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Chronic Treatment With Ethanol or Chlordiazepoxide Alters the Metabolism of Chlordiazepoxide[1]

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CHAN, A. W. K., D. L. SCHANLEY, M. C. LANGAN, F. W. LEONG AND M. L. PENETRANTE. *Chronic treatment with ethanol or chlordiazepoxide alters the metabolism of chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **35**(2) 363–366, 1990.—Although chronic ethanol administration in C57BL/6J mice did not cause an induction of ethanol metabolism, it altered the metabolism of chlordiazepoxide (CDP). Significantly lower blood levels of CDP, but higher levels of N-desmethyl CDP (NDCDP), were observed in ethanol-dependent mice compared to pair-fed controls during the first hour after CDP injection. Mice treated chronically with CDP showed significantly lower blood levels of CDP and NDCDP than pair-fed controls after a test dose of CDP. In response to an injection of ethanol, the CDP-dependent mice had lower blood alcohol levels (BAL) than the pair-fed controls, but the rate of fall of BAL was not different in the two groups. Thus, chronic CDP treatment affected the absorption and distribution of ethanol. These results provide a metabolic basis for the manifestations of CDP tolerance and ethanol cross-tolerance that have been reported in CDP-dependent mice.

Ethanol Chlordiazepoxide Chronic diet treatment Ethanol metabolism Chlordiazepoxide metabolism

ALTHOUGH chronic ethanol injection can lead to increased rates of ethanol metabolism in a variety of species including man (3, 11, 15), chronic administration of an ethanol liquid diet in mice was found not to affect ethanol elimination rates (1, 10, 16). Thus, the tolerance to ethanol observed after such chronic ethanol treatment in mice was largely functional tolerance (7). On the other hand, the effect of chronic ethanol intake on the metabolism of benzodiazepines (BZD), such as chlordiazepoxide (CDP), has not been investigated. This information would be useful in the interpretation of data concerning cross-tolerance to CDP in ethanol-dependent mice. The present study examines the effects of chronic ethanol diet treatment on the elimination of CDP and its major metabolite N-desmethyl CDP (NDCDP), as well as the effects of chronic CDP diet treatment on the elimination of ethanol, CDP and NDCDP. All BZD are biotransformed in the liver by microsomal mixed-function oxidase (17). Although BZD can induce hepatic drug metabolizing enzymes in animals (2), multiple dosage treatment with BZD in man caused neither induction nor inhibition of BZD metabolism (13). However, it has been suggested that the unusually high serum levels of N-desmethyldiazepam in a small percentage of patients treated with diazepam could be due to enzymatic induction and/or extensive metabolizer phenotypes (4).

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-hr light/dark cycle and received Teklad mouse diet (Teklad Mills, Winfield, IA) and tap water ad lib for 7–14 days before the beginning of an experiment. For the experiments described below, each treatment group had 10–12 mice.

Materials

CDP-hydrochloride, NDCDP, and diazepam 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. (Shelbyville, KY) and vitamin diet fortification mixture was from Nutritional Biochemicals (Cleveland, OH).

Ethanol Diet Administration

Mice were fed an ethanol liquid diet for 15 days as described

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previously (8). The ethanol concentrations were gradually increased from 3.5% to 8% (v/v). The average daily ethanol intake ranged from 15 to 24 g/kg/day. As reported previously (8,10), these mice showed withdrawal signs upon removal of the ethanol diet. Control mice were pair-fed an isocaloric diet with sucrose substituting for ethanol.

CDP Diet Administration

Mice were fed a liquid diet containing no CDP (control diet) for 3 days as the sole source of food and fluid (9). Thereafter, CDP was incorporated in the diet as follows [concentrations (mg/ml) and durations]: 0.6, 3 days; 0.8, 3 days; 1.0, 3 days. From then on the CDP concentration was increased by 0.1 mg/ml daily for 18–25 days. The highest daily CDP intake (near the end of treatment period) was slightly over 1200 mg/kg (9). Control mice were pair-fed the control diet.

Ethanol and CDP Metabolism

On the day of withdrawal of the ethanol or CDP diet, the drug-treated mice were fed ad lib the control diet for 2 days. The respective control mice continued to be pair-fed the control diet. On day 3 of drug withdrawal, the ethanol-dependent mice were injected with CDP (120 mg/kg), and the CDP-dependent mice were injected with either ethanol (3.5 g/kg) or CDP (80 or 120 mg/kg). The respective control mice were treated similarly. Separate batches of mice were used. Day 3 of drug withdrawal was chosen because our previous work on drug tolerance and cross-tolerance was done in this time frame (7), and because there would not have been any residual CDP or NDCDP from the previous CDP diet treatment (6). For mice injected with ethanol, serial blood samples (20 μl) were taken from the tail at hourly intervals for 5 hr. For CDP-injected mice, serial blood samples (50 μl) were taken at ¼, ½, 1, 2 and 3 hr.

Blood ethanol levels were analyzed enzymatically with an Ethanol Kit (Sigma Chemical Co., St. Louis, MO) according to published procedures (10,12). Blood CDP and NDCDP levels were determined by high pressure liquid chromatography according to previously published procedures (5,14). Diazepam was used as an internal standard.

Statistical Analysis

Results were expressed as mean ± S.E. Significance of the difference ($p<0.05$ being significant) was analyzed by ANOVA programs (Version 1.1, Human Systems Dynamics, Northridge, CA) with an Apple IIe computer. A linear regression program was used to analyze blood ethanol levels.

RESULTS

CDP Metabolism After Chronic Ethanol Treatment

The ethanol-dependent mice had significantly lower blood CDP levels at ¼, ½, and 1 hr after CDP injection than pair-fed control mice (Fig. 1A). Estimates of the $t_{½}$ values for the linear portion of each curve (Fig. 1A) were 0.83 hr for the ethanol-dependent mice and 0.52 hr for the control mice. In contrast, blood NDCDP levels were significantly higher in the ethanol-dependent mice than in the pair-fed control mice during the same time intervals (Fig. 1B). The areas under the curve for the data in Fig. 1B were 457 and 402 μg hr/ml, for the ethanol-dependent and control mice, respectively. Thus, chronic ethanol intake caused an induction of CDP metabolism, but not NDCDP metabolism. The

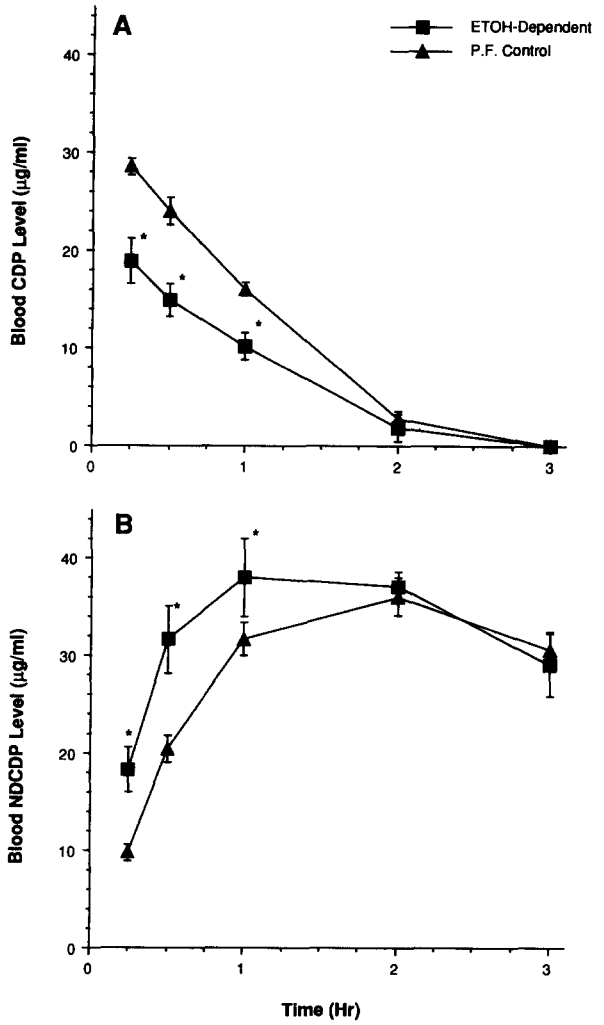

FIG. 1. Blood levels of CDP (panel A) and N-desmethyl CDP (NDCDP; panel B) after CDP injection (120 mg/kg) in ethanol-dependent and pair-fed control mice. *$p<0.005$.

metabolite of NDCDP, namely demoxepam, was not analyzed because our preliminary data indicate that after an acute injection of CDP, demoxepam levels are usually very low or undetectable.

CDP Metabolism After Chronic CDP Treatment

Blood CDP levels were dramatically lower in the CDP-dependent mice after an injection of CDP (80 mg/kg) than in the pair-fed controls (Fig. 2A). In fact, there were no detectable CDP levels in the CDP-dependent mice at ½ hr after CDP injection. Estimated $t_{½}$ values for the data (Fig. 2A) in the CDP-dependent and control mice are 1.1 and 0.18 hr, respectively. Blood NDCDP levels were transiently higher at ¼ hr, but were significantly lower at 1 and 2 hr in the CDP-dependent mice than in the control mice (Fig. 2B), with the area under the curve being much lower in the CDP-dependent mice (69 μg hr/ml) than the control mice (380 μg hr/ml). Thus, chronic CDP treatment caused an induction of metabolism of CDP and NDCDP. Similarly, decreases in blood CDP and NDCDP levels were observed when the dose of CDP was

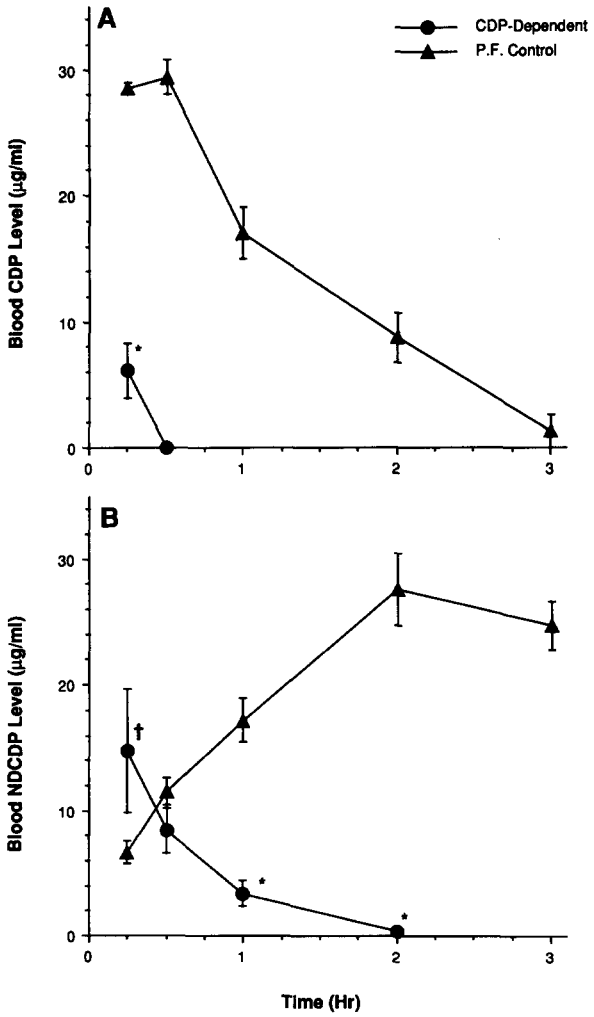

FIG. 2. Blood levels of CDP (panel A) and NDCDP (panel B) after CDP injection (80 mg/kg) in CDP-dependent and pair-fed control mice. †$p<0.05$, *$p<0.001$.

120 mg/kg (data not shown).

Ethanol Metabolism After Chronic CDP Treatment

Lower blood levels were attained in the CDP-dependent mice than in the control mice after an acute injection of ethanol (3.5 g/kg) (Fig. 3). However, a linear regression analysis indicates that the slopes of the two elimination curves are not significantly different, being −78.2 and −74.2 mg/100 ml/hr for the CDP-dependent and control mice, respectively. The extrapolated BAL at time zero are 381.2 mg/100 ml for the CDP-dependent mice and 466.4 mg/100 ml for the control mice ($p<0.001$). The data indicate that the rate of ethanol elimination was not affected by chronic CDP treatment, but the absorption and distribution of ethanol were apparently impaired. The estimated volumes of distribution for the CDP-dependent and control mice were 0.75 and 0.92 l/kg, respectively.

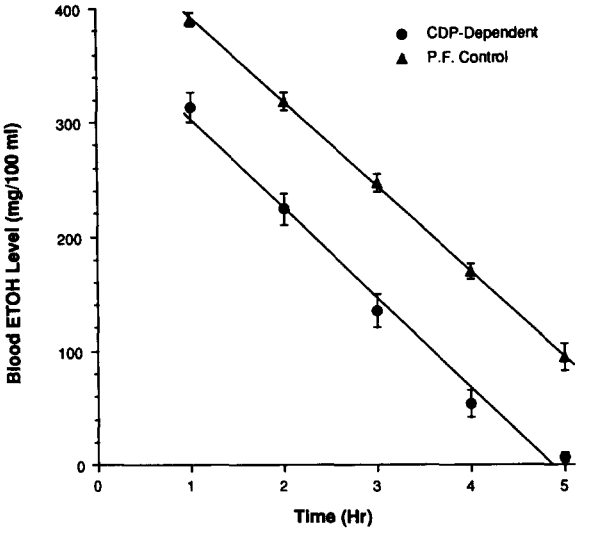

FIG. 3. Blood alcohol levels (BAL) in CDP-dependent and pair-fed control mice after ethanol injection (3.5 g/kg).

DISCUSSION

We have previously concluded (7) that the tolerance to ethanol in mice which had been treated chronically with an ethanol diet was primarily functional in nature. This is based on our data indicating that chronic ethanol treatment did not induce ethanol metabolism in the mice (10). We have also concluded (7) that, because of the short time course of the behavioral tests used to measure tolerance, the cross-tolerance to CDP in ethanol-dependent mice was largely functional in nature. Results of the present study show that ethanol-dependent mice, when given an injection of CDP, had decreased blood levels of CDP, but increased NDCDP levels. Since the pharmacological potencies of CDP and NDCDP, as determined by their abilities to increase ethanol sleep time (5), are rather similar, and since the magnitudes of decreases in blood CDP levels in ethanol-dependent mice closely matched those of increases in blood NDCDP levels (Fig. 1A and B), it is unlikely that metabolic factors played an important role in the cross-tolerance to CDP in these animals. In other words, metabolic tolerance would not be appreciable in the observed cross-tolerance to CDP in ethanol-dependent mice (7).

In CDP-dependent mice injected with CDP, blood levels of CDP and NDCDP were dramatically lower than those in control mice (Fig. 2A and B). An exception was the transiently higher NDCDP level at ¼ hr after CDP injection in the CDP-dependent mice. Therefore, in the future investigation of CDP tolerance in CDP-dependent mice, metabolic tolerance will be an important consideration besides functional tolerance. Likewise, changes in the distribution of ethanol will also contribute significantly to the phenomenon of cross-tolerance to ethanol in CDP-dependent mice. We do not know the mechanism that caused the change in the distribution of ethanol in CDP-dependent mice (Fig. 3). An analogous change in ethanol disposition has been reported in mice which had acquired environmental-dependent tolerance to ethanol, but not in mice that had developed environmental-independent tolerance to ethanol (from liquid diet treatment) (16).

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