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Impact of Acute and Chronic Ethanol Exposure on Prolactin in Both Male and Female Rats

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The deleterious effects of ethanol (EtOH) on reproduction have been well documented. This disruption is usually associated with alterations in prolactin (PRL) levels, which is relevant since this hormone is an important participant in the reproductive system. Reported EtOH-induced changes in PRL (i.e., stimulation or inhibition) have varied. These differences may have been owing to the gender or age/sexual maturity of the animal and the mode of the administration of EtOH. Therefore, to clarify the impact of EtOH on PRL, a series of experiments were conducted utilizing rats of both genders, exposed to EtOH acutely or chronically, as adults and as they progressed through puberty. In general, in younger animals of both genders, EtOH depressed serum PRL whether given acutely or chronically. In adult males, acute EtOH actually stimulated PRL levels while chronic administration had no effect. In adult females, EtOH's effect was highly dependent on the stage of the estrous cycle in which EtOH was given and during which PRL was measured. In conclusion, our studies have shown that the PRL response to EtOH is dependent on the gender and age/sexual maturity of the animals as well as on the mode of administration.

Key Words: Prolactin; ethanol; male and female rats.

Introduction

The deleterious effects of ethanol (EtOH) ingestion on rodent reproduction have been well documented in animals of both genders *(1–4)*. Also described is the impact of ethanol on bone growth and development *(5)*. A common hormonal perturbation for both problems may be an aberration in prolactin (PRL) dynamics, since this peptide is an important participant in both the reproductive and osteocyte systems *(6,7)*. Hyperprolactinemia has been inconsistently noted after both acute and chronic ethanol exposure in humans and rodents and has been incriminated in the hypogonadism associated with ethanol. The subsequent oligoovulation in females and fall in testosterone in males could be directly responsible for the osteopenia seen in ethanol-imbibing individuals and rodents *(6)*. A lowering of PRL could be equally detrimental to reproduction, lactation, and normal bone development *(6,7)*. While less common, hypoprolactinia has also been reported in the ethanol-exposed animal.

As indicated, the reported responses of PRL to EtOH have varied (see Discussion). These differences may have been owing to gender, age/sexual maturity of the organism, and/or the mode of administration of EtOH. To clarify further the impact of ethanol on PRL, a series of experiments was conducted utilizing rats of both genders exposed to ethanol acutely or chronically as adults and as they progressed through puberty.

Results

Male Rats: Acute Studies

Weight

In acute studies, there were no weight differences between the control and EtOH animals.

Blood Ethanol Level

Ninety minutes after acute EtOH exposure (2 g/kg), serum EtOH levels were 153 ± 3.5, 165 ± 4.5, and 199 ± 3.5 mg/dL in the 35-, 45-, and 55-d-old rats, respectively. There was no detectable EtOH in any of the saline-injected control animals. The older 65- to 90-d-old animals, which had received 3 g/kg of EtOH, had significantly higher levels ($p < 0.001$) than the younger animals. Ethanol concentrations were 268 ± 11 mg/dL for the 65-d-old rats and 309 ± 11 mg/dL for the 85-d-old rats.

PRL Level

As expected, there was an age-related increase in PRL levels, confirming much other data *(9–15)*. Levels were higher in 85-d-old control rats compared with the younger ages ($p < 0.001$).

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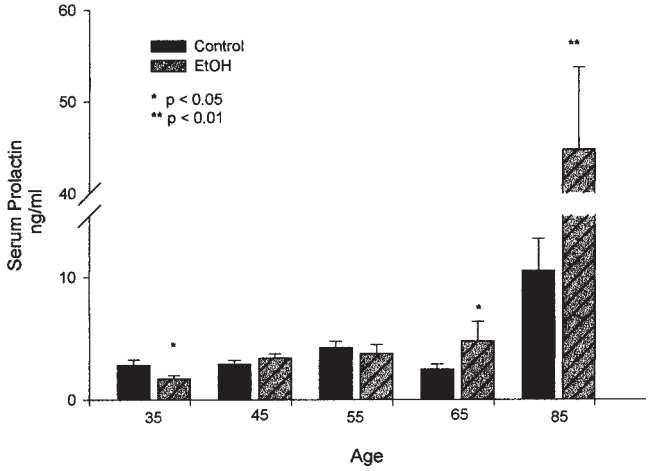

Fig. 1. PRL response to acute EtOH administration in male rats of various ages. The 35-, 45-, and 55-d-old rats received 2 g of EtOH/kg of body wt. The 65- and 85-d-old animals were given 2 g of EtOH/kg of body wt. Rats were sacrificed 90 min after injection. There were 10 per group. Data are the mean ± SEM.

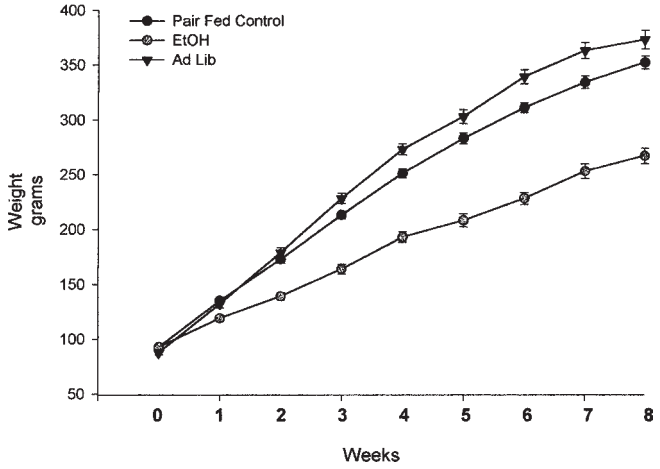

Fig. 2. Weight curves for pubertal male rats receiving chronic EtOH administration. There were 10 rats in the EtOH and control groups and 5 in the ad lib. The EtOH rats weighed significantly less ($p < 0.01$) compared with the control and ad lib rats using the least squares regression analysis. Values are mean ± SEM.

In the acute studies, the response to EtOH was age related (Fig. 1). In the 35-d-old rats, EtOH caused a significant 39% fall in PRL, from 2.83 ± 0.24 to 1.72 ± 0.24 ng/mL ($p < 0.05$). By contrast, EtOH caused no change in serum PRL in the 45- and 55-d-old animals. Finally, EtOH caused a threefold increase in PRL in the 65-d-old rats ($p < 0.05$) and a four-fold increase in the 85-d-old rats ($p < 0.01$).

Male Rats: Chronic Studies

Weight

After chronic administration of EtOH, the male rats receiving the EtOH liquid diet consistently weighed less than their pair-fed control mates, despite the fact that the animals were matched calorie for calorie. In prepubertal rats, by the end of the second week of the study, there was clear weight separation between the EtOH-fed and the other two groups. The EtOH-fed rats weighed significantly less than their pair-fed mates ($p < 0.01$) (Fig. 2). This has been a consistent finding in all our chronic feeding studies, possibly owing to subtle malabsorption, since there was always a greater number of stool pellets present in the cages of the EtOH-exposed animals. A similar pattern was seen in the adult rat study, although in this case the weight differences were only of borderline significance ($p < 0.07$) (Fig. 3). Note that EtOH animals did not lose weight; rather, the rate of weight gain was diminished. In addition, the animals were well groomed, alert, and did not appear ill in any way.

Blood EtOH Level

In the chronic studies, the serum EtOH levels were 102 ± 1.8 and 109 ± 1.5 mg/dL in the 30- and 60-d-old rats, respectively. Presumably, the lower EtOH levels in the chronic feeding studies compared with the acute studies reflect the fact that the animals were sacrificed at 10:00 AM,

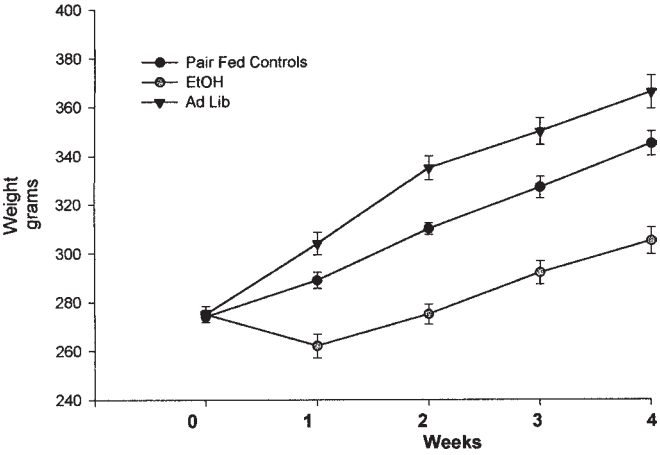

Fig. 3. Weight curves for adult male rats receiving chronic EtOH administration. There were 10 rats in the EtOH and control groups and 5 in the ad lib. EtOH, although decreased, was not quite significant compared with the control and ad lib groups using the least squares regression analysis. Values are mean ± SEM.

and most of the EtOH is consumed during the dark hours (6:00 PM to 6:00 AM).

PRL Level

Chronic ethanol exposure in the youngest animals, age 30 d at the initiation of the chronic feeding paradigm and 60 d at sacrifice, resulted in a significant decrease in PRL from 8.8 ± 4.5 to 2.4 ± 0.9 ng/mL ($p < 0.05$), as shown in Fig. 4. When feeding was continued for 2 mo, EtOH caused PRL to fall from 10.3 ± 1.5 to 4.9 ± 0.6 ng/mL ($p < 0.01$). However, chronic EtOH exposure caused no significant change in the older animals in which feeding was started at 60 d of age and continued for 1 mo. In two short-term (2-wk) studies, it appeared that the shift from EtOH inhibition of

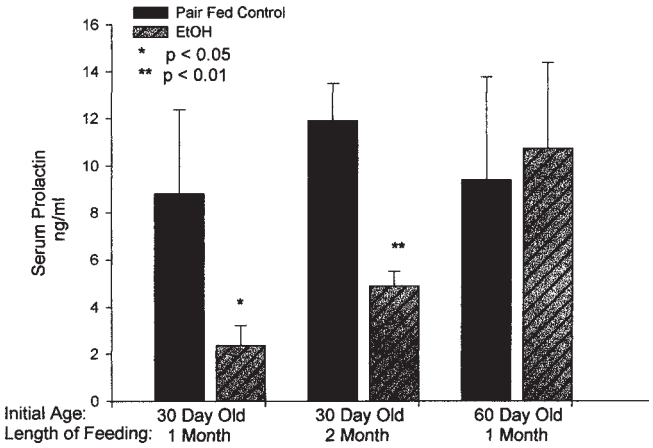

Fig. 4. PRL response to chronic EtOH administration in male rats of various ages fed for 1 or 2 mo, as indicated. There were 15 rats in the pair-fed control group and 10 in the EtOH group. Data are the mean ± SEM.

PRL to no effect occurred between 45 and 55 d of age (data not shown).

Female Rats: Acute Studies

Weight

In acute studies, there were no weight differences between the control and EtOH animals.

Blood EtOH Level

Ninety minutes after acute EtOH exposure (2 g/kg), serum EtOH levels were 147 ± 2.5 mg/dL in the 30-d-old rats. There was no detectable EtOH in any of the saline-injected control animals. The older 65- to 90-d-old animals, which had received 3 g/kg of EtOH, had significantly higher levels (213 ± 6.1 mg/dL) ($p < 0.001$) than the younger animals.

PRL Level

In prepubertal animals studied at 30 d of age, before vaginal opening, acute EtOH administration decreased serum PRL from 6.6 ± 1.4 to 2.7 ± 0.8 ng/mL ($p < 0.01$; Fig. 5A). In adult, cycling females, however, the effect of EtOH was complex.. The PRL response depended on the stage of the estrous cycle in which EtOH was administered and the stage of the cycle during which PRL was measured. Most of our data regard the PRL response at 4:00 to 5:00 PM of proestrus to EtOH given at 12 noon of proestrus. In those studies, serum PRL levels were significantly decreased after acute EtOH injection, at a time when PRL levels are known to surge *(8)*. The PRL values were 159 ± 18 ng/mL in the control animals vs 35 ± 21 ng/mL in the EtOH animals ($p < 0.001$; Fig. 5B). In contrast to this very sharp

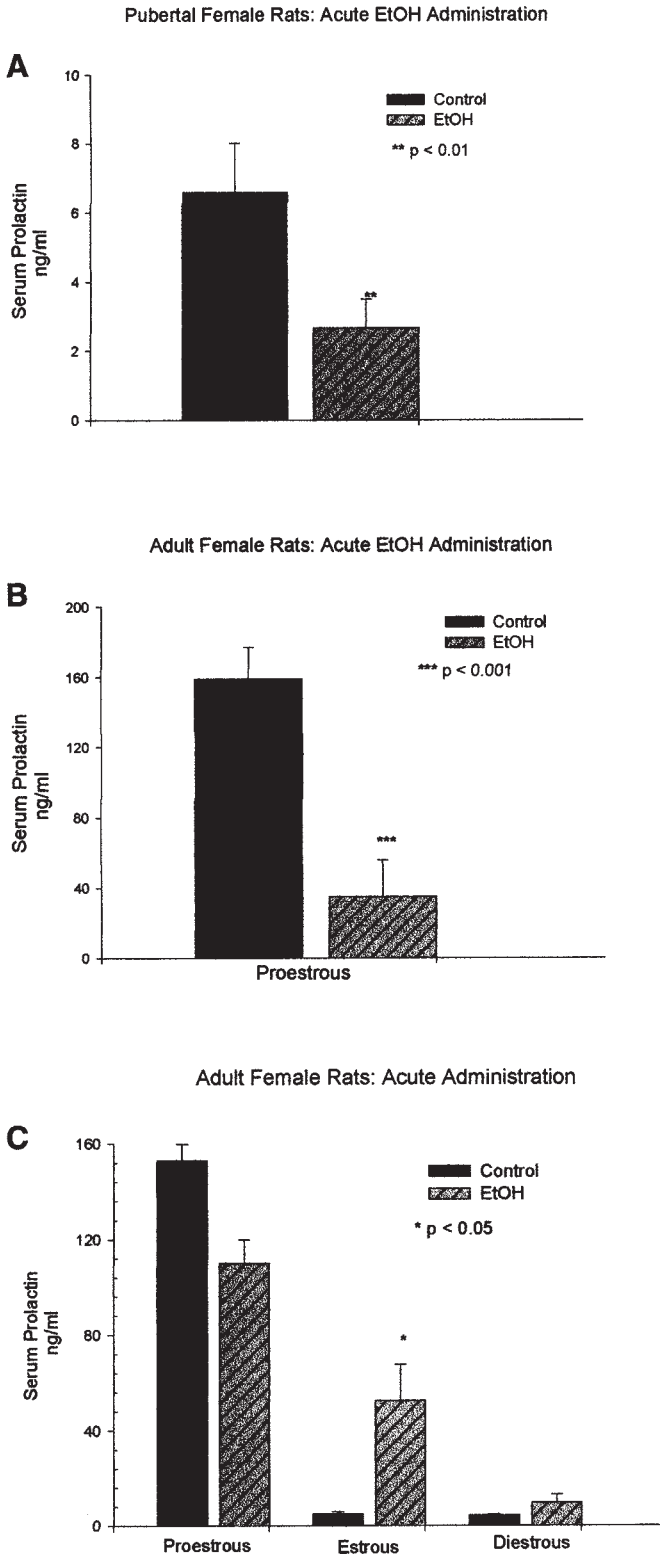

Fig. 5. (**A**) PRL response to acute administration of 2 g of EtOH/kg of body wt in prepubertal female rats. The animals were sacrificed 90 min after injection. There were 10 rats per group. Data are the mean ± SEM. (**B**) PRL response to acute administration of 3 g of EtOH/kg of body wt in adult female rats given at noon on the day of proestrus. Rats were sacrificed 4 h later. There were 10 rats in each group. Data are the mean ± SEM. (**C**) PRL response to acute administration of 3 g of EtOH/kg of body wt in adult female rats given at 4:00 PM on the subsequent proestrus, estrus, or diestrus. There were five to six rats per group. Data are the mean ± SEM.

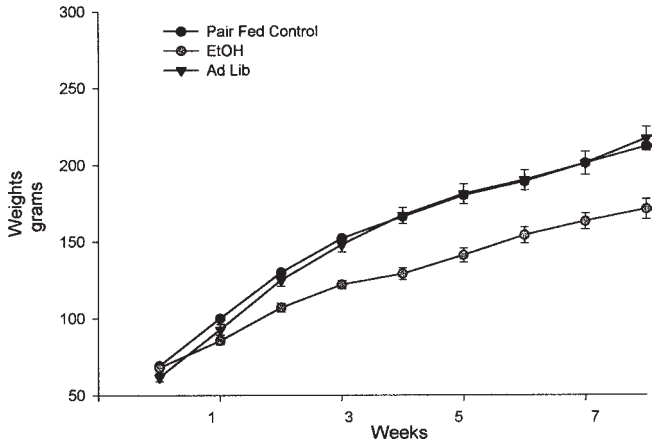

Fig. 6. Weight curves for pubertal female rats receiving chronic EtOH administration. There were 10 rats in the EtOH and control groups and 5 in the ad lib. The EtOH rats weighed significantly less ($p < 0.01$) compared with the control and ad lib rats using the least squares regression analysis. Values are the mean ± SEM.

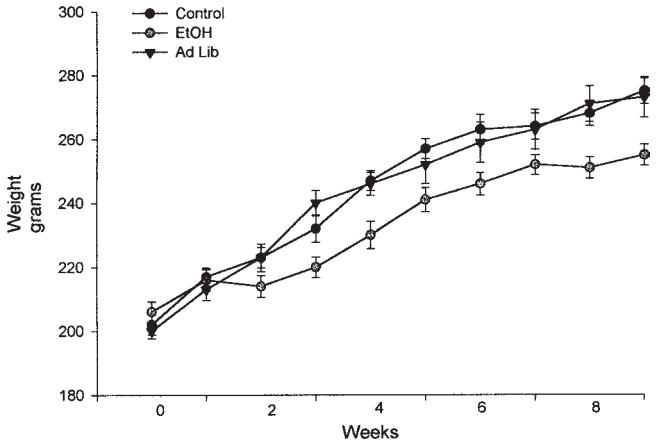

Fig. 7. Weight curves for adult female rats receiving chronic EtOH administration. There were 10 rats in EtOH and control groups and 5 in the ad lib. The EtOH rats weighed significantly less ($p < 0.05$) compared with the control and ad lib rats using the least squares regression analysis. Values are the mean ± SEM.

inhibitory effect, when EtOH was given at 4:00 PM of diestrus, there was no statistically significant attenuation of the proestrous PRL surge. Indeed, there was a slight, but significant stimulation of PRL when the animals were examined at the subsequent estrus ($p < 0.05$), and a trend toward increased PRL at diestrus (Fig. 5C).

Female Rats: Chronic Studies

Weight

As noted with the male animals, the female rats receiving the chronic EtOH diet consistently weighed less than their pair-fed control mates, although the differences were not as striking as in the male experiments. Briefly, although the EtOH-fed rats gained weight and appeared healthy, weight was consistently less than that of rats not receiving EtOH. The pubertal EtOH-fed rats' weight was significantly less than that of non-EtOH-fed rats ($p < 0.01$), while the adult female rats' weight was also significant ($p < 0.05$). Weight data for pubertal rats are shown in Fig. 6, and that for adult female rats in Fig. 7.

Blood EtOH Level

In the chronic studies, the serum EtOH levels were 50 ± 13 and 22 ± 5.0 mg/dL in the 35- and 60-d-old rats, respectively. Presumably, the lower EtOH levels in the chronic feeding studies reflect the fact that the animals were sacrificed at 4:00 to 5:00 PM on the day of proestrus, and most of the EtOH is consumed during the dark hours (6:00 PM to 6:00 AM).

PRL Level

Chronic EtOH exposure also decreased PRL levels in young animals (28 d old at the initiation of the feeding paradigm) after both 1 and 2 mo of feeding. After 1 mo of feeding, the serum PRL concentrations were 153 ± 10 ng/mL in the pair-fed control vs 46 ± 14 ng/mL in the EtOH animals ($p < 0.02$; Fig. 8). After 2 mo of feeding, PRL levels were 212 ± 18 ng/mL in the pair-fed control vs 93 ± 25 ng/mL in the EtOH-fed animals, as shown in Fig. 8 ($p < 0.01$). In older female rats (60 d old at the initiation of feeding), there was no significant difference in PRL levels after 2 wk of EtOH feeding, although there was a significant fall after 2 mo ($p < 0.05$; Fig. 9). Note that all animals were sacrificed on the afternoon of proestrus.

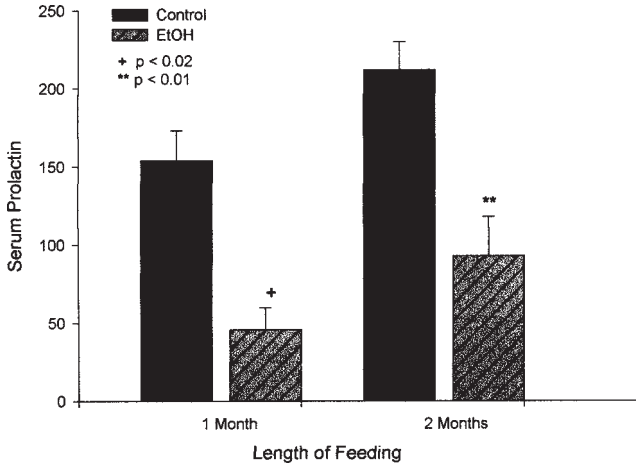

Fig. 8. PRL response to chronic 1- or 2-mo EtOH administration in pubertal female rats age 28 d at start of experiment. There were 15 rats in the pair-fed control group and 10 in the EtOH group. Data are the mean ± SEM.

Discussion

It is well known that EtOH perturbs the mammalian reproductive systems of both genders *(1–4)*. PRL is an estab-

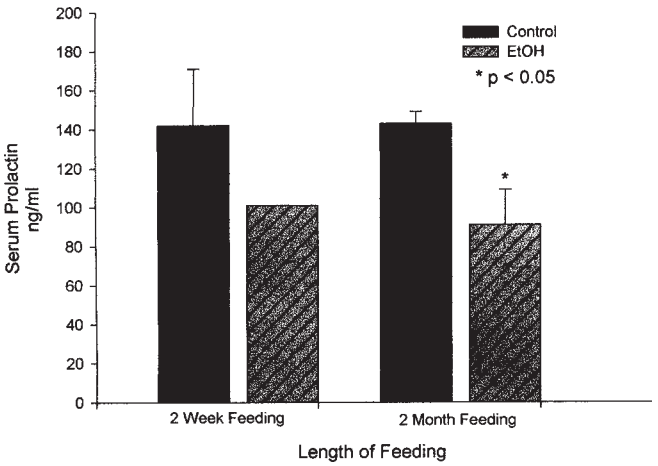

Fig. 9. PRL response to chronic 2-wk or 2-mo EtOH administration in adult female rats age 60 d at start of experiment. The rats were sacrificed between 4:00 and 5:00 PM on the day of proestrus. There were 15 rats in the control group and 10 in the EtOH group. Data are the mean ± SEM.

lished reproductive hormone, and alterations in PRL dynamics have been invoked as playing a role in this EtOH-induced reproductive dysfunction. Studies over the past 25 yr have shown divergent PRL responses to EtOH. This variability in response might be the result of several factors, including age of the animal, gender, and/or EtOH exposure paradigm. To address these possibilities, we have reported here data from several studies investigating the effect of both acute and chronic EtOH exposure in male and female rats as they progress through puberty into adulthood. Indeed, in the experiments we have reported, variable PRL changes with EtOH were noted depending on the age/sexual maturity, gender, and length of EtOH exposure, which might account for some of the discrepant PRL responses reported in the literature.

Nutritional influences are important in any study of the reproductive unit. For this reason, the weights of the animals were carefully monitored throughout the chronic feeding studies. The pair-feeding paradigm, wherein control (i.e., non-EtOH-exposed) rats are given the same number of calories as their EtOH-treated mates addresses this nutritional concern and is, in fact, the standard in EtOH studies. Despite this pair feeding, it has been a common experience that EtOH-treated rats consistently weigh less than their matched control. Inspection of the weight curves indicates that this difference in weights between EtOH and control rats is not owing to weight loss in the EtOH group; rather, it reflects slower weight gain, most noticeable in the first week of the experiment. In addition, the animals show no signs of ill health and continue to be active and appear well groomed.

In the studies of male rats, baseline PRL levels rose with age consistent with other reports in the literature *(9–15)*. The effect of EtOH seems to be age dependent. In the youngest animals (i.e., those that were prepubertal at the initiation of the study), either acute or chronic EtOH depressed PRL levels compared with control. As animals progressed through puberty into adulthood, EtOH failed to alter PRL. In the oldest animals (65 and 85 d old), acute EtOH produced a three- to fourfold increase in PRL above control. However, in 65-d-old animals, chronic EtOH did not affect PRL. The levels of EtOH differed with different age groups in the acute studies. We cannot rule out the possibility that this affected the PRL response. However, it does not seem to account for all the differences since there were age differences in PRL response to chronic EtOH despite nearly identical EtOH levels.

A review of the literature indicates that most acute studies (including one of our own) have shown an EtOH-induced increase in PRL, but all of these previous studies have been done using adult rats *(16–21)*. To our knowledge, ours was the first study of the effect of EtOH on PRL in prepubertal male rats. Taken together with the current data, the concept emerges that the PRL response to acute EtOH is markedly age dependent with young animals exhibiting decreased PRL and adults increased PRL.

In addition, a number of studies in human adult males have generally shown that EtOH increases PRL *(22–27)*, although this finding has not been invariable *(28,29)*. Thus, it appears that the phenomenon of acute EtOH-induced hyperprolactinemia is true across several species of adults. Interestingly, studies of PRL response to acute EtOH in the young adult sons of fathers with alcoholism have yielded variable and inconsistent results *(30–32)*. This may indicate that genetic factors involved in alcoholism can modify the PRL response to EtOH. We are aware of only one report of EtOH/PRL interactions in adolescent humans. In that study, PRL levels in acutely intoxicated adolescents coming to an emergency room were higher than those with no EtOH consumption *(33)*. Since we must assume that the intoxicated adolescents in the emergency room were ill and/or stressed, this is not really a study of a pure EtOH response and, thus, is not necessarily inconsistent with our findings in younger rats.

Chronic administration of EtOH in male rats has yielded variable results. Two groups (our own and Salonen and Huhtaniemi *[34]*) have found no EtOH-induced stimulation of PRL *(34)*. By contrast, two other groups *(35–37)* have reported that chronic EtOH exposure stimulated PRL. Examination of these latter three studies (from two different laboratories) reveals experimental differences, which could account for the variation in results. In the first instance, the strain of animal was different from ours—Wistar rather than Sprague Dawley *(35,36)*. In addition, there was a extraordinarily large, and unexplained, variation in baseline control PRL over a 6-wk time course during which PRL was measured weekly. Specifically, control PRL levels fell by about 85% by the third week of serial blood sampling *(35, 36)*. Thus, the difference in PRL levels between EtOH-fed

and control animals might well have been owing to a decline in control rather than a stimulation of PRL by EtOH. The study from the other laboratory was quite different from ours in that it was short term (3 d) and EtOH was force fed by a permanently implanted gastric cannula *(37)*.

Studies of chronic EtOH exposure in human adult males have generally shown an EtOH-associated increase in basal or stimulated PRL levels *(38–42)*. These have necessarily been done in patients with alcoholism. Therefore, it is difficult to conclude that any effect is purely owing to EtOH. Factors such as malnutrition, concurrent illness, effects of EtOH withdrawal, and hereditary factors in alcoholism may have been involved and could not be properly controlled.

The acute study of prepubertal (and thus noncycling) female rats demonstrated that EtOH induced a fall in serum PRL. In the acute study of adult female rats given EtOH at noon of proestrus, there was a dramatic blunting of the rise in PRL seen at this phase of the cycle. We acknowledge that, since these were single time point studies, EtOH may have caused a shift in the proestrus PRL surge. By contrast, when EtOH was given at 4:00 PM of diestrus, PRL was unaffected on proestrus and slightly stimulated at the subsequent estrus or diestrus.

We were unable to find other literature on the effects of EtOH in prepubertal female rats, so ours represents the first report of these effects in this age group. A review of the literature concerning the effect of EtOH in nonlactating, nonsuckling adult female rats seems to indicate that the response of PRL is exquisitely dependent on the phase of the estrous cycle in which EtOH is given. For example, although our studies showed that EtOH given at noon on proestrus markedly decreased or temporally shifted the PRL surge, Alfonso, Marco, and colleagues *(43–46)* reported that if EtOH is given at 6:00 PM on the day before proestrus, or 9:00 AM of proestrus, PRL is increased. The fact that our current report is not totally consistent with theirs is not surprising since the endocrine changes in proestrus are rapid. Yet, the concept emerges that the timing of EtOH exposure is crucial to the effect on PRL secretion. A large multiple time sampling study is required to define this more precisely.

There are surprisingly few data on chronic EtOH exposure in cycling female rats. Both Rettori et al. *(47)* and Sanchis et al. *(48)* showed increases in PRL after 5 d *(47)* or 5 wk *(48)* of feeding. However, these animals were not predominantly sacrificed at proestrus, as in our studies. Our work suggests that chronic EtOH (like acute) generally decreases PRL in animals sacrificed at proestrus. Taken together, these data indicate that chronic EtOH enhances the relatively low PRL levels seen at estrous cycle days other than proestrus. By contrast, EtOH blunts (or shifts the timing) of the PRL surge at proestrus.

In lactating and/or suckling rats, Subramanian and colleagues *(49–55)*, in a long series of classic studies, have consistently shown that acute or chronic EtOH decreased serum PRL. The effects of acute EtOH in ovariectomized rats are inconsistent *(56–58)*.

Of nine published studies that have examined the effects of acute EtOH exposure on PRL in adult human females, seven have shown a stimulatory effect *(59–67)*. This stimulatory result is not really at variance with our findings in adult female rats, because most of our work evaluated the effects of EtOH on the midcycle PRL surge. By contrast, all of the human studies we have cited involved postmenopausal women or women in either the follicular or luteal phases of the cycle, at times before or after the midcycle pituitary hormone surge. Moreover, we have found that when EtOH was given at 4:00 PM of diestrus, there was a slight stimulation of PRL at subsequent estrus or diestrus compatible with the human data.

There are very few chronic EtOH/PRL data in adult human females. Of two published studies, neither is directly comparable with our work. In one, Teoh et al. *(68)* showed that PRL was increased in women with chronic alcoholism. In the other, Mendelson and Mello *(69)* reported, in an observational study, that PRL was higher in women who spontaneously drank more EtOH than in those who drank less. While both of these are interesting and important observations, neither was a prospective study of controlled EtOH intake in healthy organisms, as was ours. We were unable to find any work on the effect of EtOH on PRL in human adolescent females.

The mechanism(s) and locus of action of EtOH's effect on PRL are not entirely known. It seems clear from a number of acute in vitro studies that EtOH can directly modulate PRL release, generally in a stimulatory manner *(70–76)*. This effect was dependent on protein synthesis since it was inhibited by cycloheximide *(70,75)*. Furthermore, it was also calcium dependent *(73)*. There are data to suggest that EtOH may have antidopaminergic effects at the pituitary *(17,77)*. On the other hand, EtOH probably acts by exerting hypothalamic and/or super hypothalamic effects as well. For example, EtOH was shown to act by enhancing hypothalamic vasoactive intestinal polypeptide release, which, in turn, stimulated pituitary PRL secretion *(19)*. Another study implied a mechanism whereby EtOH increased PRL via augmented hypothalamic substance P secretion, which, in turn, stimulated pituitary PRL release *(20)*. Naloxone has been shown to blunt the PRL response to EtOH in male rats, suggesting that the secretory increase in PRL might be dependent on alteration of endogenous opiate dynamics in the hypothalamus or pituitary *(21)*. On the other hand, nalxone seems to enhance the EtOH-induced PRL increase in human females *(66)* In a hormonally distinct model of the lactating rat, the studies of Subramanian and colleagues *(78–80)* have suggested that the inhibitory effect of EtOH on the suckling-induced PRL surge is exerted primarily at the hypothalamic level. He and his colleagues reported that PRL responses to a variety of pituitary PRL secretogogues (thyrotropin-releasing hormone, sulpiride, and β-endorphin) were not reduced by EtOH *(78–80)*.

Table 1
Experimental Design[a]

Gender	Age (d)	No. of control/ EtOH	Exposure protocol (time)	Day of estrous cycle sacrificed
Male	35	10/10	Acute (90 min)	NA
	45	10/10	Acute (90 min)	N/A
	55	10/10	Acute (90 min)	N/A
	65	10/10	Acute (90 min)	N/A
	85	10/10	Acute (90 min)	N/A
	30	15/10	Chronic (1 mo)	N/A
	30	15/10	Chronic (2 mo)	N/A
	45	15/10	Chronic (2 wk)	N/A
	55	15/10	Chronic (2 wk)	N/A
	60	15/10	Chronic (1 mo)	N/A
Female	30	10/10	Acute (4 h)	Not cycling
	60	10/10	Acute (4 h)	Proestrus
	60	6/6	Acute (24 h)	Proestrus
	60	5/5	Acute (24 h)	Estrus
	60	5/5	Acute (24 h)	Diestrus
	28	15/10	Chronic (1 mo)	Proestrus
	28	15/10	Chronic (2 mo)	Proestrus
	60	15/10	Chronic (2 wk)	Proestrus
	60	15/10	Chronic (2 mo)	Proestrus

[a] Acute injection was with 2 g of EtOH/kg of body wt for animals ages 30–55 d and 3 g of EtOH/kg of body wt for animals 60 d and older. Chronic feeding was with an EtOH diet containing 36% of calories as EtOH or as dextrimaltose. N/A, not available.

In conclusion, our studies show that the PRL response to EtOH is highly dependent on the mode of administration and the gender and sexual maturity of the animals. Depending on the situation, EtOH may stimulate, inhibit, or have no effect on PRL secretion. A thorough examination of the changing cellular and molecular mechanisms of these phenomena and their long-term whole organism implications seems warranted.

Materials and Methods

Animals

Male and female Sprague Dawley rats were purchased from Harlan (Indianapolis, IN). Animals arrived 5 d prior to the experiment and were housed at 22º C with a 12-hr light/dark cycle.

Table 1 summarizes the various experimental designs. In the acute experiments, male and female rats ages 30–85 d, spanning prepuberty to young adulthood, were given saline or EtOH as an ip injection. All ip injections included 2 mg/kg of lidocaine mixed in the same syringe to alleviate any pain/stress associated with the EtOH injection. There were 5–10 rats in each experimental group. Control animals received saline with ip lidocaine in a volume identical to that of the EtOH animals. Prepubertal and peripubertal rats (ages 30–55 d) received 2 g/kg of EtOH since mortality was noted at higher doses. The adult male and female animals received 3 g/kg of EtOH. Vaginal smears were taken on the female animals to determine their cycle. Male animals were sacrificed 90 min after injection, while the females were injected before noon of proestrus, or 4:00 PM of diestrus. Females were sacrificed at either 4:00 PM of proestrus, estrus, or diestrus. The 30-d-old females did not have vaginal opening and, therefore, were sacrificed 90 min after EtOH administration. Trunk blood was collected, separated, and stored at –20º C for subsequent radioimmunoassays (RIAs).

For the chronic studies, male rats were divided into three groups: ethanol exposed, pair-fed controls, and ad lib controls. There were 10 rats in each of the experimental groups. Immediately after arrival, all animals were started on a liquid diet. In the EtOH group, the Lieber DeCarli diet was used. This contained 36% of the calories as EtOH, and animals were acclimated to this diet over 3 d with gradually increasing concentrations of EtOH *(81)*. In the pair-fed control group, the animals were given the exact number of calories consumed the previous day by their EtOH-exposed mates. The control diet was identical to the EtOH diet except dextrimaltose was substituted for EtOH. The ad lib group received unlimited access to the nonEtOH-containing liquid diet. Female smears were taken daily during the entire experiment. Studies were continued for 2 wk, and 1 or 2 mo. In all chronic studies, the ad lib–fed animals had hormone levels similar to those seen in the pair-fed controls. Therefore, for clarity of presentation, the ad lib and pair-feeding data are not presented separately but are pooled. The animals were weighed weekly and at the time of sacrifice. The female animals were sacrificed on the day of late proestrus between 4:00 and 5:00 PM.

Assays

Prolactin RIA

The PRL RIA was conducted as previously described *(82)* utilizing the materials generously contributed by the National Hormone and Pituitary Program and by the National Institute of Diabetes and Digestive and Kidney Diseases. The assay sensitivity was 155 pg/mL. The interassay and intraassay coefficients of variation were 5.0 and 3%, respectively.

Blood EtOH

Blood EtOH was analyzed by a kit, using an enzymatic determination of EtOH in the serum, from Sigma (St. Louis, MO) following the suggested protocols. Blood EtOH was taken 1.5 h after the acute administration for the males and approximately 4 h after injections for the females in the acute studies.

Statistical Analyses

Statistical analyses were performed by the Mann-Whitney U-tests and two-way analysis of variance followed by the Neuman-Keuls post hoc with Tukey's follow-up and

individual group comparisons by student's unpaired *t*-test. A p value of <0.05 was considered significant. Animal weight data were analyzed by least squares regression analysis.

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