Pharmacology Biochemistry & Behavior Vol 38, pp 433–439 © Pergamon Press plc, 1991 Printed in the U S A 0091-3057/91 $3 00 + 00

The Ability of Chlordiazepoxide to Maintain Ethanol Tolerance and Dependence[1]

ARTHUR W. K. CHAN,[2] FLORENCE W. LEONG, MICHELE C. LANGAN, DONNA L. SCHANLEY AND MARIA L. PENETRANTE

Research Institute on Alcoholism, New York State Division of Alcoholism and Alcohol Abuse
1021 Main Street, Buffalo, NY 14203

Received 29 May 1990

CHAN, A W K , F W LEONG, M C LANGAN, D L SCHANLEY AND M L PENETRANTE *The ability of chlordiazepoxide to maintain ethanol tolerance and dependence* PHARMACOL BIOCHEM BEHAV **38**(2) 433–439, 1991 —Two criteria need to be satisfied in the demonstration of cross-dependence to chlordiazepoxide (CDP) in ethanol-dependent mice These are the ability of CDP to suppress ethanol withdrawal and to maintain the dependent state In this study, mice which had been fed chronically an ethanol diet followed by two days of CDP diet treatment had more severe CDP withdrawal signs induced by Ro15-1788 than drug-naive mice which were similarly exposed to the CDP diet treatment The data indicate that CDP can maintain the dependent state acquired from the prior ethanol treatment Alternatively, the prior ethanol treatment would have facilitated the development of CDP dependence, but it was not deemed likely Three behavioral tests, namely, runway, sleep time, and drug-induced hypothermia, were used to test whether CDP could maintain the ethanol tolerance acquired from the prior ethanol treatment The runway test showed that CDP could maintain the previously acquired ethanol tolerance. However, interpretations of the data from the sleep time and hypothermia tests are more equivocal because of factors such as peak tolerance, differential rates of development and dissipation of ethanol (or CDP) tolerance as determined by different behavioral tests

Ethanol Chlordiazepoxide Tolerance Dependence Withdrawal signs Cross-tolerance Cross-dependence Ro15-1788

THERE is a higher prevalence of benzodiazepine (BZD) use among alcoholics than in the general population (5, 6, 17). Although some research data suggest that the liability for BZD abuse may also be greater for alcoholics (2, 5, 6, 17), substantial methodologic problems associated with these investigations preclude such a conclusion (17). Among the possible reasons for polydrug use, an important one is that a particular drug may serve as a substitute for preferred drugs that are not available (18). The process of cross-dependence probably facilitates the substitution of one drug for another. Cross-dependence is defined as "the ability of one drug to suppress the manifestations of physical dependence produced by another and to maintain the physically dependent state" (23). Because of their effectiveness in the treatment of alcohol withdrawal and the similarity of their pharmacological properties to those of ethanol, BZD such as diazepam (DZP) and chlordiazepoxide (CDP) have often been considered as giving rise to cross-dependence with ethanol (23, 27, 31). However, evidence is lacking to support the contention that BZD can maintain the ethanol-dependent state. Besides, BZD can suppress withdrawal signs by virtue of their sedative, anticonvulsant and antianxiety properties rather than their being fully substitutable for ethanol. Anecdotal accounts of alcoholics using BZD and alcohol interchangeably (30) suggest, but do not prove, that BZD can be substituted for ethanol and vice versa.

To demonstrate cross-dependence on CDP in ethanol-dependent mice, we have previously used a bidirectional experiment in which ethanol dependence was first induced in mice by chronic treatment with an ethanol diet, and upon ethanol withdrawal, the mice were fed a liquid diet containing CDP (7,8). The substitution of CDP for ethanol fully suppressed ethanol withdrawal signs, but due to the long duration of the CDP diet treatment, both the mice treated with ethanol/CDP (first diet/second diet) and those with control/CDP showed comparable withdrawal signs following CDP withdrawal. Therefore, these data do not prove conclusively that CDP can maintain the ethanol-dependent state In the present study, we have deliberately kept the period of CDP diet treatment short The rationale is that if CDP is truly substitutable for ethanol, it should be able to maintain the dependent state acquired from the prior ethanol treatment, and CDP withdrawal signs should be manifested upon CDP withdrawal even though not much CDP dependence would have developed during the short CDP treatment in the control/CDP mice. Another objective of this study is to test the hypothesis that CDP can maintain the tolerance to ethanol and cross-tolerance to CDP developed as a result of the prior ethanol diet treatment.

[1]Supported in part by PHS grant No. AA06016 Presented in part at the Annual Meeting of the Research Society on Alcoholism, June 10–15, 1989, Beaver Creek, CO

[2]Requests for reprints should be addressed to Arthur W K Chan, Ph D , Research Institute on Alcoholism, 1021 Main Street, Buffalo, NY 14203

TABLE 1
DIET ADMINISTRATION SCHEME

Group	First Diet	Second Diet
(A) Ethanol/CDP	Ethanol diet ad lib	3 CDP diets free choice and ad lib
(B) Ethanol/Control	Ethanol diet ad lib	Control diet pair-fed with (A)
(C) Control/CDP	Control diet pair-fed with (A)	3 CDP diets free choice and ad lib
(D) Control/Control	Control diet pair-fed with (A)	Control diet pair-fed with (A)

METHOD

Animals

Male C57BL/6J mice (8 weeks old) were purchased from Jackson Laboratories, Bar Harbor, ME. They were housed singly in plastic cages in a controlled-environment room (21–22°C) on an 11/13-h light/dark cycle and received Teklad mouse diet (Teklad Mills, Winfield, IA) and tap water ad lib for 10–14 days before the beginning of an experiment.

Materials

CDP-hydrochloride and Ro15-1788 were gifts from Hoffmann-La Roche, Inc. (Nutley, NJ). Chocolate-flavored Sustacal liquid diet was purchased from Mead Johnson Nutritional Division (Evansville, IN). Ninety-five percent ethanol, USP, was from Aaper Chemical Co. (Shellbyville, KY) and vitamin diet fortification mixture was from Nutritional Biochemicals (Cleveland, OH).

Ethanol Diet Treatment

Procedures for the preparation and administration of the ethanol diet were similar to those described previously (8,12) except that the highest ethanol concentration was 7% (v/v) instead of 8%, and the durations of administration of the 3 5% and 5% diets were 4 days and 5 days, respectively, instead of 6 days and 3 days, respectively. The durations of administration of the 6.5% and 7% diets were each 3 days, making the total diet period 15 days, as reported previously. We found that these modifications helped reduce mortalities during ethanol withdrawal, but did not affect the development of tolerance to ethanol. Control mice were pair-fed an isocaloric diet (control diet) containing sucrose as a caloric substitute for ethanol. Because of the need to have subgroups of mice (N = 10 to 13 for each) for subsequent diet experiments and/or behavioral tests (see below), large numbers of mice were initially treated with either the ethanol or the control diet.

CDP Diet Administration

On day 16 of ethanol diet administration, the ethanol diet was withdrawn from the ethanol-dependent mice. Some of these mice (the ethanol/CDP group) were then given a choice of three diets containing different concentrations of CDP, namely, 0.6, 0.8 and 1.2 mg/ml, respectively. These concentrations were chosen as a result of pilot experiments which indicated that mice consumed these diets throughout the day, and that the intake of CDP was sufficient to suppress ethanol withdrawal reactions (7,8) The protocols for preparation of the CDP diets have been described previously (13) The three CDP diets were administered separately in three 50 ml plastic graduated centrifuge tubes. Tube positions were interchanged daily to avoid development of positional preference in the mice. The volume of intake in each diet was recorded daily Total daily CDP intake (mg/kg) was calculated based on the intake volume, the CDP concentration of each diet, and the mouse's body weight. Other subgroups had the following diet treatments ethanol/control, control/CDP and control/control The diet administration schemes are summarized in Table 1 Depending on the type of experiment, the duration of CDP diet administration was either two or six days

CDP Withdrawal

On the day that the CDP diets were withdrawn, mice whose diet treatment history was ethanol/CDP or control/CDP were fed ad lib the control diet, while those which had been fed ethanol/control or control/control were still pair-fed the control diet One subgroup from each major treatment group was monitored for spontaneous CDP withdrawal signs such as body weight changes, loss of appetite, and change in runway activity. Another subgroup was tested for withdrawal signs induced by injection of the BZD antagonist Ro15-1788 (25 mg/kg). The following withdrawal signs were scored at one-minute intervals during the first ten min after Ro15-1788 injection, based on the combination and modification of the methods of Gallaher et al (20) and Goldstein (21) (a) *Handling-Induced Seizures.* 0 = no seizure when mouse is turned 180°, 1 = seizure occurs when mouse is turned 180°, 2 = seizure occurs when mouse is gently "tickled," 3 = seizure occurs when mouse is picked up by the tail, 4 = spontaneous seizure in home cage. (b) *Tremor* 0 = none, 1 = fine body tremor, 2 = coarse tremor with mildly impaired locomotion, 3 = marked coarse tremor, marked impairment of locomotion, 4 = severe coarse tremor, falls during locomotion. (c) *Tail Lift* 0 = flattened to floor, 1 = horizontal, 2 = 45° lift, 3 = 90° lift, 4 = retrograde, over back. (d) *Locomotion* 0 = normal exploratory movement with rearing, 1 = slow movement, with little rearing, 2 = deliberate/slow movement, no rearing, 3 = very slow movement, virtually stationary, 4 = turning slowly in circles, or moving slowly backward. The rater was blind as to which groups the mice came from, and mice from the different treatment groups were tested in a random order Mice that were tested for withdrawal scores were not used later for testing drug tolerance (see below).

Separate batches of mice from each diet-treatment condition were used for the following behavioral tests for tolerance to either ethanol or CDP For these mice, the duration of administration of the second diet was two days. Prior to each ethanol tolerance test, the mice were injected intraperitoneally with saline, ethanol (20 w/v in saline), or CDP (1.75 and 2.5 mg/ml in saline for runway and hypothermia tests, respectively). Injection doses and testing schedules are described in the appropriate sections below.

Runway Test

The apparatus and testing procedure have been described previously (7). Basically, we recorded the number of complete runs in 5 minutes and the time it required to make the first run The mice were tested on day 3 of CDP withdrawal (day 5 of ethanol withdrawal because the CDP diet treatment period for this experiment was 2 days). They were injected with saline, ethanol (2.5 g/kg) or CDP (35 mg/kg) and tested 15 min later.

Ethanol Sleep Time

Mice from the four diet-treatment groups were injected with a high dose of ethanol (3 5 g/kg) on day 3 of CDP withdrawal. The procedures for determination of sleep onset time and sleep time

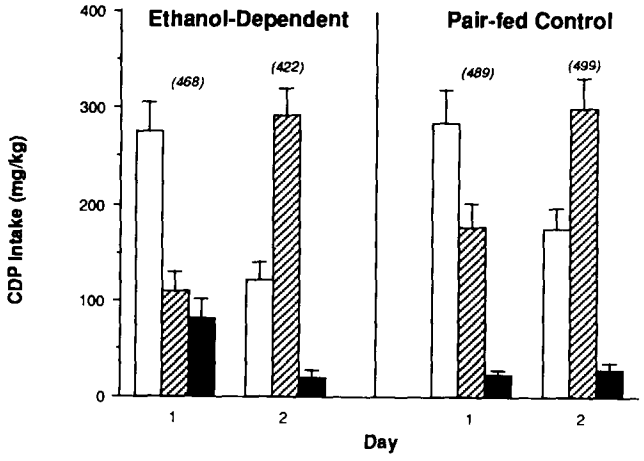

FIG 1 CDP intake after ethanol withdrawal The CDP concentrations (mg/ml) in the diet were 0 6 (open bar), 0 8 (striped bar) and 1 2 (solid bar) Values are means ± S E for N = 11 in each group Numbers in parentheses are total daily CDP intake (mg/kg).

were the same as those described previously (32) Sleep onset time was the interval between ethanol injection and loss of righting reflex, and sleep time was the interval between the loss and recovery of righting reflex. Each mouse was sacrificed at the time it regained its righting reflex, and the brain was homogenized and analyzed for ethanol levels according to published procedures (11, 16, 22).

Drug-Induced Hypothermia

On day 3 of CDP withdrawal, mice from each group were tested for their responses to an injection of ethanol (2 g/kg) or CDP (50 mg/kg). Rectal temperature (16) was determined before and at 0.5, 1 and 2 h after the drug injection.

Statistical Analysis

Statistical significance was set at the 0.05 level. Comparison of withdrawal scores was done using the Mann-Whitney U-test Other comparisons were analyzed by the ANOVA programs (Version 1.1, Human Systems Dynamics, Northridge, CA) with an Apple IIe computer.

RESULTS

CDP Intake

The data shown in Fig. 1 indicate that upon ethanol withdrawal, the ethanol-dependent mice did not have a higher CDP intake than the pair-fed control mice. Similar data (not shown) were obtained when the diet period was extended to six days. As reported previously (7,8), the intake of CDP diets by the ethanol-dependent mice suppressed the following alcohol withdrawal signs hypothermia, handling-induced seizures, tremor, and tail lift

CDP Withdrawal

After two days of CDP diet treatment, the ethanol/CDP mice had significantly more severe Ro15-1788-induced withdrawal signs (2 to 7 min) than the control/CDP mice (Fig. 2A); for example, Mann-Whitney test of the 3-min data showed $U_1 = 20.0$ and $U_2 = 101$, $Z = -2.66$ and $p<0.005$. Similar data for mice which had been treated with the CDP diet for six days also showed a trend

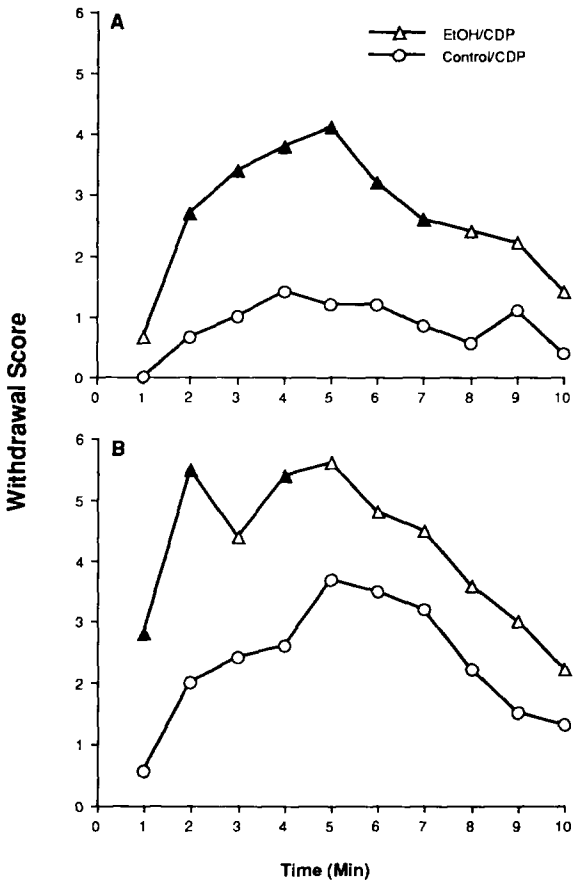

FIG 2 CDP withdrawal precipitated by injection of Ro15-1788 (25 mg/kg) after the mice had been treated with the CDP diets for 2 days (A) or 6 days (B) Mean scores for the combined withdrawal signs (see the Method section) for each group (N = 11) are plotted Closed symbols denote scores that are significantly different from those in the corresponding control group

for higher withdrawal scores in the ethanol/CDP mice, but statistical significance was reached only for scores at 1, 2 and 4 min (Fig. 2B). Mice whose diet treatment histories were ethanol/control and control/control had near zero withdrawal scores. Therefore, the data are not shown in Fig. 2A and B. Our data support the hypothesis that CDP can maintain the dependent state acquired from the prior treatment with ethanol diet.

In the experiment where the CDP treatment period was two days, the ethanol/CDP mice consumed significantly less control diet than the control/CDP mice on day 1 of CDP withdrawal, $F(1,64) = 34.6$, $p<0.001$. These ethanol/CDP mice also lost weight on day 1 of CDP withdrawal (Fig. 3A); in contrast, the control/CDP mice gained weight, $F(1,64) = 53.6$, $p<0.001$. Thus the typical spontaneous withdrawal signs, namely, loss of appetite and body weight loss (8, 20, 29), were manifested in the ethanol/CDP mice but not in the control/CDP mice. When the CDP diet treatment period was extended to 6 days, no significant difference was seen in body weight changes on day 1 of CDP withdrawal between the ethanol/CDP and control/CDP mice (Fig. 3B). Both groups lost weight on day 1 of CDP withdrawal, but the weight change on day 2 of withdrawal was significantly different between the two groups, $F(1,64) = 7.8$, $p<0.01$.

Behavioral Tests for Drug Tolerance

We have shown previously (10,16) that during the chronic

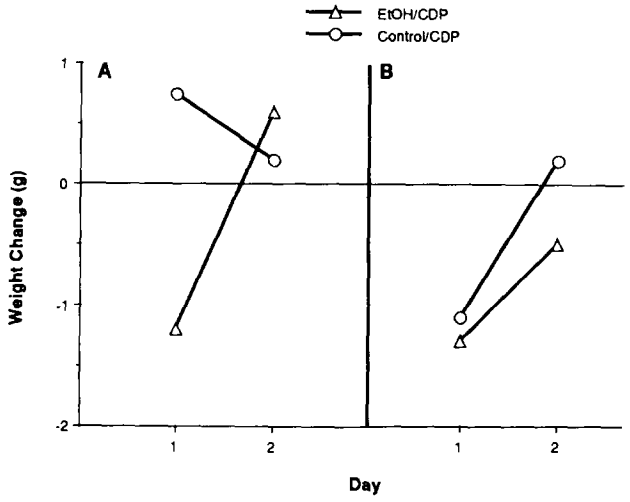

FIG 3 Body weight changes after CDP withdrawal A and B depict results for mice which had been fed the CDP diets for 2 and 6 days, respectively Values are mean differences between the weights on the morning of CDP withdrawal and those determined 24 h (day 1) or 48 h (day 2) later, respectively

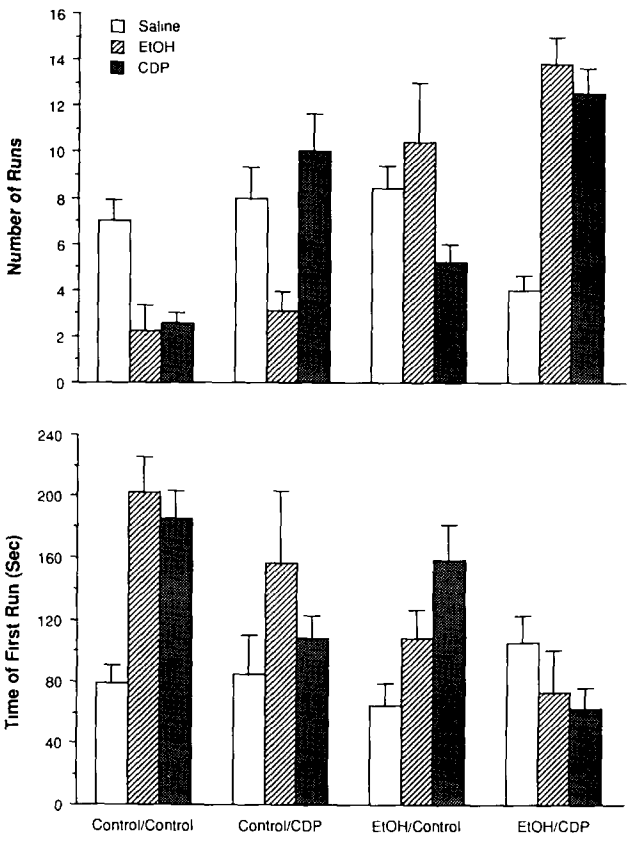

FIG 4 Runway activity after CDP withdrawal Mice were tested on day 3 of CDP withdrawal (day 5 of ethanol withdrawal) at 15 min after injection of saline, ethanol (2 5 g/kg) or CDP (35 mg/kg) Prior diet treatments (first diet/second diet) are shown on the x-axis The duration of CDP diet treatment was 2 days

ethanol diet treatment, the mice developed ethanol tolerance as well as cross-tolerance to CDP. In the present study, the subsequent CDP diet treatment in some mice could also contribute to the development of CDP tolerance and ethanol cross-tolerance. Although semantically different, the "ethanol tolerance" and "ethanol cross-tolerance" in mice treated with the two phases (ethanol followed by CDP) of diet treatment would have been manifested collectively as tolerance to ethanol when the mice were challenged with a test dose of ethanol. Likewise, the "CDP cross-tolerance" and "CDP tolerance" in these animals would have been manifested collectively as CDP tolerance when the mice were tested with a dose of CDP

Runway Test

The effects of a test dose of ethanol or CDP on runway activity in mice with the four different diet-treatment histories are shown in Fig. 4. In the control/control mice, ethanol or CDP injection significantly impaired runway activity (compared to mice injected with saline) in that the number of runs was decreased, and there was a substantial increase in the time taken for the drug-treated mice to make the first run. Compared to the control/control mice, the control/CDP mice were similarly impaired by ethanol, but showed tolerance to CDP. Thus a 2×2 ANOVA of the number of runs after saline or CDP injection in the two groups yielded a significant diet history $\times$ injection interaction, $F(1,40) = 4\ 2$, $p<0.05$ Ethanol tolerance, as evident by the increase in the number of runs, was present in the ethanol/control mice compared to the control/CDP, $F(1,39)=3.9$, $p=0.05$, and control/control mice, $F(1,39)=3.9$, $p=0\ 05$ However, the ethanol/control mice did not show any tolerance to CDP, since the degree of impairment elicited by CDP was not significantly different from that seen in the control/control mice. In terms of the number of runs, the magnitude of tolerance to ethanol in the ethanol/CDP mice was significantly much more than that in the ethanol/control, $F(1,40)=4\ 1$, $p<0.05$, control/CDP, $F(1,41)=29.6$, $p<0.001$, or control/control mice, $F(1,41)=28.0$, $p<0.001$. The data support the hypothesis that CDP can maintain the ethanol tolerance acquired from the prior ethanol diet treatment. The magnitude of CDP tolerance (as reflected by the number of runs) in the ethanol/CDP mice was significantly larger than those in the ethanol/control, $F(1,41)=30.1$, $p<0.001$, control/control, $F(1,41)=33.3$, $p<0.001$, or control/CDP mice, $F(1,41)=6.8$, $p<0.05$.

In general, a reciprocal relationship existed between the number of runs and the time for first run, namely, a smaller number of runs was usually accompanied by a longer delay in the time for first run (Fig. 4). Therefore, a comparison of drug-induced changes (compared to saline-injected mice) in the time for first run in mice with the different diet-treatment histories may provide another measure of drug tolerance. Our results indicate that this parameter is not as sensitive a measure of drug tolerance as the parameter of the number of runs (see below). The ethanol/CDP mice were the only group that showed a decrease in the time for first run (compared to saline-injected mice) when they were injected with either ethanol or CDP. These mice showed tolerance to ethanol when compared to the control/control mice [a 2×2 ANOVA showed a significant diet history $\times$ injection interaction; $F(1,41)=8.4$, $p<0\ 01$] but there were no significant interaction effects in the comparison with the control/CDP, $F(1,41)=3.2$, $p>0\ 05$, or the ethanol/control mice, $F(1,40)=2.3$, $p>0\ 1$. In terms of the CDP effects, there were significant diet history $\times$ injection interactions in the comparison of the ethanol/CDP mice with the control/control, $F(1,41)=13.6$, $p<0.001$, and ethanol/

TABLE 2

EFFECTS OF DIET TREATMENT HISTORIES ON ETHANOL SLEEP TIME

Diet Treatment (1st/2nd)	Sleep Onset Time (min)	Sleep Time (min)	Brain Ethanol Level at Awakening (mg/g)
Control/Control	1 89 ± 0 32	111 8 ± 9 36	2 67 ± 0 06
Control/CDP	2 06 ± 0 06	77 7 ± 5 27*	2 92 ± 0 05*
Ethanol/Control	1 74 ± 0 05	68 4 ± 6 58*	3 11 ± 0 06*
Ethanol/CDP	2 39 ± 0 05	43 2 ± 4 07†	3 29 ± 0 06

Mice (N=9–13 for each group) were injected with ethanol (3 5 g/kg) on day 3 of CDP withdrawal Values are means ± S E Ethanol and CDP diet treatment periods were 15 and 2 days, respectively

*$p \leq 0$ 006, compared to control/control group, †$p<0$ 01, compared to ethanol/control group, $p<0$ 001 compared to control/control and control/CDP groups, $p<0$ 05, compared to control/CDP group, $p<0$ 001, compared to control/control and control/CDP groups

control mice, $F(1,41)=10$ 2, $p=0.002$, but not in the comparison with the control/CDP mice, $F(1,41)=2.4$, $p>0.1$. In other words, the degree of CDP tolerance in the control/CDP mice, as measured by the time for first run [$F(1,40)=4.7$, $p=0.03$, compared with control/control mice], was not significantly different from that in the ethanol/CDP mice

Ethanol Sleep Time

The data in Table 2 indicate that the ethanol/CDP mice had the shortest ethanol sleep time compared to the other three groups, $F(3,42)=18.7$, $p<0.001$. Although the ethanol/CDP mice also had the slowest sleep onset time, the differences among the four groups were not statistically significant, $F(3,42)=2.6$, $p=0.06$. The brain ethanol level at awakening was also highest in the ethanol/CDP mice, and it was statistically different from the control/CDP, $F(1,20)=22$ 4, $p<0.001$, and control/control mice, $F(1,19)=46.3$, $p<0.001$, but not the ethanol/control mice, $F(1,19)=3.7$, $p=0.07$. The latter result may have been due to the smaller number of mice (N=9) in the ethanol/CDP group. Other possible factors are examined in the Discussion section. Both the ethanol/control and the control/CDP mice showed tolerance to ethanol, as reflected in the sleep time, $F(1,22)=14.4$, $p<0.001$ and $F(1,23)=10.2$, $p=0.004$, respectively, and brain ethanol level at awakening, $F(1,22)=23.4$, $p<0.001$ and $F(1,23)=9.0$, $p=0$ 006, respectively, compared to the control/control mice

Drug-Induced Hypothermia

The control/control mice had significantly more ethanol-induced hypothermia than the control/CDP, ethanol/control and ethanol/CDP mice when they were tested on day 3 of CDP withdrawal [Fig. 5A, e.g., at 0.5 h, $F(3,42)=6.6$, $p<0.001$]. Since the magnitudes of hypothermia among the latter three groups were not significantly different, these mice showed a similar degree of tolerance to the hypothermic effect of ethanol. Therefore, the results are equivocal in terms of whether CDP could maintain the ethanol tolerance developed from the prior ethanol diet treatment. With respect to the CDP-induced hypothermia, the responses from the ethanol/control and control/control mice were nearly identical, but were significantly greater than those in the control/CDP and ethanol/CDP mice (Fig. 5B). The data indicate that the CDP cross-tolerance in the ethanol/control mice had dissipated on the test day which was day 5 of ethanol withdrawal. On the other

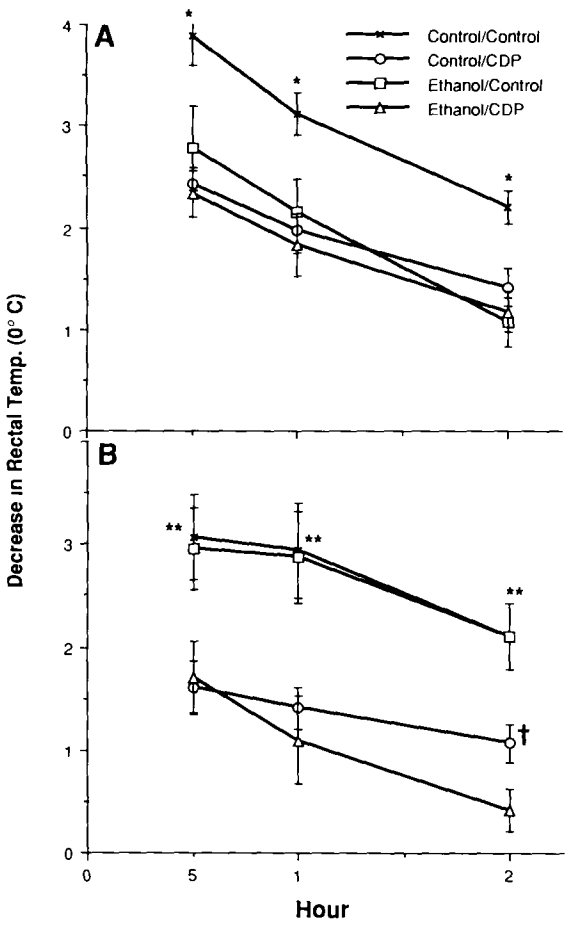

FIG 5 Effects of diet-treatment histories on hypothermic responses to injections of ethanol (2 g/kg, A) or CDP (50 mg/kg, B) Mice were tested on day 3 of CDP withdrawal (day 5 of ethanol withdrawal) Values are mean decreases (relative to zero h values) ± S E *$p<0$ 005, compared to control/CDP, ethanol/control, and ethanol/CDP groups **$p<0$ 01, compared to control/CDP and ethanol/CDP groups †$p<0$ 05, compared to ethanol/CDP group

hand, the degrees of CDP tolerance in the control/CDP and ethanol/CDP mice were not significantly different at 0 5 and 1 h, but the ethanol/CDP mice showed less CDP hypothermia than the control/CDP mice at 2 h (Fig 5B) Therefore, the two-day CDP treatment could largely account for the observed CDP tolerance in both groups

DISCUSSION

We have previously shown (7,8) that the replacement of an ethanol diet by a CDP diet suppressed ethanol withdrawal signs. In order to satisfy the criteria for full cross-dependence (23) between CDP and ethanol, the ability of CDP to maintain the ethanol-dependent state needs to be demonstrated. The present study provides an experimental support that CDP can maintain the dependent state acquired from a prior ethanol treatment Therein, mice which had been fed chronically an ethanol diet followed by two days of CDP diet treatment had more severe CDP withdrawal signs induced by Ro15-1788 than drug-naive mice which had been similarly exposed to the CDP diet treatment The more severe CDP withdrawal in the ethanol/CDP mice was not due to a dif-

ference in CDP intake between the ethanol/CDP and control/CDP mice (Fig. 1). An alternative interpretation is possible, namely, that the prior ethanol treatment facilitated the development of CDP dependence. Investigations relating to the effects of prior ethanol treatment on subsequent withdrawal severity have involved repeated cycles of ethanol administration interspersed with periods of no drug treatment (1, 3, 26). In one study, a difference in ethanol withdrawal severity was not observed until after the third or fourth cycle of ethanol treatment (26). Likewise, alcoholics with five or more previous alcohol withdrawals had more withdrawal seizures in subsequent detoxifications than alcoholics with no prior alcohol withdrawal experience (4). This was attributed to the "kindling" phenomenon (4). In the present study, the mice had their first and only treatment with ethanol, and they did not have ethanol withdrawal because of the suppression by CDP. Although we cannot rule out completely the possibility that the prior ethanol treatment could have facilitated the development of CDP dependence during the two-day CDP treatment, we favor the explanation that CDP can maintain the dependent state acquired from the prior ethanol treatment. This was manifested as CDP withdrawal when the CDP diet treatment was terminated Our results also provide credible support for anecdotal accounts of alcoholics using CDP and alcohol interchangeably.

The demonstration of full cross-dependence on CDP in ethanol-dependent mice does not necessarily prove that CDP-dependent mice are fully cross-dependent on ethanol. In fact, results of our investigation (9) indicate that CDP-dependent mice are only partially cross-dependent on ethanol, in that ethanol cannot fully suppress CDP withdrawal signs. The asymmetry of cross-dependence between ethanol and CDP may be a reflection that there are different mechanisms involved in the development of ethanol or CDP dependence

We used three behavioral tests to determine drug tolerance because Kalant et al (24) have stressed that tolerance does not necessarily develop at an equal rate to all of the actions of a given drug Thus Pohorecky et al. (28) have shown that tolerance to ethanol develops at different rates depending on the measures employed to evaluate it It has also been shown that tolerance to BZD develops at very different rates for the various behavioral effects of BZD (19). We hypothesized that if CDP could maintain the ethanol tolerance developed from the prior ethanol treatment and because of the uninterrupted drug treatments in the ethanol/CDP mice, these animals would be more tolerant to ethanol or CDP when compared to the ethanol/control, control/CDP and control/control mice. This is because the ethanol tolerance and CDP cross-tolerance developed in the ethanol/control mice (10,14) would have partially (if not fully) dissipated since the mice were tested on day 5 of ethanol withdrawal. Likewise, in the control/CDP mice, the CDP tolerance acquired from the two-day CDP treatment would have been minimal; there would have been even less ethanol cross-tolerance developed because our previous results (14) indicated that mice did not develop rapid cross-tolerance to ethanol after an acute dose of CDP.

Data from the runway test (Fig. 4) suggest that CDP can maintain the ethanol/tolerance acquired from the prior treatment with ethanol. Thus the ethanol/CDP mice were more tolerant to ethanol than the ethanol/control mice, and more tolerant to CDP than the control/CDP mice The alternative interpretation, that prior ethanol treatment facilitated the development of cross-tolerance to ethanol as a result of the subsequent CDP treatment, is deemed not very likely. Maier and Pohorecky (25) found that the acceleration of tolerance development to both ethanol-induced motor impairment and hypothermia occurred only in rats subjected to repeated withdrawal episodes, but not in rats who experienced withdrawal only once. In our study, the CDP/ethanol mice never underwent ethanol withdrawal because of suppression by the CDP diet treatment. The ethanol/control mice showed tolerance to ethanol even on day 5 of ethanol withdrawal but did not show tolerance to CDP The results suggest that the rate of dissipation of CDP cross-tolerance was faster than that of dissipation of ethanol tolerance. Similarly, the control/CDP mice showed tolerance to CDP but not cross-tolerance to ethanol This could be due to the short duration of CDP treatment which would not have allowed for the development of quantifiable ethanol cross-tolerance (14). Alternatively, the ethanol cross-tolerance which had developed as a result of the short CDP treatment could have been dissipated by day 3 of CDP withdrawal, the day of testing for drug tolerance.

Interpretations of the sleep-time data (Table 2) are less straightforward than those for the runway data. The ethanol/CDP mice had the shortest ethanol sleep time, suggesting that they were more tolerant to ethanol than the other three groups. However, the brain ethanol level at awakening in the ethanol/CDP mice was not significantly different from that in the ethanol/control mice, indicating that the degree of functional tolerance in both groups was similar. Therefore, it is not possible to conclude unequivocally that CDP can maintain the ethanol tolerance (functional) as determined by this measure. We have previously shown that chronic ethanol treatment did not induce dispositional tolerance to ethanol in mice (16), whereas chronic CDP treatment (about 30 days) could do so (Chan et al., submitted for publication). In the latter case, the rate of ethanol elimination was not different between CDP-dependent and control mice, but the CDP-dependent mice had lower brain ethanol levels at various times after an injection of ethanol (3 5 g/kg). Although we have not investigated whether two days of CDP diet treatment could induce some dispositional tolerance to ethanol, it is possible that in the present study, the ethanol/CDP mice could have developed some dispositional tolerance to ethanol. Another factor is that the functional tolerance to ethanol observed in the ethanol/control and ethanol/CDP mice might be close to or at the peak value. This would explain why there was no significant difference in ethanol tolerance between the two groups. In contrast to the runway data, the control/CDP mice showed functional tolerance to ethanol in the sleep-time test, as reflected by the higher brain ethanol level at awakening compared to the control/control mice.

Results of the hypothermia test indicate that ethanol tolerance attained from the prior ethanol treatment in the ethanol/control mice was of similar magnitude as that seen in the ethanol/CDP mice, despite the former group had two more days for the dissipation of such tolerance (Fig. 5A). The control/CDP mice also showed a similar degree of ethanol tolerance, in contrast to the results of the runway test. Future investigations need to examine the rates of acquisition and dissipation of ethanol cross-tolerance in CDP-treated mice using different measures of tolerance. The ethanol/control mice did not show any CDP tolerance in the hypothermia test (Fig. 5B). The data suggest that the rate of dissipation of CDP cross-tolerance was much faster than that of ethanol tolerance.

In summary, the runway data demonstrate that CDP can maintain the ethanol tolerance acquired from the prior ethanol treatment, but a similar conclusion cannot be reached for the sleep-time and hypothermia tests because of complicating factors discussed above.

ACKNOWLEDGEMENTS

We thank Ms. Carol Tixier for skillful typing and Dr P F Sorter of Hoffmann-La Roche, Inc for providing us with CDP and Ro15-1788

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