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Phenytoin: Similarity to Tricyclic Antidepressants

MARTIN D. SCHECHTER AND NANCY L. GREER

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

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SCHECHTER, M. D. AND N. L. GREER. *Phenytoin: Similarity to tricyclic antidepressants.* PHARMACOL BIOCHEM BEHAV 19(3) 415–417, 1983.—Rats were trained to discriminate between the stimulus properties of intraperitoneal injections of 10 mg/kg phenytoin and its pH-adjusted vehicle in a two-lever, food-motivated operant task. Once trained, rats showed a dose-related decrease in discriminative performance with lower phenytoin doses. Administration of pentobarbital and chlordiazepoxide produced vehicle-appropriate responding, whereas injection of imipramine and amitryptaline produced intermediate results. Desipramine, at an intraperitoneal dose of 10 mg/kg, produced a pattern of responding similar to that observed after the training dose of phenytoin. These results demonstrate, for the first time, the ability of a non-disruptive dose of phenytoin to act as a behavioral discriminative stimulus in the rat and suggest the possibility of a common interoceptive cue property with tricyclic antidepressants.

Drug discrimination Phenytoin Pentobarbital Chlordiazepoxide Imipramine Desipramine Amitriptyline Serotonin

PHENYTOIN (Dilantin, Parke-Davis, Morris Plains, NJ) was introduced in 1938 for the treatment of epilepsy and it was shown to be effective in controlling certain forms of epilepsy in humans [16]. Indeed, phenytoin did not cause the heavy sedation associated with previously used barbiturates which it soon replaced as the preferred antiepileptic. The effects of phenytoin on nonhuman subjects have generally been focused upon its anticonvulsant properties [21] or upon the physiological measures of its toxic effects [17]. However, the effects of phenytoin on schedule-controlled animal performance [8, 11, 12] and its use as a drug to control discriminative performance [10,15] have been reported. This latter behavioral paradigm, i.e., drug discrimination, has the advantages of not being dependent upon impairment of normal functioning as the measure of drug effect [1] and that it is stable, sensitive and specific [7].

The purpose of the present investigation was to train rats to discriminate the interoceptive cue produced after the intraperitoneal (IP) administration of a non-disruptive dose of phenytoin, to investigate the dose-response relationship of this discrimination and to test other agents, including various tricyclic antidepressants, in this behavioral paradigm. The rationale for this latter part of the study was based upon the recent lay book by Jack Dreyfus [6] detailing the efficacy of phenytoin in intractible depression and calling for experimentation to evidence the efficacy of phenytoin in disease states other than seizure disorders.

METHOD

Subjects

The subjects were 6 male ARS/Sprague-Dawley rats weighing 330–450 g at the beginning of experimentation. They were housed in individual living cages and their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80±5% of their free-feeding weights as determined by daily weighing of 2 control free-feeding rats purchased from the supplier (Zivic-Miller, Allison Park, PA) at the same time. Water was continuously available in the home cage.

Apparatus

The experimental space consisted of four standard rodent Skinner test cages (Lafayette Instruments Corp.) equipped with two operant levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the two levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9 W house-light. Solid-state programming equipment (LVB Corp.) was used to control and record the sessions and was located in an adjacent room.

Discriminative Training

Training was based upon procedures described by Overton [14] and there were two training phases. In the first phase, food deprived subjects learned to lever press on both levers for food reinforcement (45 mg Noyes pellets) on a fixed-ratio 10 (FR 10) schedule. The vehicle lever was activated first for all subjects. Animals were initially shaped to press this lever on an FR 1 schedule. This schedule was increased over 10 days until an FR 10 schedule was achieved. Throughout lever-press training, animals received daily intraperitoneal (IP) injections of 1 ml/kg phenytoin ve-

hicle 15 min prior to being placed into the 2-lever operant box. Immediately following attainment of the FR 10 schedule after vehicle administration, the opposite lever was activated and rats were trained on an FR 1 schedule after the IP administration of an equal volume of 10 mg/kg phenytoin. Daily sessions of 15 min were continued over 8 days with phenytoin administration until an FR 10 schedule was attained.

Phase II, discrimination training, then began. Subjects were trained 5 days per week with alternation of reinforcement proceeding in a pseudo-random sequence. Thus, in each 2-week period, there were 5 days with drug lever (D) correct and 5 days with vehicle lever (V) correct. The pattern was DVVDD; VDDVV. Criterion was set at 8 of 10 consecutive sessions during which the first food pellet was received within 15 or less total responses.

Dose-Response Relationships

After the rats attained the training criterion, testing and training sessions of 15 min duration with alternating administrations of 10 mg/kg phenytoin and vehicle were continued on Mondays, Wednesdays and Fridays. This procedure maintained behavioral discrimination to the training drug conditions. It was intended that if a rat was observed to make more than two incorrect lever selections in any of ten daily consecutive maintenance sessions, the data upon that rat's performance would be deleted from the results. This, however, did not occur. On Tuesdays and Thursdays, the rats were injected IP with different doses (1 ml/kg) of phenytoin than used for initial training, i.e., 5.0 and 7.5 mg/kg and, 15 minutes later, they were placed into the experimental chamber and were allowed to lever press, without receiving food reinforcement, until 10 responses were made on either lever. To preclude training at a phenytoin dose different than employed to train the animals, the rats were immediately removed from the experimental chamber upon making 10 responses on either lever. Each of the 2 lower doses of phenytoin were tested in each animal on 2 occasions with each test preceded by both a 10.0 mg/kg phenytoin and a vehicle maintenance session. The lever first pressed 10 times was designated as the "selected" lever.

Generalization to Other Compounds

Once the dose-response relationships were established, 2 doses each of pentobarbital, chlordiazepoxide, imipramine, desipramine and amitriptyline were administered and the ability of the rats to discriminate these drugs as phenytoin was tested. Each of the 2 doses of these drugs was tested on 2 occasions preceded by both a phenytoin and vehicle maintenance session and the animals were immediately removed upon making 10 responses on either lever.

Drugs

Phenytoin sodium injection (USP 50 mg/ml) was diluted to the appropriate concentration with saline (0.9% sodium chloride). The vehicle was compounded with 0.4 ml propylene glycol (USP), 0.1 ml ethanol (95% USP) and saline. The pH was adjusted to 11.8 with 10% sodium hydroxide. All other drug doses were calculated as base, were dissolved in saline and were administered intraperitoneally 15 min before testing.

TABLE 1

PHENYTOIN DOSE-RESPONSE AND TRANSFER EXPERIMENTS

	Dose (mg/kg)	No. of trials	Quantal % Phenytoin lever selection	Quantitative (±S.D.)
Saline	—	24	15.0	27.8 ± 10.5
Phenytoin	10.0	24	83.4	76.0 ± 12.2
	7.5	2	70.0	64.7 ± 14.9
	5.0	2	50.0	50.8 ± 8.2
Chlordiazepoxide	10.0	2	20.0	34.3 ± 15.4
	5.0	2	20.0	39.2 ± 26.3
Pentobarbital	10.0	2	0.0	29.6 ± 3.1
	5.0	2	0.0	24.2 ± 1.6
Imipramine	10.0	2	44.5*	51.5 ± 12.1
	5.0	2	10.0	26.4 ± 9.8
Desipramine	10.0	2	80.0	69.6 ± 9.0†
	5.0	2	60.0	51.9 ± 14.9
Amitriptyline	10.0	2	60.0	51.5 ± 1.1
	5.0	2	10.0	34.5 ± 3.2

*One rat did not press in one test session at this dose.

†Non-significant difference from quantitative measurement after administration of 10 mg/kg phenytoin (unpaired *t*-test).

Measurements

The lever pressed 10 times first was designated as the "selected" lever (quantal measurement). In addition, the total number of lever presses made before 10 presses were counted constitutes the quantitative measurement, i.e., number of responses on the phenytoin-correct lever divided by total responses made prior to 10 responses times 100. The advantages in using both measurements have been discussed by Stolerman and D'Mello [18].

RESULTS

Acquisition of Discriminative Cue

The rats learned to respond differentially under the phenytoin and vehicle training conditions in a mean (±SEM) of 29±10 training sessions.

Dose-Response Relationships

The results of testing at 2 lower doses of phenytoin in animals trained to discriminate between 10 mg/kg phenytoin and its vehicle are presented in Table 1. As the dose of phenytoin was decreased, both the quantal and quantitative measurements were seen to decrease.

Generalization to Other Drugs

Administration of 5 and 10 mg/kg of chlordiazepoxide produced 20% selected lever (quantal) responding on the phenytoin-correct lever (Table 1). Following the administration of 5 and 10 mg/kg pentobarbital, all responses were made on the vehicle-appropriate lever. Likewise, 5 mg/kg administrations of amitriptyline and imipramine produced vehicle-appropriate responding. However, the higher dose

(10 mg/kg) of these drugs produced 60 and 44.5% responses, respectively, on the phenytoin-correct lever. Desipramine at 5 mg/kg produced 60% first choice responses on the phenytoin-appropriate lever, whereas the highest dose of desipramine (10 mg/kg) produced 80% responses on the phenytoin-correct level and the quantitative measurement at this dose of desipramine was not significantly different from that seen with the training dose of 10 mg/kg phenytoin.

DISCUSSION

The present experimentation indicated that a low dose of the anticonvulsant phenytoin can readily act as a discriminable stimulus in rats trained in an operant two-lever task. This is in contrast to the report of Overton [15] who noted that phenytoin, at doses of 150–200 mg/kg, exercises only weak discriminative control over rats' behavior in a T-maze procedure. More recently, Krafft *et al.* [10] reported that pigeons trained in a two-key drug discrimination procedure, using a fixed ratio 20 schedule, learned to discriminate 5 mg/kg phenytoin from saline in 50–68 sessions. This dose (5 mg/kg) was shown to be the ED50 in the present study as decreasing doses of phenytoin produced decreased discriminative performance both in the quantal and quantitative measurements.

Generalization tests with chlordiazepoxide and pentobarbital produced responding that was essentially like the phenytoin-vehicle. Krimmer *et al.* [13] recently reported that phenytoin did not produce pentobarbital-like responding in rats trained to discriminate 10 mg/kg pentobarbital (IP) from saline and Krafft *et al.* [10] showed that neither 10 or 20 mg/kg phenobarbital nor 1 or 2 mg/kg diazepam would generalize in their phenytoin-trained pigeons.

Testing with high doses of both imipramine and amitriptyline produced discrimination unlike either phenytoin or its vehicle. Higher doses of each were precluded from use because of behavioral disruption. Interpretation of these intermediate discrimination effects is always difficult and must be done with caution [20]. Nevertheless, desipramine at 10 mg/kg produced phenytoin-like discriminative responding. This observation indicates that the interoceptive cue produced in the phenytoin-trained rats is similar to that produced by at least one tricyclic antidepressant and suggests the need for further investigations to indicate the possible effectiveness of phenytoin's use as an antidepressant agent [6].

The mechanism of action in the brain by which this common effect may have been produced is not readily apparent. However, there is experimental evidence that seizure thresholds may be modified by manipulation of central serotonin (5HT). Thus, depletion of 5HT has been associated with a fall in seizure threshold in various species [5,19] whereas a rise in brain 5HT has been associated with an elevation of seizure threshold [9,19]. Further, it has been suggested that anticonvulsants, such as phenytoin, may elevate brain 5HT in nonhumans [3] and humans [4], and that this effect may be related to its antiepileptic action. The ability of tricyclic antidepressants to increase brain serotonin is well evidenced [2] and it is possible that this common effect upon serotonin may be the explanation for the generalization of behavioral discrimination. Experiments to determine the serotonergic effects of phenytoin are currently underway in this laboratory.

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REFERENCES

1. Barry, H., III and E. C. Krimmer. Differential stimulus attributes of chlordiazepoxide and pentobarbital. *Neuropharmacology* **18:** 991–998, 1979.
2. Berger, P. A. Neurotransmitters and affective disorders. In: *Neurotransmitter Function: Basic and Clinical Aspects*, edited by W. Fields. New York: Stratton International, 1977, pp. 305–336.
3. Bonneycastle, D. D., N. J. Giarman and M. K. Paasonen. Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. *Br J Pharmacol* **12:** 228–231, 1957.
4. Chadwick, D., P. Jenner and E. H. Reynolds. Amines, anticonvulsants and epilepsy. *Lancet* **March 1,** 473–476, 1975.
5. Chen, G., C. R. Ensor and B. Bohner. A facilitation action of reserpine on the central nervous system. *Proc Soc Exp Biol* **86:** 507–510, 1954.
6. Dreyfus, J. *A Remarkable Medicine Has Been Overlooked: With A Letter to President Reagan*. New York: Simon and Schuster, 1981.
7. Glennon, R. A. and J. A. Rosecrans. Speculation on the mechanism of action of hallucinogenic indolealkylamines. *Neurosci Biobehav Rev* **5:** 197–207, 1981.
8. Goldberg, M. E. and V. B. Ciofalo. Effect of diphenylhydantoin sodium and chlordiazepoxide alone and in combination on punishment behavior. *Psychopharmacologia* **14:** 233–239, 1969.
9. Green, A. R. and D. G. Grahame-Smith. The effect of diphenylhydantoin on brain 5-hydroxytryptamine metabolism and function. *Neuropharmacology* **14:** 107–113, 1975.
10. Krafft, K., J. Cleary and A. Poling. Discriminative stimulus properties of phenytoin in the pigeon. *Psychopharmacology (Berlin)* **79:** 82–83, 1983.
11. Krafft, K., D. O. Lyon and A. Poling. Effects of phenytoin on schedule-controlled performance of rats. *Psychopharmacology (Berlin)* **78:** 93–95, 1982.
12. Krafft, K. and A. Poling. Acute and chronic effects of phenytoin on fixed-ratio performance of pigeons. *Pharmacol Biochem Behav* **16:** 843–846, 1982.
13. Krimmer, E. C., H. Barry, III and J. D. Alvin. Discriminative, disinhibitory and depressant effects of several anticonvulsants. *Psychopharmacology (Berlin)* **78:** 28–32, 1982.
14. Overton, D. A. Experimental methods for the study of state-dependent learning. *Fed Proc* **33:** 1800–1812, 1974.
15. Overton, D. A. Comparison of the degree of discriminability of various drugs using the T-maze discrimination procedure. *Psychopharmacology (Berlin)* **76:** 385–395, 1982.
16. Rall, T. W. and L. S. Schleifer. Drugs effective in the therapy of epilepsy. In: *Pharmacological Basis of Therapeutics*, 6th edition, edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: MacMillan, 1980, pp. 448–474.
17. Reinhard, J. F. and J. F. Reinhard, Jr. Experimental evaluation of anticonvulsants. In: *Anticonvulsants*, edited by J. A. Vick. New York: Academic Press, 1977, pp. 58–112.
18. Stolerman, I. P. and G. D. D'Mello. Role of training conditions in discrimination of central nervous system stimulants by rats. *Psychopharmacology (Berlin)* **73:** 295–303, 1981.
19. Wada, J. A., E. Balzamo, B. S. Meldrum and R. Naquet. Behavioral and electrographic effects of L-5-hydroxytryptophan and d,l-parachlorophenylalanine on epileptic Senegalese baboon (Papio Papio). *Electroencephalogr Clin Neurophysiol* **33:** 520–526, 1972.
20. Winter, J. C. Drug-induced stimulus control. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum, 1978, pp. 209–237.
21. Woodbury, D. M. and E. A. Swinyard. Diphenylhydantoin, absorption, distribution, and excretion. In: *Antiepileptic Drugs*, edited by D. M. Woodbury, J. K. Penry and R. P. Schmidt. New York: Raven Press, 1972, pp. 113–123.