The Effect of Acute and Chronic Ethanol Administration on Prolactin Secretion in Male Rats

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The effect of acute and chronic administration of ethanol on prolactin secretion in male rats was investigated under basal conditions and after the administration of sulpiride. The effect of ethanol on the activity of glutamic acid decarboxylase (GAD) in the hypothalamus was also examined. Acute administration of ethanol significantly increased serum prolactin levels. This increase persisted after sulpiride administration. In contrast, chronic administration of ethanol did not significantly modify serum prolactin levels. However, the elevation of prolactin levels in response to the injection of sulpiride was significantly lower in rats chronically treated with ethanol than in control animals. Pituitary concentration of prolactin was not significantly modified by acute or chronic administration of ethanol. Sulpiride administration failed to reduce the concentration of prolactin in the pituitary of rats acutely treated with ethanol, in contrast to its effect in control rats. Acute administration of ethanol significantly decreased hypothalamic GAD activity, while chronic ethanol treatment caused an increase in GAD activity in the hypothalamus. These results indicate that ethanol can alter prolactin secretion and the synthesis of GABA in the hypothalamus.

Key words: ethanol, prolactin, glutamate decarboxylase.

It has been reported that the administration of ethanol may induce alterations in reproductive functions (Hughes et al, 1980; Van Thiel et al, 1975; Van Thiel et al, 1978; Weathersbee and Lodge, 1978). In the male rat, ethanol can modify androgen metabolism and gonadotropin secretion (Badr et al, 1977; Cicero and Badger, 1977). It has been suggested that alcohol exerts its primary effects by initially affecting the hypothalamic-pituitary axis (Cicero et al, 1978). However, conflicting data have been reported on the effect of alcohol on prolactin secretion. Ethanol increased prolactin secretion in man (Majumdar, 1979) and castrated male rats (Chapin et al, 1980), while low levels of prolactin were detected during acute alcohol withdrawal (Loosen and Prange, 1977). Ylikahri et al (1978) reported decreased prolactin response to TRH during alcohol hangover in healthy volunteers. The neuropharmacologic effects of ethanol may be mediated through changes in the concentration and/or metabolism of neurotransmitters. It is well established that some neurotransmitters play an important role in the secretion of pituitary hormones. Among them, gamma aminobutyric acid (GABA) was shown to modify prolactin secretion (McCann et al, 1981; Schally et al, 1977).

In the present investigation, we have studied the effects of acute and chronic administration of ethanol on prolactin secretion and on the activity of glutamic acid decarboxylase (GAD), the key enzyme for GABA synthesis, in the hypothalamus of male rats. In addition, the pituitary response to sulpiride, a dopamine antagonist known to induce prolactin release (Debeljuk et al, 1975), was also investigated.

Material and Methods

Adult male Wistar rats were exposed to chronic treatment with alcohol for three months, as described elsewhere (Koch et al, 1976). Briefly, rats were fed laboratory chow and a drinking solution of sucrose (25%) and ethanol (32%) ad libitum in Richter type glasses. Control animals were fed a similar diet but alcohol was isocalorically replaced by sucrose. The body weight of the animals at the beginning of the treatment was approximately 120 g. The increase in body weight during

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the treatment period was not significantly different between control and ethanol-treated rats (2.02 ± 0.09 and 1.76 ± 0.18 g/day, respectively).

Acute treatment with alcohol consisted of injecting rats (mean weight 250 g) with ethanol dissolved in saline (25% p/v) intraperitonealy at a dose of 5 g/kg body weight. Control rats were injected with saline alone.

Experiment 1

All animals were lightly anesthetized with ether immediately before blood was drawn.

Sixty minutes after acute administration of ethanol, the rats received an intravenous injection of sulpiride sulphate (100 μg/rat). Blood was drawn from the jugular vein under basal conditions and before the injection of sulpiride. Twenty minutes after sulpiride injection, the animals were decapitated and blood was collected for radioimmunoassay.

In rats chronically treated with ethanol, blood samples were obtained from the jugular vein before and 20 minutes after an intravenous injection of saline and again 20 minutes after an intravenous administration of sulpiride (100 μg/rat). Sera were separated and kept frozen until assayed.

Experiment 2

Sixty minutes after acute administration of ethanol, the rats received an intravenous injection of sulpiride sulphate (100 μg/rat) or saline. Twenty minutes later, the animals were sacrificed by decapitation. The anterior pituitary glands were dissected free and weighed. The glands were then homogenized in distilled water and kept frozen until prolactin assays were performed. Hypothalami were also collected, weighed, and kept at −70 C until GAD assays were performed.

Pituitaries and hypothalami of rats receiving chronic ethanol treatment were also obtained and stored under the same conditions.

Determination of Prolactin and GAD Activity

Serum and pituitary prolactin concentrations were determined using a double antibody radioimmunoassay (Niswender et al, 1969), with kits obtained through the National Pituitary Agency, N.I.H.

The activity of GAD was determined by measuring the rate of formation of $^{14}CO_2$ from D-L (1-^{14}C) glutamic acid, using a modification of the method of Albers and Brady (1959). Hypothalami were homogenized in 50 mM potassium phosphate buffer (*p*H 6.5) containing 0.1% (v/v) Triton X-100 and 0.1 mM pyridoxal phosphate. The incubation medium contained the following components: 50 mM potassium phosphate buffer (*p*H 6.5), 0.5 mM pyridoxal phosphate, 10 mM mercaptoethanol, 0.1% (v/v) Triton X-100, 100 mM L-glutamic acid (0.25 μCi) L-D (1-^{14}C) glutamic acid, 48.1 mCi/mmol, New England Nuclear). Samples were incubated for 60 minutes. Activity of GAD was expressed as μmol/g protein/hour. Blank values, determined in parallel, were subtracted from each experimental sample.

The significance of the results was evaluated by means of Student's *t* test or analysis of variance followed by Duncan's new multiple range test (Steel and Torrie, 1960).

Results

Effects of Ethanol on Serum Prolactin Levels

Sixty minutes after acute administration of 5 g ethanol/kg body weight, serum prolactin levels were significantly increased ($P < 0.01$), compared with those measured under basal conditions and after the injection of saline (Fig. 1). Sulpiride induced an elevation ($P < 0.01$) of serum prolactin levels in both groups. Serum prolactin levels were significantly higher ($P < 0.01$) after the administration of sulpiride in ethanol-treated rats than in control rats.

Basal prolactin levels in sera of the group chronically treated with ethanol (22.53 ± 6.53 ng/ml; n = 5) and those of the control group (41.35 ± 13.48 ng/ml; n = 5) were not significantly different. The injection of saline did not significantly modify serum prolactin levels in either group. The administration of sulpiride induced significant elevations of serum prolactin levels in both groups ($P < 0.01$). However, the increase in serum prolactin after the injection of sulpiride was significantly lower ($P < 0.01$) in the ethanol-treated rats than in control animals (Fig. 2).

Effect of Ethanol on Pituitary Prolactin Concentration

Acute ethanol treatment produced a slight but not significant elevation of pituitary prolactin concentration (Fig. 3). The administration of sulpiride significantly decreased ($P < 0.01$) prolactin concentration in the saline-treated group, but did not significantly modify pituitary prolactin concentration in the ethanol-treated rats.

Chronic treatment with ethanol did not modify pituitary concentration of prolactin. However, these rats showed a significantly lower ($P < 0.01$) total content of prolactin in the pituitary (Table 1).

Effect of Ethanol on GAD Hypothalamic Activity

Acute administration of ethanol significantly decreased ($P < 0.005$) GAD activity in the hypothalamus (236.39 ± 6.90 μmol/g protein/hour; n = 5) as compared with injection of saline (283.76 ± 9.63; n = 6).

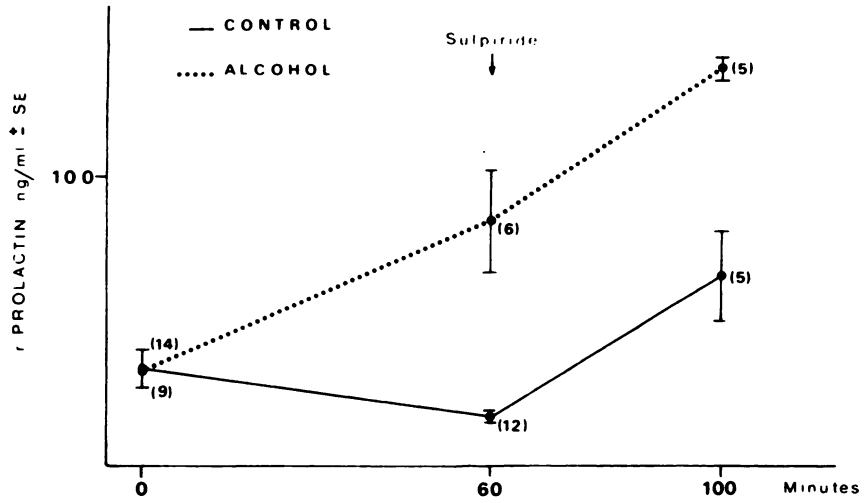

Fig. 1. Effect of acute administration of ethanol (5 g/kg body weight) on serum prolactin levels before and after the injection of sulpiride. Values represent means ± SE. The number in parentheses indicates the number of animals studied.

Rats receiving chronic ethanol treatment had significantly increased ($P < 0.001$) hypothalamic GAD activity: 343.71 ± 3.48 (n = 6) versus 287.12 ± 7.71 (n = 6) in control rats.

Discussion

This study confirms the previous finding (Chapin et al, 1980) that the acute administration of ethanol can elevate serum prolactin levels. In rats acutely treated with ethanol, serum prolactin increased even further after sulpiride administration. Ethanol induced hyperprolactinemia apparently without affecting prolactin concentration in the adenohypophysis. Furthermore, sulpiride administration to ethanol-treated rats did not decrease pituitary prolactin concentration as markedly as it did in control rats. The co-existence of high serum prolactin levels and high pituitary prolactin concentrations suggests that ethanol may stimulate prolactin synthesis and release. Ethanol could directly affect hypothalamic neuronal systems governing prolactin secretion.

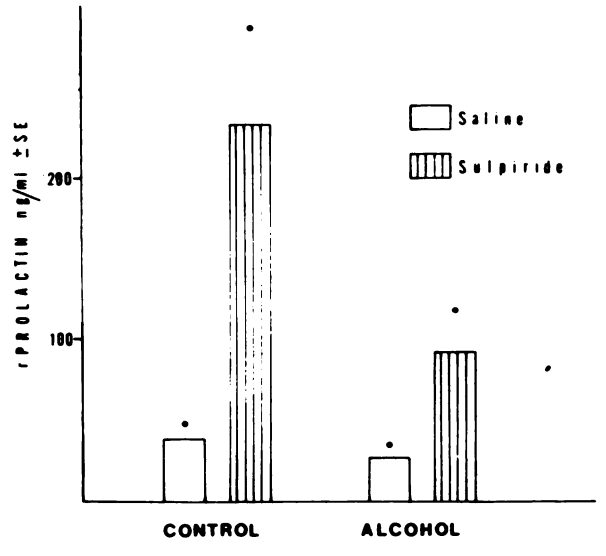

Fig. 2. Effect of chronic administration of ethanol on sulpiride-induced prolactin release. Each column represents the mean ± SE of five animals.

There is considerable evidence that brain neurotransmitters may modulate anterior pituitary secretion (Müller et al, 1977). However, there is some discrepancy in the literature regarding the effects of ethanol on central neurotransmitters. Acute ethanol administration has been reported to increase (Rawat, 1974), decrease (Sytinsky et al, 1975), or to induce no change (Sutton and Simmonds, 1973) in the concentration and/or turnover rate of rat brain GABA. Moreover, no data are available on the effect of ethanol on the activity of GABA-related enzymes in the hypothalamus. In the present experiment, acute ethanol administration induced a decrease in hypothalamic GAD activity. This finding is in agreement with a report that acute ethanol administration decreased GAD activity in some areas of the brain (Sytinsky et al, 1975). There is evidence indicating that GABA may have an inhibitory role in prolactin release (Schally et al, 1977; Vijayan and McCann, 1978; Grandison and Guidotti, 1979; Debeljuk et al, 1980). Since acute ethanol administration was effective in inhibiting GAD activity, this may

suggest that ethanol stimulated prolactin secretion, at least partially, by reducing the inhibitory influence of GABA. However, although GABAergic mechanisms could be involved in the pituitary response to ethanol administration, a direct effect of ethanol on pituitary cells cannot be ruled out. In fact, we have found that ethanol enhanced prolactin release into the incubation medium by pituitary halves *in vitro* (unpublished results).

Long-term administration of ethanol modified prolactin release, as revealed by the decreased pituitary response to sulpiride. Moreover, the total content of pituitary prolactin was also affected by chronic ethanol administration. The effect of ethanol on prolactin secretion may be mediated by alterations in the metabolism of GABA. Previous reports have evidenced increase GAD activity in cerebellum and cerebral cortex (Häkkinen and Kulonen, 1979; Sytinsty et al, 1975) in chronically alcohol-treated rats. Significant changes in hypothalamic GAD activity were evident in our studies.

In summary, it has been shown that ethanol can modify prolactin secretion in the male rat. It has also been observed that acute ethanol administration depressed GAD hypothalamic activity, while this enzymatic activity was enhanced by chronic ethanol ingestion. Although at first these effects may seem contradictory, chronic and acute treatment with ethanol was previously shown to induce opposite effects in other systems (Hunt, 1981; Ticku and Bur, 1980). Development of tolerance, nonspecific cellular toxicity, or changes in the sensitivity of receptors may account for differences between the effects of chronic and acute ethanol administration.

It can be concluded from this study that changes in prolactin secretion produced by ethanol may be dependent on modifications in GABAergic activity, on direct effects on pituitary cells, or on alterations in other neurotransmitter systems.

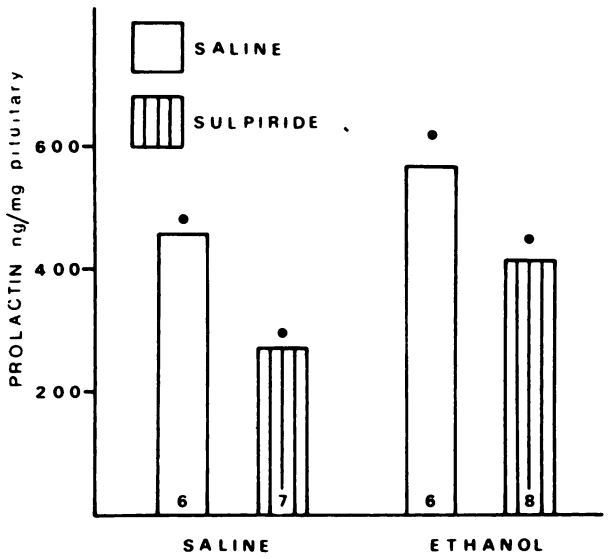

Fig. 3. Effect of sulpiride administration on pituitary prolactin concentration in rats acutely treated with ethanol. Columns represent means ± SE. The number of animals is indicated at the bottom of the column.

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TABLE 1. Effect of Chronic Administration of Ethanol on Pituitary Prolactin

Treatment	Pituitary weight (mg)	Prolactin concentration (ng/mg pituitary weight)	Prolactin content (ng/pituitary)
Control	6.04 ± 0.24 (6)	453.32 ± 77.49 (7)	2888.48 ± 264.67 (8)
Ethanol	4.90 ± 0.31 (8)	437.63 ± 89.11 (9)	1747.51 ± 234.03 (6)
	$P < 0.02$	NS	$P < 0.01$

The values are means ± SE for the number of animals given in parentheses.

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