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Influence of Chlordiazepoxide on Alcohol Consumption in Mice

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CHAN, A. W. K., F. W. LEONG AND D. L. SCHANLEY. *Influence of chlordiazepoxide on alcohol consumption in mice.* PHARMACOL BIOCHEM BEHAV 18(5) 797–802, 1983.—In a free-choice situation, chlordiazepoxide (CDP; 12.5 or 25 mg/100 ml; groups B or C), when incorporated in ethanol solutions (2 to 20%, v/v), caused a significant decrease in ethanol preference index (P.I.). This was probably due to the combined CNS effects of both drugs rather than a taste effect, since the mice did not discriminate between aqueous CDP solutions and water. However, when the mice had prior exposure to ethanol and CDP was incorporated intermittently, no significant decreases in P.I. resulted. In a no-choice situation, ethanol intake was increased only on the first day of each intermittent incorporation of CDP (3 days for each 6-day cycle), being more persistent in group B (2 to 15% ethanol) than in group C (2 to 6.5%). Ethanol intake decreased in group C when alcohol concentrations exceeded 10%. The "first-day" CDP effect also occurred in the no-choice situation of an ethanolic liquid diet. Possible factors for this effect are discussed. Thus the effects of CDP on alcohol consumption in non-deprived mice vary with experimental designs.

Chlordiazepoxide Ethanol Alcohol consumption Preference index Ethanol diet

CERTAIN inbred strains of mice are known to exhibit differences in preference for alcohol solutions [25]. The C57BL mice have a high preference for, and the DBA mice avoid, a 10% ethanol solution [25,32]. These traits are highly inheritable [15,30], suggesting a genetic basis of alcohol selection. Other factors that have been implicated as capable of influencing ethanol selection in these mice include the capacity to metabolize alcohol and acetaldehyde [31, 32, 35], environmental influences [28,29], neurochemical manipulations [19,33], sensitivity to alcohol [21], and hormonal actions [16].

We have recently reported that chlordiazepoxide (CDP), when administered together with ethanol, caused a supra-additive effect on the ethanol-induced loss of righting reflex [2]. This effect was shown not to be due to an alteration of the rate of elimination of blood ethanol, but was probably due to some neurochemical actions of the combined drugs [3]. This paper tests the hypothesis that if the selection of ethanol in mice can be influenced by their sensitivity to the drug, then CDP, when combined with ethanol, is expected to decrease their preference for the alcohol/CDP solution. The consumption of both the benzodiazepines and alcohol together in humans is not uncommon [17].

Benzodiazepines have been shown to facilitate feeding behavior in different mammalian species [5, 7, 10, 20, 30, 34]. These drugs are also known to influence drinking responses, for example, CDP increases water consumption in rats and mice [8, 22, 24, 26]. Soubrié *et al.* [36] demonstrated that CDP, diazepam and lorazepam increase the time devoted to drinking in a 10-min test period in these animals.

Little data is available on the effects of chronic benzodiazepine treatment on drinking responses. It was reported that chronic diazepam treatment (2.5 mg/kg given on alternate days) did not stimulate water intake in non-deprived rats [38]. Falk and Burnidge [12] failed to observe any change in water intake with daily administration of CDP (15 mg/kg) in rats adapted to a 24-hr water-deprivation schedule. In contrast, Cooper and Francis [8] reported that CDP (10 mg/kg), when given to rats acutely or for 9 consecutive days, stimulated water consumption and enhanced the frequency of occurrence of drinking in a 15-min drinking test.

The effects of benzodiazepines on consumption of ethanol solution have not been investigated extensively. It has been reported that diazepam increased the tendency to maintain ethanol preference during ethanol withdrawal in rats, and this has led to the speculation that the use of diazepam to treat alcoholism might be counterproductive [11]. However, using another experimental design, Ferko *et al.* [13] did not find any increase in ethanol preference during postwithdrawal drinking by rats that exhibited physical dependence on alcohol. Phenazepam, when given by daily intraperitoneal injection (1 mg/kg) to rats for 3 weeks, has been reported to depress a previously-developed preference for ethanol [39].

We have investigated the effect of CDP on the consumption of ethanol solutions (2 to 20%, v/v) in non-deprived mice in no-choice and free-choice situations. Another aspect of the study deals with the influence of CDP on the intake of an ethanolic liquid diet commonly used in animal models of alcoholism. We chose to use different schedules of ethanol administration because the type of ethanol exposure has been known to influence voluntary intake [23,37]. A preliminary report of this investigation has appeared [1].

METHOD

Animals

Male C57BL/6J mice (9 weeks old; Jackson Laboratories, Bar Harbor, ME) were acclimated for a week in a controlled-environment room (22°), with automatic light-/dark (12/12 hr) cycle before use. They were housed singly in plastic cages throughout the experiments.

Procedure

Experiment 1. Five groups of mice (N=12 in each group) were given a choice of water and one of the following: (1) aqueous ethanol solution, commencing with an ethanol concentration of 2% (v/v; from 95% ethanol) and increasing the concentration (successively to 5, 8, 12.5, 15 and 20%) every 3 days until it reached 20%; (b) aqueous ethanol solution containing CDP (12.5 mg/100 ml). The concentration of ethanol was increased gradually as in (a); (c) same as (b) except that the concentration of CDP was 25 mg/100 ml; (d) aqueous solution of CDP (12.5 mg/100 ml). This was offered for the entire duration as in (a); (e) same as (d) except that the concentration of CDP was 25 mg/100 ml. Food pellets were available ad lib for all groups. The drinking tube was a 15 ml graduated, plastic conical centrifuge tube equipped with a size 0 stopper and a 2 1/2 in. metal drinking tube. The positions of the drinking tubes were interchanged every day. Tubes containing CDP solutions were wrapped with aluminum foil, since CDP is sensitive to light. Stability of CDP in the ethanol solutions at room temperature during a 24-hour period was confirmed by high pressure liquid chromatography [18]. Fresh solutions were prepared each day and the daily intake of each mouse was recorded. Preference index for solutions other than water is defined as the ratio: volume of solution consumed/total volume of fluid (water plus the other solution) consumed, and is expressed as a percentage.

Experiment 2. Mice (N=30) were given a choice of a 2% ethanol solution and water for 3 days. Bottle positions were interchanged daily. On day 4, the mice were rearranged into 3 groups (A,B,C, N=10 each). Group A continued to receive the choice of 2% ethanol solution and water for another 3 days. During this period, mice in groups B and C were offered the choice of water and a 2% ethanol solution which contained CDP (12.5 and 25 mg/100 ml, respectively). The same schedule of administration of ethanol solution for 3 days (A,B,C) followed by another 3 days of either continued ethanol treatment (A only) or a combination of CDP and ethanol treatment (B and C) were repeated in succession with different concentrations (%, v/v) of ethanol solution, namely, 5, 8, 10, 12.5, 15 and 20. Daily fluid intake of each mouse was recorded and the mice were individually weighed each week.

Experiment 3. This was similar in design to Experiment 2 except that mice received only the ethanol solution (with or without CDP) as the sole drinking fluid. The same schedule of administration of these solutions was followed.

Experiment 4. Mice (N=30) were fed ad lib a chocolate-flavored nutrament liquid diet (distributed by the Drackett Products Co., Cincinnati, OH), fortified with a vitamin mixture (ICN Pharmaceuticals, Inc., Cleveland, OH) and containing 3.5% (v/v) ethanol (from 95% ethanol). Fresh diet was prepared daily according to published procedure [14]. Daily diet intake of each individual animal was recorded. After 3 days, the mice were arranged into 3 groups (A,B,C) such that each did not differ from the other two in terms of the mean intake of the diet. Group A mice continued to receive the 3.5% diet ad lib for another 3 days. The concentration of ethanol diet was then increased by 1.5% every 3 days up to 8% (total of 12 more days after group arrangements). Mice in groups B and C were fed ad lib the same ethanol diets except that CDP (3.2 mg and 6.4 mg/100 ml, respectively) was incorporated each day. The hydrochloride of CDP was dissolved in the volume of water used to solubilize the vitamin mixture [14]. The decrease in CDP dosage was to compensate for the volume of intake of liquid diet (sole source of food) compared to ethanol intake in other experiments. Daily diet intake was recorded and the ethanol intake was computed.

Student's *t*-test or analysis of variance was used for statistical evaluations of the data.

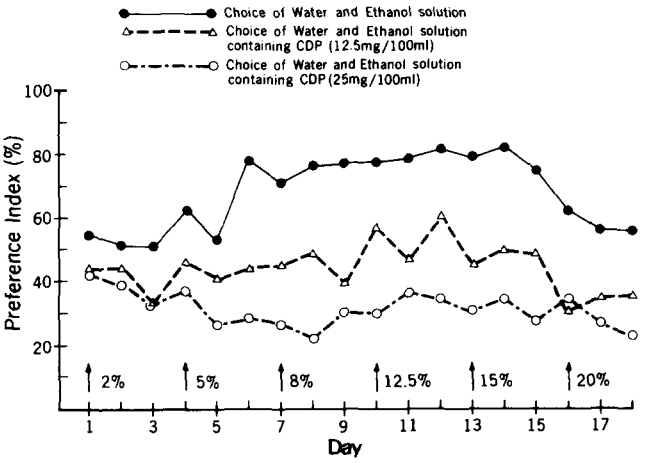

FIG. 1. Effects of CDP on preference index for ethanol solutions. Preference index is the ratio: Intake of ethanol solution (with or without CDP)/total fluid intake (water plus ethanol). Each point represents the mean of 12 observations. Arrows indicate the days when ethanol concentrations were changed.

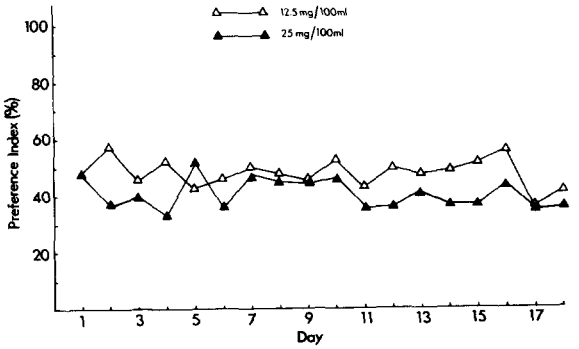

FIG. 2. Preference indices for aqueous CDP solutions. Mice were given the choice of water and an aqueous solution of CDP (concentrations shown in figure). Each point represents the mean of 12 observations.

RESULTS

Experiment 1

Figure 1 depicts the daily preference index (P.I.) for solu-

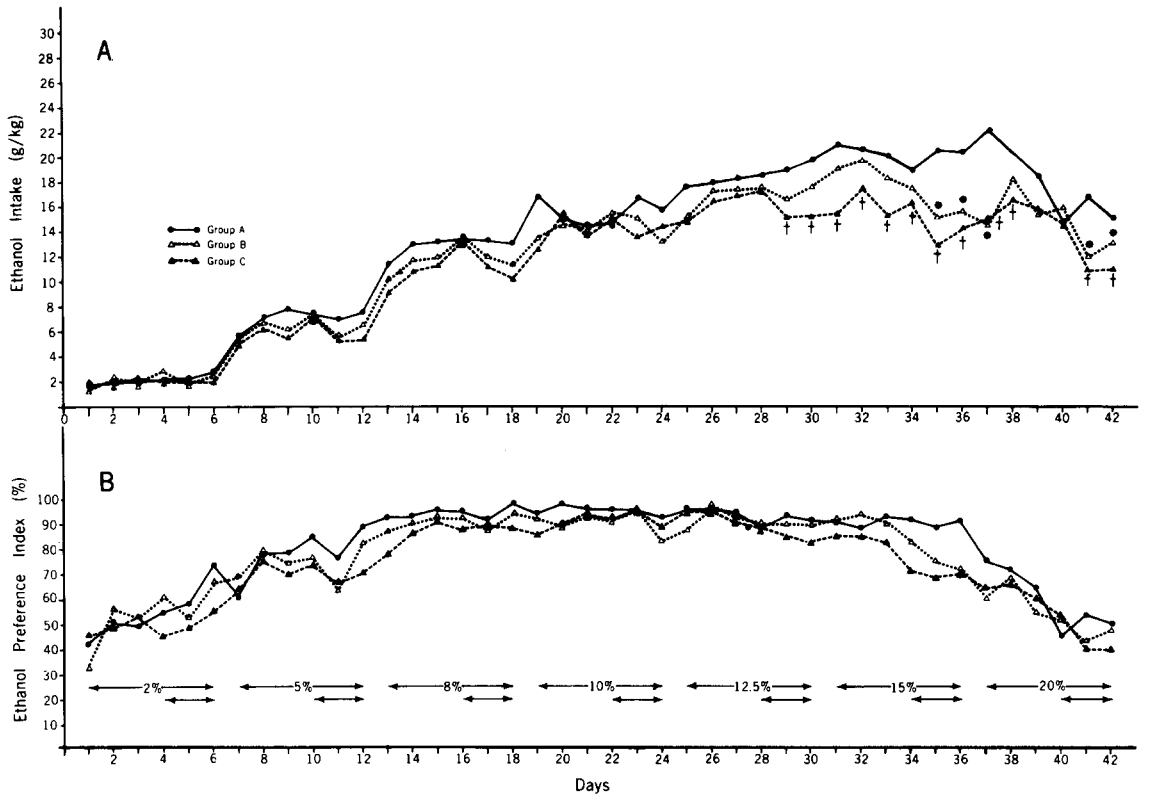

FIG. 3. Effects of intermittent administration of CDP on ethanol intake (A) and preference index (B). Double arrows with % values represent durations when the indicated ethanol concentrations were administered. Group A: Choice of water and ethanol solutions for the entire period. Group B: Choice of water and either ethanol solutions or ethanol solutions containing CDP (12.5 mg/100 ml). Group C: Choice of water and either ethanol solutions or ethanol solutions containing CDP (25 mg/100 ml). Durations for the inclusion of CDP in groups B and C were marked by uninterrupted double arrows. *$p<0.001$, Group B vs. Group A; †$p<0.001$, Group C vs. Group A.

tions (other than water) consumed by groups A, B and C. In group A the P.I. for ethanol solution was 50–60% for the 2 and 5% solutions, and this increased to over 80% for solutions with higher ethanol concentration (8–15%), with a return to the lower P.I. for the 20% solution. Incorporation of CDP (12.5 mg/100 ml; group B) in the ethanol solutions resulted in a significant ($p<0.001$) decrease in P.I., the effect being even more pronounced ($p<0.001$, compared with group B) in group C in which the mice received ethanol solutions containing the higher CDP concentration (25 mg/100 ml). In general there were no significant differences in the daily total fluid intake among the three groups. The daily intake of CDP, resulting from consumption of the ethanol/CDP solution, ranged from 9–18 and 14–29 mg/kg for B and C, respectively.

Figure 2 shows the daily preference index for an aqueous solution of CDP (low and high concentrations, D and E, respectively). The results indicate that for the 12.5 mg/100 ml solution, the P.I. (about 50%) was similar to that for water. The lower P.I. (about 40%) for the 25 mg/100 ml solution reflects a slight preference for the mice to select water. The mean daily intake of CDP ranged from 13–20 and 26–40 mg/kg for groups D and E, respectively.

The mean body weight for each of the five groups of mice was not significantly different from those of the other at the beginning and end of the experiment, e.g., group A had a mean body weight of 23.64 g (±0.36, SEM). A gradual increase in weight occurred with the mean weight of this group reaching 24.71±0.27 g at the end of the experiment.

Experiment 2

Mice which were exposed alternately (every 3 days) to ethanol solutions or ethanol/CDP solutions (plus water in each case) exhibited a higher preference index for the CDP-containing ethanol solutions than those mice which were exposed to the ethanol/CDP solutions continuously (Fig. 3B compared to Fig. 1). The differences were significant ($p<0.001$) for the 5–15% ethanol/CDP solutions. In other words, the inhibiting effects of CDP on P.I. for ethanol solutions seen in Experiment 1 were not operative under this particular experimental design, although a trend for lower P.I.'s for groups B and C still existed (Fig. 3B). The amounts of ethanol consumed by mice in these two groups at high ethanol concentrations (12.5 to 20%) were significantly ($p<0.005$) less than those ingested by group A (Fig. 3A).

Experiment 3

Figure 4 depicts the daily consumption of ethanol in the no-choice situation. The mean volume of ethanol intake each day remained fairly constant (range 3–6.5 ml) except for a 10–15% decrease on the days when the 20% solution was present (not shown). However, the absolute amount of ethanol consumed increased gradually from about 3 to 24

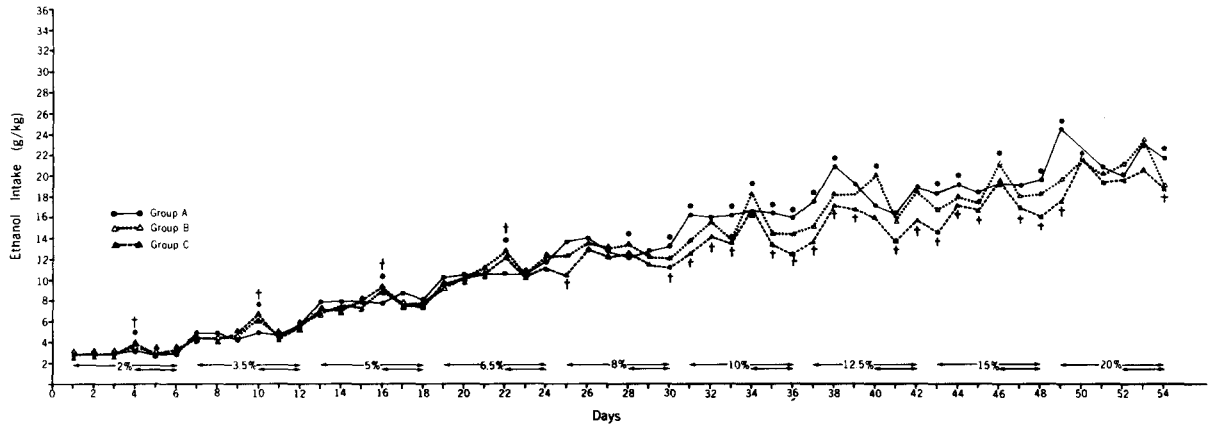

FIG. 4. Effects of intermittent administration of CDP on ethanol intake under the no-choice situation. Group A: ethanol solutions. Group B: ethanol solutions with or without CDP (12.5 mg/100 ml). Group C: ethanol solutions with or without CDP (25 mg/100 ml). The double arrows have the same denotations as those shown in Fig. 3. *$p<0.005$, group B vs. group A; †$p<0.005$, group C vs. group A.

g/kg per day (group A), due to the increasing concentrations of ethanol solution. The intermittent incorporation of CDP in alcohol solutions (3-day cycle) caused significant increases (8 to 38%; $p<0.005$) in ethanol intake only on the first day that CDP was present (e.g., days 4, 10, 16, etc.; Fig. 4) but not on the other two days; this pattern of increased intake was more persistent with group B (lower CDP content, 12.5 mg/100 ml), being maintained up to the 15% solution, whereas group C (higher CDP content, 25 mg/100 ml) showed this pattern only up to the 6.5% solution. For ethanol concentration above 8%, groups B and C showed significantly less ($p<0.01$) ethanol intake than group A, not only on the second and third days that CDP was incorporated, but also on the days when just the alcohol solutions were introduced. These effects were more pronounced in group C than group B. The daily CDP intake, when CDP was present in the ethanol solution, for group B mice amounted to 20–30 mg/kg and for group C, double this amount.

Experiment 4

Addition of CDP in the ethanolic liquid diet caused a sharp increase in ethanol intake (20–25%) by mice in groups B and C only on the first day that CDP was present in the 3.5% diet (Fig. 5). The magnitude of increase was the same in these two groups. However, there was no significant difference in the ethanol intake among mice in groups A, B and C on the second and third days that CDP was included in the 3.5% diets. The same was true for the six days when CDP was incorporated in the 5 and 6.5% diets (3 days each). On the following 3 days when the diet contained 8% ethanol, groups B and C showed an increase (7–14%, compared with group A) in the intake of diets containing CDP. Since the volume of diet consumed decreased as the ethanol concentration increased to 8%, the daily CDP intake actually decreased towards the end of the experimental period. Thus for group B, the highest intake was on day 4, ranging from 20–25 mg/kg, and the value dropped to 10–15 mg/kg on the last 3 days. The daily intake of CDP for group C mice was approximately doubled that for group B.

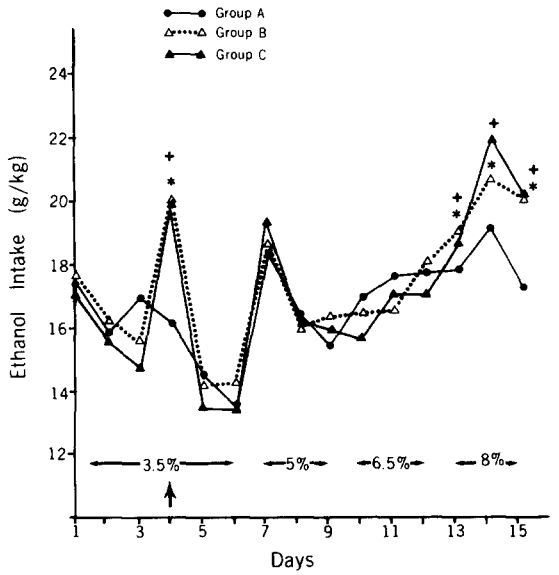

FIG. 5. Effect of incorporation of CDP in ethanolic liquid diets on ethanol intake. Double arrows with % values represent durations when the indicated ethanol concentrations were present in the liquid diet. Vertical arrow denotes the beginning of incorporation of CDP in ethanol diets for groups B and C. Group A: ethanol diet only. Groups B and C: ethanol diet only for 3 days followed by ethanol diet containing CDP (3.2 mg/100 ml for B and 6.4 mg/100 ml for C). *$p<0.001$ group B vs. group A; †$p<0.001$ group C vs. group A.

DISCUSSION

Our results (Fig. 1) confirm the well-known preference for ethanol solutions (especially 8–15%) in C57BL mice [25]. This strain of mice is therefore not averse to the taste of dilute ethanol solutions (8–15%). The decrease in preference index (P.I.) for ethanol solutions containing CDP shown in Fig. 1 was not likely to be due to a taste effect of CDP. This is because the P.I. for aqueous CDP solutions was around

50% (Fig. 2), suggesting a lack of discrimination between water and aqueous CDP solutions in the animals. Since there were no significant changes in the total daily fluid intake (water and aqueous ethanol) throughout the experiment, the decrease in P.I. represents an inhibition in ethanol selection. We have previously shown [2] that the combination of ethanol and CDP in mice renders the animals more sensitive to the central actions of ethanol. Therefore, the inhibition in ethanol consumption in Experiment 1 may be due to the combined CNS effect of CDP/ethanol. This effect, if operative, should be more evident with alcohol solutions containing a higher concentration of CDP. Our results (Fig. 1) do provide support for this hypothesis. The CNS effects of CDP/ethanol experienced by the mice following ingestion of the combined drug solution during a 24-hour period would no doubt be much less than those induced by the intraperitoneal injection of both drugs, even though a large proportion of drinking was done in the dark hours. This may explain why a substantial inhibition of the preference index was not observed during the first two days that the 2% ethanol/CDP solution was offered (Fig. 1). Another factor seems to be the schedule of CDP administration since the prior exposure to ethanol and the subsequent intermittent administration of CDP did not result in any significant reduction in P.I. for ethanol solutions (Fig. 3B compared with Fig. 1), although there was a trend for a lower P.I. This suggests that there might be a carry-over effect due to the uninterrupted administration of CDP (Experiment 1) which could lead to the gradual accumulation of CDP or its metabolites in the animals. The magnitude of inhibition of ethanol selection did not increase with time (except for 20% ethanol; Fig. 1), contrary to what would be expected to occur if the carry-over effect was a dominant factor. It is possible that the mice developed tolerance to the central effects of CDP and ethanol during chronic drug administration. On the other hand, these mice also have the ability to tolerate and maintain preference to a wide range of ethanol concentrations (5–15%) in the absence of CDP (Fig. 1), despite increases in the intake of the absolute amount of ethanol (intake volume remained relatively constant). It is not very likely that there could have been a substantial carry-over effect due to CDP itself because this drug is known to be metabolized very rapidly in the mouse [2]. We have reported that after C57BL mice had been fed an ethanolic liquid diet containing CDP (6.4 mg/100 ml) for 12 days, blood and brain samples taken at $2^1/_2$ hr after withdrawal of diet showed no measurable concentration of CDP [4]; however, the N-demethyl metabolite of CDP (NDCDP) was detectable in blood (about 3 μg/ml). We have also shown that NDCDP was mainly responsible for the supra-additive effect of CDP on ethanol-induced loss of righting reflex [2]. Thus, a carry-over effect due to NDCDP might be possible in Experiment 1, while the intermittent administration of CDP in Experiment 2 might have been insufficient to elicit appreciable accumulation of NDCDP in the mice.

In the no-choice situation (Fig. 4), the intermittent introduction of CDP resulted in an increase in ethanol intake only on the first day of each 3-day cycle that CDP was incorporated in the ethanol solutions. The effect was more persistent in group B (lower CDP concentration) than in group C, occurring from 2 to 15% ethanol in the former group; but in group C, the effect lasted only up to 6.5% ethanol. This phenomenon of increased alcohol consumption on the first day of introduction of CDP also occurred when an ethanolic liquid diet was the sole source of food and fluid (Fig. 5). These data are suggestive of a novelty effect; however, such an effect was not observed on the "first days" in the free-choice situations such as those in Experiments 1 and 2. The major experimental difference between the free and forced choice paradigms was the constant presence of a "non-novel" fluid, namely, water, in the former situation. Perhaps this had an inhibiting influence on the "novelty effect" elicited by the ethanol/CDP combination. The increased intake of ethanol/CDP on the first day that CDP was present (Figs. 4 and 5) could have resulted in a carry-over effect of CDP or NDCDP, the latter being more important as discussed above. Thus the combined central effects of CDP/ethanol or NDCDP/ethanol experienced by the mice might become a dominant factor in controlling the intake of ethanol/CDP on the second and third day of each 3-day cycle. This can explain why the novelty effect occurred up to 15% ethanol for group B, but only up to 6.5% ethanol for group C. In fact an opposing effect, namely, inhibition of ethanol intake, occurred when the ethanol concentration reached 10% (Fig. 4). This was more severe in group C, where the inhibition persisted even on the days when CDP was not present in the ethanol solution. It has been reported [9] that after a single, oral administration of CDP (20 mg/kg) in mice, blood CDP could be detected up to 4 hr, but brain CDP was measurable up to 2 hr only; NDCDP was present in the blood and brain up to 18 hr, and the polar conjugates could not be detected at this time; lactam metabolite (LCDP) was not present in the brain at 18 hr, but could still be measured in the blood at 24 hr, having a mean value of 0.85 μg. The consumption of ethanol solutions in Experiment 3 was spread out mostly during the dark hours, and the average daily intake of CDP (when present) for group C mice amounted to no more than 40 mg/kg in cases involving higher alcohol concentrations. Therefore, it is not likely that appreciable amounts of metabolites of CDP would have been present in the blood or brains of those animals two or three days after consuming the CDP-containing alcohol solution. In the absence of supporting data, we cannot rule out the possibility that the inhibition in the intake of ethanol or ethanol/CDP might be a learned phenomenon.

In Experiment 4, the incorporation of CDP was uninterrupted, thereby increasing the likelihood of development of tolerance to the behavioral effects of both alcohol and CDP in the mice. Our results indicate that after repeated exposure to CDP, the mice had significant increases in ethanol diet when the alcohol concentration reached 8% (Fig. 5). This may be due to the development of tolerance to the combined central effects of ethanol/CDP, such that the mice could ingest more diet without being more affected by the drugs. We are not sure what caused the reproducible decline in intake of the 3.5% diet in group A (Fig. 5, days 4–6), but it might be related to the fact that the mice showed less preference to ethanol solutions of low concentrations (Fig. 1). This gave rise to the apparent increase in intake on day 7, although the absolute intake was comparable to those at the beginning of the experiment.

Although CDP is known to have other behavioral actions, e.g., antineophobic [6, 20, 27] and hyperphagic [6,30] effect, their relevance to this work is unclear and requires further investigation.

This paper represents the first study dealing with the effects of CDP on alcohol consumption in non-deprived mice. Although the protocols of drug administrations utilized here avoided the nonphysiological effects of the commonly employed 23-hr water deprivation regimen or stimulus cues due

to drug injections, they generated problems such as carry-over effects and inability to control the pattern of drug intake. In summary, the effects of CDP on alcohol consumption vary with the experimental designs.

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