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Cellular and Learned Tolerances for Ethanol Hypothermia

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MACKENZIE-TAYLOR, D. AND R. H. RECH. *Cellular and learned tolerances for ethanol hypothermia.* PHARMACOL BIOCHEM BEHAV **38**(1) 29–36, 1991.—Four groups of rats received ethanol: 1) intermittently while experiencing hypothermia, 2) chronically while experiencing hypothermia, 3) intermittently while protected from hypothermia, and 4) chronically while being protected from hypothermia. On postchronic testing, Group 1 showed tolerance to 2.0 and 2.3 but not 2.7 g/kg ethanol, Group 2 was tolerant to all 3 doses, Group 3 was tolerant to none, and Group 4 was tolerant only to 2.7 g/kg. On withdrawal of chronic ethanol or vehicle, Groups 1 and 2 showed trends to lose tolerance which became significant after subsequent extinction training. The treatments were repeated in other rats up to the postchronic test for tolerance, after which they were killed at 15–120 min after ethanol to assay serum and brain concentrations. Serum and brain levels of ethanol were higher in Groups 2 and 4 despite less intense hypothermia (i.e., no metabolic tolerance). Analysis of covariance indicated less tolerance in Group 1 vs. Group 2 and Group 3 vs. Group 4 for the same brain levels of ethanol (i.e., cellular tolerance in Groups 2 and 4). Therefore, both learned and cellular tolerances were observed in these subjects and appeared to be separable phenomena according to the various treatments imposed.

Ethanol Learned tolerance Cellular tolerance Body temperature Brain ethanol

CLASSICAL concepts of tolerance development to central nervous depressants such as ethanol have stressed the relationships of this phenomenon to either enhanced dispositional (metabolic) factors or states of physical dependence (16,26). These types of tolerance (metabolic or cellular) are generally considered to require chronic intoxication for at least several weeks to perhaps several months or more in various mammalian species for full development. Once a prominent tolerance is developed the sudden discontinuation of the drug is followed by a loss of tolerance over 5–10 days, accompanied by a withdrawal syndrome related to cellular tolerance mechanisms but not to metabolic tolerance aspects (9, 16, 24, 28).

More recent studies have emphasized the development of a tolerance with drugs depressing various central nervous functions that is based upon conditioning or learning processes. Chen (5,6) suggested that tolerance to behavioral effects of chronic ethanol incorporates two separate mechanisms, the one based upon learned adaptation with repeated experiences of the behavioral deficit in the drugged state and the other representing cellular biochemical/physiological changes to chronic intoxication constituting a state of physical dependence. Subsequent investigations have explored Pavlovian (11,32) and Skinnerian (13,34) bases for conditioned types of tolerance. Indeed, some investigators have stressed learned behavioral adaptations as a pervasive mechanism in all types of drug tolerance for behavioral deficits (17, 30, 31, 35, 36), while others (18,22) have pictured the learned tolerance as an augmentation of cellular tolerance components. The proposal of separable mechanisms was supported by Rech et al. (29), Commissaris and Rech (7,8) and Commissaris et al. (9). These authors and others (3,25) stressed the persistence of the learned type of tolerance in the absence of chronic drug exposure, as opposed to loss of metabolic or cellular tolerances over some days after discontinuing a chronic drug treatment in the absence of learned tolerance patterns.

Despite the many studies that have been performed on these drug tolerances, the relationships between cellular and behavioral (learned) tolerances remain enigmatic. Much of the problem lies in the fact that experimental designs employed in these investigations have usually been inadequate to isolate treatment influences calculated to develop either cellular tolerance (chronic drug exposure) or behavioral tolerance (repeated experiences of drug-impaired behavior). The present study attempts to do this by a design in which some subjects receive chronic ethanol treatment but are protected from manifestations of its effects, while other subjects receive intermittent drug treatments that cause the behavioral decrement (hypothermia) to be expressed repeatedly.

METHOD

Subjects

Male Sprague-Dawley rats of consistent genetic stock (Harlan Inc., IN) were acquired at 175–225 g (about 3 months of age) and maintained in approved animal quarters on a 12-h light-dark cycle (lights on 7 a.m. to 7 p.m.). Food (Lab Blox® or Sego® liq-

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TABLE 1

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

	Period Schedules Days						
Rat Group	1–6: IP Vehicle	7–12: Prechronic IP Drug Test	13–48: Chronic Drug or Vehicle	49–51: Postchronic Drug Tolerance Testing	52–61: Postwithdrawal Test	62–71: Extinction Training	72: Postextinction Test
1 (INT. EXP)†	Towel-Wrap Heat Lamp, BT* monitored over 2 h	Measure BT Effects over 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 Drug (2.3 g/kg) on Day 61 on BT	Vehicle; Test BT Each Day	IP Drug Test Dose (2.3 g/kg) on BT
2 (CHR. EXP)†	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 Drug (2.3 g/kg) on Day 61 on BT	Same as Group 1	Same as Group 1
3 (INT. NONEXP.)†	Test BT Over 2 h	Towel-Wrap Heat Lamp, BT monitored over 2 h	IP Vehicle and Towel-Wrap, 3 days; IP Vehicle on BT Every 4th Day, then IP Drug with Towel-Wrap, Heat Lamp, BT monitored over 2 h	Same as Group 1	Same as Group 1	Same as Group 1	Same as Group 1
4 (CHR. NONEXP.)†	Same as Group 3	Same as Group 3	Drug in Diet; IP Drug and Towel-Wrap daily; Also IP Vehicle on BT first Every 4th Day	Same as Group 1	Same as Group 2	Same as Group 1	Same as Group 1

*BT = body temperature; †INT = intermittent drug treatment; CHR. = chronic drug treatment; EXP. = repeated experience with ethanol effects on body temperature; NONEXP. = protected from ethanol effects on body temperature.

uid diet) and water were available ad lib.

Rectal Temperature Measurements

Temperature was determined with mild restraint (for which rats were adapted beforehand) and by insertion of a flexible plastic probe 4.0 cm into the rectum, readings obtained from a Yellow Springs Instrument telethermometer (Model 2100) after 40 s (9).

Ethanol Administration

Test doses and a portion of the chronic ethanol treatment were administered by IP injection (10% w/v in 0.9% NaCl), ranging from 2.0 to 2.7 g/kg. The remainder of the chronic treatment was supplied via a liquid diet of Sego® supplemented with vitamins, delivered from 5 p.m. to 7 a.m. as 5–10% ethanol (w/v) in Wahman® calibrated drinking tubes. Body weights were monitored during chronic treatment so that weights did not fall below 85% of ad lib weight of subjects maintained on the normal rat laboratory diet. If a subject's weight decreased to this level, supplementary food as Lab Blox® was offered. Despite the above precautions, a number of subjects (approximately 5%) showed prominent loss of weight and a sickly demeanor, at which time they were promptly euthanized. When these subjects were examined, postmortem signs of liver toxicity and ascites were apparent. Additional animals were scheduled with the treatments of the euthanized rats to maintain the balance of subjects in each group. For the majority of chronically treated rats body weight was maintained and tolerance developed to the hypothermic effects of test doses of ethanol. As tolerance developed the maintenance doses of chronic drug were gradually increased to insure some decremental effect to foster development of cellular tolerance, as done with barbiturates by Okamoto (27).

Treatment Schedules

Four groups of randomly assigned rats were exposed to 7 sequential periods of treatment, as shown in Table 1. Group 1 (INT. EXP.) received ethanol only intermittently as injected test doses at 4-day intervals and was allowed to experience the resulting hypothermic effects. Group 2 (CHR. EXP.) was given ethanol in the liquid diet and by daily injections during the chronic period and was also allowed to experience hypothermia after the test doses. Group 3 (INT. NONEXP.) received ethanol intermittently by injection, but was protected from experiencing hypothermia

[modified from (2,12)] by lightly restraining them in a towel wrap (for which they were adapted beforehand), placing them under heat lamps and maintaining the control body temperature (within ±0.5°C) over 2 h. Group 4 (CHR. NONEXP.) was administered chronic ethanol in the same manner as Group 2, but was protected from experiencing hypothermia from test doses in the same way as done for Group 3.

During the first period (Table 1, days 1–6) Groups 1 and 2 received drug vehicle at 10 a.m., after which they were restrained in a towel and placed in the area of a heat lamp for 2 h while monitoring body temperature and adjusting each animal's position to maintain it at normal levels. On days 1–6 Groups 3 and 4 were injected with drug vehicle at 8 a.m. and tested for body temperature at 5–15-min periods while being maintained in home-type cages at 21°C (ambient room temperature). During period 2 (days 7–12), Groups 1 and 2 received 3 test doses of ethanol in random order, 2 injections of each dose at 3-day intervals, after which body temperature was measured at 5–15-min intervals for 2 h with the subjects maintained at room temperature. Groups 3 and 4 also received 2 injections each of the 3 test doses during the second period, but were towel-wrapped and kept under heat lamps to maintain body temperature at normal levels. Therefore, this period, the prechronic testing, established the initial hypothermic effects only in Groups 1 and 2.

During the 3rd period (days 13–48, the chronic treatment phase) Groups 1 and 3 received chronic vehicle as liquid diet and injections, while Groups 2 and 4 were given the ethanol liquid diet and ethanol injections as chronic maintenance. Since Groups 2 and 4 ingested the ethanol liquid diet during the night and received ethanol injections during the day, they were exposed to the drug regularly over each 24 h. On every fourth day of this period Groups 1 and 2 were injected with an IP test dose of ethanol, kept at room temperature, and body temperature measured over 2 h. On every fourth day Groups 3 and 4 received IP ethanol test dose, followed by 2 h of towel-wrap and heat lamp exposure to maintain normal body temperature. Also on every fourth day Groups 3 and 4 received IP vehicle injection followed by measurement of body temperature over 2 h. In addition, Groups 1 and 2 were exposed to towel-wrap/heat lamps and vehicle injections as well as to temperature measurements outside the towel-wraps so that the total ranges of experiences were equivalent in all subjects, but with different relationships as to contiguity.

During the 4th period (days 49–51, Table 1, the postchronic test period) maintenance treatments of the 3rd period were continued and all groups received 3 test dose injections of ethanol in random order over the 3 days. Body temperature was measured with all rats maintained at room temperature for 2 h postinjection. During the 5th period, days 52–61, all subjects were withdrawn from chronic vehicle or chronic ethanol and maintained in home cages. On day 61, the postwithdrawal test, all animals received an injection of the middle dose (2.3 g/kg). Body temperature was measured for 2 h thereafter, with all rats maintained at room temperature. Period 6 (days 62–71) involved "extinction training," with all subjects receiving IP vehicle injections followed by measurement of body temperature over 2 h on each day. Finally, day 72 represented the 7th period (postextinction test), when the middle test dose was again injected in all subjects and body temperature measured at 5–15-min intervals over the next two h, the animals being maintained at room temperature.

Blood and Brain Ethanol

The treatment schedules in Table 1 were repeated in other rats up to day 49 (postchronic test period). On day 49 all animals received IP injection of the same test-dose of ethanol (middle dose, 2.3 mg/kg) and were killed serially at various times up to 2 h after drug, being maintained during the time at room temperature. Just prior to death by decapitation body temperature was determined, and immediately after death a blood sample was taken and the brain was removed for analysis. Serum and brain samples were stored at −90°C until the time of assay for ethanol content. The brains were homogenized in 2 volumes of deionized (Milli-Q®-filtered) water. Blood and brain homogenate samples were protein-filtered (Amicon, MPS-1). Twenty μl aliquots were added to 20 μl of 0.1% methanol, which served as the internal standard. Two μl of this mixture was introduced onto a 4 mm (i.d.), 6 ft. glass column packed with Chromosorb 101® and maintained at 160°C in a Perkin Elmer 4200 gas chromatograph using flame ionization detection. The injection port and detector temperatures were set at 300°C. The ethanol concentrations were estimated by the peak-area ratio method with reference to the internal standard.

Statistics

The data were analyzed using a repeated measures ANOVA design for body temperature measurements across the various groups for each test period. The relationship of ethanol hypothermia to blood and brain levels of ethanol in the 4 groups was explored using analysis of covariance, and comparison of sets of individual determinations was done using Tukey's test (4). Statistical significance was indicated by $p<0.05$.

RESULTS

After the chronic treatment period, the hypothermic effects of 3 doses (2.0, 2.3, 2.7 g/kg) of ethanol were determined in all 4 groups of subjects over the 3 days (49–51) of the postchronic test period. Figure 1 compares these effects with those obtained during the prechronic determinations (in Groups 1 and 2 only) for the peak hypothermia to ethanol. Group 1, intermittently treated (INT.) with ethanol and drug-effect experienced (EXP.) during the chronic period, showed less hypothermia as compared to prechronic values or postchronic values of Group 3 (INT. NONEXP.) only at the middle dose. Group 2, chronically treated with ethanol (CHR.) and drug-effect experienced (EXP.), suffered less hypothermia than for the prechronic testing at all 3 dose levels. Group 4, which received chronic ethanol but was protected from experiencing ethanol hypothermia (CHR. NONEXP.), did not differ in hypothermia from the prechronic test for the low or middle doses but showed significant reduction for the high dose. The reductions in body temperature for Groups 2 and 4 treated with the high dose were almost identical. Therefore, as to the peak effect in the INT. groups, EXP. appeared to promote greater tolerance. As to CHR. groups, EXP. also seemed to induce greater tolerance, except at the high dose, at which EXP. and NONEXP. groups were equally tolerant. The latter finding can be interpreted to indicate the development of cellular/metabolic tolerance.

The durations of ethanol hypothermia during the postchronic dose-response determinations are illustrated in Fig. 2. For the low and middle doses of ethanol Group 1 showed significantly shorter duration of hypothermia than observed for the prechronic measures or the values from Group 3. Group 2 was also significantly different from prechronic duration of hypothermia at the low and middle doses, while Group 4 was not. At the high dose only Groups 2 and 4 differed in duration of hypothermia from prechronic levels, and Group 2 values were essentially identical to those of Group 4. Thus results in Fig. 2 show that experience promoted greater tolerance in both INT.- and CHR.-treated groups at the two lower doses. While only a suggestion of cellular/metabolic tolerance was seen at the two lower doses, it was apparent at the high dose of ethanol. Additionally, the hypothermia to the middle dose in Group 2 just missed being significantly different

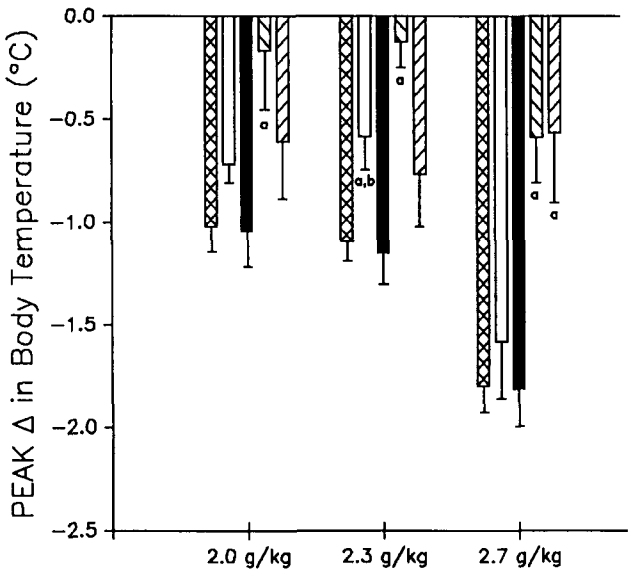

FIG. 1. Peak hypothermia to ethanol during Prechronic and Postchronic tests. Cross-hatched columns: Prechronic values; open columns: Postchronic values in Group 1 (INT. EXP), solid columns: Postchronic values in Group 3 (INT. NONEXP.), back-slashed columns: Postchronic values in Group 2 (CHR. EXP.), slashed columns: Postchronic values in Group 4 (CHR. NONEXP.). Body temperature measures are relative to baseline controls (0.0). The letter a below the bar denotes a significant difference from Prechronic values; the letter b denotes a significant difference from NONEXP. subjects (Groups 3 and 4).

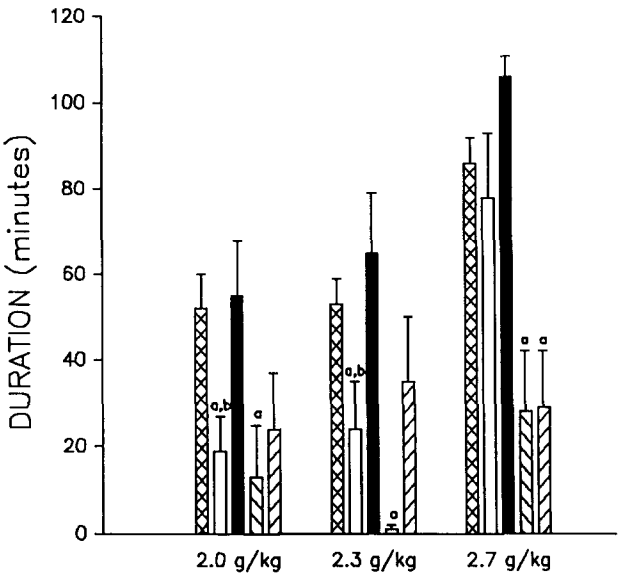

FIG. 2. Duration of ethanol hypothermia during Prechronic and Postchronic testing of dose-response effects. See Fig. 1 legend for further clarification.

from that of Group 4 both for peak (Fig. 1) and duration (Fig. 2) effects. This suggests that the use of a slightly larger number of subjects in each group would have demonstrated a greater tolerance in Group 2, as a consequence of drug experience, as compared to Group 4.

The influence of the withdrawal phase (Period 5, days 52–61, Table 1) and extinction training (Period 6, days 62–71) procedure on all groups for peak and duration of hypothermia to 2.3 g/kg ethanol is depicted in Fig. 3 and Fig. 4, respectively. "Withdrawal" in Group 1 (INT. EXP) showed a nonsignificant trend for loss of tolerance, while extinction caused little additional change, relative to postchronic readings. Group 2 (CHR. EXP.)

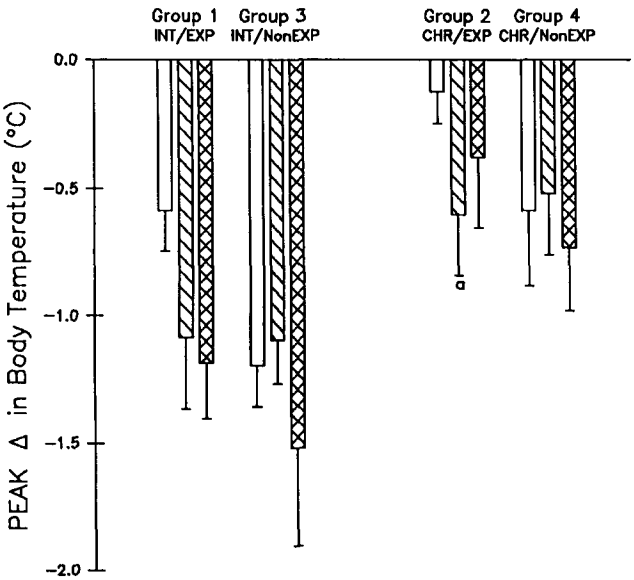

FIG. 3. Peak hypothermia to 2.3 g/kg ethanol, comparing values of the Postchronic (open columns), Postwithdrawal (hatched columns), and Postextinction (cross-hatched columns) tests. The letter a below the bar denotes a significant difference from Postchronic measures.

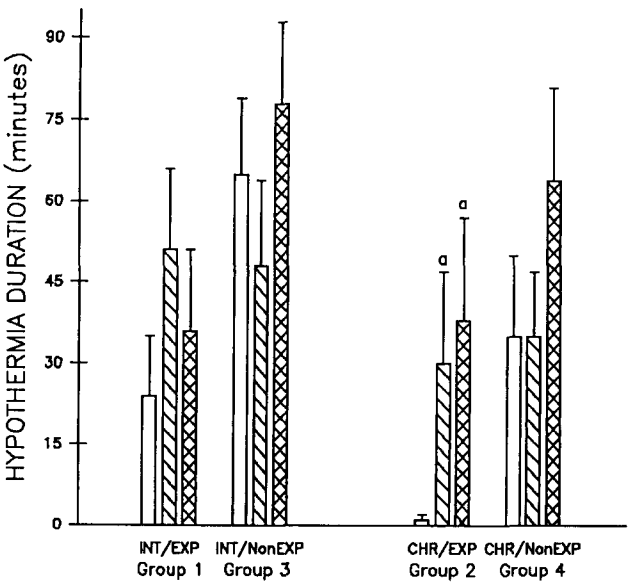

FIG. 4. Duration of ethanol (2.3 g/kg) hypothermia, comparing values of Postchronic (open columns), Postwithdrawal (hatched columns), and Postextinction (cross-hatched columns) tests. The letter a above the bar denotes a significant difference from Postchronic measures.

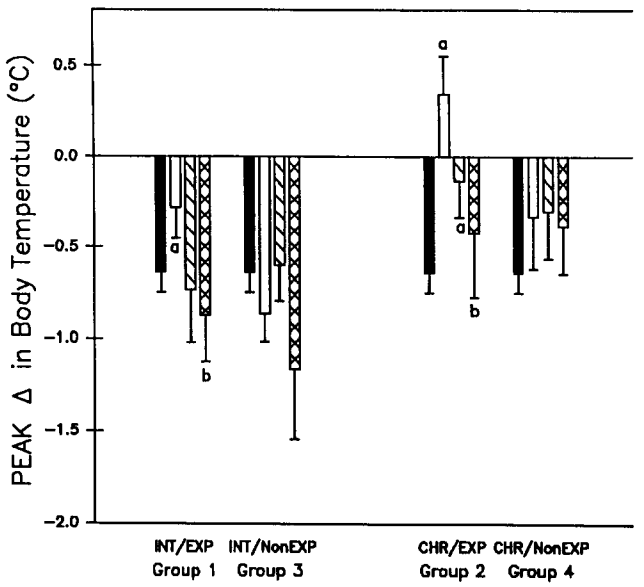

FIG. 5. Ethanol (2.3 g/kg) hypothermia at 50 minutes after injection, comparing Prechronic (solid columns), Postchronic (open columns), Postwithdrawal (hatched line columns), and Postextinction (cross-hatched columns) test results. The letter a above or below the bar denotes a significant difference from Prechronic values. The letter b below the bar denotes a significant difference from Postchronic values.

was less tolerant as to peak and duration after withdrawal; after extinction only the duration continued to be significantly greater than the prechronic test value. Group 4 showed no significant loss of tolerance. These results demonstrate that the withdrawal and extinction procedures were not very effective in causing the loss of behavioral or cellular/metabolic tolerance developed by repeated drug experiences or by chronic drug administration. Nevertheless, Group 2, for which the combined chronic treatment and drug experience should have promoted the greatest total tolerance, did lose a significant portion of the tolerance over the withdrawal period (Figs. 3 and 4).

In examining time-courses of postwithdrawal and postextinction ethanol hypothermia, it appeared that the shape of the effect-duration curves may have changed from that observed at the prechronic and postchronic testing period. To attempt to address this issue we have compared the extent of ethanol hypothermia by 2.3 g/kg ethanol at the prechronic, postchronic, postwithdrawal, and postextinction test periods at 50 min after drug administration (Fig. 5). The time of 50 min was chosen since it marked the point of peak hypothermia to this dose at the prechronic test. Group 1 (INT. EXP.) showed significant tolerance postchronically relative to the prechronic drug effect. After withdrawal this tolerance was lost, although the variability of the postwithdrawal test scores was too large to show a significant difference from postchronic values. However, the postextinction test scores did show a significant loss of tolerance from the postchronic measures. Group 3 (INT. NONEXP.) showed no significant changes across tests, though there was a trend for increased hypothermia at the postextinction test. Group 2 (CHR. EXP.) actually showed a trend for hyperthermia, relative to baseline controls, at the postchronic test. The postchronic value was significantly less than the prechronic level for Group 2 and remained so, though tending to reverse, at the postwithdrawal test. At the postextinction test the tolerance to ethanol effects seen postchronically in Group 2 was lost to a significant degree. Group 4 (CHR. NONEXP.) showed no significant changes across tests, though some trend for a lessened degree of hypothermia was seen on postchronic, postwithdrawal, and postextinction measures relative to prechronic values. The pattern in Fig. 5 may be explained as a development of tolerance relating to repeated drug experiences, whether or not CHR. treatment was involved, and that some cellular/metabolic tolerance had developed at least in Group 2.

The replication of treatments for the 4 groups to correlate ethanol hypothermia with brain and serum concentrations of the drug at the postchronic test (see the Method, and the Blood and Brain Ethanol sections) is shown in Table 2. Since this middle dose of ethanol (2.3 g/kg) almost invariably resulted in higher serum drug levels in CHR.-treated subjects (Groups 2 and 4) compared to INT.-treated rats (Groups 1 and 3) at all times after the test dose, a metabolic tolerance was not observed. Brain levels of ethanol were also almost always higher in the CHR. groups than in the INT. groups. Despite this relationship, the degree of tolerance to hypothermia was considerably greater in Groups 2 and 4 than in Groups 1 and 3. An analysis of covariance of brain concentrations and hypothermic effects of ethanol proved that there was a lesser hypothermia at comparable brain levels, or the establishment of cellular tolerance, in the chronically treated rats (Group 1 vs. Group 2, Group 3 vs. Group 4). There is also a trend, from 30 min onward, for EXP. animals (Groups 1 and 2) to show less hypothermia than comparable NONEXP. subjects (Groups 3 and 4, respectively), which is consistent with the presence of behavioral tolerance.

DISCUSSION

The original concepts of functional tolerance to drug effects stressed the necessity of chronic drug exposure that somehow altered biochemical/physiological mechanisms to be resistant to depressant effects (1, 14, 16, 26, 27). The concept that practice of a behavior decremented by a drug effect may add to the tolerance developed was introduced in the 1960's by several investigators [see (30)], notably Chen (5) with regard to alcohol. Chen suggested that conditioning-type experiences with drug effects represented a learned tolerance that was separable from the classical cellular types of tolerance. However, a series of reports over several decades by Kalant and colleagues (18,22) have advanced a different thesis. These latter investigators presented evidence that behavioral practice only accelerated the process of cellular tolerance due to chronic exposure to ethanol, and that the fundamental underlying mechanisms for the tolerances were essentially identical. Nevertheless, Le et al. (20) extended their studies to indicate that a conditioned tolerance to ethanol hypothermia might be a separable mechanism under some conditions. Other investigators have proposed that all drug tolerance phenomena may be explained as examples of Pavlovian (31) and/or instrumental conditioning (30,33).

The controls usually imposed in an experimental design to assess learned vs. unlearned drug tolerance utilize the administration of drug before the behavioral session (contiguous) or after the measurement of behavior (noncontiguous), each day, or every 3rd day, or every week, etc. (5, 10, 21). Less attention has been given to parameters of chronic drug treatment with protection from behavioral effects to attempt to develop a "pure" cellular tolerance in the absence of learned components (1, 2, 8, 15, 25, 26, 28). Shortcomings of most of these studies have related to an inadequate separation of behavioral training or testing from chronic drug treatments or testing, insufficient chronicity of drug treatment in behaviorally protected subjects (i.e., not experiencing specific or related behavioral effects of the drug), or failure to test for loss of tolerance upon withdrawal of chronic drug treat-

TABLE 2

ETHANOL HYPOTHERMIA AT THE POSTCHRONIC TEST (2.3 g/kg) AND CORRELATED BRAIN AND SERUM ETHANOL CONCENTRATIONS

Group*	N	Body Temp.	(Brain)	(Serum)
		15 Minutes		
1	4	−1.75 ± 0.37†	1.50 ± 0.15‡	2.89 ± 0.16§
2	4	−1.00 ± 0.47	1.79 ± 0.27	3.35 ± 0.40
3	3	−1.67 ± 0.46	1.42 ± 0.23	2.37 ± 0.28
4	4	−0.44 ± 0.36	1.82 ± 0.23	2.94 ± 0.55
		30 Minutes		
1	4	−1.00 ± 0.20	1.43 ± 0.16	2.43 ± 0.38
2	3	−0.58 ± 0.17	1.71 ± 0.44	3.55 ± 0.75
3	4	−1.44 ± 0.26	1.33 ± 0.14	2.16 ± 0.19
4	4	−0.88 ± 0.38	1.31 ± 0.14	2.24 ± 0.14
		60 Minutes		
1	4	−0.88 ± 0.30	1.71 ± 0.10	2.19 ± 0.37
2	2	0.00 ± 0.25	1.59 ± 0.18	2.91 ± 0.30
3	3	−1.25 ± 0.38	1.14 ± 0.16	2.16 ± 0.26
4	3	−0.25 ± 0.50	1.38 ± 0.06	2.55 ± 0.03
		120 Minutes		
1	4	−1.13 ± 0.63	0.86 ± 0.17	1.55 ± 0.35
2	3	−0.75 ± 0.14	1.23 ± 0.19	2.32 ± 0.60
3	3	−2.00 ± 0.38	1.09 ± 0.03	1.79 ± 0.60
4	3	−0.83 ± 0.30	1.20 ± 0.19	1.84 ± 0.26

*Group 1 = INT. EXP, Group 2 = CHR. EXP., Group 3 = INT. NONEXP., and Group 4 = CHR. NONEXP. (see the Method section).

†Change in body temperature relative to baseline control value, °C. ‡Expressed as μg/g of brain tissue. §Expressed as μg/ml.

ment or training for extinction of a presumably conditioned tolerance. Some success has been achieved in protecting ethanol-treated subjects from hypothermia and thus preventing the development of a behavioral tolerance (2,12). We recognize the great difficulty in formulating an experimental design completely separating a "pure" cellular tolerance from an attenuated drug effect related only to a learned adaptation. Nevertheless, we utilized the design presented here to separate as much as possible the presentation of chronic drug exposure without appreciable behavioral experience for the drug effect (Group 4) from the treatment of subjects only with intermittently administered drug under conditions allowing repeated manifestations of the drug effect (Group 1). Table 1 lists the treatments and time periods for the 4 groups, indicating that the same overall treatments were received by all animals, but the full expression of ethanol hypothermia prior to postchronic testing was experienced only by Groups 1 and 2. Note that Groups 3 and 4 never experienced the full extent of drug-induced hypothermia prior to the postchronic test period. Group 2 represented the control calculated to develop both types of tolerance (if there be such), and Group 3 should have developed neither. Furthermore, the later testing for a loss of cellular tolerance upon withdrawal of chronic drug and/or the dissipation of a learned tolerance upon extinction training represented additional criteria for examining the relationships between the two types of tolerance, ones that are seldom included. Figures 1 and 2 demonstrated that ethanol hypothermia in intermittently treated and experienced rats (Group 1) was less than in animals treated intermittently and protected from drug experience (Group 3), at least for the low and middle doses of ethanol. Group 2, treated chronically and experiencing drug effect, did show the greatest overall level of tolerance at the low and middle doses. However, the trend for greater tolerance in Group 2 compared to Group 4 (chronic treatment without drug experience) was not significant, nor could hypothermia in Group 4 be shown to be significantly less than prechronic values at the low and middle doses. These determinations may have reflected a marginal degree of cellular tolerance at the lower test doses that combined with a behavioral tolerance in Group 2. The high test dose of ethanol evoked no indication of tolerance in Group 1 (INT. EXP), which may suggest that the learned tolerance mechanism has a "ceiling effect" as to dose tested. That is, it can only be expressed during the tolerance test at lower doses, higher doses somehow masking the learned adaptation. On the contrary, the high dose demonstrated a clear tolerance in both chronically treated groups (2 and 4), with no differences related to the factor of drug experience. Le et al. (20) have also presented data on ethanol hypothermia indicating that learned tolerance was observed after lower doses but not after higher doses of ethanol. Therefore, data of Figs. 1 and 2 appear to distinguish between the types of cellular and behavioral tolerances on the basis of prior treatment and dose parameters. Behavioral (learned) tolerance was observed without significant evidence of cellular tolerance in Groups 1 and 2 at the low and middle doses of ethanol, while cellular tolerance was observed without significant evidence of behavioral tolerance in Groups 2 and 4 at the high dose of ethanol. As anticipated, intermittent treatment without behavioral experience (Group 3) failed to promote tolerance at any dose. Le et al. (19) indicated that a behaviorally augmented tolerance for ethanol motor impairment did not generalize to hypothermia or sleep-time, and the practiced tolerance to motor decrements faded little over 2 weeks of withdrawal.

Testing for tolerance loss at the postwithdrawal and postextinction periods (Figs. 3 and 4) yielded less of a reduction in tolerance than anticipated when the overall peak and duration of hypothermia were the criteria. There was a significant loss of tolerance in Group 2 (CHR. EXP.) after withdrawal, which may have resulted from some dissipation of cellular tolerance combined with partial extinction of learned tolerance (perhaps by interrupted practice rather than by specific training). It must be noted that the postwithdrawal and postextinction drug tests employed only the middle dose of ethanol, which by results in Figs. 1 and 2 did not reflect a significant cellular tolerance. The postwithdrawal and postextinction testing may have been more informative if we have tested the high dose of ethanol, at which a cellular tolerance was demonstrated during the postchronic test. Le et al. (18) demonstrated rapid loss of tolerance to ethanol hypothermia after 3 days of discontinuing chronic treatment, and these subjects also experienced hypothermia during chronic treatment. Le et al. apparently did not examine withdrawal in subjects exposed to chronic ethanol but prevented from experiencing hypothermia.

Another complication in comparing the pattern of hypothermia by ethanol at the prechronic, postchronic, postwithdrawal, and postextinction tests is that the shape of the effect-duration curve appeared to have changed to alter the time of peak effect. The data in Fig. 5 addressed this issue by comparing ethanol-induced hypothermia for all the tests at the time of peak hypothermia as observed during the prechronic determinations (50 min). These results supported the development of a behavioral tolerance in Group 1 relative to Group 3 and Group 2 relative to Group 4, experience being the dependent variable. The trend for hyperthermia seen in Group 2 at the postchronic test period is likely a reflection of conditioned compensatory hyperthermia, as reported by several other investigators (12, 23, 25). In this analysis extinction did cause a significant loss of the tolerance developed postchronically, in both Groups 1 and 2. The withdrawal procedure did not uncover a loss of cellular tolerance, again probably because only the middle ethanol dose was tested, at which a cellular tolerance was not readily demonstrable.

Repetition of all treatments up to the postchronic test, with groups of rats terminated at various times after ethanol injection to analyze blood and brain drug levels, afforded critical data to this investigation. First, ethanol serum levels of chronically treated rats were higher than comparable intermittently treated subjects, whereas hypothermia was greater in the latter groups. This indicates that a dispositional (metabolic) tolerance was not responsible for the reduced hypothermia in the chronically treated rats. Second, brain levels of ethanol were greater in the chronically treated groups, and an analysis of covariance demonstrated the presence of cellular tolerance in the chronically treated animals. Thus, drug experience being factored out, chronically treated subjects were less hypothermic than intermittently treated rats at the same brain concentrations of ethanol. Therefore, the prominent tolerance observed after the high ethanol dose in Figs. 1 and 2 is not explainable on the basis of a metabolic or learned tolerance, but appears most likely to be an expression of a classical type of cellular tolerance.

In conclusion, the results of this investigation support the premise of Chen (6), Commissaris and Rech (8), Jorgensen et al. (15), Melchior (25), Hjeresen et al. (12) and Holloway et al. (13), that cellular and behavioral tolerances are separate entities involving different causative mechanisms and longevities as related to chronic or nonchronic drug exposure and drug-behavioral experiences. They do not support the thesis of LeBlanc et al. (22) that both tolerances are served by a common mechanism, nor the proposals of Siegel (31), implying that all tolerances to behavioral effects of drugs represent primarily conditioning or learning phenomena.

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