EFFECTS OF ALCOHOL ON THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN THE MALE RAT[1]

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ABSTRACT

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The effects of acute and chronic alcohol administration on serum testosterone and luteinizing hormone (LH) levels were examined in the male rat. Chronic alcohol administration resulted in depressed serum testosterone and LH levels when alcohol-fed rats were compared with rats maintained, *ad libitum*, on rat chow and water. However, neither testosterone nor LH levels were significantly lower in alcohol-treated rats when comparisons were made to pair-fed control animals, indicating that the nutritional deficits imposed by the chronic alcohol-feeding regimen contributed heavily to the observed reductions in the two hormones. To avoid the problems associated with a chronic drug delivery model, we injected rats with a single acute injection of alcohol. LH levels dropped significantly within 2 hours after the injection of a 2.5 g/kg dose of alcohol and remained depressed, at a level between 25 and 30% of control values, from 2 to 4 hours. By 6 hours after the injection, LH levels had returned to base-line levels. Testosterone levels were also reduced by alcohol, but this drop was not significant until at least 3 hours after the injection. Testosterone levels did not return to control levels throughout the 6-hour course of the experiment. Dose-response determinations revealed that alcohol produced a biphasic effect on serum testosterone and LH: low doses of alcohol significantly increased testosterone and LH, whereas high doses decreased the levels of both hormones. The results of these studies suggest that the ability of alcohol to depress serum testosterone levels, and thus produce symptoms of hypogonadism in the male of several species, is due to a primary effect of alcohol on the hypothalamic-pituitary aspect of the hypothalamic-pituitary-gonadal axis.

Chronic exposure to alcohol markedly disrupts the function of the hypothalamic-pituitary-gonadal axis in several species. Perhaps the most pronounced effect is on the biosynthesis, release and metabolism of testosterone. For example, it has been repeatedly shown that male alcoholics have many clinical manifestations of hypogonadism, such as gynecomastia, testicular atrophy and low serum testosterone levels (Lloyd and Williams, 1948; Fahim *et al.*, 1970; Galvao-Teles *et al.*, 1973; Southren *et al.*, 1973; Mendelson and Mello, 1974; Van Thiel *et al.*, 1974; Gordon *et al.*, 1975). In addition,

Rubin *et al.* (1976) have recently shown that chronic alcoholics have substantially elevated hepatic activities of 5-α-reductase, an enzyme involved in the metabolism of testosterone. Many of these alcohol-induced alterations in reproductive endocrinology have also been observed in animals (Kieffer and Ketchel, 1970; Badr and Bartke, 1974; Symons and Marks, 1975; Van Thiel *et al.*, 1975). Consequently, it appears that alcohol exerts important effects on the regulation of testosterone levels in a wide range of species.

The primary issue raised in the foregoing studies is whether the effect of alcohol on testosterone represents a primary effect on the gonads or liver or whether this effect is secondary to a more direct action on the hypothalamic-pituitary-axis. The data regarding this issue at present are equivocal. For example, in man luteinizing hormone (LH) levels have been reported to be slightly elevated, unaltered or slightly decreased in chronic alcoholics (*e.g.*, Galvao-Teles *et al.*, 1973; Van Thiel *et al.*, 1974; Gordon *et al.*, 1975). Since no consistent or prominent effect of alcohol on LH levels has been observed, it has been suggested by most investigators that alcohol exerts its effects chiefly on peripheral testosterone release and/ or metabolism. However, this conclusion must be interpreted cautiously since, for the most part, only the chronic alcoholic has been examined. As a model to study drug-specific effects, chronic alcoholism has many limitations, not the least of which is the intrusion of a number of confounding variables, such as poor nutritional status and concurrent liver damage. Consequently, it would be unwise to conclude on the basis of the limited data available that alcohol exerts a specific effect on the hypothalamic-pituitary-gonadal axis or that its effects are restricted to either the peripheral or central aspects of this system.

The purpose of the studies described in this paper was to systematically examine the effects of alcohol on the hypothalamic-pituitary-gonadal axis in the male rat. To circumvent the problems associated with a chronic drug delivery system, we have examined the effects of a single, acute injection of alcohol on serum LH and testosterone levels in the adult male rat, particularly with regard to time-response and dose-response relationships. We have also examined the effects of chronic alcohol administration, using a liquid diet reported to be nutritionally adequate (Freund, 1969; Freund and Walker, 1971), on these same hormones.

Methods

Experimental procedure. Male Sprague-Dawley derived rats, 60 to 75 days of age, were used in all experiments. The rats were housed in groups of three under a 12-hour light-dark cycle. In each experiment, rats were injected intraperitoneally with sufficient amounts of a 25% (v/v) solution of alcohol to yield the grams per kilogram doses specified below. Control rats were injected with distilled water only. In the time-response study, groups of rats ($N = 8$) were injected with alcohol or water intraperitoneally and were then killed at 1, 2, 3, 4, 5 or 6 hours after the injection. The time of injection was staggered to insure that the rats were always killed between the hours of 3 and 5 P.M. in order to minimize any cyclic changes in serum LH or testosterone levels (Cicero *et al.*, 1976b). In the dose-response studies, rats were injected with various doses of alcohol or water and were then killed 3 hours (for LH) or 4 hours (for testosterone) later. Again, the rats were always killed between 3 and 5 P.M. The rats were killed by decapitation, the blood was collected from the trunk and was allowed to clot at 0–4°C for 3 to 4 hours. The bloods were then centrifuged at 1600 × *g* for 30 minutes, and the sera were collected and frozen at −20°C until the radioimmunoassays were performed.

Alcohol liquid diet regimen. The alcohol liquid diet regimen employed was similar to that previously described (Freund, 1969; Freund and Walker, 1971; Hunter *et al.*, 1973). The food intake of the rats was limited each day until they had been reduced to 80% of their initial body weight and they were then divided into four groups: lab chow control (group 1); liquid diet control (group 2); ethanol liquid diet (group 3); and pair-fed control (group 4). The following procedure was employed for each group. Group 1 rats ($N = 10$) were maintained on Purina rat chow and water *ad libitum* throughout the 20-day experimental period. Group 2 rats ($N = 10$) were given a Metrecal-Shape liquid diet *ad libitum* for 20 days. The composition of the diet has been fully described elsewhere (Freund, 1969; Freund and Walker, 1971). Group 3 rats ($N = 10$) were maintained on the same liquid diet as group 2, except that the calories derived from sucrose were replaced with ethanol on the following schedule. Days 1 to 5: 37% of the calories in the diet were derived from alcohol; days 6 to 10: 38%; days 11 to 15: 39%; and days 16 to 20: 40% of the calories were derived from alcohol. Group 4 rats ($N = 10$) were pair-fed to group 3. On each day the group 4 rats were allocated the liquid control diet at the mean level consumed by group 3 animals on the preceding day. At the end of the 20-day pe-

riod, the rats were decapitated and their blood was collected. In half of the animals, LH and testosterone levels were determined as described below, whereas in the other half, blood chemistries consisting of alkaline phosphatase, total protein, blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT), bilirubin and creatinine were obtained.

Blood alcohol levels. Blood was collected from the tail vein of each animal in the alcohol-fed group (group 3) the day prior to an increase in ethanol-derived calories in the liquid diet. Blood was collected at 8:30 P.M. to correspond to the time after the greatest amount of alcohol had been consumed. Blood alcohol levels were determined by gas chromatography as described elsewhere (Perez *et al.*, 1971).

Radioimmunoassay of luteinizing hormone and testosterone. Testosterone levels were determined in serum by a sensitive and specific radioimmunoassay which has been described elsewhere (Cicero *et al.*, 1974). For the LH determinations, the double antibody radioimmunoassay procedure of Niswender *et al.* (1969), as modified slightly in our laboratory (Cicero *et al.*, 1976a), was used.

Statistical analysis. Student's *t* tests were used to examine all statistically significant differences.

Results

Effects of a single injection of alcohol on testosterone and LH levels. The effects of a 2.5 g/kg dose of alcohol on serum testosterone and LH levels are presented in figure 1. In this figure only a single control value is presented since there were no statistically significant differences between control groups killed at any of

the postinjection time intervals and, therefore, the data were pooled. Two hours after the injection of alcohol, LH levels were significantly depressed below control levels. From 2 to 4 hours, LH levels remained significantly depressed at approximately 30% of control levels. Six hours after the injection of alcohol, LH levels had returned to control levels. Testosterone levels also dropped after the injection of alcohol, but this drop was not statistically significant until 3 hours after the injection. The maximal depression in testosterone levels occurred 4 hours after the alcohol injection. Testosterone levels did not return to normal over the 6-hour time course examined.

Dose-response characteristics. To determine the relationship between the dose of alcohol and changes in testosterone and LH levels, rats were injected with various doses of ethanol and were killed 3 or 4 hours later, the times at which the peak depression of LH and testosterone occurred, respectively, in the preceding experiments. The results of these studies are shown in figure 2. Low doses of alcohol significantly increased serum testosterone and LH levels over control levels; as the dose of ethanol was increased, however, both LH and testosterone dropped significantly below control levels. A dose of 1.5 to 2.0 g/kg was sufficient to depress LH and testosterone by over 50%. To examine the possibility that the large increase in testosterone and LH levels observed 4 hours after the injection of low doses of alcohol represented a "rebound" after an initial depression,
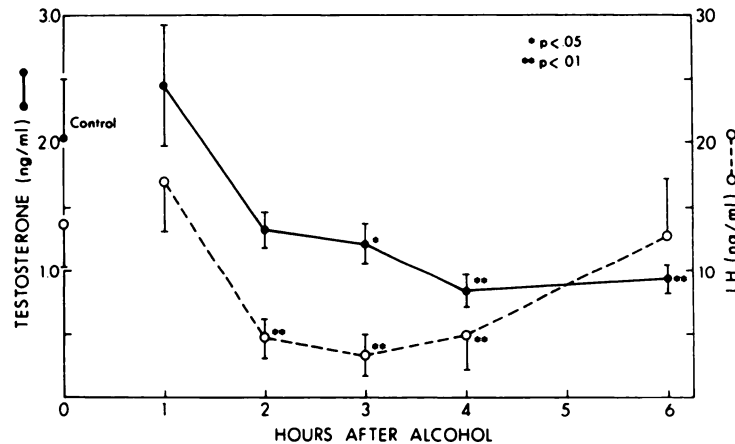

FIG. 1. The effects of alcohol (2.5 g/kg) on the mean (±S.E.M.) testosterone and LH levels in male rats at intervals after its injection. All control testosterone and LH levels, sampled at 1, 2, 3, 4, 5 and 6 hours after the injection of distilled water, were pooled since there were no statistically significant differences between the groups. The control values have been plotted as a zero time point in this figure.
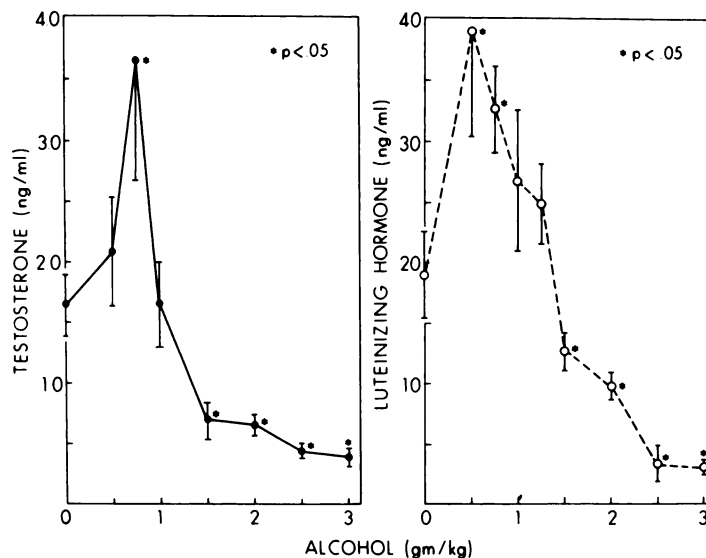
FIG. 2. The effects of various doses of alcohol on the mean (±S.E.M.) testosterone (left panel) and LH (right panel) levels in the serum of male rats 4 and 3 hours after the injection, respectively.

we injected rats with 0.75 g/kg of ethanol, a dose which produced a large increase in serum testosterone and LH levels in the preceding studies. The rats were then killed at various intervals up to 4 hours. The results of this study showed that there was no decrease observed in LH or testosterone levels at any of the time intervals examined. Rather, the only change in either LH or testosterone was a marked increase in serum levels occurring 3 to 4 hours after the injection of alcohol.

Chronic ethanol administration. Rats maintained on ethanol consumed over 13 g/kg of ethanol daily and their blood alcohol levels averaged 207.3 (±32.4) mg/100 ml throughout the 20-day period, when measured after the peak daily consumption had occurred. With respect to the physical condition of these rats, they consumed significantly less nutrients than the liquid diet controls throughout the experiment and, as a result, failed to grow at the same rate as lab chow or liquid diet controls. Consequently, by the end of the study, the alcohol-fed rats weighed 25% less than the two control groups. The results of the blood chemistry analyses are shown in table 1. In general, all animals maintained on the Metrecal-Shape liquid diets, particularly the pair-fed controls, had significantly lower alkaline phosphatase, SGPT, BUN, bilirubin, total protein and creatinine levels than the lab chow controls.

The effects of chronic alcohol administration on serum LH and testosterone levels are shown in table 2. As can be seen, the ethanol-fed animals had significantly lower serum testosterone and LH levels when compared with the lab chow or liquid diet control animals. However, the levels of these hormones were not significantly lower in ethanol-fed animals than in the pair-fed control animals. These experiments were repeated several times and, in each case, the same results were obtained.

Discussion

A number of previous reports indicate that alcohol exerts profound effects on reproductive function in the male (Lloyd and Williams, 1948; Southren *et al.*, 1973; Van Thiel *et al.*, 1974; Rubin *et al.*, 1976). However, because of the problems inherent in utilizing the human alcoholic (*e.g.*, poor nutritional status and liver damage), it has been difficult to conclude with any certainty that alcohol exerts direct, specific effects on the hypothalamic-pituitary-gonadal axis in the human.

In an attempt to avoid the problems associated with using the human alcoholic, a number of investigators have employed animal models of chronic alcohol administration to examine the effects of alcohol on reproductive endocrinology. Unfortunately, these studies have produced conflicting data. Although the results

TABLE 1

Blood chemistries of animals (N = 5) in each of the four experimental groups

Values are mean ± S.E.M.

Group	Alkaline Phosphatase	SGPT	BUN	Bilirubin	Total Protein	Creatinine
	I.U./l	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*g/100 ml*	*mg/100 ml*
Lab chow control	177 ± 21.9	41 ± 6.4	25.4 ± 2.61	0.17 ± .01	6.3 ± 0.19	0.70 ± 0.15
Liquid diet control	126 ± 15.0[a]	31 ± 5.0	16.6 ± 3.13[a]	0.22 ± .05	6.0 ± 0.44	0.90 ± 0.17
Alcohol liquid diet	144 ± 23.7	40 ± 5.2[b]	18.6 ± 3.78	0.11 ± 1.0[a]	5.0 ± 0.15[a]	0.54 ± 0.13
Pair fed control	123 ± 7.9[a]	20 ± 1.9[a]	12.4 ± 2.30[a]	0.16 ± .02	5.1 ± 0.07[a]	0.50 ± 0.05

[a] P < .05 when compared with lab chow control animals.

[b] P < .05 when compared with pair-fed control animals.

TABLE 2

Effects of chronic alcohol administration on the testosterone and LH levels in the male rat

Values are mean ± S.E.M. See text for further details.

Group	N	Testosterone	LH
			ng/ml
Lab chow control	5	3.79 ± 0.96	40.5 ± 8.48
Liquid-diet control	5	3.91 ± 0.79	36.7 ± 15.89
Ethanol-fed	5	1.58 ± 0.78[a]	11.4 ± 2.09[a]
Pair-fed	5	2.52 ± 1.05	27.1 ± 7.39

[a] P < .05 when compared with lab-chow control or liquid-diet control animals.

presented in this paper appear to indicate that chronic alcohol treatment reduces both LH and testosterone levels, the poor nutritional status (as indicated by severe weight loss, restricted food intake and abnormal blood chemistries) of animals maintained on the alcohol-containing liquid diet, appears to be the major cause of the reductions in LH and testosterone levels. Symons and Marks (1975) have also shown that an apparent alcohol-induced alteration in LH levels in the male rat after prolonged alcohol consumption may also be due to nutritional variables rather than alcohol alone. However, Van Thiel *et al.* (1975) have reported that rats, maintained on a liquid diet containing ethanol, had evidence of testicular degeneration, atrophy of secondary sex organs and low serum testosterone levels when compared with *ad libitum* chow-fed rats or "isocaloric" control animals. Thus, these authors have apparently demonstrated a specific effect of alcohol, apart from a nutritional difficulty. The reasons for

the apparent conflict between the results of these investigators and those reported in this paper are unclear and should be clarified in further experiments. However, it appears that chronic alcohol administration, in both the rat and human, has thus far not provided the best model with which to investigate the effects of alcohol on the hypothalamic-pituitary-gonadal axis.

The present results indicate that an acute injection of alcohol in the normal, well nourished and drug-naive rat, can significantly depress serum LH and testosterone levels, indicating that, at least in the rat, alcohol can exert specific effects on reproductive endocrinology. We are aware of few previous reports examining acute alcohol administration on the hypothalamic-pituitary-gonadal axis. In the human, Ylikahri *et al.* (1974) showed that a rather high dose of alcohol in normal, healthy volunteers depressed serum testosterone, but not LH, levels 16 to 20 hours after its injection. Toro *et al.* (1973) found that alcohol produced no changes in circulating serum testosterone or LH levels after its ingestion by healthy volunteers. However, only a single, low dose of alcohol and relatively short intervals were examined in those studies. Finally, in agreement with the results presented in this paper, Symons and Marks (1975) showed that an acute injection of alcohol produced a slight depression in LH levels 60 minutes (the only interval examined) after its injection in the male rat.

The results of the studies described in this paper contrast to most published reports which conclude that the effect of ethanol on the hypothalamic-pituitary-gonadal axis represents a primary effect on the gonads or liver (*e.g.*, Galvao-Teles *et al.*, 1973; Southren *et al.*, 1973;

Van Thiel *et al.*, 1974; Gordon *et al.*, 1975; Rubin *et al.*, 1976). Our results and some previous research indicate that the changes in serum testosterone levels and gonadal function observed after alcohol treatment are probably secondary to a primary effect of alcohol on the hypothalamic-pituitary axis. This conclusion is based on the following facts. Firstly, in the present studies, LH levels fell significantly after an acute injection of alcohol and this fall preceded a drop in testosterone levels by approximately 1 hour. Since LH is responsible for regulating testicular steroidogenesis (Ahluwalia *et al.*, 1974; Moyle and Ramachandran, 1973), this time-response relationship is compatible with the interpretation that a reduction in LH is the necessary intermediate step leading to a reduction in serum testosterone levels. Secondly, one would expect that if alcohol lowered serum testosterone levels *via* a peripheral mechanism, then LH should rise dramatically on the basis of a release of negative feedback control (*e.g.*, Shin and Jowitt, 1975; Gomes and Van Demark, 1974). Since this does not occur, the conclusion that alcohol must, in some way, disrupt this feedback control system seems unquestionable. Consequently, our results suggest that alcohol, at the very least, affects the hypothalamic-pituitary link in the hypothalamic-pituitary-gonadal axis, as well as the gonads. Moreover, our data suggest that the apparent alcohol-induced aberrations in gonadal function are probably secondary to a primary effect of alcohol on the hypothalamic-pituitary axis.

The failure of previous investigators to demonstrate an effect of alcohol on LH levels in the human is difficult to understand but could be due to several pharmacological considerations, particularly time-response and dose-response relationships. Specifically, in the present studies we found that whether one would observe any effect of alcohol on serum LH or testosterone levels would depend on the time after injection when blood samples were obtained (fig. 1). Moreover, our data suggest that the direction of change in testosterone or LH would depend on the dose of alcohol employed (fig. 2). These time-response and dose-response problems could play a significant role in previous failures to observe changes in LH levels in the human after acute or chronic alcohol treatment.

The effects of ethanol on testosterone and LH

levels appear to be biphasic: low doses of alcohol increased testosterone and LH, whereas high doses decreased both hormones. Alcohol has biphasic effects on many processes, such as neuronal firing patterns, sensory processing and more complex behavioral functions (Wallgren and Barry, 1970), so that its biphasic effect on LH and testosterone is not too surprising. We, of course, at present can offer no biological or neurochemical explanation for this biphasic response but a number of experiments are in progress in an attempt to more fully examine this issue. One interpretation can, however, be excluded: specifically, that increases in LH and testosterone are due to a "rebound" after an initial depression by alcohol. Careful time-response studies indicated that low doses of alcohol had only one effect on LH and testosterone—an increase in their levels 2 to 4 hours after its injection.

In conclusion, the results of the foregoing studies indicate that alcohol markedly disrupts the function of the hypothalamic-pituitary-gonadal axis in the adult male rat. Moreover, our data suggest that alcohol exerts its primary effects by initially disrupting the hypothalamic-pituitary axis.

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