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BRIEF COMMUNICATION

The Effects of Cadmium Exposure on Ethanol Pharmacokinetics

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NATION, J. R., R. T. BURKEY, C. A. GROVER AND G. R. BRATTON. *The effects of cadmium exposure on ethanol pharmacokinetics.* PHARMACOL BIOCHEM BEHAV **48**(2) 543-546, 1994.—Twenty-four adult male rats were exposed in the home cage to water containing 100 ppm added cadmium chloride. An additional 24 animals were pair-watered with water containing no added cadmium. Following 60 days of exposure to their respective watering regimens, one third of the animals in each exposure group (N = 8/condition) received IP injections of 1.0, 2.0, or 3.0 g/kg ethanol (20% v/v). Serum alcohol concentrations were measured at 15, 30, 60, 120, 180, 240, and 360 min postinjection. Although serum alcohol concentrations increased with dose for both cadmium-exposed and control animals, there was no indication at any dose of group differences. The lack of differences in ethanol pharmacokinetics reported here is instructive with respect to improving our understanding of the mechanisms underlying cadmium/ethanol interactions.

Cadmium Ethanol Metabolism

PREVIOUS investigations of cadmium/ethanol interactions have shown that exposure to the toxicant increases volitional ethanol intake (12-14), yet responsiveness to acute applications of the drug is diminished among cadmium-treated animals (3,8). Although several factors may contribute to this curious pattern of results, the possibility that cadmium may alter the pharmacological profile of ethanol is especially prominent. Since cadmium is known to preferentially block L-type calcium channels that are implicated in the neurobehavioral effects of ethanol (1,5,11,17), it is plausible that the competitive action of the metal at the membrane site may decrease the drug's pharmacological impact. The resulting hyposensitivity and functional decrease in dose may be expressed as compensatory drinking in the free-access situation or reduced behavioral reactivity when ethanol is administered acutely in the animal model [see (3) for a more detailed discussion of this rationale).

A reasonable alternative to the notion of cadmium-induced changes in the pharmacological potency of ethanol relates to kinetic mechanisms and attendant drug action. Differential patterns of ethanol absorption, distribution, and/or metabolism among cadmium-exposed and control rats may underlie the aforementioned effects. Given the prominent role of metalloenzyme function in ethanol catalysis (16), cadmium may alter hepatic oxidative processes via ionic-based disturbances in alcohol dehydrogenase (ADH) activity and/or drug metabolites (9). Along these lines, we note that the demonstration of cadmium-related changes in ethanol pharmacokinetics would favor what is arguably the simplest and most straightforward interpretation of cadmium/ethanol interactions.

Accordingly, the present experiment examined serum alcohol concentration in separate groups of cadmium-treated and control rats administered 1.0, 2.0, or 3.0 g/kg ethanol (IP). The aim was to clarify the role of cadmium as an ethanol buffer or biochemical antagonist.

METHOD

Animals

The animals used in this study were 48 viral disease-free adult male Sprague-Dawley rats obtained from the Holtzman Company (Madison, WI). The animals arrived at the labora-

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tory ranging in weight from 175 to 199 g and were approximately 50 days old when the experiment began. The animals were stratified by weight and assigned to either group-cadmium (N = 24) or group-control (N = 24) according to a strategy which insured that body weights across groups were equal before the exposure regimen was introduced. Animals in group-cadmium were exposed ad lib in the home cage to distilled water that contained 100 ppm added cadmium chloride and 0.45% (w/v) sodium saccharin. The saccharin amendment was necessary to render the solution more palatable. Group-control animals were exposed in the home cage to distilled water that contained an identical concentration of sodium saccharin with no added cadmium. Animals were pair-watered across groups to insure equivalency in overall fluid intake. Standard laboratory chow (Teklad, Harlan Sprague-Dawley, Madison, WI) was continuously available in the home cage throughout the duration of the experiment.

Procedure

The animals were maintained on a 12-h dark/light cycle throughout the experiment. Animal body weights were recorded weekly and fluid intakes were recorded daily. Experimental operations commenced on day 60 of exposure for each animal, with the respective exposure regimens continuing until 1 min prior to testing.

Animals were tested in squads of four at 0800 or 1300, counterbalancing for group assignment and drug dose. Group-cadmium and group-control rats were subdivided into three dose conditions (N = 8/condition) creating a 2 (Groups) × 3 (Doses) × 7 (Recording Times) design. Animals from group-cadmium and group-control received a single IP injection of 1.0, 2.0, or 3.0 g/kg ethanol at a concentration of 20% (v/v), with saline serving as the vehicle.

The procedures used here were modeled after those reported by Bonthius, Goodlett, and West (2). Serum alcohol concentrations were determined for each animal from tail-blood samples that were taken successively at 15 min, 30 min, 1 h, 2 h, 3 h, and 4 h postinjection. A final reading was taken from trunk blood that was collected following decapitation 6 h after the ethanol injections had been administered.

Immediately following the appropriate IP injection of ethanol each animal was placed in a restraining tube that was perforated to allow for ventilation but rendered the animal immobile. The full length of the tail of each animal protruded from the restraining tube and permitted 100–150-μl samples of whole blood to be drawn from the tip of the tail. This was accomplished by cutting the very tip of the tail, collecting the appropriate blood volume, and then reopening the clot as successive samples were collected over time. Between recordings bleeding was stopped by applying silver nitrate to the tip of the tail. Each sample of blood was collected in 1.5-ml non-heparin-coated microcentrifuge tubes and immediately placed into a centrifuge (centrifuge 5415, Eppendorf [Brinkmann], Westbury, NY), where the samples were spun down for 2 min at 12 000 rpms. A micropipette was then used to extract 10 μl of serum that was placed in cuvets with optical properties suitable for use with a spectrophotometer (Varian Analytical Instruments, series 634) set at 340 nm. Alcohol was determined enzymatically (Sigma Chemical Co., St. Louis, Catalog No. 333-B) using a prepared Alcohol Reagent Solution containing nicotinamide adenine dinucleotide (NAD^+), ADH (yeast), buffer salts, and stabilizers. Reduced NAD (NADH) formation was measured with the spectrophotometer set at 340 nm, and the alcohol concentration in serum was calculated accordingly. A set of ethanol standards (0.05%, 0.10%, 0.30% [w/v]) were run to establish a standard curve and confirm the reliability of the test.

Blood Cadmium Analyses

As indicated above, animals were decapitated 6 h after they received their respective injections of ethanol. In addition to the 100–150-μl samples that were taken for the final serum alcohol concentration reading, 5 ml of trunk blood was collected in heparinized vials and stored at <0°C for later analysis of cadmium residues in blood. Cadmium determinations were made from whole blood via an atomic absorption assay, the procedural details of which have been described elsewhere (12).

RESULTS

Water Intake

The pair-watering procedure produced essentially identical fluid consumption for group-cadmium animals and group-control animals. Over the course of the experiment group-cadmium animals consumed an average of 168.35 ml/kg body weight/day and group-control animals consumed an average of 171.75 ml/kg body weight/day. Thus, differential fluid intake was not an issue in this study. Based on body weights (see below), the exposure regimen used here resulted in an average burden of 10.29 mg cadmium/kg body weight/day.

Body Weight

The analysis of body weight data failed to show any evidence of group separation at any point during the experiment. Both the main effect for groups, $F(1, 46) = .14, p > .10$, and the Groups × Weeks interaction, $F(8, 368) = 2.06, p > .10$, did not reach acceptable levels for statistical significance. A significant main effect for weeks, $F(8, 368) = 1007.45, p < .01$, was obtained, reflecting uniform increased weight gains for both groups across the duration of the study.

Serum Alcohol Concentrations

With respect to the analysis of serum alcohol concentrations there was no indication that group-cadmium animals were different from group-control animals at any dose. At each dose, serum alcohol concentration levels increased sharply after the IP administration of ethanol and then declined over the course of the 6-h recording period. For both groups, a dose-dependent increase in serum alcohol concentration was evident.

The results of this experiment are presented in Fig. 1. Statistical analysis of the serum alcohol concentration readings failed to reveal any evidence of group separation at any dose. Specifically, a 2 (Groups [group-cadmium and group-control]) × 3 (Doses [1.0, 2.0, and 3.0 g/kg]) × 7 (Recording Times [15, 30, 60, 120, 180, 240, and 360 min]) repeated-measures analysis of variance (ANOVA) was performed on the data, with recording times serving as the within factor. Both the main effects for doses, $F(2, 42) = 56.64, p < .01$, and recording times, $F(6, 252) = 15.09, p < .01$, reached acceptable levels for statistical significance. Individual comparisons (Tukey's) of means indicated that the serum alcohol concentration values increased with increasing dose ($ps < .01$) and that ethanol concentration peaked within 60 min and declined thereafter to a low point at 360 min ($ps < .01$). The

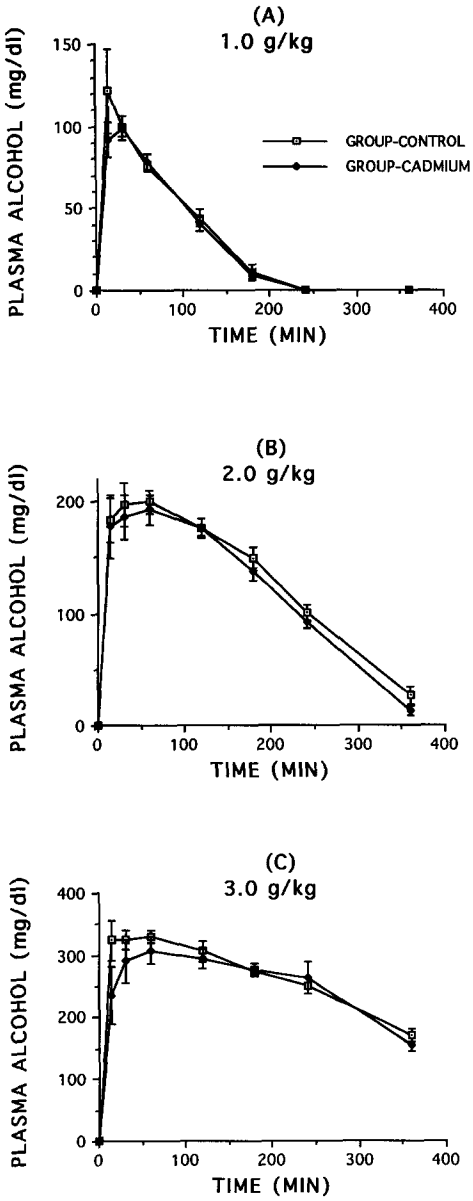

FIG. 1. The serum alcohol concentrations for group-cadmium and group-control animals across the 6-h period that followed a single IP injection of 1.0 (A), 2.0 (B), or 3.0 g/kg ethanol (C).

main effect for groups failed to show significant separation ($p > .10$).

In terms of the interaction findings, only the Doses × Recording Times interaction was found to be significant, $F(12, 252) = 2.45$, $p < .01$. Subsequent individual comparisons of means, collapsed over groups, showed that the declines in serum alcohol concentration readings for animals given a 1.0-g/kg dose were greater over the course of the recording period than those of animals administered a 3.0-g/kg dose. That is, at 360 min the serum alcohol concentrations remained elevated in the highest dose condition, whereas they essentially returned to baseline levels (zero) in the low dose condition.

The lack of a significant main effect for groups coupled with a failure to observe any significant interaction with groups affirms the parallel pattern that is visually apparent in Fig. 1. Indeed, the most striking aspect of the data is the virtual overlap of serum alcohol concentration readings for group-cadmium and group-control animals at each of the three ethanol doses used here.

Blood Cadmium Residues

The analysis of cadmium residues in blood indicated that the concentration of cadmium in trunk blood was significantly greater for group-cadmium animals ($\bar{X} = 0.142$ ppm) relative to group-control animals ($\bar{X} = 0.004$ ppm), $t(19) = 5.21$, $p < .01$. This analysis, which assumed unequal variances, simply confirmed that the exposure regimen was effective in terms of creating greater body burdens of cadmium among treated animals.

DISCUSSION

The results of this investigation failed to reveal any differences between cadmium-exposed and control animals with respect to serum alcohol concentration following acute administration of ethanol. Specifically, adult male rats presented with 100 ppm added cadmium chloride in the drinking water for 60 days, and their control counterparts, showed parallel postinjection serum alcohol concentration patterns following IP administration of 1.0, 2.0, or 3.0 g/kg ethanol (20% v/v). Although there was no indication of group separation at any point during the experiment, dose-dependent increases in serum alcohol concentration were observed, and there was evidence that ethanol clearance was slower to occur with higher doses. Finally, treated and control animals were not substantially different in overall fluid intake or body weight.

The absence of group differences in serum alcohol concentration readings in this study is exceedingly important with respect to interpretive issues relating to cadmium/ethanol interactions. As noted, chronic cadmium exposure is associated with increased voluntary consumption of a 10% ethanol solution (12–14), yet the toxicant lowers an animal's sensitivity to acute administrations of the drug across a variety of experimental settings (3,6,8,15). For treated animals, it is possible that ethanol may occasion less physiological and psychological change than would be expected normally. The resulting attenuated impact of the drug may induce elevated drinking then, since greater quantities are needed before detectable alterations in the neurobehavioral status of the animal are experienced. In this regard, environmental contamination may serve as an antecedent to excessive alcohol consumption by compromising drug efficacy.

At least for the cadmium/ethanol protocol defined here and in previous investigations (3,8,12–15), metal-based disturbances in ethanol pharmacokinetics do not seem to be an issue. Although extracellular concentrations of ethanol at the neural membrane site may be affected by local disturbances in ethanol-related mechanisms, it is clear from the present experiment that cadmium is not producing its effect on ethanol by disrupting overall metabolic function.

Future efforts in this area might focus on possible neurochemical substrates for the observed cadmium/ethanol interaction effects. Inasmuch as the behavioral array exhibited by cadmium-treated animals given ethanol mirrors tolerance effects to the drug, some consideration must be given to those mechanisms that mutually define these phenomena. Noteworthy is the fact that ethanol tolerance is associated with the proliferation of dihydropyridine binding sites (4). Since such

sites play a modulatory role in L-type calcium channel activity integral to ethanol effects (4,7,11), the demonstrated antagonism of these channels by cadmium takes on added meaning (1,17). It may be that cadmium-related disturbances in intracellular and/or extracellular calcium dynamics produce a functional tolerance to ethanol similar to that produced by recurrent drug administration.

Whatever the mechanism, the environmental ubiquity and increased burden of cadmium residues in humans creates concerns for the health industry in general (10,18) and for at-risk alcoholic populations in particular. At present, hardly any data on cadmium/ethanol interactions at the human level are available. Based on the animal model, perhaps the fields of human toxicology and pharmacology would profit from such information.

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