Pharmacology Biochemistry and Behavior, Vol. 44, pp. 717–725, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Cellular and Learned Tolerances to Chlordiazepoxide Hypothermia and Ataxia

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

Department of Pharmacology and Toxicology, B440 Life Sciences, Michigan State University, East Lansing, MI 48824

Received 26 May 1992

MACKENZIE-TAYLOR, D. R. AND R. H. RECH. *Cellular and learned tolerances to chlordiazepoxide hypothermia and ataxia.* PHARMACOL BIOCHEM BEHAV 44(3) 717–725, 1993.—Four groups of rats received chlordiazepoxide (CDP): a) intermittently, experiencing hypothermia and rotarod performance (RR) deficit after test doses (contingency); b) chronically, experiencing hypothermia and RR deficit after test doses; c) intermittently, RR preceding test doses and with protection against hypothermia afforded by exposure to heat lamps (nonexperienced, noncontingency); and d) chronically, RR preceding test doses and with protection against hypothermia. After 36 days of chronic CDP (groups 2 and 4) or vehicle (groups 1 and 3), all groups experienced RR and body temperature (BT) drug deficits after test doses of CDP at the postwithdrawal test. Group 1 but not group 3 was tolerant to peak hypothermia of the drug. Both chronic groups (2 and 4) showed marked tolerance to hypothermia. At the postwithdrawal test, after discontinuing chronic CDP or vehicle for 9 days, only groups 2 and 4 lost drug tolerance to hypothermia. After extinction training (daily testing of RR and BT after injecting vehicle over 9 days), group 2 but not group 4 was again less sensitive to CDP-induced hypothermia at the postextinction test. Regarding CDP-induced RR ataxia, group 1 was more tolerant than group 3 at the postchronic test, while group 4 but not group 2 also showed tolerance to ataxia. At the postwithdrawal test, only group 4 lost tolerance to peak RR ataxic effects of CDP. At the postextinction test, only group 1 lost tolerance for ataxia relative to postchronic test results. These findings differ from those of previous studies with alcohol and pentobarbital that matched learned tolerance [contingent tolerance (CT)] with contingent drug behavior presentation and cellular and/or dispositional tolerances [noncontingent tolerance (NCT)] with chronic drug treatment, but without need of drug-behavior contingency. In the present study, chronic CDP administration, while promoting cellular/dispositional tolerances (NCT), appeared to interfere with development of learned or behavioral tolerance (CT) even when contingent drug behavior training was done (in group 2). That this interference related to chronic CDP is assured because intermittent CDP at 4-day intervals with contingent drug behavior training (group 1) caused prominent CT whereas there was little evidence of CT in rats receiving intermittent CDP but not tested contingently (group 3). Therefore, CT does develop with spaced doses of CDP, and NCT develops dramatically with chronic CDP. But, chronic dosing may somehow nullify development of CT when these subjects (group 2) are also exposed to the contingent training procedure.

Chlordiazepoxide Body temperature Rotarod Learned tolerance Cellular/dispositional tolerance

EARLY concepts of tolerance to drugs depressing the nervous system emphasized the requirement of chronic exposure to induce hepatic metabolizing enzymes, reducing concentration of the drug at the target site (dispositional tolerance), or alterations in molecular components of brain neurons, decreasing sensitivity of nerve cells to the drug effects ["cellular" tolerance; (15,26)]. Only the latter type (pharmacodynamic or cellular tolerance) has been associated with development of physical dependence after chronic treatment with drugs such as ethanol and barbiturates. Dispositional and cellular tolerance will be identified herein as noncontingent tolerance (NCT).

Prominent tolerance to central depressant actions of various substances has been described over the last three decades that did not require chronic drug exposure (3,16,19,23,29). This type of tolerance relates to a conditioning or learned adaptation dependent upon the subject experiencing the drug-induced behavioral deficit (contingency), best demonstrated with multiple trials spaced in time. This learned or behavioral type of tolerance will be referred to as contingent tolerance (CT) in this article, "contingent" referring to simultaneous occurrence of drug effect and behavioral response pattern.

Chen (3) believed that NCT and CT were formed through separate mechanisms and was supported by several other laboratories in this proposal (1,4,14,23). In contrast, others implied that all types of tolerances involve mechanisms of selective classic (Pavlovian) or instrumental conditioning (11,16, 29,33). A third viewpoint considered CT to be a form of augmentation of NCT, enhancing the rate or intensity of NCT development as related to the initial behavioral decrements (18,19). In an effort to resolve these issues, we utilized the

[1] To whom requests for reprints should be addressed.

following experimental design to study ethanol and pentobarbital tolerances (20–22). Some subjects experienced repeated drug behavioral deficits (contingent presentation) with time-spaced drug administrations (intermittent) while others received chronic escalating doses of drug without the opportunity to experience drug-evoked deficits of the measured behaviors (noncontingent presentation). Other animals received intermittent/noncontingent or chronic drug/contingent treatment protocols. Postchronic tests indicated that only drug behavior-experienced (contingently trained) subjects developed CT (learned tolerance), and this tended to be reversed with extinction training. On the other hand, only chronic treatment developed NCT (dispositional/cellular tolerances), which tended to be lost after a period of drug withdrawal. The combination of chronic drug/contingent training protocols resulted in development of both NCT and CT in the same rats, the former being dissipated by withdrawal while the latter was lost after extinction trials.

The experimental design described above was used to examine tolerance aspects of chlordiazepoxide (CDP) in the present study. This drug and other benzodiazepines have been controversial with regard to development of an experience-contingent tolerance (6,7,8,10,12,13), whereas chronic high doses have had variable effects in inducing tolerance with or without evidence of physical dependence phenomena or may even have interfered with tolerance development (8,9,11, 12,24).

METHOD

Subjects

Male Sprague-Dawley rats of consistent genetic stock (Harlan-Sprague-Dawley, Indianapolis, IN) were acquired at 200 ± 25 g body weight and maintained in humidity- and temperature-controlled animal quarters on a 12 L : 12 D cycle (lights on 7:00 a.m.–7:00 p.m.). Food (Lab Blox® or ground chow) and water were available ad lib.

Training

Rectal temperature (BT) was measured as described by MacKenzie-Taylor and Rech (20,21), adapting rats to mild restraint (see below) for inserting the rectal thermosensing probe. Temperatures (°C) were read from a Yellow Springs telethermometer (Model 2100, Yellow Springs Instruments, Yellow Springs, CO) at 10-min intervals.

All subjects were trained on the rotarod (RR) as described previously (22). A cylinder 10 cm wide and 10.5 cm in diameter was evenly rotated at nine revolutions per minute 90 cm above the floor. A rat that slipped off would fall onto a well-padded platform to avoid possibility of injury. The training criterion required the rat to stay on the rotating cylinder for at least 180 s on three consecutive trials. During this training, rats were also adapted to drug–vehicle injections IP, a snug towel-wrap, and monitoring of BT between RR trials while being positioned under heat lamps (20–22). Adjustment of the locus of towel-wrapped subjects under the heat lamps was such as to maintain normal BT. The towel-wrap was designed to hold the rat's position under the heat lamps as well as deny drug-treated animals the experience of attempting to ambulate while intoxicated.

Drug Schedules

Test doses of 15, 30, and 60 mg/kg CDP and part of the chronic drug treatments were administered by IP injection. The remainder of the chronic treatments was provided orally at night in ground laboratory chow with an initial drug concentration of 2 mg/g. Body weights were monitored during chronic drug administration to assure maintenance of 85% of ad lib body weights compared to rats that were on plain ground chow. As tolerance occurred over the 36 days of chronic drug treatment, doses of chronic drug were increased gradually to promote greater NCT as practiced by Okamoto (25). These increases in dosage in the ground chow were implemented only when consumption by the chronic groups (2 and 4) was at least 80% of the amount of control chow eaten by the intermittently treated groups of rats (1 and 3). In fact, on only a few days during chronic treatment was the planned increment in dosage delayed for a day when this criterion was not met.

Treatment and testing Schedules

Treatment and testing schedules were designed as in previous studies with ethanol and pentobarbital (20–22). Four groups of 12 rats each, adapted for BT monitoring and trained on the RR, were randomly assigned to four variants of seven sequential treatment periods, as summarized in Table 1. During period 1 (days 1–6), intermittent drug/contingently trained and chronic drug/contingently trained rats (groups 1 and 2) received IP vehicle daily followed by heat-lamp exposure for 2 h, monitoring BT. Intermittent drug/noncontingently trained and chronic drug/noncontingently trained subjects (groups 3 and 4) also received injections of vehicle during period 1 but were tested thereafter on the RR at 15-min intervals and for BT every 10 min for 2 h while being maintained at room temperature (21°C) and without heat lamps or towel-wrap. During period 2 (days 7–12), all rats received three test doses of CDP IP in random order, each dose injected twice at 3-day intervals. Groups 1 and 2 were tested for RR and BT over 2-h postdrug while unrestrained and at room temperature (without towel-wrap and heat lamps) during this period. However, groups 3 and 4, while receiving the same drug dosing, were protected from hypothermia by towel-wrap and heat-lamp exposure and were tested on RR before the drug injections during period 2 (prechronic IP drug test).

During period 3 (days 13–48), the chronic drug or vehicle treatment period, all groups were subjected to comparable treatments with the exceptions that only groups 2 and 4 were exposed to chronic drug administration and only groups 1 and 2 were allowed to experience hypothermia and RR deficits resulting from the test drug injections. Thus, groups 2 and 4 received chronic drug and groups 1 and 3 received vehicle in their diet and as IP injections during period 3 (Table 1). On every fourth day, animals undergoing contingent training (groups 1 and 2) received 30 mg/kg CDP IP and then were tested for BT and RR over 2 h while unrestrained and at 21°C. Conversely, rats trained noncontingently (groups 3 and 4) were towel-wrapped and placed under heat lamps after the test drug injection every fourth day for 2 h, BT being maintained in the normal range; these animals were tested on RR only after vehicle injection and not during the time course of the test drug effect. Based upon results of previous studies (20–22), it was anticipated that groups 1 and 2 would develop a learned tolerance (CT) and that groups 2 and 4 would develop dispositional/cellular (NCT) types of tolerance.

The postchronic drug tolerance test (period 4, Table 1) took place on days 49–51, during which maintenance treatments of period 3 were continued. All groups received the three test doses of drug in random order over the 3 days,

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/CE (1)	TW,† HL,† BT† monitored for 2 h	Measure BT and RR† effects for 2 h	IP vehicle and TW daily; test Ip drug every fourth day on BT and RR	Test 3 IP drug doses on BT and RR	Stop chronic vehicle; test IP drug on BT and RR on day 61	Daily vehicle, test BT and RR	Test IP drug on BT and RR
CHR/CE (2)	Same as group 1	Same as group 1	Drug in diet; IP drug and TW, 3 days; IP vehicle and TW every fourth day, then test IP drug on BT and RR	Same as group 1	Stop chronic drug; test IP drug on BT and RR on day 61	Same as group 1	Same as group 1
INT/NCE (3)	Measure BT and RR effects for 2 h	TW, HL, BL monitored for 2 h	IP vehicle and TW, 3 days; IP on BT and RR every fourth day, then IP drug with TW and BT monitored for 2 h	Same as group 1	Same as group 1	Same as group 1	Same as group 1
CHR/NCE (4)	Same as group 3	Same as group 3	Drug in diet; IP drug and TW daily; IP vehicle on BT every fourth day	Same as group 1	Same as Group 2	Same as group 1	Same as group 1

*INT, intermittent drug treatment; CE, repeated experience (contingency training) with drug effect on BT and RR; CHR, chronic drug treatment; NCE, protected from experiencing drug effects (noncontingently trained) on BT and RR; see the Method section for details.

†TW, restraint with body towel-wrap; HL, maintenance of normal BT by placing TW animals in the vicinity of heat lamps; BT, body temperature measurement; RR, rotarod performance.

following which BT and RR were assessed in unrestrained animals at 21°C. During the next 9 days (52–61), all chronic maintenance and test dosing of CDP were discontinued and all subjects remained in their home cages through day 60 (withdrawal period). On day 61, the intermediate test dose (30 mg/kg) of drug was injected into all rats, followed by evaluation of BT and RR without towel-wrap or heat-lamp exposure for the postwithdrawal test. During period 6 (days 62–71), all rats were subjected to extinction training by injecting vehicle daily with subsequent BT and RR determinations while subjects were unrestrained and at 21°C. On day 72, the postextinction test consisted of injecting 30 mg/kg CDP and then determining effects on BT and RR in all groups maintained unrestrained and at 21°C.

Statistics

BT measurements were analyzed by factorial and repeated-measures analysis of variance (ANOVA) across the four groups for each test period. Factorial and repeated-measures ANOVA were used to analyze duration of drug impairment of BT (time to last significant measure of hypothermia) and of RR (time to 50%, 90 s or more, recovery of ability to remain on the rotating cylinder). The nonparametric Kruskal–Wallis and Mann–Whitney *U*-tests were employed in analysis of RR time course and peak effects, respectively, due to presence of nonhomogeneous variances. Individual differences were evaluated by Tukey's test (2). Statistical significance was set at $p < 0.05$.

RESULTS

Hypothermia

Peak changes in BT after the three test doses of CDP (15–45 min postdrug) are compared for the four groups of rats for the prechronic and postchronic tests in Fig. 1. A dose-related increase in hypothermia was observed during the prechronic test. During the postchronic test, group 1 rats (intermittent drug/contingent training) showed less hypothermia to the 15- and 60-mg/kg doses as compared to the prechronic test results. Group 3 (intermittent drug/noncontingent training) did not differ significantly in hypothermic effects for all three drug doses at the postchronic test. Both group 2 (chronic drug/contingent training) and group 4 (chronic drug/noncontingent training) demonstrated marked tolerance to all three doses of CDP at the postchronic test and, in fact, showed trends for a hyperthermia.

The durations of hypothermia to prechronic test and postchronic test drug effects are illustrated in Fig. 2. A dose-related increase in hypothermia was seen during the prechronic test. In the postchronic test, group 1 subjects showed a trend for a lessened effect for all three doses of CDP, but this was significant only for the 60-mg/kg dose. Group 3 animals also showed this trend but without significant differences between prechronic test and postchronic test results for any dose. Groups 2 and 4 were markedly tolerant with brief durations of hypothermia from all three doses at the postchronic test, in agreement with the peak drug effects (Fig. 1).

After withdrawal of chronic drug or vehicle, BT effects of the 30-mg/kg test dose of CDP were determined during the postwithdrawal test (day 61, Table 1). All subjects were then exposed to extinction training and administered 30 mg/kg CDP at the postextinction test (day 71). Comparison of peak drug and duration effects on BT at the postchronic, postwithdrawal, and postextinction tests is seen in Table 2. Groups 1 and 3 showed no significant differences over these three test periods. Group 2 lost tolerance at the postwithdrawal test compared to the postchronic test values, but again showed

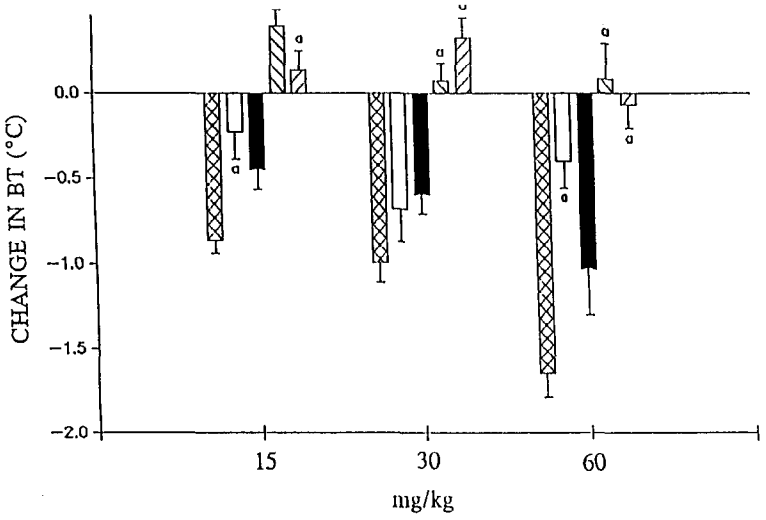

FIG. 1. Peak hypothermia to chlordiazepoxide during prechronic and postchronic tests. Cross-hatched bars, prechronic values; open bars, postchronic values in group 1 rats; solid bars, postchronic values in group 3 rats; right-hatched bars, postchronic values in group 2 rats; left-hatched bars, postchronic values in group 4 rats. Only groups 1 and 2 received contingency training and only groups 2 and 4 received chronic chlordiazepoxide. Body temperature measures are relative to baseline controls (±SEM). Test doses of drug = 15, 30, and 60 mg/kg IP. The letter *a* above or below a bar denotes postchronic measures that are significantly different ($p < 0.05$) from prechronic controls. See the Method section and Table 1 for details of treatments.

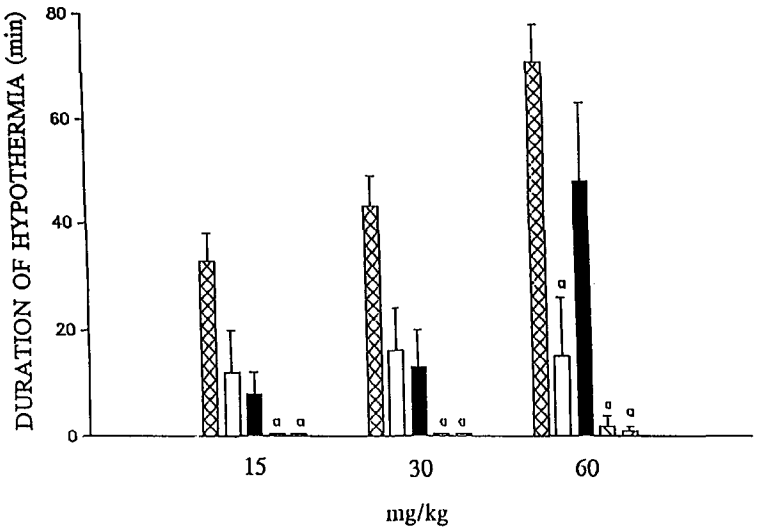

FIG. 2. Duration of chlordiazepoxide hypothermia during prechronic and postchronic tests. See Fig. 1 legend for further details.

less hypothermia to 30 mg/kg CDP during the postextinction test. This latter determination (−0.26°C) was also a significantly smaller drop in BT than that caused by 30 mg/kg CDP during the prechronic test (−1.0°C, Fig. 1). Group 4 rats also lost a significant degree of tolerance to peak hypothermia from 30 mg/kg CDP at the postwithdrawal test. However, in group 4 the fall in BT at the postextinction test, while showing a trend for a lesser effect, remained significantly different from the value of the postchronic test and did not differ from the postwithdrawal test BT reading.

Ataxia

Prechronic and postchronic test scores for peak ataxia after 15, 30, or 60 mg/kg CDP are depicted in Fig. 3. Although not shown, RR scores were not altered from control values following injection of vehicle. Prechronic values indicated a dose-related decrement in RR. Postchronic test scores showed that groups 1 and 3 developed tolerance for the 15-mg/kg dose, but only group 1 was also tolerant to the 30- and 60-mg/kg doses. Group 2 showed a nonsignificant trend for tolerance at the postchronic test for peak ataxia, while group 4 showed significant tolerance at this test for the 30- and 60-mg/kg doses, relating to the prechronic test scores.

The durations (time to recover to 50% of control RR, or 90 s) of CDP disruption of RR during the prechronic and postchronic tests are illustrated in Fig. 4. Prechronic scores indicated a dose-related increase in duration of impaired RR by the initial CDP test doses. During the postchronic test, group 1 subjects were tolerant to all three test doses of drug whereas group 3 animals were not. Groups 2 and 4 failed to show significant tolerance for RR duration effects at the postchronic test, with the exception of the 60-mg/kg dose of CDP in group 4.

Table 3 compares the RR scores from postchronic, postwithdrawal, and postextinction tests relating to the effects of

TABLE 2

COMPARISON OF POSTCHRONIC, POSTWITHDRAWAL, AND POSTEXTINCTION TEST RESULTS FOR CHLORDIAZEPOXIDE EFFECTS ON BODY TEMPERATURE

	Groups			
Drug Test	INT/CE (group 1)	INT/NCE (group3)	CHR/CE (group2)	CHR/NCE (group 4)
Peak BT effects (°C ± SEM)				
Postchronic	−0.69 (± 0.16)	−0.55 (±0.12)	+0.14 (± 0.14)	+0.32 (±0.11)
Postwithdrawal	−0.38 (± 0.18)	−0.51 (±0.14)	−0.52 (± 0.19)*	−0.44 (±0.18)*
Postextinction	−0.68 (± 0.14)	−0.59 (±0.31)	−0.26 (± 0.18)	−0.35 (±0.15)
Duration of BT effects (min ± SEM)				
Postchronic	15.7 (± 7.9)	12.9 (±6.8)	0.4 (± 0.1)	0.4 (±0.2)
Postwithdrawal	17.9 (±11.1)	12.1 (±7.9)	14.6 (± 5.7)*	24.6 (±8.9)
Postextinction	19.6 (± 8.9)	10.0 (±7.9)	16.8 (±11.1)	12.8 (±6.8)

See Table 1 for keys to abbreviations.
*Significantly different from postchronic value, $p < 0.05$. Test dose of drug = 30 mg/kg IP.

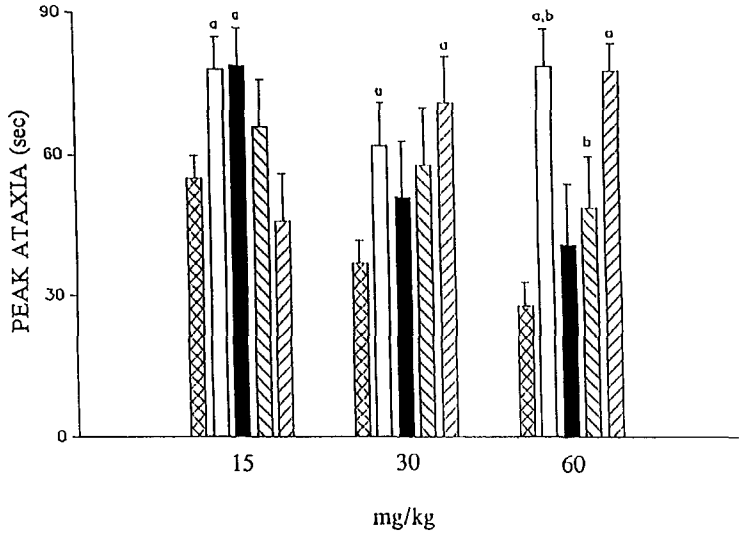

FIG. 3. Peak rotarod impairment by chlordiazepoxide during prechronic and postchronic tests. Peak ataxia, mean time (seconds ± SEM) rats remained on the rotating cylinder. The letter *a* above a bar denotes postchronic values that are significantly different ($p < 0.05$) from prechronic controls. The letter *b* above a bar denotes contingently trained rats that are significantly different from the matched intermittent or chronic drug group without contingency training. See Fig. 1 legend for further details.

30 mg/kg CDP. For peak effects, only group 4 showed a loss of tolerance comparing the postchronic test and postwithdrawal test scores; only group 1 lost tolerance when comparing postwithdrawal test and postextinction test scores. Regarding duration of RR deficits caused by CDP, only group 3 showed significant changes: At the postwithdrawal test, the RR score was prominently decreased from the postchronic test value, but at the postextinction test RR sensitivity to CDP once more increased to be significantly greater than at the postwithdrawal test score.

DISCUSSION

Hypothermia induced by CDP was dose related for both peak effects and duration. Earlier studies with ethanol, barbiturates, and benzodiazepines also demonstrated dose-related

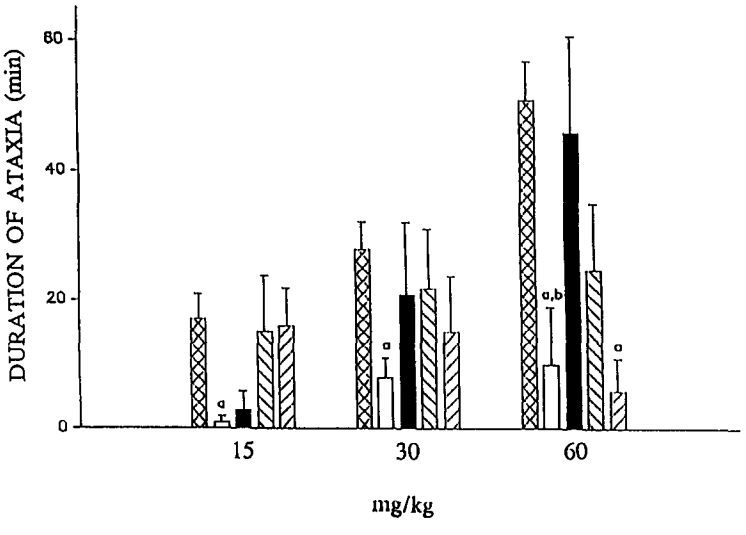

FIG. 4. Duration of rotarod ataxia (time to 50% recovery, min ± SEM) by chlordiazepoxide during prechronic and postchronic tests. See Fig. 1 and 3 legends for further details.

TABLE 3

COMPARISON OF POSTCHRONIC, POSTWITHDRAWAL, AND POSTEXTINCTION TEST RESULTS FOR CHLORDIAZEPOXIDE EFFECTS ON ROTAROD PERFORMANCE

	Groups			
Drug Test	INT/CE (group 1)	INT/NCE (group3)	CHR/CE (group2)	CHR/NCE (group 4)
Peak RR effects (seconds ± SEM)				
Postchronic	62.7 (±8.7)	51.4 (±13.0)	56.2 (±10.5)	77.8 (± 5.7)
Postwithdrawal	77.8 (±8.1)	63.2 (±11.4)	66.1 (±11.4)	42.2 (± 9.7)*
Postextinction	55.1 (±8.9)†	50.9 (±11.6)	55 (±11.3)	51.9 (±10.5)
Duration of RR effects (min ± SEM)				
Postchronic	7 (±3.4)	21.5 (±11.1)	23.3 (± 9.6)	16.1 (±10.2)
Postwithdrawal	5 (±3.3)	8.3 (± 3.8)*	21.1 (±11.4)	20.0 (± 7.8)
Postextinction	15 (±5.0)	27.2 (±11.7)†	26.1 (±12.2)	20.0 (±10.0)

See Table 1 for keys to abbreviations.
*Significantly different from Postchronic value, $p < 0.05$. Test dose of drug = 30 mg/kg IP.
†Significantly different from postwithdrawal value, $p < 0.05$.

hypothermia (5,13,17,20–22). Group 1 rats, with time-spaced contingent experiences of CDP hypothermia, showed CT (learned, or behavioral, tolerance). This was best illustrated by the difference in hypothermia between groups 1 and 3 at the 60-mg/kg dose (Figs. 1 and 2). This agrees with our findings for ethanol (20) and pentobarbital (21,22), where contingency training with spaced dosing also induced CT. However, in those studies the lowest test dose afforded the clearest evidence of CT, as was also true for ethanol in studies by Le et al. (17) and Tabakoff et al. (31). Greeley and Cappell (12) claimed to establish CT for CDP hypothermia but did not explore dose-response parameters. On the other hand, Griffiths and Goudie (13) established a dose-related hypothermia to a benzodiazepine but failed to develop a CT. Griffiths and Goudie criticized the conclusion of Greeley and Cappell in that intrinsic stimulus factors unrelated to tolerance phenomena may have led to a faulty interpretation. Nonetheless, our results show clearly that contingency training with a benzodiazepine can develop CT.

Chronic drug treatment in the present study (groups 2 and 4) resulted in a marked tolerance (assumed to be primarily NCT, cellular/dispositional types) to hypothermia at all doses, so that any expression of CT in group 2 would have been obscured. Actually, a trend for hyperthermic responses was noted in these groups (Fig. 1). Trends for hyperthermia after chronic ethanol (20) and pentobarbital (21) were also noted, and a compensatory hyperthermia to chronic ethanol has been reported by others (12,14,23).

Upon withdrawal of chronic drug or vehicle, only the chronic drug groups lost tolerance to hypothermia at the postwithdrawal test (Table 2), indicating that only these subjects had developed significant NCT. After extinction training, 30 mg/kg CDP induced a lower level of peak hypothermia in group 2 at the postextinction test but not in group 4. This is paradoxical because any CT present in group 2 should have dissipated and resulted in a greater fall in BT. Therefore, the duration or quality of extinction training may have been insufficient, a proposal that gains credence by the failure of group 1 to lose significant tolerance at the postextinction test. Further, the different patterns of peak BT changes for the tests in Table 2 for groups 2 and 4 may be explained by the presence of an occult CT in group 2 that was masked by chronic drug treatment. After sufficient withdrawal and testing at the postextinction test, this CT may have finally been expressed.

In our earlier studies of ethanol and pentobarbital hypothermia (20,21), group 2 did not lose significant tolerance at the postwithdrawal test while group 4 did show a loss (presumably, loss of NCT). In those studies, the presence of CT in group 2 appeared to mask, as a redundant influence, the loss of NCT at the postwithdrawal test. This was affirmed by significant loss of tolerance in group 2 to ethanol or pentobarbital test doses at the postextinction test, but without further loss of tolerance at this test in group 4. Differences between patterns of tolerance to CDP hypothermia and those seen previously with ethanol and pentobarbital suggest that benzodiazepines act differently due to peculiar aspects of their psychopharmacology or perhaps due to formation of long half-life active metabolites [(28); see also below].

The groups receiving intermittent CDP (1 and 3) were not expected to lose significant tolerance for hypothermia at the postwithdrawal test, as was the case (Table 2). Group 1 (contingently trained) should have lost any CT that had developed during extinction, but there was no significant loss of tolerance for CDP hypothermia in this group at the postextinction test, as indicated above. However, the CT expressed by group 1 (but not group 2) at the postchronic test occurred with the 15- and 60-mg/kg test doses of CDP, whereas the postwithdrawal and postextinction tests utilized the 30-mg/kg test dose. If 15 or 60 mg/kg rather than 30 mg/kg had been injected before the postextinction test, a loss of CT might have been observed in group 1. Another complication of this experimental design was the treatment of group 1 with vehicle injections during the chronic treatment period (Table 1, days 13–48), which may have introduced a partial reinforcement influence that would increase resistance to extinction.

Acute ataxia (RR) effects of CDP (prechronic test, Figs. 3 and 4) were dose related for both peak and duration, as true for the previous study of pentobarbital ataxia (22). At the postchronic test, group 1 showed CT (compared to drug effects in group 3). Surprisingly, group 2 showed no significant tolerance to RR impairment by CDP at the postchronic test, although group 4 did show tolerance at this test. Hypothetically, group 2 should have demonstrated the greater tolerance, with CT relating to contingency training and NCT from chronic dosing. Therefore, contingency training in group 2 for RR influences appeared not to promote CT, as well as to interfere with expression of NCT that was anticipated for this group. In the earlier pentobarbital study (22), the towel-wrap

appeared to complicate experimental influences for RR, that is, a "learned helplessness" (27) seemed to nullify development or expression of CT in group 1 subjects to pentobarbital test doses and actually enhance RR disruption to pentobarbital in group 3 at the postchronic test in this previous study. Nevertheless, groups 2 and 4 still expressed significant tolerance to pentobarbital impairment of RR, without a significant difference between them. Therefore, the artifact associated with towel-wrap cannot fully explain the unexpected RR results with CDP in group 2 in the present study.

Upon withdrawal of chronic vehicle, group 1 showed no change for peak ataxia to CDP, comparing postwithdrawal test scores to postchronic test results. However, group 1 lost tolerance at the postextinction test, as the CT that this group had developed at the postchronic test (Fig. 3) was attenuated by extinction training. Moreover, group 3, which was not contingently trained and showed little evidence of CT at the postchronic test, was not significantly different in peak RR scores going from postwithdrawal to postextinction test scores (Table 3). Peak ataxia in group 2 did not change significantly going from the postchronic test to the postwithdrawal test and then to the postextinction test, in accord with the lack of expression of tolerance in Fig. 3. Group 4 had shown tolerance (presumably NCT) at the postchronic test to peak ataxia and was significantly more ataxic for peak effects at the postwithdrawal test (loss of NCT), while showing no further loss of tolerance at the postextinction test (i.e., no CT was present as these subjects had not been contingently trained). Scores for the duration of RR effects of CDP at the postwithdrawal and postextinction tests (Table 3) did not coincide with anticipated results. This was in particular true for group 3, which lost "tolerance" at the postwithdrawal test (although these rats were not tolerant at the postchronic test) and then showed again a greater duration of RR effects at the postextinction test.

Other behaviors that have been examined in rats for development of CT with CDP treatment include general activity levels. File (7) showed that acquisition of tolerance was not related to contingency training but retention of tolerance was. Greeley and Cappell (12), failing to demonstrate CT for CDP hypnosis, claimed to show CT to a less prominent CNS depression ("inactivity time"). Cook and Sepinwall (6) indicated a CT for sedation of CDP in a conflict-punishment procedure ("initial treatment phenomenon"). Mokler and Rech (24) described an initial treatment phenomenon for diazepam in another type of punishment paradigm in which some elements appeared to involve CT while others were consistent with NCT. Triet (32) found NCT for the anxiolytic effects of diazepam that developed after 10 days of 1 mg/kg daily dosing. Therefore, it appears that NCT to benzodiazepines can develop rapidly even with minimal chronicity. Failure to promote CT to benzodiazepines under some conditions has been attributed to insufficient discriminating cues or "overshadowing" or masking influences of the environments or treatment protocols (12,13). Nevertheless, CT has been described for many behavioral effects of other CNS depressant drugs with reasonable facility, suggesting that CT aspects of the benzodiazepines are more complex than for other drug classes. Perhaps amnestic properties of chronic benzodiazepines are involved (28), or rapid development of NCT may reduce the impetus to develop CT.

In summary, results from both BT and RR measures showed that: a) spaced dosing of rats with CDP administered contingently to affect behaviors resulted in the development of CT, whereas spaced dosing noncontingently in other animals showed little tendency to promote CT; b) chronic CDP, with or without contingency training, was effective in developing NCT, excepting the RR measure in chronic drug, contingently trained subjects (group 2); c) contingency training in subjects treated chronically with CDP did not develop a clear-cut CT. Points a) and b) agree with previous conclusions from studies of the same design with ethanol (20) and pentobarbital (21,22). But the CDP data relating to point c) differs from that of the previous studies and suggests something unique about the psychopharmacology of chronic CDP.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge support of this study by NIDA Grant DA03822, technical assistance of C. Casey, and the manuscript preparation by M. Vanderlip.

REFERENCES

1. Bird, D. C.; Holloway, F. A.; Carney, J. M. Schedule-controlled behavior as an index of the development and loss of ethanol tolerance in the rat. Psychopharmacology (Berl.) 87:414–420; 1985.
2. Bruning, J. L.; Kintz, B. L. Computational handbook of statistics: A handbook. 3rd ed. Glenview, IL: Scott, Foresman & Co.; 1987.
3. Chen, C. S. A further note on studies of acquired behavioral tolerance to alcohol. Psychopharmacologia 27:265–274; 1972.
4. Commissaris, R. L.; Rech, R. H. tolerance to pentobarbital and ethanol following chronic pentobarbital administration in the rat. Subst. Alcohol Actions/Misuse 2:331–339; 1981.
5. Commissaris, R. L.; Semeyn, D. R.; Rech, R. H. Dispositional without functional tolerance to the hypothermic effects of pentobarbital in the rat. J. Pharmacol. Exp. Ther. 220:536–539; 1982.
6. Cook, L.; Sepinwall, J. Behavioral analysis of the effects and mechanisms of action of benzodiazepines. In: Mechanisms of action of benzodiazepines. Costa, E.; Greengard, P., eds. New York: Raven press; 1975;1–28.
7. File, S. E. Development and retention of tolerance to the sedative effect of chlordiazepoxide: Role of apparatus cues. Eur. J. Pharmacol. 816:637–643; 1982.
8. File, S. E.; Mabbutt, P. S.; Hitchcott, P. K. Characterisation of the phenomenon of "one-trail tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology (Berl.) 102:98–101; 1990.
9. Galpern, W. R.; Lumpkin, M.; Greenblatt, D. J.; Shader, R. I.; Miller, L. G. chronic benzodiazepine administration VII. Behavioral tolerance and withdrawal and receptor alterations associated with clonazepam administration. Psychopharmacology (Berl.) 104:225–230; 1991.
10. Garcia, J.; Carson, V., Tewari, S. Impairment of memory consolidation and inhibition of protein synthesis following lorazepam treatment. Pharmacologist 33:167; 1991.
11. Goudie, A. J. Behavioral influences on benzodiazepine tolerance: When do they occur and what do they mean? Psychopharmacology (Berl.) 95:546–549; 1988.
12. Greeley, J.; Cappell, A. Associative control of tolerance to the sedative and hypothermic effects of chlordiazepoxide. Psychopharmacology (Berl.) 86:487–493; 1985.
13. Griffiths, J. W.; Goudie, A. J. Analysis of the role of drug-predictive environmental stimuli in tolerance to the hypothermic effects of the benzodiazepine midazolam. Psychopharmacology (Berl.) 90:513–521; 1986.
14. Hjeresen, D. L.; Reed, D. R.; Woods, S. C. tolerance to the

hypothermia induced by ethanol depends on specific drug effects. Psychopharmacology (Berl.) 89:45–51; 1986.
15. Kalant, H.; LeBlanc, A. E.; Gibbons, R. J. tolerance to, and dependence on, some nonopiate psychotropic drugs. Pharmacol. Rev. 23:135–191; 1971.
16. Krasnegor, N. A., ed. Behavioral tolerance: Research and treatment implications. NIDA Research Monograph 18. Washington, DC: U.S. Government Printing Office; 1978.
17. Le, A. D.; Khanna, J. M.; Kalant, H. Role of Pavlovian conditioning in the development of tolerance and cross-tolerance to the hypothermic effect of ethanol and hydralazine. Psychopharmacology (Berl.) 92:210–214; 1987.
18. LeBlanc, A. E.; Gibbins, R. J.; Kalant, H. Behavioral augmentation of tolerance to ethanol in rat. Psychopharmacologia 30: 117–122; 1973.
19. LeBlanc, A. E.; Gibbins, R. J.; Kalant, H. Generalization of behaviorally augmented tolerance to ethanol and its relation to physical dependence. Psychopharmacologia 44:241–246; 1975.
20. MacKenzie-Taylor, D. R.; Rech, R. H. Cellular and learned tolerances for ethanol hypothermia. Pharmacol. Biochem. Behav. 38: 29–36; 1991.
21. MacKenzie-Taylor, D. R.; Rech, R. H. Cellular and learned tolerance for pentobarbital hypothermia. Pharmacol. Biochem. Behav. 39:249–256; 1991.
22. MacKenzie-Taylor, D. R.; Rech, R. H. Cellular and learned tolerances to pentobarbital ataxia. Pharmacol. Biochem. Behav. 39: 257–264; 1991.
23. Mansfield, J. G.; Cunningham, C. L. Conditioning and extinction of tolerance to ethanol in rats. J. Comp. Physiol. Psychol. 94:962–969; 1980.
24. Mokler, D. J.; Rech, R. H. Mechanisms of the initial treatment phenomenon to diazepam in the rat. Psychopharmacology (Berl.) 87:242–246; 1985.
25. Okamoto, M. Barbiturates and alcohol: Comparative overviews on neurophysiology and neurochemistry. In: Lipton, M. A.; DiMascio, A.; Killam, K. F., eds. Psychopharmacology: A generation of progress. New York: Raven press; 1978:1575–1590.
26. Okamoto, M.; Rosenberg, H. C.; Boisse, N. R. tolerance characteristics produced during the maximally tolerable chronic pentobarbital dosing in the cat. J. Pharmacol. Exp. Ther. 192:555–569; 1975.
27. Porsolt, R. D.; Ahton, G.; Blavet, N.; Jalfre, M. Behavioral despair in rats: A new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47:379–391; 1978.
28. Rall, T. W. Hypnotics and sedatives; ethanol. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. Goodman and Gilman's the pharmacological basis of therapeutics. 8th ed. New York: Pergamon press; 1990:345–382.
29. Siegel, S. Evidence from rats that morphine tolerance is a learned response. J. Comp. Physiol. Psychol. 89:498–506; 1975.
30. Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. Science 193:323–325; 1976.
31. Tabakoff, B.; Melchoir, C. L.; Hoffman, P. Factors in ethanol tolerance. Science 224:523–524; 1984.
32. Treit, D. Evidence that tolerance develops to the anxiolytic effect of diazepam in rats. Pharmacol. Biochem. Behav. 22:383–387; 1985.
33. Wenger, J. R.; Tiffany, T. M.; Bombardier, C.; Nicholis, K.; Woods, S. C. Ethanol tolerance in the rat is learned. Science 213: 575–577; 1981.