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Barbiturate Dependence in Mice: Effects of Propranolol on the Withdrawal Syndrome (40798)[1]

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The pharmacologic treatment of barbiturate withdrawal usually involves the administration of one of the barbiturates, most often pentobarbital or phenobarbital, in gradually decreasing doses (1, 2). This approach involves the substitution of one drug (used in therapy) for another drug (originally abused by the patient), under conditions where both drugs are similar in their drug effects, and exhibit cross-tolerance and cross-dependence with each other (2). However, a search has continued for drugs which are equally effective as those commonly in use, but are not cross-tolerant or cross-dependent with the original drug of abuse. Along this vein, one drug which has attracted attention in the treatment of alcoholics during withdrawal is propranolol, a β-receptor antagonist, which has been reported to be effective in some aspects of the alcohol withdrawal syndrome, particularly in the treatment of high cardiac output, anxiety, and tremor (3–5). The overall reduction in withdrawal symptomatology seen with propranolol compared favorably to that seen with the use of benzodiazepines (3, 4). However, propranolol has not been tested for its possible usefulness in treating barbiturate withdrawal. The purpose of the present report was to test the effects of propranolol on the barbiturate withdrawal syndrome using a laboratory animal model (mice).

Methods. Physical dependence on phenobarbital (PB) was produced by the method of Belknap *et al.* (6, 7) using male C57BL/6J mice (The Jackson Laboratories, Bar Harbor, Me.). Animals from a single shipment were used for each experiment. Chronic intoxication was produced by providing a milled diet (Purina Lab Chow) thoroughly mixed with phenobarbital (free acid). Mice were given 3.0 mg phenobarbital (PB) per gram diet on Days 0–4, followed by 3.4 mg/g on Days 5–7. The mean daily dosage of PB per group in all experiments was 365–400 mg/kg.

Animals in Experiment 1 (3 months old) were divided into four groups ($N = 10$ each) at the time of withdrawal (7 AM, light onset) and they each received one of the following treatments: 0.9% saline vehicle ip (control), 15 or 45 mg/kg (±) propranolol HCl ip, or 16 μg (±) propranolol HCl intracranially (ic), respectively. All doses were given at 13 hr and again at 21 hr after withdrawal of PB. Following the injections, the animals were left undisturbed for 0.8 to 1.0 hr. The ip doses were given in an injection volume of 10 ml/kg (saline vehicle), while the ic doses were given in a fixed 4-μl volume in Ringer's solution by the method of Schlesinger *et al.* (8). Beginning 0.8 to 1.0 hr after the first injection, each animal was scored for withdrawal signs (on a blind basis) at 4-hr intervals for the next 12 hr. This time span (14 to 26 hr after withdrawal) encompasses the peak in withdrawal sign frequency (6, 9). Scoring was accomplished by suspending each animal by the tail for 10 sec (with gentle twirling), returning it to its home cage, and observing it for 1 min to determine the presence or absence of four withdrawal signs (9). These were: convulsions on handling—myoclonic spasms of the face, usually extending to the rest of the body; tremor—marked tremulousness of the entire body; Straub tail—arching of the

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tail over the back, a sign of muscular rigidity; seizures—mild clonic seizures of the anterior part of the body or tonic–clonic seizures involving all of the body. Hyperreactivity and wild running were also observed, but these occurred too infrequently for statistical analyses. Convulsions on handling (10–12) were scored while the mouse was suspended by the tail; all other symptoms were scored while the mouse was in its home cage.

A withdrawal score (WS) was also determined on the basis of the following 3-point rating scale (6): 0–no appreciable symptomatology; 1—the presence of one or more of the following: convulsions on handling, Straub tail, tremor, mild clonic seizures; 2—tonic–clonic seizures. The withdrawal scores were summed across all four observations for each animal, and differences between groups assessed by the Mann–Whitney *U* test (two tailed). The frequencies of each withdrawal sign were analyzed by the Fisher exact probability test (two tailed).

In Experiment 2 the mice (8 months old) were treated identically as in Experiment 1 except that three groups were formed (N = 10 each) which were given either the 0.9% saline vehicle (controls), 45 mg/kg (+) propranolol or 45 mg/kg (−) propranolol. The stereoisomers were obtained from Ayerst. All doses (ip) were given under the same conditions and the withdrawal syndrome assessed in the same manner as in Experiment 1.

Results. The relative frequencies of withdrawal signs after propranolol administration are shown in Table I. In Experiment 1, (±) propranolol administered to C57BL/6J mice either ip (45 mg/kg) or ic (16 μg) significantly reduced the incidence of seizures (vs controls), but only when animals were observed within 1 (0.8 to 1.0) hr of an injection. This finding is consistent with the short half-life of propranolol of 45 min in mice as measured in brain tissue (13). No other symptom category was significantly affected (vs controls), either 0.8 to 1.0 hr subsequent to the two injections or for all four observations. The 45 mg/kg (±) propranolol ip dose also produced a significant reduction in the mean withdrawal score. The 15 mg/kg ip (±) propranolol dose produced no significant effects, although the same trends were evident as in the 45 mg/kg dose. Crude impressions were that these doses of propranolol produced no obvious effects on general activity or response to handling at 1 hr after injection.

In Experiment 2 (Table I), both stereoisomers of propranolol (45 mg/kg)

TABLE I. EFFECTS OF PROPRANOLOL ON BARBITURATE WITHDRAWAL SIGNS IN EXPERIMENTS 1 AND 2[a]

	Tremor	Convulsions on handling	Straub tail	Seizures	Mean withdrawal score (WS)
Experiment 1					
Saline (control)	23	13	23	33 (35)	4.6
45 mg/kg ip (±)	18	10	13	20 (5)*	3.1†
15 mg/kg ip (±)	25	5	20	28 (15)	3.7
16 μg ic (±)	30	10	23	20 (5)*	3.9
Experiment 2					
Saline (control)	3	43	0	25	2.3
45 mg/kg ip (−)	15	45	3	0**	0.6†
45 mg/kg ip (+)	3	25	6	8*	0.9

[a] Animals rendered physically dependent on phenobarbital were observed following withdrawal for the frequency of four withdrawal signs (see Methods). Data shown represent the percentage of total observations in which a given sign occurred. For each group shown at left (N = 10 mice each), there were four observations per mouse at 4-hr intervals for a total of 40 observations per group. Numbers in parentheses refer to the percentage of total observations made within 1 hr after each of the two injections for a total of 20 observations per group (seizures only). An overall composite withdrawal score (WS) is shown in the far right hand column.

* $P < 0.05$, Fisher test.

** $P < 0.01$, Fisher test.

† $P < 0.05$, U test.

produced a significant reduction in seizure frequency relative to control values. The withdrawal scores were also reduced, but significance was achieved only with the (−) isomer. No other withdrawal sign category was significantly affected by either isomer.

The control group for Experiment 1 showed a surprisingly different profile of withdrawal sign frequencies than the control group in Experiment 2 in spite of identical treatment. Why this occurred is not clear, but the two experiments were performed 6 months apart, with different shipments of mice, and the ages of the animals were also much different.

Discussion. The results from Experiments 1 and 2 suggest that propranolol may be of some value in the treatment of barbiturate withdrawal. The major effect was the reduction of seizures, which is consistent with the anticonvulsant effects of this drug reported by Saelens *et al.* (13) with respect to strychnine-, pentylenetetrazol-, and electroshock-induced seizures in mice. The intracranial dose, although representing only 1.5% of the 45-mg/kg ip dose, was equally effective as this ip dose in reducing the frequency of seizures. This suggests that the anticonvulsant effects were due primarily to actions in the CNS, and that the propranolol itself is responsible rather than a hepatically derived active metabolite such as propranolol glycol. The latter is known to be an effective anticonvulsant in mice (13). None of the other signs of barbiturate withdrawal were appreciably affected by propranolol administration.

In Experiment 2, both stereoisomers were found to be effective in reducing seizure frequency. This suggests that β-receptor antagonism probably does not account for much of the observed anticonvulsant effect, since the (−) isomer is many times more potent as a β-receptor antagonist than the (+) isomer, while both isomers are about equipotent in their nonspecific membrane-stabilizing actions (14–16). Thus, the latter appears to be the most likely mediating mechanism for the observed seizure reduction. This is consistent with the results of Jaeger *et al.* (17), who showed that the anticonvulsant effects of propranolol (with regard to electroshock-induced seizures) are unrelated to the β blockade.

With respect to alcohol withdrawal in mice, Goldstein (11) found that 50 mg/kg (±) propranolol produced a transient enhancement of convulsions on handling: the effects on other withdrawal signs were not reported. Freund (18) reported that (+) propranolol (which has minimal β-blocking actions) prevented seizures and reduced tremors associated with alcohol withdrawal in C57BL/6J mice. This anticonvulsant effect compared favorably to that produced by phenobarbital.

The principal finding of the present work is that propranolol is an effective anticonvulsant in an animal model of barbiturate withdrawal. This effect is probably mediated by membrane-stabilizing actions similar to those produced by lidocaine (18).

Summary. C57BL/6J mice were made physically dependent on phenobarbital by means of a powdered diet mixed with the drug. During withdrawal, propranolol was found to reduce the frequency of seizures, while no effect was seen on other signs of withdrawal from barbiturates. Propranolol appears to be an effective anticonvulsant with respect to withdrawal-induced seizures.

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