ± 7.7	654.3 ± 28.0

The control scores are given at the bottom of the table, and for the scores for the 70 mg/kg groups, see Figs. 1, 2 and 3.
*Sig. diff. from acute group, $p<0.05$.

Phenobarbital produced a dose-related increase in rearing; only the increase with 70 mg/kg reached significance ($p<0.01$), but on Duncan's post hoc tests no individual group differed significantly from control and nor did any of the chronic treatment groups differ significantly from the acute treatment (see Table 2).

On withdrawal from phenobarbital treatment there was a significant reduction in the time spent head-dipping 24 hr after the last dose of 70 mg/kg (see Table 3). There was a significant reduction in locomotor activity, $F(2,76) = 6.3$, $p<0.005$, on withdrawal from phenobarbital, and on post hoc tests this reached significance at 48 hr after the 70 mg/kg dose (see Table 3).

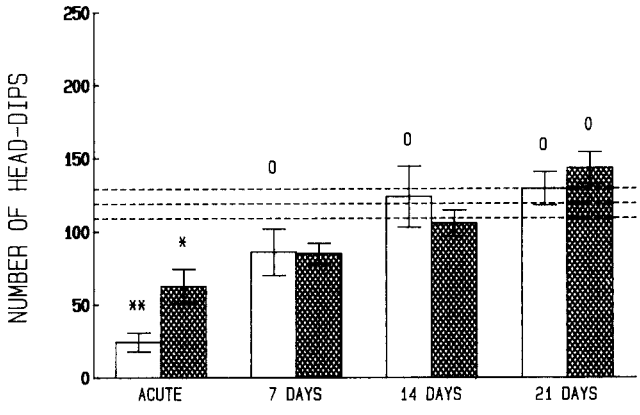

FIG. 1. Mean number (± sem) of head-dips made by rats injected with sodium phenobarbital (70 mg/kg) and tested 0.5 hr (clear columns) or 8 hr (hatched columns) after a single administration (acute) or after 7, 14 or 21 days administration. The control mean (± sem) is shown by the dotted lines. 0: Sig. diff. from acute group, $p<0.05$; sig. diff. from control *$p<0.05$, **$p<0.01$.

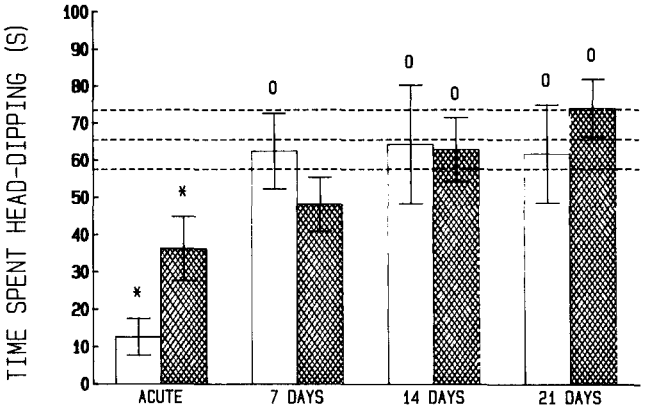

FIG. 2. Mean (±sem) time (sec) spent head-dipping by rats injected with sodium phenobarbital (70 mg/kg) and tested 0.5 hr (clear columns) or 8 hr (hatched columns) after a single administration (acute) or after 7, 14 or 21 days administration. The control mean (±sem) is shown by the dotted lines. 0: Sig. diff. from acute group, $p<0.05$; sig. diff. from control *$p<0.05$.

Withdrawal from phenobarbital (70 mg/kg) also caused a significant reduction in rearing ($p<0.05$), but on post hoc tests this did not reach significance for any individual time-point.

DISCUSSION

Sodium phenobarbital had both stimulant and depressant effects on behavior in the holeboard. However, this was not exactly as we had expected. Both doses significantly enhanced locomotor activity, and whereas 70 mg/kg significantly reduced head-dipping, 20 mg/kg was without significant effect. It is, therefore, of greatest interest to discuss the development of tolerance to the effects of the higher dose.

Tolerance developed by 7 days of treatment to the decrease in head-dipping, yet at the same time (0.5 hr) there was no development of tolerance to the locomotor stimulant effects. It is, therefore, difficult to advance a pharmacokinetic explanation for

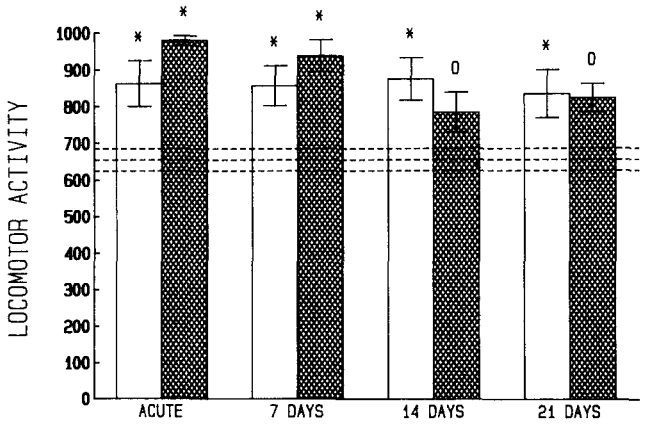

FIG. 3. Mean (±sem) locomotor activity of rats injected with sodium phenobarbital (70 mg/kg) and tested 0.5 hr (clear columns) or 8 hr (hatched columns) after a single administration (acute) or after 7, 14 or 21 days administration. The control mean (±sem) is shown by the dotted lines. 0: Sig. diff. from acute group, $p<0.05$; sig. diff. from control *$p<0.05$.

TABLE 2

MEAN (±sem) NUMBER OF REARS MADE BY MICE INJECTED WITH SODIUM PHENOBARBITAL (20 AND 70 mg/kg) AND TESTED 0.5 OR 8 HR AFTER AN ACUTE INJECTION OR AFTER 7, 14 OR 21 DAYS OF TREATMENT

Days of Treatment	Time	Phenobarbital 20 mg/kg	Phenobarbital 70 mg/kg
1	0.5 hr	105.0 ± 4.2	118.4 ± 7.4
7		115.6 ± 6.0	109.6 ± 6.4
14		104.2 ± 5.0	107.1 ± 8.2
21		101.4 ± 4.6	109.0 ± 2.5
1	8 hr	108.5 ± 4.1	118.4 ± 6.8
7		105.2 ± 11.1	111.0 ± 4.5
14		102.5 ± 6.9	110.3 ± 7.9
21		85.2 ± 7.8	103.5 ± 9.1

Control scores = 100.4 ± 4.1.

the observed behavioral tolerance. However, the partial tolerance that was observed to the locomotor effects 8 hr after 20 and 70 mg/kg is most likely to result from the more rapid metabolism of phenobarbital with chronic treatment (8). A similar development of tolerance to the anticonvulsant effects of 70 mg/kg was observed 8 hr, but not 0.5 hr, after injection (6).

It therefore seems that for drugs acting at both the benzodiazepine binding site (chlordiazepoxide, diazepam, CL 218,872), and for those acting at a site associated with the chloride channel on the GABA-benzodiazepine receptor complex (tracazolate, phenobarbital), tolerance does not develop to the stimulant effects, but does rapidly develop to the sedative effects. The effects of sodium phenobarbital (20 mg/kg) differ from those of tracazolate (2.5 mg/kg) in that the former increased locomotor activity, but not exploratory head-dipping, whereas the latter increased head-dipping, but not locomotor activity (12). However, it is not possible to conclude that a dose of phenobarbital cannot be found that will increase head-dipping. Indeed, it is likely that a lower dose might have done so, since head-dipping was increased in the rat 12 hr after 20 mg/kg (brain concentrations of 8.7±0.5 g/g), whereas it was not increased at 8 or 1 hr after 20 mg/kg (brain concentrations 10.9±0.8 and 14.8±0.2, respectively) (15). Brain concentrations have previously been reported to be important in determining the stimulant versus depressant effects of phenobarbital (11).

The pattern of results seen with phenobarbital (70 mg/kg) provides further evidence for the independence of locomotor activity and exploratory head-dipping in the holeboard (1, 2, 5, 9). Not only did this dose decrease exploration at the same time as increasing locomotor activity, but tolerance developed only to the former effect.

When phenobarbital was withdrawn following a period of chronic treatment there was a decrease in both locomotor activity and in head-dipping. The decrease in locomotor activity suggests that it is possible to observe withdrawal responses even when tolerance has not developed to that particular behavioral effect (i.e., locomotor stimulation) of the drug. This is similar to the pattern observed for the anticonvulsant action of phenobarbital (70 mg/kg) where there was no tolerance after 21 days, but a decrease in seizure threshold was detected on drug withdrawal (6). Tolerance did develop to the decrease in exploratory head-dipping and there was a change in this behavior on drug withdrawal; however, the change seen on drug withdrawal was in the *same* direction as

TABLE 3

MEAN (±sem) NUMBER OF HEAD-DIPS, TIME (SEC) SPENT HEAD-DIPPING, LOCOMOTOR ACTIVITY AND REARS MADE BY MICE 24, 48 AND 72 HR AFTER WITHDRAWAL FROM 21 DAYS OF TREATMENT WITH SODIUM PHENOBARBITAL (20 OR 70 mg/kg/DAY)

		Hours After Drug Withdrawal		
		24	48	72
Number of Head-Dips	Control	91.3 ± 4.9	87.2 ± 10.1	105.7 ± 7.1
	PHB 20	90.7 ± 8.3	80.1 ± 6.9	98.0 ± 6.6
	PHB 70	86.5 ± 11.3	75.3 ± 6.9	109.0 ± 9.6
Time Spent Head-Dipping	Control	65.5 ± 10.9	55.3 ± 7.2	63.2 ± 4.7
	PHB 20	53.0 ± 6.8	49.5 ± 5.6	54.1 ± 3.0
	PHB 70	39.5 ± 7.0*	48.7 ± 5.8	73.3 ± 9.5
Locomotor Activity	Control	418.6 ± 33.5	402.2 ± 22.9	415.1 ± 22.2
	PHB 20	381.6 ± 22.3	351.6 ± 24.3	407.7 ± 32.0
	PHB 70	343.2 ± 28.5	302.6 ± 25.4*	361.9 ± 19.1
Number of Rears	Control	71.3 ± 6.7	68.7 ± 4.6	79.7 ± 5.6
	PHB 20	68.3 ± 4.4	70.2 ± 4.4	60.4 ± 4.2
	PHB 70	58.0 ± 5.3	62.1 ± 7.0	65.8 ± 9.3

*Sig. diff. from control, $p<0.05$.

the original drug effect. This is unusual, in that withdrawal responses are usually in the opposite direction to the initial effects. However, this is not universally the case and withdrawal from a dose of ethanol that reduces motor activity also results in hypoactivity (13,14). It has also been suggested that a decrease in head-dipping seen 4 hr after an acute dose of lorazepam in the rat may represent an acute withdrawal response (10). In the mouse a decrease in head-dipping was observed 6 hr after an acute dose of lorazepam or oxazepam that also reduced head-dipping 1–1.5 hr after administration, but whereas the reductions at 1–1.5 hr could be reversed by flumazenil, the later appearing reductions could not (7). Thus, reduced exploration may represent a withdrawal response after both acute and chronic drug treatments.

In conclusion, it is clear that after 21 days of treatment, tolerance develops to some, but not all, of the behavioral effects of sodium phenobarbital. However, the development of tolerance to a particular behavioral effect does not determine whether that response will change on drug withdrawal. This suggests that at least for the barbiturates, independent mechanisms may underlie the development of functional tolerance and the incidence of withdrawal.

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REFERENCES

1. Durcan, M. J.; Lister, R. G. Does directed exploration influence locomotor activity in a holeboard test? Behav. Neural Biol. 51: 121–125; 1989.
2. File, S. E. Variability in behavioral responses to benzodiazepines in the rat. Pharmacol. Biochem. Behav. 18:303–306; 1983.
3. File, S. E. Tolerance to the behavioral actions of benzodiazepines. Neurosci. Biobehav. Rev. 9:113–122; 1985.
4. 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.
5. File, S. E.; Wardill, A. G. Validity of head-dipping as a measure of exploration in a modified holeboard. Psychopharmacologia 44:53–59; 1975.
6. File, S. E.; Wilks, L. J. Changes in seizure threshold and aggression during chronic treatment with three anticonvulsants and on drug withdrawal. Psychopharmacology (Berlin), in press; 1989.
7. File, S. E.; Wilks, L. J.; Mabbutt, P. S. The role of the benzodiazepine receptor in mediating long-lasting anticonvulsant effects and the late-appearing reductions in motor activity and exploration. Psychopharmacology (Berlin) 97:349–354; 1989.
8. Frey, H. H. Experimental evidence for the development of tolerance to anticonvulsant drug effects. In: Frey, H. H.; Froscher, W.; Koella, W. P.; Meinardi, H., eds. Tolerance to beneficial and adverse effects of antiepileptic drugs. New York: Raven Press; 1986:7–16.
9. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 92:180–185; 1987.
10. Lister, R. G.; File, S. E. A late-appearing benzodiazepine-induced hypoactivity that is not reversed by a receptor antagonist. Psychopharmacology (Berlin) 88:520–524; 1986.
11. Middaugh, L. D.; Blackwell, A.; Boggan, W. O.; Zemp, J. W. Brain concentrations of phenobarbital and behavioral activation or depression. Pharmacol. Biochem. Behav. 15:723–728; 1981.
12. Pellow, S.; File, S. E. Lack of cross-tolerance in mice between the stimulatory and depressant actions of novel anxiolytics in the holeboard. Behav. Brain Res. 23:159–166; 1987.
13. Pohorecky, L. A. Withdrawal from ethanol: simple quantitative behavioral tests for its evaluation. Psychopharmacology (Berlin) 50:125–129; 1976.
14. Rogers, J.; Weiner, S. G.; Bloom, F. E. Long-term ethanol administration methods for rats: advantages of inhalation over intubation or liquid diets. Behav. Neural Biol. 27:466–486; 1979.
15. Wilks, L. J.; File, S. E.; Dingemanse, J. Hyperactivity and increased aggression in the rat several hours after a single dose of phenobarbital. Pharmacopsychoecologia 1:23–31; 1988.