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Corticosterone concentrations in mice during ethanol drinking and withdrawal

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Consumption of an ethanol-containing diet by mice resulted in a significant increase in circulating concentrations of corticosterone which was maintained for 8 days. There were no changes in the concentrations of plasma corticosterone binding globulin. Ethanol withdrawal symptoms followed the removal of ethanol from the diet and circulating corticosterone concentrations were further increased. There was no correlation between blood ethanol and glucocorticoid concentrations during the chronic ethanol treatment. Stress related to ethanol consumption may be of greater importance than the circulating ethanol concentrations in producing the elevation in plasma glucocorticoids.

To our knowledge there have been no reports in which circulating glucocorticoid concentrations were compared with blood ethanol concentrations in animals during a period of chronic consumption of ethanol nor have measurements of corticosterone binding globulin (CBG) concentrations been made at the times when the glucocorticoids were measured. Biologically effective concentrations of glucocorticoids are dependent on the equilibrium between the free hormone and that bound to CBG in plasma (Goldstein & Motulsky, 1974), and chronic ingestion of ethanol may alter CBG concentrations by altering liver protein synthesis (Baroana & Lieber, 1976). We have therefore set out to ascertain whether, throughout a period of chronic ethanol intake in mice, 1) high concentrations of circulating glucocorticoids remained evident, 2) changes in the circulating concentration of plasma corticosterone binding globulin (CBG) occurred, 3) the total corticosterone concentration correlated with blood ethanol concentrations, and 4) alterations in the diurnal pattern of corticosterone concentrations, such as noted by Kakihana & Moore (1976), were evident throughout. Previous methods of administration of ethanol (Ellis, 1966; Kakihana, Butte & others, 1971; Kakihana & Moore, 1976) in experiments in which glucocorticoid concentrations were measured in animals would not have been expected to produce physical dependence (Goldstein, 1975). In the present experiments we chose a method which would result in the development of tolerance to and physical dependence on ethanol (Goldstein, 1975; Ritzmann & Tabakoff, 1976) and we also monitored the glucocorticoid concentrations during and after the withdrawal syndrome.

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MATERIALS AND METHODS

Corticosterone and florisil (magnesium silicate) were obtained from Sigma Chemical Company; [1,2-^{3}H]-corticosterone (54·5 Ci m mol^{-1}) was obtained from New England Nuclear, and male rhesus monkey plasma was obtained from Pel-Freeze Biologicals, Inc.

Male C57B1/6 mice (22–24 g) (ARS/Sprague Dawley) were housed, before use for at least seven days at 23 $\pm$ 1° with 7·00–19·00 light cycle and had free access to Purina Lab Chow and water. On the first day of the experiment mice were divided into two groups; both received a liquid diet consisting of sucrose (96·8 g litre^{-1}), Vitamin Supplement (ICN Pharmaceuticals, Inc., 3 g litre^{-1}) and Carnation Slender. On the second day, ethanol (59·6 g litre^{-1}) was substituted for the sucrose in one group's diet; the diet of the control animals was unchanged but was restricted so that consumption matched the mean amount of the ethanol-containing diet consumed. This restriction resulted in corticosterone concentrations which were no different from those found in animals feeding freely on the sucrose containing diet. The animals continued on their respective diets for eight days, and on the morning of the ninth day all animals again received the sucrose-containing diet (withdrawal). Body weight was recorded immediately before and daily during the experiment and plasma corticosterone and blood ethanol concentrations were measured each day between 8.30–9.30 and 17.00–18.00 h. Tail blood (50 μl) from three or four animals of each group on each occasion was collected within 2 min of initial handling. Plasma was prepared by centrifugation and 10 μl used for corticosterone determination by a competitive binding assay using male rhesus monkey plasma as the source of binding protein (Murphy, 1967). To control for carryover of

stress-induced increases in glucocorticoid concentrations, no animal was used for consecutive samplings, and individual animals were not sampled more than four times during the experiment. The dissociation constant and the number of CBG binding sites were determined by a modification of the method of Pegg & Keane (1969). Ethanol concentrations in whole blood were determined by a gas chromatographic procedure (Tabakoff, Anderson & Ritzmann, 1976). The concentrations of corticosterone and ethanol are expressed throughout as the mean $\pm$ s.e.m.

The animals' behaviour during the period following ethanol withdrawal was scored as previously described (Ritzmann & Tabakoff, 1976). Rectal temperature was also monitored in some animals since Ritzmann & Tabakoff (1976) have shown that the severity and time course of withdrawal in mice can be assessed by monitoring withdrawal hypothermia. Animals in which body temperature was measured were not used subsequently for determination of plasma corticosterone concentrations.

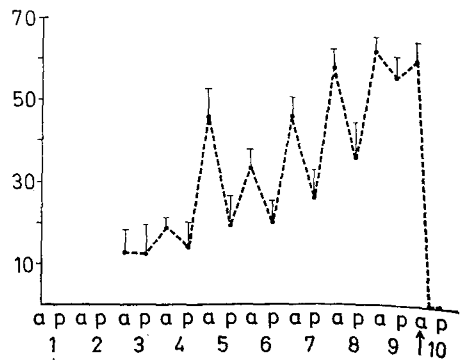

FIG. 1. Blood ethanol concentrations during chronic ethanol intake by mice. Mice were acclimated to the sucrose-containing (control) liquid diet during the first day of the experiment. On the evening of the second day, the ethanol-containing liquid diet was substituted for the control diet and mice were fed the ethanol-containing diet until the morning of the tenth day. At this time, the mice were again given the sucrose-containing control diet. The time of withdrawal of the ethanol diet is marked by an arrow. The blood ethanol concentrations (μmol ml^{-1}) (ordinate) are expressed as the mean $\pm$ s.e.m. Values are from 6–10 animals at each time. Abscissa: Time (days) of experiment. a: a.m. p: p.m.

RESULTS

The amount of ethanol-containing diet consumed daily by mice was 10·5 $\pm$ 0·9 ml. This is equivalent to an ethanol dose of 26 g kg^{-1} day^{-1} by a 24 g mouse. The ethanol-fed mice lost an average of 14% of their starting body weight during the eight days of drinking while control mice lost 10%. Mean blood ethanol concentrations ranged from 14·5 to 48·4 μ mol ml^{-1} during the third to ninth day of the experiment (Fig. 1). Evening blood ethanol concentrations were lower than the morning concentrations. The morning ethanol values increased during the course of the experiment.

After withdrawal of ethanol, its concentration in the blood decreased rapidly and was not detectable within 5 h at which time behavioural symptoms of withdrawal and hypothermia were evident: of 10 animals, 3 were in category 1; 2, in category 2; and 5, in category 3, and the mean temperature decrease was 2·1 $\pm$ 0·3° (n = 10). Twenty-four h after withdrawal, neither parameter differed significantly from control values.

Control animals had morning corticosterone concentrations which were significantly lower than the concentrations in the evening (t = 12·8, df = 128, P <0·001, t-test). The cycle was maintained throughout the 12 days the mice consumed the sucrose-containing diet (Fig. 2).

Differences between morning and evening corticosterone concentrations were less regular in the mice consuming ethanol. Over the period of ethanol consumption mean plasma corticosterone concentrations were lower in the morning than in the evening (t = 3·1, df = 53, P <0·01) (Fig. 2), but the mean morning values on certain days were higher than mean evening values. The mean morning values from the third to ninth days (Fig. 2) were

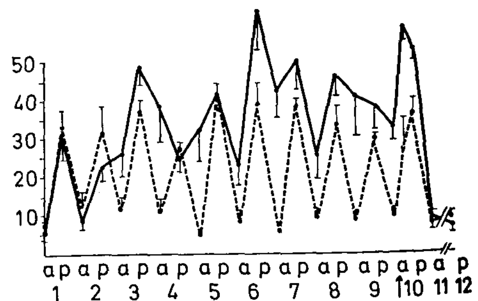

FIG. 2. Plasma corticosterone concentrations in control and ethanol-drinking mice. Control mice received the sucrose-containing liquid diet throughout the experiment while the diet regimen for the ethanol-drinking mice was as described in the text and Fig. 1. The arrow indicates the time of withdrawal of ethanol from the diet of the ethanol-fed mice. Corticosterone concentrations (μg/100 ml) (ordinate) were determined as described and the results are expressed as the mean $\pm$ s.e.m. Values at each time were from 6–10 animals. (●——● ethanol-drinking group; ● - - - - ● control group.) Abscissa: Time (days) of experiment. a: a.m. p: p.m.

consistently and significantly (t = 7·8, df = 89, $P < 0{\cdot}01$, *t*-test) higher than the control values; over the period of ethanol feeding the value was 29·1 ± 3·5 μg/100 ml (n = 28), while the control value was 8·7 ± 1·0 μg/100 ml (n = 63). The evening corticosterone values of the ethanol group were also significantly higher than the control values (t = 2·6, df = 92, $P < 0{\cdot}01$, *t*-test). There was no correlation between the morning blood ethanol and plasma corticosterone concentrations when these were simultaneously determined (r = 0·37). The data in Table 1 indicate that the chronic ingestion of ethanol, or its withdrawal, has no significant effect on the dissociation constant or binding capacity of plasma CBG for corticosterone compared with control values.

Table 1. *Morning blood corticosterone concentrations and characteristics of corticosterone binding globulin (CBG).*

Day*	Group	Corticosterone (μg/100 ml)	CBG	
			$K_D \times 10^{-9}$ M	$N \times 10^{-6}$ M
1	Control (4)	14·7 ± 4·5	3·2 ± 0·4	0·9 ± 0·1
	Ethanol (4)	8·8 ± 2·5	2·2 ± 0·2	1·0 ± 0·2
5	Control (4)	8·9 ± 3·3	2·5 ± 0·3	1·4 ± 0·2
	Ethanol (4)	26·7 ± 7·5	2·5 ± 0·1	1·3 ± 0·1
	Control (4)	2·7 ± 1·2	2·7 ± 0·4	1·4 ± 0·2
	Ethanol (4)	57·4 ± 8·1	5·5 ± 2·5	1·5 ± 0·3
10**	Control (4)	14·4 ± 1·6	3·2 ± 0·6	1·6 ± 0·1
	Ethanol (4)	61·5 ± 8·2	5·7 ± 1·2	1·6 ± 0·2

* Mice were acclimated to the liquid diet, fed ethanol and withdrawn from ethanol diet as described in Fig. 1. Values are the mean ± s.e.m.

** Values obtained 6 h after withdrawal of animals from ethanol-containing diets.

The withdrawal of ethanol from the diet and the decrease in circulating ethanol concentrations (Fig. 1) resulted in an increase in circulating corticosterone during a time which coincided well with the peak behavioural symptoms of withdrawal and withdrawal hypothermia. Thus, on the day of withdrawal, a peak in corticosterone values was noted at 13.00 h which was significantly higher than the control (t = 3·2, df = 12, $P < 0{\cdot}01$). On the first and second days after withdrawal of ethanol, the morning plasma corticosterone concentrations were similar to control concentrations (Fig. 2).

DISCUSSION

The chronic intake of ethanol by mice resulted in a significant rise in plasma corticosterone concentrations both morning and evening with the greater increase being in the morning values. The effects of continued consumption differed from those obtained by Kakihana & Moore (1976) who found that while morning corticosterone values were higher in DBA/2J mice consuming ethanol in their drinking water compared with those not drinking ethanol; there was no rise in the evening. However blood ethanol concentrations after intake in drinking water (Kakihana & Moore, 1976) were much lower than those obtained with the ethanol-containing liquid diet (Fig. 1). The higher blood ethanol concentrations as well as strain differences may have been responsible for the high plasma corticosterone concentrations maintained throughout the day in our animals. Since there was little change in plasma CBG between treated and control mice (Table 1), the higher plasma corticosterone concentrations in the treated mice could be expected to provide sufficient hormone for combination with cellular receptor proteins (Feldman, Funder & Edelman, 1972).

We found no correlation between morning plasma corticosterone and blood ethanol concentrations in mice. However changes in plasma glucocorticoid values may not correspond quantitatively with blood ethanol at a particular time but may be dependent on the rate of change of blood ethanol (Czaja & Kalant, 1961). Our results indicate that little tolerance develops to this effect of ethanol through the eight-day feeding cycle. Our previous studies have, however, indicated that tolerance to the behavioural effects of ethanol develops in five days under this feeding schedule (Ritzmann & Tabakoff, 1976).

The daily rhythm of circulating glucocorticoids may be influenced by the pattern of daily food intake (Krieger, 1974). The results of Freund (1970) as well as those from our laboratory (Ritzmann & Tabakoff, 1976) have demonstrated that the intake of the liquid diet containing ethanol is distributed evenly throughout the day while control animals consumed their diet primarily during the dark period. Although such feeding differences may result in differences in the normal circadian pattern of adrenocortical function, they do not explain the sustained high concentrations of circulating corticosterone found in the ethanol-consuming animals. Krieger (1974) demonstrated that restrictions in food intake did not affect the absolute concentrations of circulating glucocorticoids during the peak or trough phases.

We found that when ethanol was withdrawn and had declined to undetectable concentrations in blood, a rise in plasma corticosterone was noted which did not follow the usual daily pattern of change in corticosterone seen in controls (Fig. 2). Since this peak in plasma corticosterone in the

ethanol-withdrawn animals corresponded to the peak in behavioural and physiologic symptoms of the withdrawal syndrome, it is probable that the rise in plasma glucocorticoids was related to the symptoms associated with the withdrawal syndrome. Twenty-four h after withdrawal of ethanol, when the behaviour and body temperature had returned to normal, plasma corticosterone values were no different from those in controls indicating that the changes in circulating glucocorticoid concentrations produced by chronic ingestion of ethanol and its withdrawal are reversible and rapidly normalized.

The increases in plasma corticosterone concentrations during ethanol ingestion may reflect the direct effects of ethanol on various tissues, including the cns, and such increases of circulating glucocorticoids may in turn contribute to the homeostatic alterations which develop in response to chronic ethanol intake. Glucocorticoids may serve a "permissive" function as suggested by Sze, Yanai & Ginsberg (1974) for the development of physical dependence on ethanol and metabolic (Sze, 1975) or cns tolerance to (Wood, 1977) ethanol. 'Permissive' is used to indicate that the hormone does not by itself initiate the effect but its presence is necessary if the response to ethanol is to occur.

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