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CIRCADIAN RHYTHMS OF ALCOHOL DEHYDROGENASE AND MEOS IN THE RAT[1,2]

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Abstract. The circadian peak in alcohol dehydrogenase (ADH) activity fell near the time of maximal blood ethanol clearance rates both in groups of rats injected with a single ethanol dose (acute group) and in rats continuously exposed to ethanol for 22 weeks (chronic group). However, at all timepoints investigated ADH activity levels were lower and fluctuated less in the chronic group than in either the acute or control (ethanol naive) groups. In contrast, activity levels of the microsomal ethanol oxidizing system (MEOS) revealed a prominent rhythm that was 180° out of phase with the ADH rhythm in the chronic group, while MEOS activity showed very low levels in the acute and control groups and did not vary over the circadian span.

Circadian variation in ethanol metabolic rate has been established for the rat (1-4) and for man (5,6). However, possible mechanisms underlying this chronovariation in pharmacokinetic response have not been investigated to date. The present report presents the results of a preliminary mechanistic study in which the activity changes of hepatic alcohol dehydrogenase (ADH) and the microsomal ethanol oxidizing system (MEOS) were assessed over the 24-hr span in groups of rats singly or chronically exposed to ethanol.

Materials and Methods. Three groups of male rats (Charles River Breeding Labs, stock COBS(SD)BR, n=24) 62-70g initially, were housed in environmental chambers maintained on a 12 hr: 12 hr light-dark schedule (light 0800 - 2000 hr). Food (rat chow) and drinking fluid were continuously available for all animals. For 1 group (chronic rats) the drinking fluid was 15% ethanol (v/v) sweetened with 0.05% aspartame[1]. For the other 2 groups (acute and control rats), drinking fluid consisted of 0.05% aspartame in tap water. Fluid consumption was recorded daily.

After 22 weeks each of the 3 groups was divided into 4 subgroups (n=6) to determine hepatic enzyme levels at 4 equally spaced circadian timepoints (1 hr after the onset of light and dark phases and the 2 midpoints between them). Each rat in the acute subgroups was injected with 1.5 g/kg ethanol (i.p.) 3½ hr before sacrifice. Blood ethanol concentrations were determined by gas chromatographic analysis of 7 tail vein blood samples taken at 20 min intervals beginning 60 min after dosing. Blood ethanol levels of the chronic group were measured at the time of sacrifice. The control group remained ethanol naive.

At 0900, 1500, 2100 and 0300 hr, 1 subgroup from each of the 3 groups was decapitated. Livers were quickly removed, rinsed, blotted dry, chilled and weighed. Approximately 1 g of liver

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was homogenized in 9 ml of 0.15M KCl and centrifuged at 9000 x g for 30 min at 4°C. ADH levels were determined on this supernatant fraction according to the method of Anderson et al. (7). The remainder of the supernatant fluid was centrifuged at 100,000 x g for 60 min. The resultant microsomal pellet was washed, resuspended in KCl and the activity level of MEOS determined by using the method of Lieber and DeCarli (8). Protein levels were determined by the method of Lowry et al. (9). All assays were performed in duplicate.

Results. ADH activity levels, expressed as nmol substrate oxidized/min/mg protein of the 9000 x g fraction, are shown in Fig. 1. In all 3 groups, peak levels were noted at 0300, with minimal levels at 1500 hr. Although enzyme activity levels in the acute and control groups did not differ appreciably from each other, they were considerably decreased (22-55%) in the chronic group. Mean 24-hr values (±SE) for the acute, chronic and control groups were 8.46 ± 0.80, 4.8 ± 0.32 and 7.85 ± 1.01, respectively. Similar curves were found when ADH levels were calculated per minute/g liver or per Kg body weight. In all cases a significant circadian variation was seen in ADH levels for acute ($p<0.05$) and control ($p<0.01$) groups. Although this was not the case for the chronic group, a Student's t-test showed a difference ($p<0.05$) between the 1500 and 0300 hr determinations in these rats.

MEOS activity levels, assessed as nmol acetaldehyde formed/min/mg protein, are shown in Fig. 2. The low levels seen in both the acute and control groups showed no significant change over the 24-hr span. In contrast, MEOS activity levels in the chronic group were approximately 5-fold greater at 1500 than at 0300 hr. It is apparent that the circadian rhythms of the 2 enzyme systems were 180° out of phase with each other.

Blood ethanol clearance rates of the acute group ranged from 5.56 ± 0.76 mg/ml/min for the subgroup injected with ethanol late in the light phase to 9.32 ± 0.11 mg/ml/min for the subgroup injected late in the dark phase. Blood ethanol concentrations in the chronic group (as measured at the time of sacrifice) ranged from 1.8 mg/ml early in the light phase to 0.2 mg/ml early in the dark phase of the LD schedule.

The volume of daily fluid consumption of the control-acute rats was approximately twice that of the chronic (ethanol) rats. At the end of the study the mean body weight of the former group was 21% greater than that of the latter (mean ± S.E. 652 ± 2.3g, 518 ± 7g, respectively).

Discussion. In the ethanol naive (control) and singly dosed (acute) groups, ADH peak activity levels fell near the circadian phase of maximal blood ethanol clearance rates (1-3). The chronically dosed rats showed suppressed ADH levels, which varied little over the 24-hr span. MEOS activity levels, however, did vary in a circadian fashion. This cannot be attributed to alterations in the normal 24-hr pattern of eating and drinking episodes, since the greatest food and fluid intake occurred during the dark hours for all groups. Blood ethanol levels in the chronic group at the time of sacrifice provided additional evidence that a normal circadian drinking pattern was maintained in this group. Clearly, the long-term continuous exposure to ethanol had altered not only the metabolic processes by which hepatic degradation of ethanol occurred, but also the circadian timing of the reactions.

Soliman et al. (10) reported a nocturnal peak in hepatic ADH but a diurnal peak in brain ADH in groups of (presumably) ethanol-naive rats. Although North et al. (11) detected circadian variation in mouse liver ADH (as well as in 12 other hepatic enzymes), Deimling and Schnell (12) found that the ADH activity levels were invariant over the 24-hr span in their mice, as was the rate of disappearance of ethanol from the blood. Others (13) have also reported a lack of chronovariation in blood ethanol decay rates in mice. These findings suggest that the laboratory rat is a better model than the mouse for investigations of the mechanisms subserving the chronopharmacokinetics of ethanol and the changes effected by long-term exposure.

A recent review of the literature (14) noted a disparity in the effects of chronic ethanol ingestion on ADH

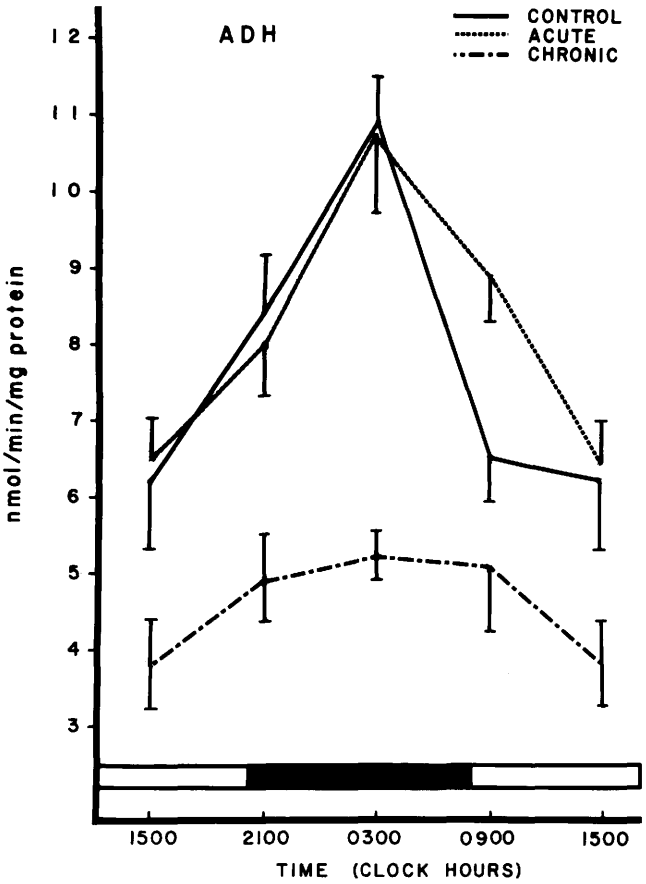

Fig. 1. Circadian variation in hepatic ADH activity levels assessed indirectly by monitoring the rate of NADH formation. The ordinate indicates nmol substrate (9000 x g hepatic supernatant fraction) oxidized per minute per mg protein of that fraction. Clock hours of light and darkness as indicated by the bar along the abscissa. Vertical bars indicate 1 SE. n=6 rats per group per timepoint.

activity levels. The levels were reported as increased, decreased or unchanged in various studies. One possible explanation for this confusion might be related to the time of day of sample collection. As shown in Fig. 1, had ADH levels been measured only in the morning (in accordance with the usual laboratory work schedule), little or no significant difference would have been seen between the chronic and control groups. While a single liver sample obtained near the middle of the dark period would have revealed considerably decreased ADH levels in the chronic group as compared to the control and acute groups (Fig. 1), it would not have shown the notable increase in MEOS levels effected by chronic ethanol ingestion (Fig. 2). Only a mid-afternoon sample would have shown this relationship. In any case, no single enzyme determination, regardless of the time of day of sampling, could have revealed the chronovariation in activity levels. Clearly, failure to consider circadian variation in biological variables may lead to gross errors in interpretation of experimental data and may have played a role in confusion of results as reported in the literature.

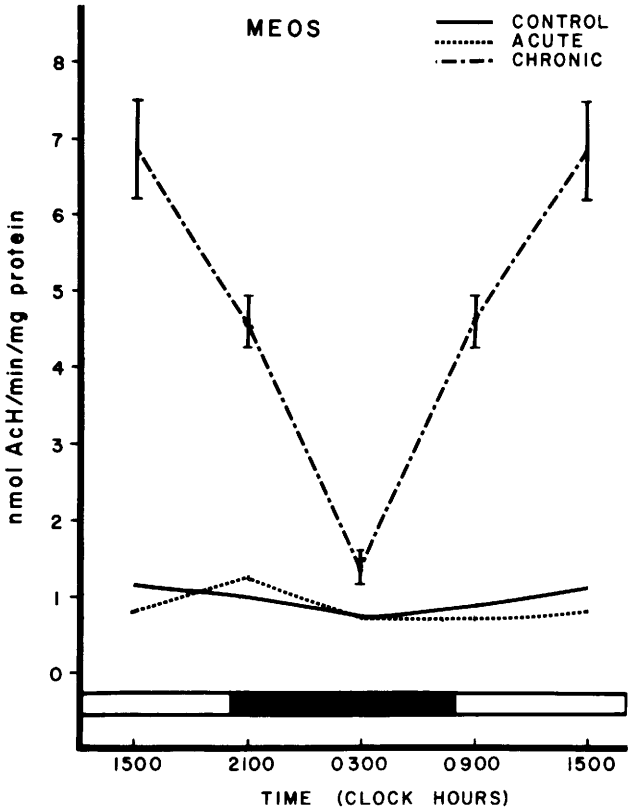

Fig. 2. Circadian variation in hepatic MEOS activity levels estimated by determinating nmol acetaldehyde formed per min per mg protein on the 100,000 x g supernatant fraction. Details as in Fig. 1.

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