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Cellular and Learned Tolerances for Pentobarbital Hypothermia

D. R. MacKENZIE-TAYLOR AND R. H. RECH[1]

Department of Pharmacology and Toxicology, B 440 Life Sciences Bldg. Michigan State University, East Lansing, MI 48824

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MacKENZIE-TAYLOR, D. R. AND R. H. RECH. *Cellular and learned tolerances for pentobarbital hypothermia.* PHARMACOL BIOCHEM BEHAV **39**(2) 249–256, 1991.—The development of behavioral tolerance to pentobarbital-induced hypothermia, as separable from cellular and metabolic tolerance, was established. Pentobarbital (PB) was administered to 4 groups of rats, 2 groups of which received intermittent (INT) IP PB treatment. One of these groups, INT/EXP, experienced the hypothermic (measured as rectal body temperature) drug effect after PB injection. The other group, INT/NONEXP, was monitored for body temperature functions (room temperature) before receiving PB (vehicle administration) and then prevented from experiencing PB-induced hypothermia by maintenance of body temperature with a towel wrap restraint and a heating lamp. The INT/EXP group also received equivalent exposure to this towel wrap after vehicle administration. Two other groups received chronic PB treatment (IP and in ground chow), one with experience for hypothermia after injections (CHR/EXP) and one prevented from experiencing the hypothermia (CHR/NONEXP). These groups also received equivalent exposure to the body temperature (at room temperature) testing and towel wrap restraint, EXP rats after vehicle injections and NONEXP after drug injections. A postchronic test of all groups compared the extent of PB hypothermia to prechronic test effects to assess the degree of tolerance. The INT/EXP group demonstrated behavioral tolerance for PB-induced hypothermia, as contrasted with the INT/NONEXP group which demonstrated little or no tolerance. Prominent tolerance was noted in both chronic groups for PB hypothermia, without a significant difference between them. After the postchronic test, chronic treatment was discontinued for 9 days (withdrawal) followed by 9 days of extinction training (vehicle behavioral testing). The two intermittent groups demonstrated no change in the hypothermic drug response during the postwithdrawal and postextinction drug tests. However, in CHR/EXP rats tolerance to hypothermia was decreased at the postwithdrawal test, with a greater loss at the postextinction test. CHR/NONEXP animals showed a prominent loss of tolerance at the postwithdrawal test only. Brain concentrations of PB in identically treated rats (up to the postchronic test) yielded evidence of metabolic tolerance in the two chronic treatment groups. Evidence for cellular tolerance was also produced in these two groups when the brain concentrations were correlated with the extent of drug-induced hypothermia. Behavioral tolerance to drug effects was expressed in EXP groups after both chronic and intermittent pentobarbital treatment, compared to effects in the NONEXP groups, and existed separate from cellular and metabolic tolerance.

Pentobarbital Learned tolerance Cellular tolerance Metabolic tolerance Body temperature

ACQUIRED tolerance, the decrease in effects of a drug on repeated administration, was first considered to be dispositional or cellular in nature. Barbiturates show a limited dispositional (pharmacokinetic) tolerance after several weeks of chronic use resulting from enhanced metabolism (11, 15, 30). Cellular (pharmacodynamic or "functional") tolerance to barbiturates requires a longer period of time and attains a much higher degree than that deriving from increased drug metabolism (22,30). Only cellular tolerance is associated with physical dependence and withdrawal phenomena (22,29). A third type of tolerance, described in the last several decades, is based on learned adaptations to repeated functional deficits by various drugs, particularly for ethanol (6, 12, 18, 25, 39) and morphine (8, 14, 20, 35). Schuster et al. (33) proposed that amphetamine-induced decreases in rates of reinforcement may furnish the impetus for the learned adaptive response patterns of a behavioral tolerance. However, LeBlanc et al. (25) and others (16, 36, 37) have questioned this mechanism as a single or primary one capable of inducing tolerance.

Chen (7) proposed that cellular and learned tolerances incorporated separate mechanisms and gained support from investigations by Rech et al. (31), Commissaris and Rech (9), Bird et al. (4), and Melchior (28). However, some investigators have implied that all types of drug tolerance involving behavior are based on rather selective classical (Pavlovian) or instrumental conditioning (35,39). Therefore, the relationships between cellular and behavioral modes of tolerance remain enigmatic, it being difficult to design experiments to isolate treatments that theoretically would develop only one or the other. In a recent study of ethanol tolerance (27), we utilized a design in which some rats received chronic ethanol either with (CHR/NONEXP) or without protection (CHR/NONEXP) from the hypothermia of test doses. Other groups received intermittent test doses with (INT/NONEXP) or without (INT/EXP) protection from the hypothermia. With this design, INT/NONEXP animals developed no tolerance, INT/EXP animals developed behavioral tolerance only, CHR/NONEXP animals developed cellular and metabolic tolerance only, and CHR/EXP animals developed behavioral, cellu-

[1]Requests for reprints should be addressed to Dr. R. H. Rech.

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 BT Effects for 2 h	IP Vehicle and Towel Wrap Daily; Test IP Drug Every 4th Day on BT	Test 3 IP Drug Doses on BT	Stop Chronic Vehicle; Test IP Drug‡ on Day 61	Daily Vehicle and Test BT	Test IP Drug‡ on BT
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 BT	Same as Group 1	Stop Chronic Drug; Test IP Drug‡ on BT on Day 61	Same as Group 1	Same as Group 1
INT/ NONEXP (3)	Measure BT Effects for 2 h	Towel Wrap, Heat Lamp, BT monitored for 2 h	IP Vehicle and Towel Wrap, 3 Days; IP Vehicle on BT 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 BT 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 body temperature, CHR = chronic drug treatment, NONEXP = protected from drug effects on body temperature.

†BT = Body temperature.

‡Drug dose adjusted for each animal to a dose causing significant hypothermia for at least 15 min but no longer than 45 min at the postchronic drug test.

lar and metabolic tolerance. Since all groups received the same handling, these different drug treatment parameters were found to promote behavioral tolerance as a consequence of experience with drug-induced hypothermia and cellular tolerance as a function of chronic ethanol treatment. This same design is used in the present study to explore behavioral and cellular tolerance aspects of hypothermia to pentobarbital.

METHOD

Subjects

Male Sprague-Dawley rats of consistent genetic stock (Harlan Inc., IN) were acquired 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.

Rectal Temperature Measurements

Temperature was determined as described by MacKenzie-Taylor and Rech (27), adapting rats to mild restraint for insertion of a rectal thermosensing probe. Values were read on a Yellow Springs telethermometer (Model 2100) at 10-min intervals for the studies to be presented below.

Pentobarbital Administration

Test doses and a portion of the chronic drug treatment were administered by IP injection, test doses initially being 20, 28 and 40 mg/kg but eventually ranging up to 80 mg/kg in the chronically treated animals. The remainder of the chronic treatment was provided at night in ground laboratory chow with an initial drug concentration of 2 mg/g. Body weights were monitored during chronic treatment to assure that they did not fall below 85% of ad lib weights of rats on regular ground chow. As tolerance developed in chronically treated subjects, doses of both the test drug and chronic treatment were gradually increased to insure continued decrement, as practiced by Okamoto (29). However, increases in drug concentration in the ground chow were contingent upon the chronic rats (CHR/EXP and CHR/NONEXP) consuming at least 80% of the amount taken in by rats (INT/EXP and INT/NONEXP) on the control diet.

Treatment Schedules

Four groups of 12 rats each, randomly assigned, underwent 7 sequential periods of treatment, as depicted in Table 1. Dur-

ing the first period (days 1–6, Table 1) INT/EXP (Group 1) and CHR/EXP (Group 2) animals received IP vehicle at 10 a.m. These animals were then towel wrapped and placed under heat lamps for 2 h while body temperatures were monitored, and the animals distance from the lamps adjusted to keep the temperatures at control (normal) levels. INT/NONEXP (Group 3) and CHR/NONEXP (Group 4) animals were injected with vehicle at 8 a.m. and body temperatures monitored over 2 h with the subjects unrestrained and at room temperature (21°C) for each day of the first period. During the second period, the prechronic drug test period (days 7–12), INT/EXP and CHR/EXP animals received 3 test doses of pentobarbital (1 dose/day) in random order, then the order was repeated on the second three days of this period. The subjects' body temperatures were measured over 2 h with the animals unrestrained and at room temperature. Both NONEXP groups (3 and 4) received the same doses of pentobarbital on days 7–12, but were protected from experiencing hypothermia by lightly restraining them in a towel wrap, placing them under heat lamps, and maintaining normal body temperature (within ± 0.5°C) over at least 2 h [adapted from the method of Alkani et al. (2)]. Therefore, during the prechronic drug test period only the EXP groups were allowed to experience the pentobarbital-induced hypothermia and only these animals contributed to the prechronic dose-response determinations of this effect. This was necessary to assure minimal experience with hypothermia in the NONEXP groups prior to postchronic testing.

The INT/EXP animals received pentobarbital by IP injection at 4-day intervals (intermittently) during the chronic drug or vehicle treatment period (days 13–48) and were allowed to experience hypothermia following these test doses. The INT/NONEXP animals were injected with pentobarbital test doses every 4 days, but were protected from the hypothermic effect by a towel wrap restraint and maintenance of their normal body temperature by placing them under heat lamps and monitoring their rectal temperature every 10 minutes. On the other three days of the 4-day interval, INT/EXP and INT/NONEXP animals received vehicle administration and then exposure to the towel wrap maintenance for 2 h. The CHR/EXP group was given pentobarbital in the diet and by daily injections during the chronic period and also experienced hypothermia after the test doses. The CHR/NONEXP animals were administered chronic drug in the same manner as CHR/EXP animals but were protected from experiencing hypothermia from pentobarbital test doses at 4-day intervals in the same way as done for the INT/NONEXP animals. A previous study had demonstrated that these concentrations of pentobarbital in ground chow caused negligible alterations in the body temperature of rats (11). On the three nontest days of the 4-day cycle the CHR/EXP and the CHR/NONEXP animals were towel wrapped and maintained at control body temperatures for 2 h after pentobarbital administration.

To ensure that all subjects received the same nondrug experiences, during the nontest days all groups underwent the towel wrap restraint after vehicle (INT groups) or pentobarbital (CHR groups) administration for two hours. On the test days the NONEXP groups were tested after vehicle but prior to pentobarbital administration and towel wrap, and the EXP groups were towel wrapped after vehicle but prior to pentobarbital and testing. The EXP groups also had equivalent amounts of hypothermia experience, since both groups were allowed to experience pentobarbital-induced hypothermia only during every 4th day of testing during this chronic treatment period.

The postchronic drug tolerance tests were done next (days 49–51), during which all rats were continued on their third period chronic maintenance, as well as receiving 3 test doses of pentobarbital in random order over the 3 days. As explained above, CHR/EXP and CHR/NONEXP subjects received larger test doses (28, 40 and 80 mg/kg) during days 49–51, since similar chronic treatment of animals in a pilot study had produced prominent tolerance to pentobarbital. INT/EXP and INT/NONEXP subjects were tested with the original doses of 20, 28, and 40 mg/kg pentobarbital. Following these test doses all animals were measured for body temperature changes over 2 h while maintained unrestrained at room temperature.

Utilizing the data obtained from the postchronic drug tolerance testing, the dose, among the three test doses, was established for each subject that produced significant hypothermia for at least 15 minutes but no longer than 45 minutes. Therefore, the dose was chosen for each subject that induced about the same level of hypothermia across all subjects at the postchronic test period [a procedure adopted from Okamoto et al. (30) to facilitate comparisons as to effects of these same doses during the postwithdrawal and postextinction tests]. The mean test doses were 25.0 ± 1.8 mg/kg for the INT/EXP group, 22.2 ± 1.1 for INT/NONEXP, 52.3 ± 6.0 for CHR/EXP, and 48.7 ± 6.2 for CHR/NONEXP.

During the subsequent 9 days (52–61, withdrawal period, Table 1) all chronic maintenance was discontinued and subjects remained in their home cages through day 60. On day 61 all subjects were tested unrestrained at room temperature for pentobarbital hypothermia to determine loss of tolerance consequent to withdrawal (postwithdrawal test).

After completion of the postwithdrawal test, all rats were trained for "extinction" [days 62–71, Period 6 in Table 1; see Roffman and Lal (32) for efficacy of extinction of behavioral tolerance to barbiturate hypothermia]. During these 9 days, vehicle was injected each day, after which body temperature was measured in unrestrained subjects kept at room temperature. On day 72, the postextinction test was performed in each animal by injecting the same doses as utilized for the postwithdrawal test and measuring the extent of hypothermia in the unrestrained rats maintained at room temperature to determine any additional tolerance loss consequent to extinction trials.

These same subjects were also tested for drug-induced impairment of rotarod performance and tolerance aspects for this behavior; results are being prepared separately in a subsequent manuscript.

Blood and Brain Pentobarbital

The treatment schedules in Table 1 were repeated up to day 49 (postchronic test period), on which day all subjects were injected with 40 mg/kg pentobarbital. Rats from each group (4–6) were killed serially at 15, 30, 60, and 120 min intervals, just after the determination of body temperature, all animals having been unrestrained and kept at room temperature. Immediately after death, trunk blood samples were taken and brains were removed for drug analysis. Serum and brain samples were stored at -90°C until the time of assay for pentobarbital concentrations (11). The brains were homogenized in 2 volumes of 0.1 N HCl; 500 μl of this mixture was added to 1 ml distilled water, 2.5 μg amobarbital in 100 μl methanol (internal standard), and 2 ml of 0.4 N HCl, after which the sample was sonicated. Fifteen ml of chloroform was added, the mixture vortexed for 30 s, centrifuged, and the bottom layer separated and filtered through anhydrous sodium sulfate into a second 50 ml silanized centrifuge tube. Five ml of 1.0 N NaOH was added, the sample vortexed and centrifuged, and the chloroform layer removed and discarded. Five ml of 1.0 N HCl was added to the remainder, this mixture vortexed, 10 ml of chloroform added, and the complex vortexed and centrifuged again. The chloroform layer was filtered through anhydrous sodium sulfate in a 15 ml conical

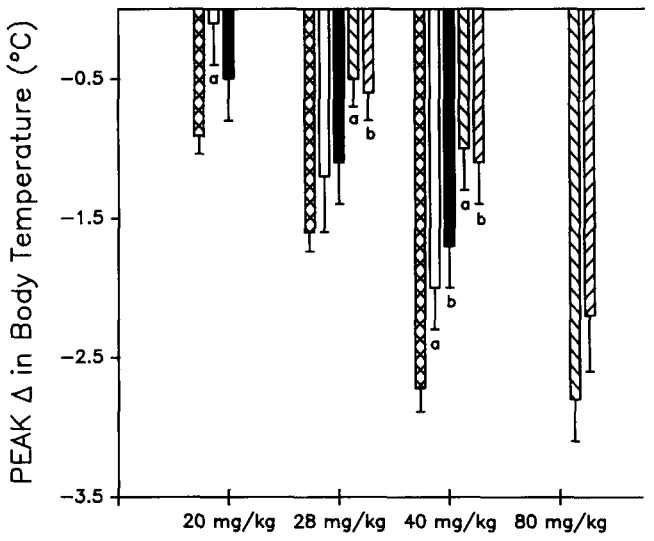

FIG. 1. Peak hypothermia to pentobarbital during prechronic and postchronic tests. Cross-hatched bars: prechronic values; open bars: postchronic values in INT/EXP rats (Group 1); solid bars: postchronic values in INT/NONEXP rats (Group 3); right-hatched bars: postchronic values in CHR/EXP rats (Group 2); left-hatched bars: postchronic values in CHR/NONEXP rats (Group 4); body temperature measures are relative to baseline controls (±SEM). The letter *a* below a bar denotes EXP subjects sign. diff. ($p<0.05$) from prechronic controls; the letter *b* denotes NONEXP subjects sign. diff. from prechronic controls. See the Method section for details of treatments.

tube, followed by drying gently with a stream of nitrogen before adding 25 μl of trimethylanilinium hydroxide (Meth Elute, Pierce Biochemicals). This solution was injected onto the column of a Varian Aerograph 2400 gas chromatograph with an HP 3392A integrator. SP2250 (Supelco) was the column matrix, with nitrogen carrier gas at 60 ml/min, injection port at 250°C and column temperature at 180°C. Detection was by flame ionization at

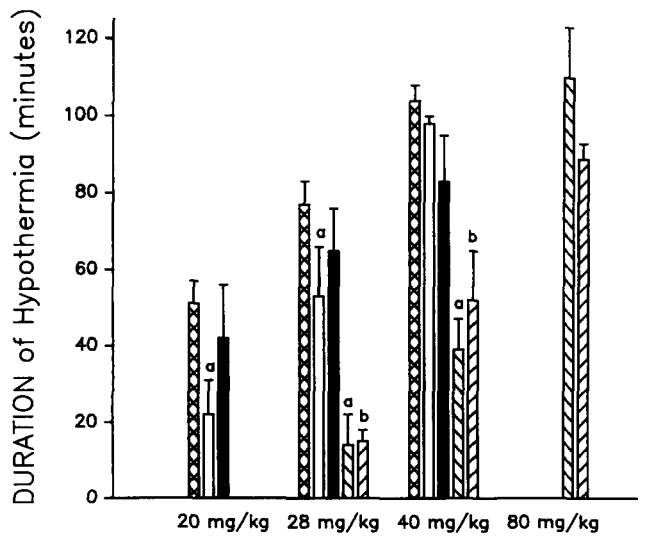

FIG. 2. Duration of pentobarbital hypothermia during prechronic and postchronic tests. See Fig. 1 legend for further details.

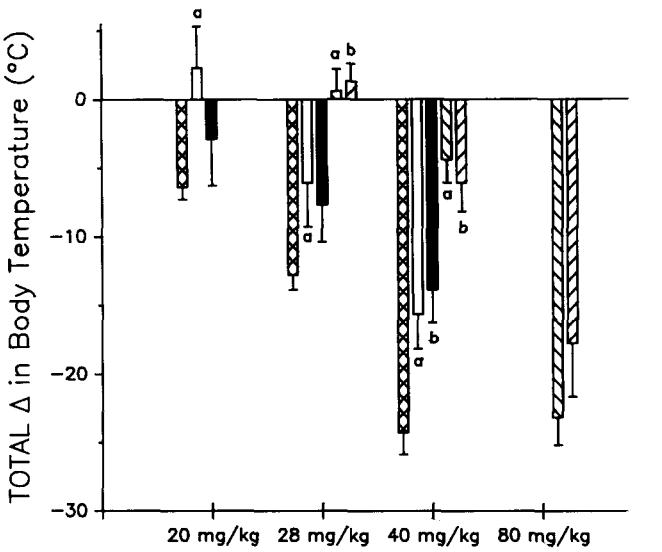

FIG. 3. Total hypothermia (area under the curve) to pentobarbital during prechronic and postchronic tests. See Fig. 1 legend for further details.

250°C with a mixed dry air and hydrogen flame. Pentobarbital was determined by peak area ratio method referred to the amobarbital internal standard. The blood samples were handled in a similar fashion following separation into serum and cells, after which 500 μl of serum was added to 1 ml distilled water

TABLE 2

PENTOBARBITAL HYPOTHERMIA AND BRAIN CONCENTRATION AT VARIOUS TIMES AFTER ADMINISTRATION: DUPLICATION OF POSTCHRONIC TESTING PROCEDURE

Group	n	Body Temperature (°C)	Brain (μg/g)
		15 minute	
INT/EXP (1)	4	−0.85 ± 0.16	40.1 ± 4.7
CHR/EXP (2)	5	−1.26 ± 0.08	40.7 ± 1.4
INT/NONEXP (3)	5	−0.38 ± 0.30	27.7 ± 2.2
CHR/NONEXP (4)	5	−0.42 ± 0.29	25.0 ± 3.0
		30 minute	
INT/EXP (1)	6	−1.05 ± 0.16	34.8 ± 5.7
CHR/EXP (2)	6	−0.13 ± 0.45	19.0 ± 2.3
INT/NONEXP (3)	5	−2.00 ± 0.22	40.9 ± 4.1
CHR/NONEXP (4)	6	−0.62 ± 0.44	22.8 ± 3.2
		60 minute	
INT/EXP (1)	5	−2.14 ± 0.79	33.0 ± 5.9
CHR/EXP (2)	5	−0.06 ± 0.13	14.7 ± 2.2
INT/NONEXP (3)	6	−2.07 ± 0.30	26.7 ± 3.2
CHR/NONEXP (4)	5	0.10 ± 0.17	9.9 ± 0.3
		120 minute	
INT/EXP (1)	4	−0.50 ± 0.64	16.0 ± 3.9
CHR/EXP (2)	4	0.83 ± 0.15	7.7 ± 1.6
INT/NONEXP (3)	5	−0.44 ± 0.37	14.5 ± 3.1
CHR/NONEXP (4)	4	0.18 ± 0.20	8.8 ± 1.9

*INT = intermittent drug treatment, EXP = repeated experience with drug effect on body temperature, CHR = chronic drug treatment, NONEXP = protected from drug effects on body temperature.

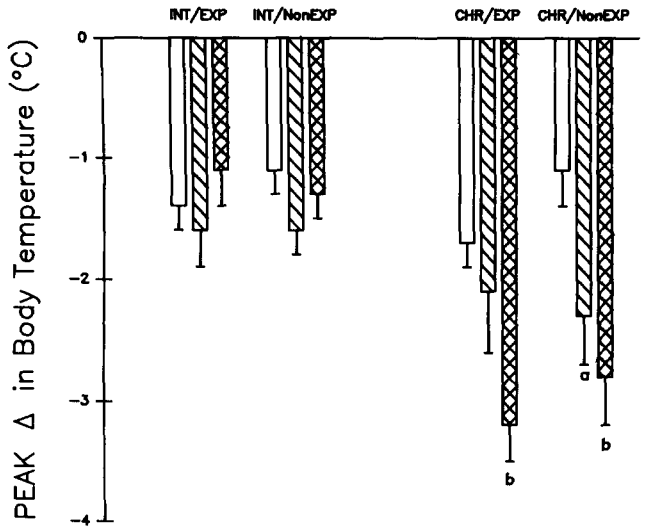

FIG. 4. Peak hypothermia to the pentobarbital test dose, comparing values of the postchronic (open bars), postwithdrawal (hatched bars), and postextinction (cross-hatched bars) tests. The letter *a* below a bar denotes that the postwithdrawal value is sign. diff. ($p<0.05$) from the postchronic value; the letter *b* denotes the postextinction value is sign. diff. from the postchronic value.

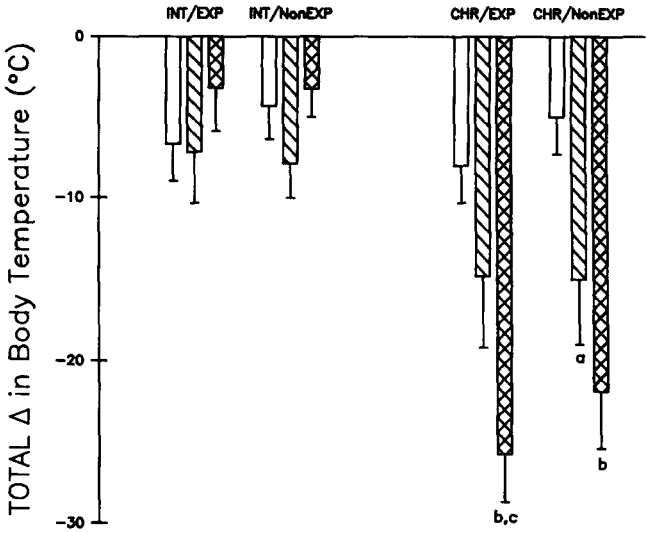

FIG. 6. Total hypothermia (area under the curve) to the pentobarbital test dose, comparing postchronic (open bars), postwithdrawal (hatched bars), and postextinction (cross-hatched bars) test values. See Figs. 4 and 5 legends for meaning of letters *a*, *b*, and *c* below the bars.

and the internal standard (2.5 μg amobarbital in 100 μl methanol). Two ml of 0.4 N HCl was then added and the sample was sonicated. Fifteen ml of chloroform was added, then the sample was vortexed and centrifuged. The chloroform layer was filtered through anhydrous sodium sulfate and dried by a gentle stream of nitrogen. The serum samples were then handled in the same manner as the brain samples for gas chromatographic analysis.

Statistics

The data were analyzed by factorial and repeated measures ANOVA designs for body temperature determinations across the various groups for each test period. The relationship of pentobarbital-induced hypothermia to blood and brain concentrations was examined by analysis of covariance. Comparisons of sets of individual determinations were done by Tukey's test (5). Statistical significance was set at $p<0.05$.

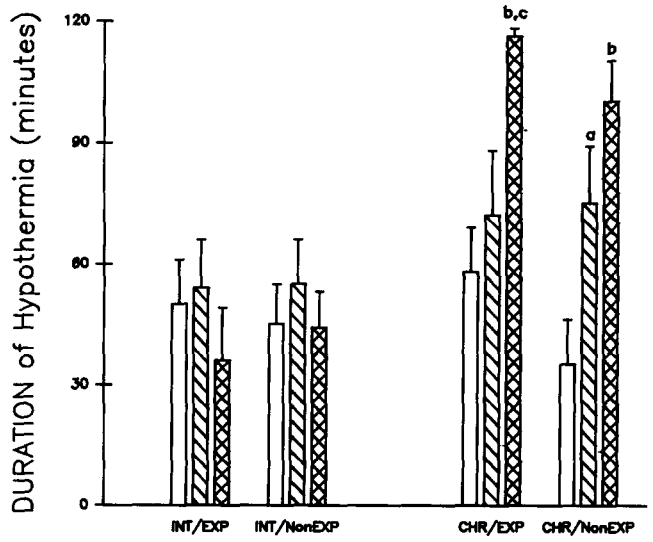

FIG. 5. Duration of pentobarbital hypothermia, comparing postchronic (open bars), postwithdrawal (hatched bars), and postextinction (cross-hatched bars) test values. See Fig. 4 legend for meaning of letters *a* and *b* above bars. The letter *c* above the bar denotes postextinction values sign. diff. ($p<0.05$) from postwithdrawal values.

RESULTS

The hypothermic effects of 3 doses of pentobarbital were determined in the 4 experimental groups of rats, following a period of chronic drug or vehicle treatment period (Period 3, days 13–48, Table 1). INT/EXP rats (Group 1) had received only intermittent drug injections (every 4 days) and had experienced drug-induced hypothermia during the chronic treatment period. CHR/EXP animals (Group 2) were administered chronic drug as well as experiencing hypothermia after the test doses. INT/NONEXP subjects (Group 3) also received intermittent drug injections but were protected from experiencing drug-induced hypothermia. CHR/NONEXP rats (Group 4) were exposed to chronic drug treatment while being protected from the hypothermia of test doses of pentobarbital. Peak changes in body temperature to the various doses at the postchronic drug tolerance test (Period 4, Table 1) are illustrated in Fig. 1. After 20 mg/kg, the INT/EXP group demonstrated significant tolerance at the postchronic test, relative to the prechronic effect. The CHR groups did not receive the 20 mg/kg dose, since dose levels for these subjects had been increased as explained in the Method section. INT/EXP and INT/NONEXP groups were little changed in response to the 28 mg/kg dose as compared to the prechronic effect, but the CHR groups exhibited a prominent tolerance to this dose. At 40 mg/kg all four groups showed a tolerance to the peak hypothermia, the CHR groups tending toward a greater change. The 80 mg/kg dose, tested only in the CHR groups, had no prechronic dose available for comparison. However, the hypothermia induced was about the same as the 40 mg/kg prechronic test effect. Since 80 mg/kg in naive rats is in the lethal range (3), there is no doubt of prominent tolerance at this high-

est dose. The results in Fig. 1 for peak effects indicate that a behavioral tolerance developed to the 20 mg/kg dose in INT/EXP animals, but no behavioral tolerance was evident in CHR/EXP rats at any dose (relating effects in the CHR/EXP group to those in the CHR/NONEXP group).

The durations of hypothermia for the postchronic drug tests are shown in Fig. 2. INT/EXP subjects displayed less hypothermia after 20 and 28 mg/kg of pentobarbital as compared to the prechronic effect. CHR groups demonstrated a prominent tolerance at 28 and 40 mg/kg, not differing from one another, and it can be presumed that they were also highly tolerant at the 80 mg/kg dose, as indicated above (no fatalities were observed). Therefore, drug experience with intermittent treatment resulted in a decreased duration of hypothermia at the two lowest doses tested. However, drug experience with chronic treatment (CHR/EXP) did not demonstrate an augmentation of the considerable tolerance induced by chronic treatment, relating to effects in CHR/NONEXP animals, regardless of the test dose of pentobarbital administered.

An analysis of the time course (reported here as total hypothermia, an approximation of the area under the curve or sum of the body temperatures from each time point) is depicted in Fig. 3. At the two lowest doses (20 and 28 mg/kg) the INT/EXP animals showed significant tolerance development at the postchronic tolerance test as compared to the prechronic effect, measuring total hypothermia. Both the INT/EXP and INT/NONEXP groups demonstrated significant tolerance to the 40 mg/kg dose. The CHR groups showed prominent tolerance to both the 28 and 40 mg/kg doses as compared to the prechronic effects. Hypothermia induced by the 80 mg/kg dose in the CHR groups during the postchronic test was equivalent to the 40 mg/kg dose effect during the prechronic test. There was no significant increase in tolerance developed after chronic drug treatment attributable to experience (CHR/EXP), relating to effects in the CHR/NONEXP animals.

Following the postchronic tests, all subjects underwent "withdrawal," a cessation of chronic treatments over 9 days (Table 1). Subjects were then tested with a dose of pentobarbital that produced a moderate and roughly equivalent duration of hypothermia during the postchronic testing. Results of the postwithdrawal testing for all groups are depicted in Fig. 4 (peak hypothermia), Fig. 5 (duration of hypothermia) and Fig. 6 (total hypothermia). All groups were then subjected to "extinction training" (Table 1, days 62–71, see the Method section) and tested once again with the dose level administered at the postwithdrawal test. INT/EXP and INT/NONEXP demonstrated no significant changes in peak, duration or total hypothermia to this test dose of pentobarbital when comparing postchronic effects with those of the postwithdrawal and postextinction tests. The CHR/EXP animals did not display a significant loss of tolerance for the peak, duration and total hypothermia at the postwithdrawal test as compared to the postchronic effects (Figs. 4, 5, and 6). However, hypothermia was increased (significant tolerance loss) in these CHR/EXP animals at the postextinction test, for peak (Fig. 4) only relative to the postchronic value, but for duration (Fig. 5) and total hypothermia (Fig. 6) relative to both the postchronic and postwithdrawal values. CHR/NONEXP subjects, on the contrary, showed significantly greater hypothermia (significant tolerance loss), compared to postchronic measures, at the postwithdrawal test for all three measures (peak, duration and total). Extinction training in these CHR/NONEXP animals did not further enhance the hypothermia induced by pentobarbital at the postextinction test.

Other rats were divided into 4 groups and the treatments indicated in Table 1 were replicated up to the postchronic test period. On day 49 all subjects were injected with a 40 mg/kg test dose of pentobarbital, body temperature measured at 15, 30, 60, or 120 min (the rats kept unrestrained and at room temperature), and the animals killed immediately thereafter to obtain brains for analysis of drug concentrations. As listed in Table 2, hypothermia in INT/EXP and INT/NONEXP was greater than in CHR/EXP and CHR/NONEXP at all time periods, confirming that greater tolerance was induced by the chronic drug treatment. This greater tolerance in CHR animals is partially attributable to metabolic tolerance, as determined by significantly reduced brain concentrations in the CHR groups as compared to the INT groups. Determination of a cellular tolerance component in the CHR animals was accomplished by analysis of covariance using brain drug concentrations and the degree of hypothermia that occurred just before death in each subject. By comparing INT/EXP to CHR/EXP ($F=4.618$, $p<0.05$) and INT/NONEXP to CHR/NONEXP ($F=7.571$, $p<0.01$) with this analysis, it was possible to determine that cellular tolerance was present in both CHR groups. There is also a trend, at least for the first 2 time periods, for the hypothermia to be less in EXP animals than in NONEXP animals, consistent with the development of a behavioral tolerance.

DISCUSSION

Early tolerance studies of barbiturates (13,34) stressed the need for chronic high dose treatments to promote high levels of tolerance of a type (cellular, pharmacodynamic) associated with physical dependence. Later, barbiturates were found to induce a limited degree of metabolic tolerance in the initial weeks of chronic use (22,30). More recently, learning factors were shown to be involved in the tolerance to many behavioral decrements of psychoactive drugs (1, 6, 8, 18, 25, 33, 38). The present study examined the role of these three tolerance factors in effects of pentobarbital on body temperature.

The INT/EXP group developed tolerance to peak hypothermia, duration of hypothermia and total hypothermia at the postchronic test. This tolerance was hypothermia experience dependent at the lower doses, since INT/NONEXP animals did not demonstrate significant tolerance at these doses. This is consistent with previous studies demonstrating that behavioral tolerance development is best manifested at low doses (24,36).

The CHR groups developed a prominent tolerance to pentobarbital peak hypothermia, duration of hypothermia and total hypothermia. This would be expected after chronic administration produced both cellular and metabolic types of drug tolerance. Evidence for behavioral tolerance was not present at the postchronic testing in the CHR/EXP animals, however. It is likely that the prominent cellular/metabolic tolerance in these animals masked a behavioral tolerance produced in these animals. However, at the postwithdrawal test, CHR/EXP animals did not demonstrate a significant tolerance loss. Though the loss of a cellular/metabolic tolerance was expected, a "reserve" of behavioral tolerance may have replaced it. This persistent tolerance component was significantly diminished after extinction trials, consistent with a mechanism of learned tolerance. On the contrary, the CHR/NONEXP animals showed a significant tolerance loss after withdrawal, but with no additional loss after extinction trials.

In a recent study using the same design as this one (27), ethanol tolerance for hypothermia was greater in experienced subjects, for both INT and CHR groups. In contrast to the pentobarbital results above, tolerance development in the CHR/NONEXP ethanol group was in the same range as that in the INT/EXP ethanol group. This may have allowed for enough decrement in the CHR/EXP ethanol group for the clear expression of the additional component of behavioral tolerance. The lack of an expressed behavioral tolerance by the CHR/EXP group (relative to

that in the CHR/NONEXP group) in the current study at the postchronic test (Figs. 1, 2, and 3) may be due to a "floor" effect. The metabolic/cellular tolerance level of pentobarbital may have been so prominent as to completely overshadow presence of behavioral tolerance. Uncovering of the behavioral tolerance could then occur during the withdrawal period. It would be interesting in future studies to try to determine how much of that total postchronic tolerance is experience dependent by doing extinction trials prior to withdrawal, comparing CHR/EXP subjects to CHR/NONEXP.

Extinction trials appeared to cause a loss of behavioral tolerance in CHR/EXP animals. Why was the same not observed in INT/EXP subjects? These INT/EXP animals may have become resistant to extinction due to partial reinforcement by the testing procedure. The drug dosing cues (IP administration, laboratory environment) were partially reinforced in these animals since they received saline injections during the chronic drug treatment period. Also, the INT/EXP animals did not experience the drug outside of the testing procedure, as contrasted with CHR/EXP subjects which experienced the pentobarbital within the towel wrap. A different test day environment or drug experience outside of the testing procedure during the extinction period might have been more successful in extinguishing the tolerance developed in INT/EXP animals.

The presence of both cellular and metabolic tolerance in the CHR animals was verified by combined analysis of hypothermic dose relationships and brain pentobarbital concentrations. A significant reduction in brain concentrations in CHR animals compared to INT animals confirmed the presence of metabolic tolerance in CHR animals. Analysis of covariance corrected for the difference in brain concentrations, allowing for comparison of the hypothermia produced in CHR animals to that of INT animals. The EXP factor was compensated for by comparing CHR/EXP to INT/EXP and CHR/NONEXP to INT/NONEXP, the resulting significant difference being attributed to a cellular tolerance.

In summary, this study supports conclusions of Chen (7), Commissaris and Rech (9,10), Jorgensen et al. (21), Tabakoff et al. (36), Holloway et al. (19) and MacKenzie-Taylor and Rech (27), that cellular and behavioral (learned) tolerances are separate entities with distinct modes of causation and dissipation. It does not support the concept of a behaviorally augmented tolerance factor relating to a single type of cellular (physiological) tolerance (23,26). These results are also not consistent with proposals of Hinson and Siegel (17) and Wenger et al. (39), that all tolerance to behavioral effects of psychoactive drugs generally involves a conditioning or learning mechanism.

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