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BRIEF COMMUNICATION

Attenuation of Phenobarbital-Induced Deficits in Coordinated Locomotion During Subacute Exposure[1]

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KULIG, B. M. *Attenuation of phenobarbital-induced deficits in coordinated locomotion during subacute exposure.* PHARMACOL BIOCHEM BEHAV 24(6) 1805–1807, 1986.—The effects of phenobarbital on coordinated locomotion were measured in rats trained to criterion to avoid electric shock by running treadmill fashion along a motor driven belt. Two phenobarbital groups (35 and 50 mg/kg) and saline controls were injected for 29 consecutive days. Testing was carried out on Drug Days 1, 15, and 29. On Drug Day 1, disturbances in gait and balance were reflected by an increased time off belt for the phenobarbital groups. In addition, both groups demonstrated a significant improvement in performance over the three 2-min trial test sessions. Deficits were further reduced on Drug Day 15, and by Drug Day 29, the performance of rats treated with 35 mg/kg was equivalent to control values while that of the 50 mg/kg group had improved by more than 90%. There was no corresponding change in plasma levels of phenobarbital during subacute dosing. After a one month drug withdrawal period, treatment with phenobarbital again produced coordination deficits which were equivalent to those seen on Drug Day 1. These data demonstrate that an attenuation of the effects of phenobarbital on coordination can begin within minutes of the initial test session and is most likely due to behavioral processes (i.e., acute functional tolerance). In addition, results suggest that further drug exposure may play a role in the carry-over of functional tolerance from one drug test session to the next.

Phenobarbital Tolerance Rats Motor coordination Chronic administration

PHENOBARBITAL is a widely prescribed antiepileptic drug which, in addition to its anticonvulsant properties, also produces a variety of behavioral effects. In the clinic, phenobarbital intoxication is characterized by sedation and often accompanied by ataxia and dysarthria [7]. In animals, phenobarbital produces changes in spontaneous activity [8], coordinated movement [6] and the performance of schedule-controlled behavior [9,10].

Despite the fact that the treatment of epilepsy involves the chronic administration of antiepileptic drugs, there is little systematic information regarding the changes in the behavioral effects of these compounds over time. It is a general clinical impression that for most patients, complaints regarding behavioral disturbances diminish with continued exposure. However, for other patients drug-related impairments may persist and even be exacerbated with prolonged drug use [12].

Because of the widespread use of phenobarbital in the treatment of epilepsy and its prominent effects on motor function, a study was carried out to assess the effects of this compound on coordinated movement during subchronic administration. Using a treadmill task originally described by Gibbins *et al.* [2], previous studies [6] have indicated that small, but reliable deficits in coordination occur in this task with the acute administration of phenobarbital when plasma levels exceed 20 μg/ml, a value well within the therapeutic range.

Since experiments with other ataxia-producing compounds have indicated that acute behavioral tolerance in this task can begin to develop during the first drug exposure

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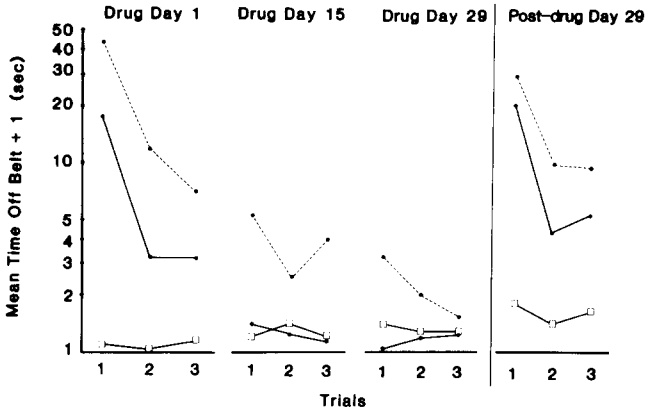

FIG. 1. The effects of phenobarbital on treadmill performance measured on Days 1, 15 and 29 of the chronic treatment period and Day 29 of drug withdrawal. ●— —●, Phenob 50; ●——●, Phenob 35; □——□, Saline. The values are the group means (+1 sec) for each trial (3 trials per session) plotted logarithmically.

[4,5], the present study examined whether similar within-session changes also occur during acute phenobarbital administration. Further, the course of changes in drug response was followed at two-week intervals over a one month drug administration period and one month after drug withdrawal.

METHOD

Animals

Eighteen adult (200–250 g) female Wistar rats (TNO Labs, Zeist) were maintained under ad lib food and water conditions in a temperature and humidity controlled animal colony under a 12:12 hr lighting regimen. Behavioral testing was carried out during the light-on phase.

Apparatus

The apparatus consisted of a transparent Plexiglas® chamber (outer dimensions: 61×30×26 cm) with an opaque cover and a stainless steel grid floor. A 5.4 cm wide belt made of conducting rubber (developed by the Plastics and Rubber Institute TNO, Delft) was stretched over the grid floor and over two stainless steel cylinders leaving 41 cm of the belt exposed in the test compartment. One of the cylinders was rotated by a low-torque servo-motor which moved the belt at a constant speed. The belt made contact with a metal strip connected to the first rod of the grid floor, but insulated from the rest of the grid. The grid was electrified with constant-current scrambled shock (400 V DC). The apparatus was constructed in such a way that an animal was forced to run treadmill fashion completely on the belt to avoid the shocked grid. An animal was considered off the belt if one or more paws touched the grid floor. Time off belt was noted by visual observation and recorded by means of a stopwatch.

Initial Training Procedures

In order to insure a high degree of stable performance, a graded difficulty training approach was employed. Rats were first trained to remain on the belt when it was stationary and the shock level low (60 μA). In subsequent training sessions, the belt was moved at a slow speed (80.5 cm/min; shock, 90 μA) and immediately stopped if the rat lost its balance and came in contact with the grid floor. As the rat demonstrated the ability to regain its balance and reposition itself on the moving belt, halting the belt was discontinued and the belt speed and shock level were gradually increased to their final values of 434.5 cm/min and 240 μA. Halting the belt was never employed except during this initial training phase. Rats received daily training sessions consisting of three 2-min trials spaced 30 sec apart until they reached a criterion of 98% time on belt for at least five consecutive sessions. The duration of the training phase to criterion was approximately 15 sessions.

Drug Testing

Following the stabilization of performance, rats were randomly divided into three groups and received sodium phenobarbital 35 mg/kg (Phenob 35), sodium phenobarbital 50 mg/kg (Phenob 50), or an equivalent volume of saline (1 ml/kg). Animals were injected IP daily for 29 consecutive days.

On Drug Days 1, 15, and 29 the effects of phenobarbital on treadmill performance were measured in three 2-min trials spaced 30 sec apart. On these days, animals received their daily dosing 30 min prior to testing. On the remaining drug days, animals were injected with the appropriate drug but were not tested. In addition, on Drug Days 1 and 29, a 400 μl blood sample was collected from the tail vein from all animals immediately following testing. The samples were centrifuged at 9000 rpm and the separated plasma was stored frozen at −14°C until analysis. Plasma concentration determinations were carried out using routine enzyme-multiplied immunoassay techniques (EMIT,® Merck) performed with a Gilford 300N microsample spectrophotometer [11].

Drug Withdrawal

After completion of the subchronic drug treatment phase, the drugs were withdrawn for 28 days. During the drug withdrawal period, animals were left undisturbed in their home cages: they were not injected, nor did they receive any training. On Post-Drug Day 29 of the withdrawal period, the performance of the three groups was again measured in three 2-min trials 30 min following injection.

Data Analysis

Overall performance, defined as total time off belt on three trials, and trial-by-trial performance were analyzed using analyses of variance (ANOVA) and *t*-tests (significance level of $p<0.05$) suitable for multiple comparisons. For the sake of graphic presentation, 1 was added to the mean time off belt for each group and the values plotted logarithmically, however, all statistics were performed on the raw data.

RESULTS

Figure 1 shows the effects of phenobarbital on treadmill performance during the one month drug period and on Post-Drug Day 29 of the withdrawal period. On Drug Day 1, the overall performance for the phenobarbital groups was significantly impaired compared to saline controls (one-way ANOVA, $p<0.001$). Effects were greatest on Trial 1 and diminished across the three 2-min trial test sessions (ANOVA, repeated measures design, $p<0.001$). On Drug

Day 15, both phenobarbital groups demonstrated a significant improvement in overall performance compared to Drug Day 1 (ANOVA, repeated measures design, $p<0.001$). Compared to saline controls, performance of the Phenob 35 group was equivalent (t-test, two-tailed, $p>0.05$) whereas that of the Phenob 50 group still showed a significant deficit (t-test, two-tailed, $p<0.01$). On Drug Day 29, the mean time off belt on Trial 1 for the Phenob 50 group was still slightly, but significantly, higher than that of the saline controls. However, by Trial 3, there were no significant differences between any of the groups (ANOVA, $p>0.05$).

The administration of phenobarbital following a one month drug-free period again produced marked deficits in coordinated locomotion (ANOVA, $p<0.001$). Moreover, overall performance on Post-Drug Day 29 was equivalent to that seen in both drug groups on Drug Day 1 (related t-tests, $p>0.05$).

Finally, examination of the plasma concentrations of the two phenobarbital groups revealed that there were no differences in phenobarbital concentration between Drug Day 1 and Drug Day 29 for either of the phenobarbital groups. For the Phenob 50 group, mean plasma concentration (±S.E.M.) on Drug Day 1 and Drug Day 29 were 55.7±4.3 and 57.3±5.2 μg/ml respectively; for the Phenob 35 group 42.1±1.7 and 45.0±3.2 μg/ml respectively. Further, all animals appeared in good health throughout these experiments and there were no significant differences in body weight between the groups on either Drug Day 1 or Drug Day 29. Mean body weight for the saline, Phenob 35 and Phenob 50 groups on Drug Day 1 were 217.2 g, 219.0 g and 210.8 g respectively: on Drug Day 29, 230.7 g, 230.8 g, and 222.0 g respectively.

DISCUSSION

The present study demonstrates that the acute administration of phenobarbital produced marked deficits in coordinated movement with corresponding plasma levels in the upper end of the human therapeutic range. Moreover, there was a significant attenuation of drug response over the seven minute drug test session. These data are in keeping with the results of previous investigations of the development of acute tolerance to the effects of other drugs on motor coordination [4,5] and are most likely due to behavioral processes whereby the animals can learn to overcome the drug-induced deficits by practicing the task while in the drug state (i.e., functional or behavioral tolerance).

With respect to the changes in drug effects during subacute administration, the present study suggests that acute functional tolerance to phenobarbital can be retained for up to two weeks in the absence of training and in the presence of daily drug treatment and, further, that continued drug exposure leads to a further reduction in drug response. It is well-known that chronic barbiturate administration is accompanied by a decreased duration of action due to the induction of hepatic microsomal drug-metabolizing systems [1]. Therefore, if one were to carry out a time course study of phenobarbital concentrations, one might well expect the half-life on Drug Day 29 to be shorter than that on Drug Day 1. In the present study, however, testing was carried out when phenobaribital concentrations were at peak levels and there were no differences in drug concentrations between the first and last day of subacute treatment when measured 30 min post-injection. Thus, it does not appear likely that an increased rate of metabolism could have played a significant role in the reduction in drug response observed over the 29 day treatment period.

What is not clear from the present study is the role played by subacute drug exposure in mediating the carry-over of functional tolerance from one drug test session to the next. Previous studies suggest that the opportunity to practice in the drug state is the *sine qua non* for the development of chronic behavioral tolerance [3]. Our findings that functional tolerance was maintained during prolonged exposure, but lost during drug withdrawal suggest that exposure *per se* may play some role in helping to maintain functionally acquired behavioral tolerance. Clearly, further studies employing experimental designs other than the one used here are necessary to address this point directly.

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REFERENCES

1. Bush, M. T. and E. Sanders. Metabolic fate of drugs: barbiturates and closely related compounds. *Annu Rev Pharmacol* **7**: 57–76, 1967.
2. Gibbins, R. J., H. Kalant and A. E. LeBlanc. A technique for accurate measurement of moderate degrees of alcohol intoxication in small animals. *J Pharmacol Exp Ther* **159**: 236–242, 1968.
3. Harris, R. A. and D. Snell. Effects of acute and chronic administration of phenobarbital and d-amphetamine on schedule-controlled behavior. *Pharmacol Biochem Behav* **12**: 47–52, 1980.
4. Kulig, B. M. Acute functional tolerance to the motor impairment effects of ethanol. *Physiol Psychol* **5**: 3–6, 1976.
5. Kulig, B. M. Acute functional tolerance to the motor impairment effects of di-propylacetate. *Pharmacol Biochem Behav* **5**: 511–514, 1976.
6. Kulig, B. M. The evaluation of the behavioral effects of antiepileptic drugs in animals and man. In: *Epilepsy and Behavior '79*, edited by B. M. Kulig, H. Meinardi and G. Stores. Lisse: Swets and Zeitlinger, 1980, pp. 47–62.
7. Meinardi, H. and L. M. Stoel. Side-effects of antiepileptic drugs. In: *Handbook of Clinical Neurology*, Vol 15, edited by P. Vinken and G. Bruyn. Amsterdam: Elsevier, 1974, pp. 705–738.
8. Middaugh, L. D., L. A. Blackwell, W. O. Boggan and J. W. Zemp. Brain concentrations of phenobarbital and behavioral activation or depression. *Pharmacol Biochem Behav* **15**: 723–728, 1981.
9. Picker, M. and A. Poling. Effects of anticonvulsants on learning: performance of pigeons under a repeated acquisition procedure when exposed to phenobarbital, clonazepam, valproic acid, ethosuximide and phenytoin. *J Pharmacol Exp Ther* **230**: 307–316, 1984.
10. Picker, M., J. Thomas, C. Koch and A. Poling. Effects of phenytoin, phenobarbital, and valproic acid, alone and in selected combinations, on schedule-controlled behavior of rats. *Pharmacol Biochem Behav* **22**: 389–393, 1985.
11. Schmidt, D. Measurement of diphenylhydantoin and phenobarbital by enzyme immunoassay and gas-liquid chromatography. *J Neurol* **213**: 41–46, 1976.
12. Stores, G. Behavioural effects of anti-epileptic drugs. *Dev Med Child Neurol* **17**: 647–658, 1975.