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Cellular and Learned Tolerances to Pentobarbital Ataxia

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MacKENZIE-TAYLOR, D. R. AND R. H. RECH. *Cellular and learned tolerances to pentobarbital ataxia.* PHARMACOL BIOCHEM BEHAV **39**(2) 257–264, 1991.—Pentobarbital was administered to 4 groups of rats: 1) intermittently before testing on the rotarod (RR) (experienced, EXP), 2) chronically (CHR) before testing on the RR (EXP), 3) intermittently (INT) after being tested on the RR (NONEXP), and 4) chronically (CHR) after being tested on the RR (NONEXP). On postchronic testing, Group 1 (INT/EXP) failed to show tolerance to the RR decrement, related to prechronic scores, while Group 3 (INT/NONEXP) actually showed an enhanced RR decrement. Group 2 (CHR/EXP) and Group 4 (CHR/NONEXP) both exhibited prominent tolerance to RR impairment at the postchronic test, with a nonsignificant trend for greater tolerance in Group 2. The lack of an expressed behavioral tolerance in INT/EXP rats and the enhanced RR decrement in INT/NONEXP subjects at the postchronic test was attributed to repeated use of a towel wrap restraint during the chronic treatment period. When the prechronic tests for INT/EXP animals were separated into the first 3 and last 3 days, pentobarbital impairment of RR during days 4–6 was significantly less than during days 1–3. This tolerance in INT/EXP rats was lost at the postchronic testing, while INT/NONEXP subjects had by then developed an enhanced RR impairment to pentobarbital. Following postchronic testing, chronic pentobarbital (CHR/EXP and CHR/NONEXP groups) and chronic vehicle (INT/EXP and INT/NONEXP groups) were discontinued for 9 days (withdrawal), after which an intermediate dose of the drug was tested on RR performance. Next, 9 days of extinction training involved vehicle injection daily before testing RR performance, after which the intermediate drug dose was again tested. INT/EXP and INT/NONEXP groups showed no change in RR impairment at the postwithdrawal and postextinction tests. However, in CHR/EXP rats pentobarbital tolerance was partly lost at the postwithdrawal test, with a significantly greater loss at the postextinction test. The CHR/NONEXP group showed a prominent loss of tolerance at the postwithdrawal test and no significantly greater loss at the postextinction test. Analysis of serum and brain concentrations of drug in other rats identically treated up to the postchronic period yielded evidence of metabolic and cellular tolerances in the chronically treated groups (2 and 4). Additionally, behavioral tolerance appeared to form as a function of drug experience in the CHR/EXP group. INT/EXP subjects (Group 1) failed to express behavioral tolerance during the postchronic test despite drug experience and the INT/NONEXP (Group 3) showed enhanced RR decrement, probably as the result of an interfering factor of repeated episodes of towel wrap restraint.

Pentobarbital Learned tolerance Cellular tolerance Ataxia Rotarod

TOLERANCE to central nervous depressant drugs after chronic exposure was explained initially as the enhanced metabolic disposition of the agent and/or induction of latent hyperexcitability in brain neurons to persistent impairments of cellular processes (13, 20, 29). More recently, a role of repeated experiences with drug-impaired behavior has been demonstrated in the development of a type of tolerance based upon learned adaptations (1, 6, 8, 12, 24, 32, 35, 38). This latter type of tolerance has been considered variably as (a) different from the classical cellular/metabolic types of tolerance (6, 8, 19, 27), (b) an augmentation of cellular tolerance by drug-intoxicated practice (23,24), or (c) a predominant mode of tolerance development operating in all types of tolerance to drug-induced behavioral deficits (35,40). Since these distinctions have considerable theoretical as well as some practical significance for drug effects on behavior, we [(25,26), this study] attempted a design to test the hypothesis that a pharmacodynamic tolerance was distinct from a learned adaptation to repeated experiences with drug-impaired behavior. Some rats were exposed to intermittent drug challenges and allowed to experience behavioral deficits, while others so treated were denied these experiences. Chronic drug treatment was imposed on other subjects, some of which experienced behavioral decrements of test doses while another group did not. In previous articles we presented evidence that tolerances to ethanol and pentobarbital hypothermias were developed as learned or cellular/metabolic types, according to the treatments presented (25,26). That is, behavioral tolerance acquisition and loss was dependent upon repeated drug experiences and extinction training, while cellular/metabolic tolerance related to chronic drug treatment and drug withdrawal. In the present study, tolerance factors for pentobarbital ataxia are examined by utilizing this same experimental design of drug treatments and measuring the effects on rotarod behavior.

METHOD

Subjects

Male Sprague-Dawley rats of consistent genetic stock (Har-

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TABLE 1

SCHEDULE OF TREATMENT PERIODS AND PENTOBARBITAL EXPOSURE FOR ALL FOUR EXPERIMENTAL GROUPS

	Period Schedule (Days)						
Rat Group*	1–6: IP Vehicle	7–12: Prechronic IP Drug Test	13–48: Chronic Drug or Vehicle Treatment	49–51: Postchronic Drug Tolerance Test	52–61: Withdrawal Period	62–71: Extinction Training	72: Postextinction Test
INT/EXP (1)	Towel-Wrap, Heat Lamp, BT† monitored for 2 h	Measure RR Effects for 2 h	IP Vehicle and Towel Wrap Daily; Test IP Drug Every 4th Day on RR; IP Vehicle Every 4th Day on RR	Test 3 IP Drug Doses on RR	Stop Chronic Vehicle; Test IP Drug‡ on RR on Day 61	Daily Vehicle and Test RR	Test IP Drug§ on RR
CHR/EXP (2)	Same as Group 1	Same as Group 1	Drug in Diet; IP Drug and Towel Wrap, 3 Days; IP Vehicle and Towel Wrap Every 4th Day, Then Test IP Drug on RR	Same as Group 1	Stop Chronic Drug; Test IP Drug§ on RR on Day 61	Same as Group 1	Same as Group 1
INT/ NONEXP (3)	Measure RR‡ Effects for 2 h	Towel-Wrap, Heat Lamp, BT monitored for 2 h	IP Vehicle and Towel Wrap, 3 Days; IP Vehicle on RR every 4th Day, Then IP Drug and Towel Wrap and BT monitored for 2 h	Same as Group 1	Same as Group 1	Same as Group 1	Same as Group 1
CHR/ NONEXP (4)	Same as Group 3	Same as Group 3	Drug in Diet; IP Drug and Towel Wrap Daily; IP Vehicle on RR Every 4th Day	Same as Group 1	Same as Group 2	Same as Group 1	Same as Group 1

*INT = intermittent drug treatment, EXP = repeated experience with drug effect on RR performance, CHR = chronic drug treatment, NONEXP = drug test doses administered after conducting the RR test.

†BT = Body temperature.

‡RR = Rotarod performance.

§Drug dose adjusted for each animal to a dose causing significant RR decrement for at least 30 min but no longer than 60 min at the Postchronic Drug Test.

lan, Inc., IN) were purchased at 200 ± 25 g body weight and maintained in humidity- and temperature-controlled animal quarters on a 12-h light-dark cycle (lights on 7 a.m. to 7 p.m.). Food (Lab Blox® or ground chow) and water were available ad lib, except during chronic drug treatment, when ground chow with or without pentobarbital admixed was available only from 5 p.m. to 7 a.m. These same subjects were used to test tolerance aspects of pentobarbital hypothermia; results were presented in an earlier paper (26).

Training

All subjects were trained over 3–5 days on the rotarod (RR). This device consists of a cylinder (cm width, 10.5 cm diameter) rotated at 9 revolutions per min (8, 9, 31). The subject was placed on the cylinder and required to walk in a counter-rotating direction to avoid slipping off and falling 3 feet onto a padded platform. The training criterion was met when the rat walked the cylinder for at least 180 s on 3 consecutive trials. During these training days, the animals were also adapted to a towel wrap and monitoring of rectal temperature, while being placed under heat lamps (26). The towels were snugly but not tightly secured around the subjects with Acco® clips to render the animal relatively immobile. The subjects were adjusted in placement under the heat lamps to maintain body temperature within normal limits. The towel wrap procedure aided to maintain the rat's position as well as to deny a drug-treated animal the experience of attempting to ambulate while intoxicated.

Pentobarbital Administration

Test doses and a part of the chronic drug treatment were administered by IP injection, doses being 20, 28 and 40 mg/kg to start but eventually ranging up to 80 mg/kg. The remainder of the chronic drug treatment was provided in ground laboratory chow, as detailed in the previous publication (26). Body weights were monitored during chronic treatment to assure that they did not fall below 85% of ad lib weights of rats on regular food. Pentobarbital added to the food was only increased in dosage as subjects consumed 80% or more of that consumed by rats on a control diet. Test doses and chronic maintenance doses were gradually increased as tolerance developed to maintain depres-

sant effects, by the method of Okamoto (28).

Treatment and Testing Schedules

Four groups of 12 rats each were randomly assigned to 7 sequential periods of treatment as listed in Table 1. During the first period (days 1–6) INT/EXP and CHR/EXP animals (Groups 1 and 2) received IP vehicle daily, following which they were towel wrapped and maintained under heat lamps for 2 h; body temperature was monitored over this period. INT/NONEXP and CHR/NONEXP subjects (Groups 3 and 4) also received injections of vehicle during the first period, but were then tested on the RR at 15-min intervals for 2 h. Body temperature was also determined at 10-min intervals with the rats unrestrained and maintained at room temperature in this and later periods of RR testing. During the prechronic test period (days 7–12, 2nd period) all rats received 3 test doses of pentobarbital IP in random order, twice for each dose at 3-day intervals. INT/EXP and CHR/EXP groups were tested for RR performance and body temperature measured over the next 2 h, while INT/NONEXP and CHR/NONEXP groups were towel wrapped and maintained under heat lamps with body temperature monitored. Avoiding prechronic tests of drug effect on the RR in the nonexperienced groups follows the design of Cunningham et al. (11) to optimize the learned tolerance differences between experienced and nonexperienced subjects.

The third period (days 13–48) was the chronic treatment period, when INT groups (1 and 3) received daily vehicle injections and ground chow, whereas CHR groups (2 and 4) received daily drug injections and ground chow containing pentobarbital (injections during the day and food available during the evening). On every 4th day of this period the INT/EXP and CHR/EXP groups were injected with an IP test dose of drug (starting at 30 mg/kg and increasing as tolerance developed), and the subjects were tested for RR and body temperature over the next 2 h. The INT/NONEXP and CHR/NONEXP groups also received drug test doses every 4th day during this period, but were towel wrapped under heat lamps with body temperature monitored over the next 2 h. Additionally, Groups 1 and 2 received IP vehicle daily followed by 2 h of towel wrap, heat lamps, and body temperature monitoring. The NONEXP groups also received IP vehicle every 4th day followed by testing over 2 h on the RR and measurements of body temperature while unrestrained at room temperature. Thus, during the chronic treatment period, all subjects were exposed to comparable treatments with the exceptions that only CHR/EXP and CHR/NONEXP groups (2 and 4) received chronic drug and only INT/EXP and CHR/EXP groups (1 and 2) were allowed to experience RR deficits and hypothermia after pentobarbital intoxication. The INT/NONEXP subjects (Group 3) received neither chronic drug nor the RR and hypothermic experiences.

The postchronic drug tolerance tests were done during days 49–51 (4th period, Table 1), all subjects being continued on maintenance treatments of the 3rd Period and injected with 3 test doses of drug in random order over the 3 days. Since CHR animals had by now developed a prominent tolerance, their test doses were increased to 28, 40 and 80 mg/kg. INT subjects continued on original test doses of 20, 28 and 40 mg/kg. Over the 2 h following drug injection, all rats were tested on the RR (body temperature also measured without restraint and at room temperature). During the subsequent 9 days (52–61) all chronic maintenance was discontinued and subjects remained in their home cages through day 60 (withdrawal period). On day 61 a test dose of pentobarbital was administered to all subjects, after which RR performance and body temperature changes were measured over 2 h in unrestrained animals at room temperature (postwithdrawal test). The test dose of drug here was tailored to each rat, based on the postchronic test results, to induce a modest duration of RR impairment (30–60 min). Most animals in the INT/EXP group received 28 mg/kg (the remainder injected with 20 mg/kg; mean dose $= 25 \pm 1.8$ mg/kg). Most rats in the INT/NONEXP group received 20 mg/kg (a few being given 28 mg/kg; mean $= 22.2 \pm 1.1$ mg/kg). Most in the CHR/EXP and CHR/NONEXP groups received 40 mg/kg (a few being injected with 80 mg/kg; means $= 52.3 \pm 6.0$ and 48.7 ± 6.2, respectively). This procedure was adopted from Okamoto et al. (29) to facilitate comparisons of the postwithdrawal and postextinction (see below) test results using comparable levels of RR impairment as observed during the postchronic test period.

On completion of the postwithdrawal tests, all rats were subjected to extinction training (days 62–71, Period 6 in Table 1). During these 9 days vehicle was injected daily, followed by RR determinations and body temperature measurements while unrestrained, for all subjects. On day 72 the postextinction test was done using the same drug test doses as injected for the postwithdrawal tests, measuring changes in RR performance in unrestrained animals.

Blood and Brain Pentobarbital

The treatment schedules shown in Table 1 were repeated to day 49 (postchronic test period) in other rats, on which day all subjects received IP pentobarbital, 40 mg/kg. Animals from each of the 4 groups were killed serially at 15, 30, 60, and 120 min after drug, and just after determining the RR decrement and hypothermia while the rats were unrestrained at room temperature. Details of the drug analytic procedure were described previously (26); only a brief outline is furnished here. Immediately after death, trunk blood was taken and the brain quickly removed from the cranium. Brains were homogenized in acid, measured amounts of amobarbital added as an internal standard, and chloroform added prior to vortexing and centrifuging. The bottom layer was filtered through anhydrous sodium sulfate, NaOH added, the sample vortexed and centrifuged and the chloroform layer discarded. The remainder was acidified with HCl, chloroform added, and the chloroform layer again filtered through anhydrous sodium sulfate after mixing. The chloroform layer was dried in a stream of nitrogen and 25 μl of trimethylanilinium hydroxide (Meth Elute®, Pierce Biochemicals) was added. This solution was injected onto the column of a Varian Aerograph 2400 gas chromatograph with a HP 3392A Integrator, SP2250® (Supelco) as the column matrix, nitrogen as the carrier gas, and detection done by flame ionization. Pentobarbital was quantified by the peak area ratio method referred to amobarbital. The blood samples were separated into serum and cells, after which the serum was prepared for analysis in a manner similar to that for brain homogenates.

Statistics

Factorial and repeated measures ANOVA designs were used to analyze duration of drug impairment of RR and the time course of brain concentration of drug. The nonparametric Kruskal-Wallis and Mann-Whitney U-tests were employed in analysis of RR time course and peak effects due to the presence of nonhomogeneous variances. Comparisons of sets of individual determinations were done by Tukey's test (5). Statistical significance was set at $p<0.05$.

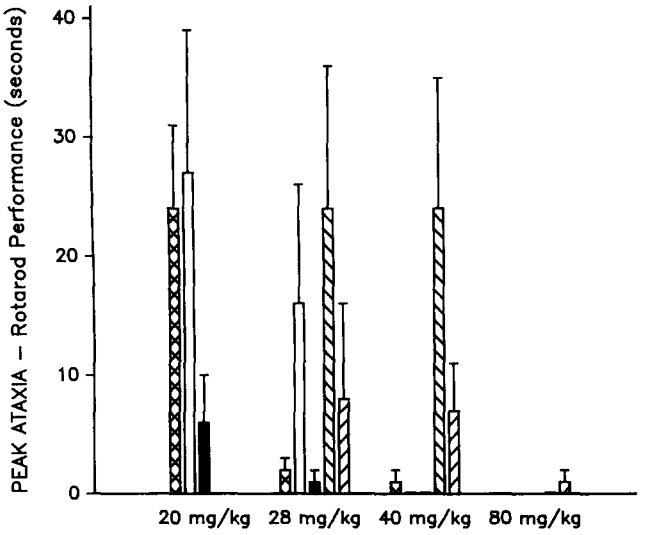

FIG. 1. Peak rotarod impairment by pentobarbital during prechronic and postchronic tests. Cross-hatched bars: prechronic values; open bars: postchronic values in INT/EXP rats (Group 1); solid bars: postchronic values in INT/NONEXP rats (Group 3); right-hatched bars: postchronic values in CHR/EXP rats (Group 2); left-hatched bars: postchronic values in CHR/NONEXP rats (Group 4). Times represent the lowest scores (±SEM) recorded 15–30 min after injection (IP) of the drug doses indicated. See the Method section for details of treatments.

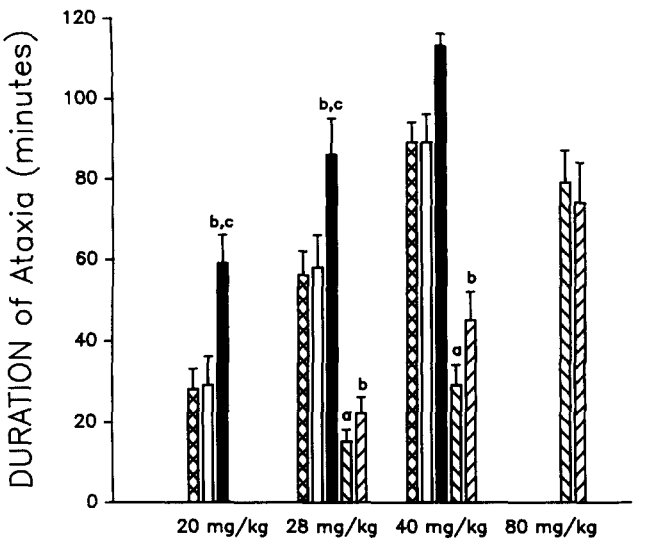

FIG. 2. Duration of rotarod impairment (±SEM) by pentobarbital during prechronic and postchronic tests. The letter *a* above a bar denotes EXP subjects sign. diff. ($p<0.05$) from prechronic controls; the letter *b* denotes NONEXP subjects sign. diff. from prechronic controls; the letter *c* denotes EXP subjects sign. diff. from NONEXP subjects. See Fig. 1 legend for further details.

RESULTS

The results relating to pentobarbital hypothermia were presented in a previous paper (26). This manuscript presents results relating to rotarod (RR) impairments and tolerance patterns induced by intermittent (INT) and chronic (CHR) drug treatments.

The prechronic and postchronic RR scores at peak ataxia from pentobarbital (20–40 mg/kg in INT groups and 28–80 mg/kg in CHR groups; see the Method section) are compared for all groups in Fig. 1. Although there appear to be large differences in drug effect among groups (lower scores indicate greater impairments), large variances and the requirement to use nonparametric statistics resulted in a lack of statistical differences. There are some interesting and surprising trends, however. INT/EXP rats, which should have developed a behavioral tolerance but not cellular/metabolic tolerance, were about the same at the 20 mg/kg postchronic test, relative to prechronic test scores, but did show a trend for tolerance at the 28 mg/kg dose level. INT/NONEXP animals, which should have developed no tolerance, actually showed a trend for greater impairment at the 20 mg/kg dose. The CHR/EXP and CHR/NONEXP groups both showed trends for prominent tolerance, which was most evident at the 40 mg/kg dose. CHR/EXP subjects also tended toward less RR impairment at both 28 and 40 mg/kg as compared to CHR/NONEXP animals, suggesting that CHR/EXP rats had developed some behavioral tolerance in addition to cellular/metabolic tolerance. Although the scores of both CHR groups at 80 mg/kg show too much impairment to afford comparisons, there is little doubt that prominent cellular/metabolic tolerance was present. All of these subjects survived in spite of the fact that 80 mg/kg of pentobarbital represents about an LD_{50} in naive rats (2).

The duration of pentobarbital-induced rotarod impairment for all groups on comparing prechronic and postchronic test scores is illustrated in Fig. 2. It was anticipated that the INT/EXP group would reflect a behavioral tolerance by manifesting lower postchronic scores than prechronic values. However, postchronic scores for this group did not differ from prechronic times at any dose. The INT/NONEXP group was expected to be unchanged in RR performance, comparing prechronic to postchronic scores. What actually occurred was an enhanced duration of impairment, which for the 2 lower doses of drug was significantly greater than either this group's prechronic or the postchronic values of INT/EXP animals. The CHR groups developed marked tolerance at all doses, as expected from the chronic drug exposure (development of cellular/metabolic tolerance), but the CHR/EXP rats did not express a behavioral tolerance relative to the CHR/NONEXP subjects, though a trend was present at the 2 lower doses.

In comparing the time course curves for RR impairment by pentobarbital, the shape of the temporal patterns appeared to shift at the postchronic tests in the various groups of rats. In an attempt to derive a more accurate measure of RR disruption by the drug, we cumulated scores where all values were significantly different from baseline performance over 2 h postdrug for each group (the "area under the curve"). Results of this analysis are shown in Fig. 3. Once again the INT/EXP group yielded postchronic scores that did not differ from prechronic performance, but INT/EXP animals posted postchronic values showing consistently greater impairment, for all doses, than prechronic measures. This impairment was also greater, for the 2 lower doses, than observed in the INT/EXP group. Postchronic values for CHR/EXP and CHR/NONEXP groups reflected once again a prominent tolerance as related to prechronic times, but with no differences based on the presence or absence of experience.

The lack of tolerance in INT/EXP animals and the enhanced impairment in INT/NONEXP subjects for pentobarbital effects on RR were surprising, since our previous studies (8) demonstrated significant behavioral tolerance for pentobarbital RR impairment after only 3 repetitions. The major difference in procedures between this earlier study and the present one was the use of the towel wrap in an effort to reduce experience with drug-induced ataxia. This procedure does represent a restraint of locomotor functions, which may induce a type of learned help-

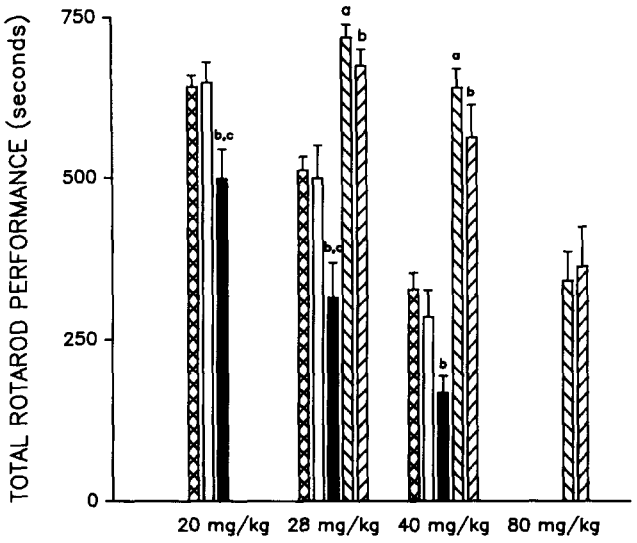

FIG. 3. Cumulative rotarod performance (area under the time course curve ± SEM) over the 2 h after pentobarbital dosing during prechronic and postchronic tests. See legends for Figs. 1 and 2 for further details.

lessness or response apathy (3,30). Such a state may interact with a depressant drug to potentiate its actions. The enhanced RR impairment in Group 3 could have resulted from such an interaction. Furthermore, the lack of tolerance expressed in INT/EXP rats could relate to a masking of the behavioral tolerance that had developed by the tendency for an enhanced effect due to the towel wrapping experiences [see discussions in Griffiths and Goudie (14), Hinson and Siegel (17), and Hinson and Rhijnsburger (16)]. With this in mind, we reexamined the RR deficits during the prechronic tests, analyzing separately the first 3 and the last 3 drug treatment days of this period on RR performance and utilizing again the cumulated scores for all values significantly different from baseline control over the 2 h postdrug. In Fig. 4 these results are compared for the INT/EXP and INT/NONEXP groups relating to postchronic test effects. The 4–6-day prechronic testing showed a trend for tolerance at the 28 mg/kg dose and a significant difference, relative to the 1–3-day prechronic tests, at the 40 mg/kg dose. Moreover, this tolerance during the second set of 40 mg/kg prechronic doses was lost at the postchronic test period in the INT/EXP group. During the postchronic test the INT/NONEXP group showed significantly greater RR impairment, relative to the 1–3- or 4–6-day intervals of the prechronic test period for all 3 test doses. Although not indicated in Fig. 4, the postchronic scores of the INT/NONEXP rats were also significantly lower than those of the INT/EXP group for the 2 lower doses.

After completing the postchronic tests all groups were subjected to "withdrawal," a cessation of chronic treatments over 9 days (Table 1). At the end of this period each animal was tested on the RR after a dose of drug that produced a moderate and roughly equivalent duration of RR impairment during the postchronic testing. Results of this postwithdrawal test are illustrated in Fig. 5. All groups then underwent "extinction training" (Table 1, days 62–71; see the Method section) and were again tested with the same doses as utilized in the postwithdrawal tests. The postextinction test scores also are shown in Fig. 5. Both INT groups showed no change in RR decrement after withdrawal, as anticipated since they had not received chronic drug. However, the INT/EXP group also did not express a loss of tolerance after extinction, which we would have expected if behavioral tolerance was present. Following withdrawal, the CHR/EXP group lost some portion of the tolerance

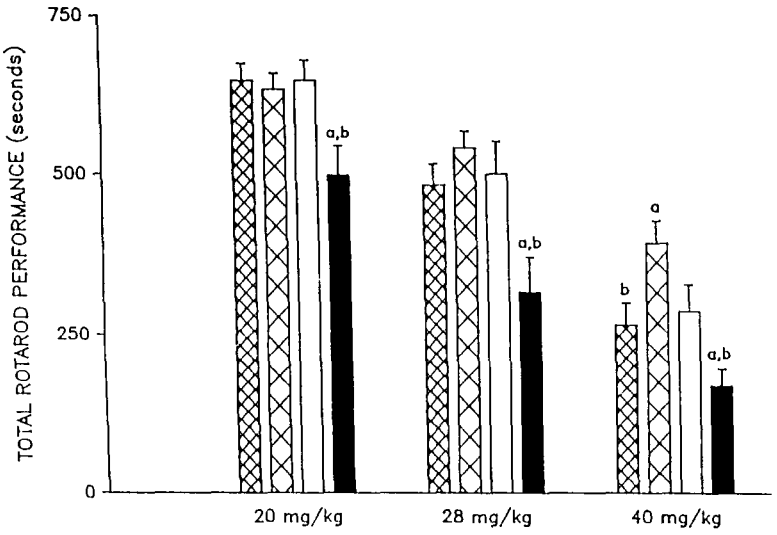

FIG. 4. Cumulative rotarod performance (area under the time course curve ± SEM) over the 2 h after pentobarbital dosing during prechronic (1–3 vs. 4–6 days) tests and postchronic tests in INT subjects (Groups 1 and 3). Double-hatched bars: prechronic 1–3 day test values; cross-hatched bars: prechronic 4–6 day test values; open bars: postchronic values in INT/EXP rats (Group 1); solid bars: postchronic values in INT/NONEXP rats (Group 3). The letter *a* above a bar denotes sign. diff. ($p<0.05$) from prechronic 1–3 day value; the letter *b* denotes sign. diff. from prechronic 4–6 day value. See the Method section for details of treatment.

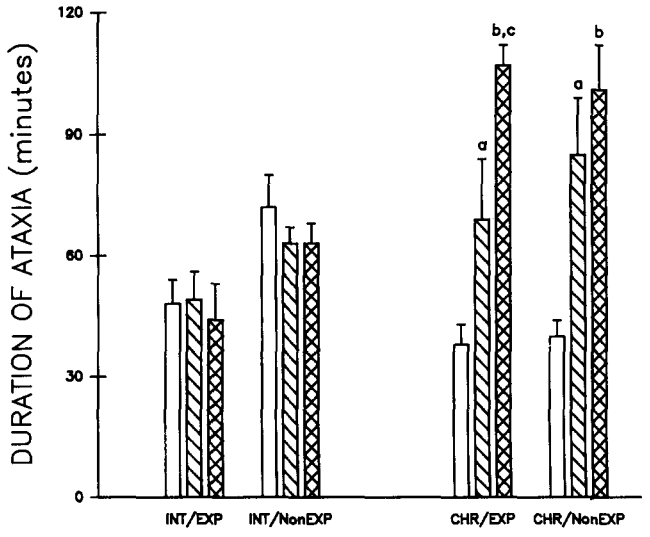

FIG. 5. Duration of rotarod impairment (±SEM) by the test dose of pentobarbital, comparing postchronic (open bars), postwithdrawal (hatched bars), and postextinction (cross-hatched bars) test values for all groups. The letter *a* above a bar denotes sign. diff. ($p<0.05$) of postwithdrawal values from postchronic values; the letter *b* denotes sign. diff. of postextinction values from postchronic values; the letter *c* denotes sign. diff. of postextinction values from postwithdrawal values. See the Method section for details of treatments.

TABLE 2

PENTOBARBITAL RR IMPAIRMENT AND BRAIN CONCENTRATION AT VARIOUS TIMES AFTER ADMINISTRATION; DUPLICATION OF POSTCHRONIC TESTING PROCEDURE

Group*	n	RR† Performance (sec ± S.E.)	Brain‡ (μg/g ± S.E.)
		15 minute	
INT/EXP (1)	4	0 ± 0	40.1 ± 4.7
CHR/EXP (2)	5	15 ± 14	27.7 ± 2.2
INT/NONEXP (3)	5	0 ± 0	40.7 ± 1.4
CHR/NONEXP (4)	5	19 ± 18	25.0 ± 3.0
		30 minute	
INT/EXP (1)	6	3 ± 3	34.8 ± 5.7
CHR/EXP (2)	6	70 ± 15	19.0 ± 2.3
INT/NONEXP (3)	5	0 ± 0	40.9 ± 4.1
CHR/NONEXP (4)	6	53 ± 18	22.8 ± 3.2
		60 minute	
INT/EXP (1)	5	19 ± 18	33.0 ± 5.9
CHR/EXP (2)	5	90 ± 0	14.7 ± 2.2
INT/NONEXP (3)	6	0 ± 9	26.7 ± 3.2
CHR/NONEXP (4)	5	90 ± 0	9.9 ± 0.3
		120 minute	
INT/EXP (1)	4	69 ± 21	16.0 ± 3.9
CHR/EXP (2)	4	90 ± 0	7.7 ± 1.6
INT/NONEXP (3)	5	90 ± 0	14.5 ± 3.1
CHR/NONEXP (4)	4	90 ± 0	8.8 ± 1.9

*INT = intermittent drug treatment, EXP = repeated experience with drug effect on RR performance, CHR = chronic drug treatment, NONEXP = drug test doses administered after conducting the RR test.

†RR = Rotarod.

‡These values were also reported in MacKenzie-Taylor and Rech (26).

demonstrated at the postchronic test, and subsequently lost a significantly greater amount after extinction training. This is consistent with the dissipation of cellular tolerance by drug withdrawal and the attenuation of behavioral tolerance through extinction. To reinforce this interpretation, note that the CHR/NONEXP group lost the majority of tolerance after withdrawal and no significantly greater degree after extinction.

Other rats were divided into 4 groups and treated as indicated in Table 1 up to the postchronic test period. On day 49 all animals received 40 mg/kg pentobarbital IP and RR performance was measured at 15, 30, 60, or 120 min thereafter (subgroups of 4–6 rats randomly selected from each of the original 4 groups). Immediately after the RR testing each animal was killed by decapitation, a trunk blood sample obtained, and brains removed. The brain drug concentrations found at analysis, along with RR scores just before death, for all 4 time periods are listed in Table 2. RR decrement was greater in the INT groups but so were the brain levels of pentobarbital. The presence of prominent metabolic tolerance was confirmed by comparing serum/brain ratios of drug concentrations in each group (not shown in Table 2). These ratios were similar for all groups, with CHR animals having lower serum as well as brain drug levels. This was anticipated, since a previous study using similar chronic drug treatment schedules for a shorter period demonstrated prominent metabolic tolerance (10).

DISCUSSION

A multitude of studies over the last several decades has emphasized the importance of experience or previous environmental history relating to drug exposure in determining the types and intensities of tolerance observed following repeated drug administration (1, 6, 7, 36, 37). The phenomenon of behavioral tolerance has been described by one group as essentially an augmentation of the classical form of pharmacodynamic (cellular) tolerance (23,24). It has also been considered as a mechanism operating in all behavioral expressions of pharmacodynamic types of tolerance (32, 34, 40); that is, all "cellular-based" tolerances have been considered by some to involve learned behavior. Other investigators have concluded that cellular and behavioral tolerances may be separable, the cellular type following temporal factors of drug exposure and the behavioral type requiring experience with the altered behavior and opportunity to develop adaptive learned responses (6, 8, 19, 21, 37). In 2 previous articles using the same design as in this study (25,26), we showed tolerance to hypothermia by lower doses of ethanol or pentobarbital administered at 4-day intervals, but only in subjects allowed to experience repetitions of the drug effects. Chronic treatment with larger doses of pentobarbital produced more prominent tolerance to hypothermia in both experienced and nonexperienced rats. Some of the chronic pentobarbital tolerance was lost only by extinction in the experienced group, while a significant degree of tolerance was lost only by drug withdrawal in the nonexperienced animals. These data were interpreted to support separate types of tolerance based directly on chronic drug exposure to promote cellular tolerance and on repeated drug experiences to generate adaptive learning that resulted in behavioral tolerance. In both studies, analysis of brain drug levels and associated behavioral deficits after chronic drug showed a lesser deficit at the same brain levels even when behavioral tolerance was not involved. Note that the subjects described previously for tolerance to pentobarbital-induced hypothermia are the same ones presented here regarding tolerance to pentobarbital RR performance.

The peak effects of pentobarbital ataxia (Fig. 1) showed a

trend for behavioral tolerance in INT/EXP rats compared to INT/NONEXP subjects (at 28 mg/kg), and in the CHR/EXP group vs. the CHR/NONEXP group (at 40 mg/kg), but the variability was too great to allow for significant differences. On exploring the duration of RR impairment by this drug (Fig. 2), marked tolerance was noted in both CHR groups (2 and 4) at all doses, with only a trend for behavioral tolerance at the two lower doses. The INT/EXP (Group 1), which was expected to show behavioral tolerance, did not. The INT/NONEXP (Group 3), which at the postchronic test should have shown no tolerance, actually showed an enhanced RR decrement. These effects were more clearly displayed in Fig. 3, indicating cumulative RR scores showing significant ataxia over the 2 h following drug (the "area under the curve"). Previous studies in this laboratory demonstrated tolerance in as few as three replications of rotarod disruption by pentobarbital (8), and others (15,38) have found greater tolerance to barbiturate motor impairments resulting from repeated experiences. Therefore, this drug class does readily promote the behavioral tolerance phenomenon in regard to motor deficits. Knowing this, we sought a complicating factor in the current research design that could have interfered with the expression of behavioral tolerance in the INT/EXP group.

We made use of a towel wrap restraint in this study, which appears to have complicated the experiential influences relating to motor impairments. Daily towel wraps may have enhanced the RR deficit by pentobarbital in the INT/NONEXP rats, perhaps as a form of learned helplessness (3,30). This same tendency in INT/EXP animals probably counteracted the expression of behavioral tolerance during the postchronic testing (Fig. 3). These types of interfering factors have been discussed by Griffiths and Goudie (14). These authors proposed that altered drug response shaped by positively conditioning experiences can be counteracted by further exposure to negatively conditioning stimuli. Evidence for this presumption in our study was obtained by expressing the RR impairment during the prechronic period for the first 3 days of treatment vs. the last 3 days. As seen in Fig. 4, tolerance developed to the drug-induced RR impairment during days 4–6 of the prechronic period, at least for the 40 mg/kg dose. Then, after repeated drug-RR couplings and multiple towel wraps, INT/EXP subjects actually lost this index of tolerance at the postchronic test period. INT/NONEXP animals, without the mechanisms to develop behavioral tolerance, showed only an enhanced decrement of RR performance at the postchronic test. Recall that these same INT/EXP rats did manifest a clear tolerance to pentobarbital hypothermia that was not observed in INT/NONEXP rats, as reported in the previous article (26).

Withdrawal and extinction demonstrated the presence (CHR/EXP) and absence (CHR/NONEXP) of behavioral tolerance in the chronically treated subjects (Fig. 5). The CHR/EXP group lost part of the postchronic tolerance after withdrawal, but lost an additional amount after extinction. Therefore, the initial loss appears to relate to dissipation of cellular tolerance and the latter loss to attenuation of behavioral tolerance. The CHR/NONEXP group showed a significant loss of tolerance only after withdrawal and not after extinction, as expected since this group should have developed only cellular tolerance. The results of the postwithdrawal and postextinction tests in the INT groups were as anticipated excepting the failure of extinction in the INT/EXP group to cause a loss of tolerance. This failure might reflect too short an extinction training (4); however, the extinction procedure was effective in Group 2. The failure is more likely related to the vehicle treatment followed by RR testing which was done in INT/EXP rats on nondrug test days during the chronic treatment period. This experience was essentially no different than the extinction training given later to these subjects. This partial reinforcement training appears to have induced a resistance to extinction at the postextinction test [cf. (33)]. Also, the proposed "learned helplessness" due to towel wrapping may have dissipated during the extinction trials, the loss of this effect counterbalancing a loss of behavioral tolerance. In the previous studies of behavioral tolerance development to ethanol and pentobarbital hypothermia (25,26), we also failed to reverse the tolerance in INT/EXP animals after extinction training, presumably as a consequence of these same design parameters.

On replicating the treatments up to the postchronic tests and analyzing serum and brain concentrations of pentobarbital, we found evidence for both metabolic and cellular tolerances. Metabolic tolerance in the CHR groups was evident by lower blood and brain drug levels at the same time after the drug, compared to levels in INT groups. In the previous study measuring hypothermia (26), cellular tolerance unrelated to a behavioral tolerance influence was evident as a reduced behavioral effect at comparable brain drug concentrations, comparing INT/EXP vs. CHR/EXP animals and INT/NONEXP vs. CHR/NONEXP subjects (analysis of covariance).

In conclusion, the results of this study support our previous findings (8, 25, 26, 39) and those of others (6, 19, 21, 32, 37, 38) that behavioral tolerance can develop as a learned adaptation in the absence of a cellular/metabolic tolerance. They do not support the proposal that behavioral tolerance only augments cellular tolerance that must develop simultaneously (22–24). Nor are they consistent with concepts developed by Siegel (34), Hinson and Siegel (17), and Wenger et al. (40), implying that most drug tolerances to behavioral effects are generally explainable as conditioned adaptations dependent upon specific experiential effects. These results further support the development of a behavioral (learned) tolerance as a function of repetitions and intensity of the drug-impaired experiences.

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