Studies on the Site of Ethanol Action in Inducing Prolactin Release in Male Rats

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Hypersecretion of prolactin (PRL) has been implicated as one of the factors that mediate ethanol-induced hypogonadism, but the site(s) in the central nervous system where ethanol acts to lead to the stimulation of PRL secretion is unknown. To clarify the site(s) of ethanol action, medial basal hypothalamic deafferentation (MBHD) or medial basal hypothalamic ablation (MBHA) were performed stereotaxically in male rats, and their PRL secretory capacity in response to acute ethanol administration was compared with that of intact or sham-operated controls. In intact control rats, plasma immunoreactive PRL concentration increased markedly ($P < .001$ *v* saline injection) following ethanol 400 to 500 mg/100 g body weight (BW) intraperitoneally (IP). The PRL response was dose-related and reached a maximum plateau level at 15 minutes. Plasma PRL returned to a near-basal level by 60 minutes. The response was blocked completely ($P < .001$) by pretreatment with dopamine (1 mg per rat), a specific inhibitor of adenohypophyseal PRL secretion. In sham-operated rats and in MBHD and MBHA rats, ethanol (500 mg/100 g BW IP) induced a significant ($P < .001$ to .05) elevation of PRL relative to the respective saline treatment. The basal level was significantly ($P < .005$) lower in the MBHD group (5.3 ± 0.9 ng/mL) and significantly ($P < .001$) higher in the MBHA group (101.1 ± 15.7 ng/mL) than in the sham group (17.2 ± 5.9 ng/mL). These results suggest the following: (1) acute ethanol administration stimulates PRL secretion from the pituitary in a dose-related manner, (2) ethanol appears to have direct stimulatory effects on adenohypophyseal PRL secretion, and (3) extrahypothalamic brain areas exert a stimulatory influence and the hypothalamus an inhibitory influence on basal PRL secretion.

ACUTE OR CHRONIC ethanol (EtOH) ingestion affects the functions of the endocrine system including the hypothalamic-pituitary-gonadal (HPG) axis. It has been well documented that chronic alcohol ingestion leads to gonadal atrophy and a decrease in testosterone synthesis.[1] Several mechanisms at multiple levels of the HPG axis have been proposed to explain the EtOH-induced suppression of testosterone secretion and hypogonadism.[2-3] Prolactin (PRL) involvement is among the mechanisms proposed to date. PRL is known to affect the HPG axis presumably through its suppressive effects on hypothalamic-pituitary luteinizing hormone dynamics and also through interference with luteinizing hormone action at the gonadal level.

Serum PRL level has been reported to be elevated in chronic alcoholics,[4] as well as in EtOH-treated experimental models.[5-7] However, only a few reports are available on the effects of acute administration of EtOH on PRL dynamics, and their results are conflicting. Moreover, the site(s) within the central nervous system where EtOH acutely acts to lead to stimulation of PRL secretion is unknown. Although the mechanisms of the acute PRL-stimulating effect of EtOH might be different from those of the hyperprolactinemia that may be seen in chronic alcoholics, it is nevertheless interesting to know the mechanism of the PRL secretion caused by acute EtOH administration. It may not be irrelevant, considering that the outcome of chronic alcoholism is determined by the cumulative sum of the effects of habitual drinking binges, each of which is, so to speak, an "acute episode" of excess EtOH intake. In the present studies, experiments were designed primarily to sort out the site(s) of the PRL-stimulating action of acutely administered EtOH, using medial basal hypothalamic deafferentation (MBHD) and medial basal hypothalamic ablation (MBHA) models in the rat.

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MATERIALS AND METHODS

Animals

Male Wistar rats aged 13 to 16 weeks (280 to 330 g) were used. In one experiment, female rats of the same strain and of comparable age were also used. With female rats vaginal smears were examined daily, and only those rats in the estrus stage were used. They were housed two to three animals per cage and maintained under a controlled light-dark cycle (lights on 6:30 AM to 8:30 PM) and temperature (24°C). Food and water were available ad libitum at all times. The rats were acclimatized for 2 to 3 weeks before each experiment with daily handling.

Surgery

Stereotaxic surgery to establish complete MBHD was performed under ether anesthesia according to the original methods of Halász and Pupp,[8] but with some modifications[9] to ensure completeness of deafferentation. The knife was attached to the stereotaxic instrument (Natsume, Tokyo, Japan) in a sagittal alignment and inserted into the brain with the tip positioned 1.5 mm behind the bregma. The knife had a radius of 1.5 mm and a height of 2.9 mm. After lowering the knife to the point where the knife tip lightly touched the skull base, it was rotated 180° anteriorly, moved back 1 mm while the knife was held at a 90° position, rotated 180° posteriorly, and then moved forward 1 mm such that a complete island of isolated medial basal hypothalamus was produced. Sham surgery consisted of opening the cranium and lowering the knife 4 mm below the surface of the brain cortex and withdrawing it without rotation.

A C-shaped modified Halász-Pupp knife was used to establish MBHA.[10,11] The shape and dimension of the knife were designed such that rotation through 360° produced enucleation of the medial basal hypothalamic tissue.

EtOH Experiments

All experiments were performed between 9:00 and 11:30 AM. The animal quarters were not entered for 16 hours before each

experiment, to standardize the experimental conditions. All blood samples were obtained in a room adjacent to the animal quarters between 9:00 and 11:30 AM.

The animals were injected intraperitoneally (IP) with either 200 to 500 mg/2 mL/100 g body weight (BW) EtOH dissolved in saline or equal volumes of saline. After injection, the rats were returned to their cages and killed by rapid (within 30 seconds after removal from the cages) decapitation 15 to 60 minutes later. Blood samples were collected into heparinized test tubes for subsequent radioimmunoassay of PRL. The basal control rats were decapitated without injection. In some experiments, the animals were pretreated with subcutaneous injections of either dopamine (1 mg per rat) or saline 5 minutes before IP EtOH. When dopamine was given, it was dissolved with saline immediately before the injection to yield a concentration of 2 mg/mL.

Blood samples were centrifuged at 1,600 × *g* for 30 minutes, and the plasma was separated. Plasma samples were kept frozen at −40°C until assayed for PRL. Brains were fixed with 15% Formalin, decalcified with hydrochloric acid, formic acid, and aluminium chloride solution, and processed for histological examination to confirm the completeness of MBHD and MBHA. Data from rats in which brain histology showed unsatisfactory surgery were omitted. Plasma PRL concentration was determined by a double-antibody radioimmunoassay using kits kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases. All samples were measured in duplicate, and samples from a given set of experiments were analyzed in the same assay to avoid interassay variation. Statistical probabilities were determined by ANOVA.

RESULTS

Effects of Acute Administration of Graded Doses of EtOH on Plasma PRL Concentrations in Intact Male Rats

A total of 37 intact male rats were divided into five groups of seven to eight rats each. Rats were administered 200, 300, 400, or 500 mg/100 g BW EtOH or saline and decapitated 30 minutes later. There was a dose-related increase of plasma PRL after EtOH administration. There was a 7.3-fold elevation ($P < .001$) of plasma PRL relative to the level obtained with saline treatment following 500 mg/100 g BW EtOH (Fig 1).

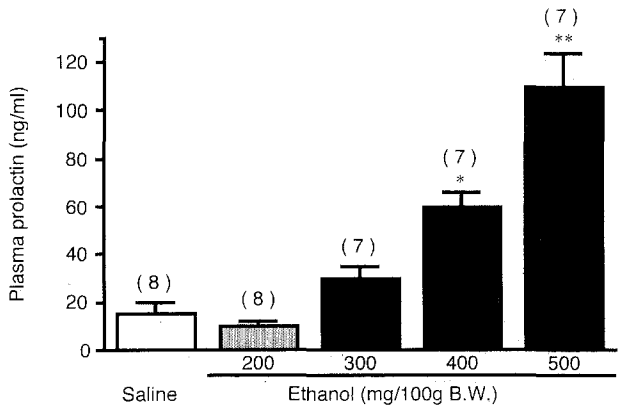

Fig 1. Plasma PRL concentration in response to IP administration of graded doses of EtOH or saline in intact male rats. Each bar and line represents the mean ± SEM plasma PRL concentration for 7 to 8 rats; number of animals used is indicated in parentheses. *$P < .01$, **$P < .001$: *v* saline control.

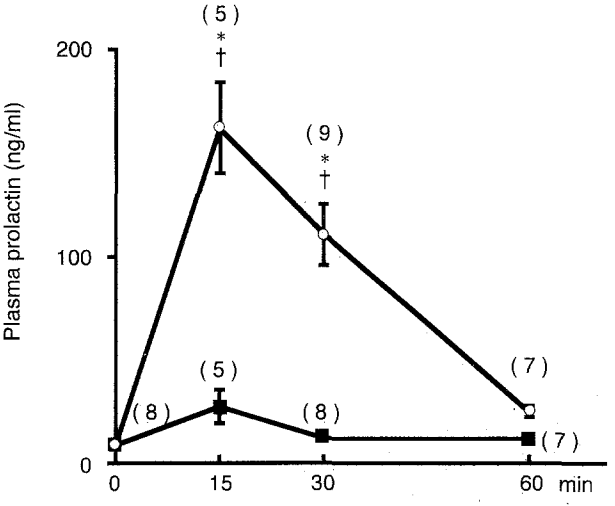

Fig 2. Temporal pattern of plasma PRL concentration following IP administration of 500 mg/100 g BW. EtOH (○) or saline (■) in intact male rats. Each point represents the mean ± SEM plasma PRL for 5 to 9 rats; number of animals used is indicated in parentheses. *$P < .001$ *v* baseline PRL. †$P < .001$ *v* corresponding saline controls.

Temporal Course of PRL Secretion in Response to EtOH 500 mg/100 g BW

Forty-one intact male rats were administered 500 mg/100 g BW EtOH or saline, and groups of five to nine rats were decapitated 15, 30, or 60 minutes later. Basal control rats were decapitated without injection. Plasma PRL concentration increased unequivocally and reached a maximum plateau level ($P < .001$ *v* basal control) at 15 minutes. It then returned to a near-basal level by 60 minutes. Injection of saline did not cause significant changes of plasma PRL (Fig 2).

Sex Comparison of the Effects of EtOH 500 mg/100 g BW

Thirteen intact male rats and 17 intact female rats were administered EtOH 500 mg/100 g BW IP and decapitated 30 minutes later. EtOH induced a significant elevation of plasma PRL in both sexes, but the degree of elevation was much greater with males than with females. In the males, EtOH induced a 20.4-fold elevation of PRL relative to the saline treatment level ($P < .001$). In contrast, only a 4.7-fold ($P < .05$) elevation was observed in the females (Fig 3). Basal levels of plasma PRL were not different ($P > .1$) between males (8.5 ± 3.2 ng/mL) and females (8.2 ± 1.4 ng/mL).

Effects of Pretreatment with Dopamine on EtOH-Induced PRL Release

Thirteen intact male rats were pretreated with dopamine 1 mg per rat or saline subcutaneously 5 minutes before EtOH (500 mg/100 g BW IP) and decapitated 30 minutes later. EtOH-induced PRL release was blocked completely ($P < .001$) by dopamine pretreatment (Fig 4).

Effects of MBHD on EtOH-Induced PRL Release

Groups of MBHD and sham-operated rats were administered EtOH (500 mg/100 g BW IP) or saline and decapi-

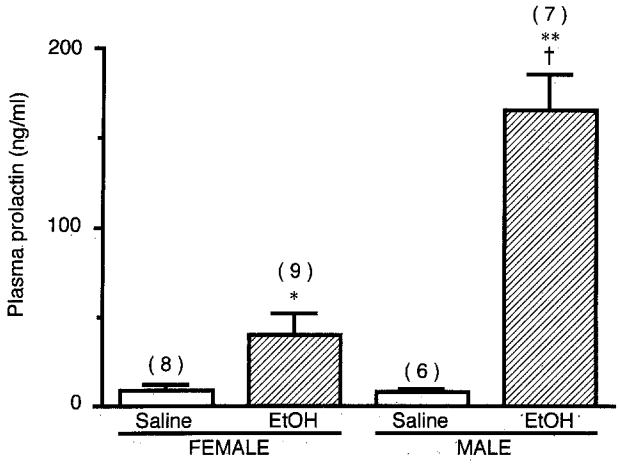

Fig 3. Plasma PRL concentration in response to IP administration of 500 mg/100 g BW EtOH or saline in intact male and female rats. Each bar and line represents the mean ± SEM plasma PRL for 6 to 9 rats; number of animals used is indicated in parentheses. *$P < .05$, **$P < .001$: *v* respective saline controls. †$P < .001$ *v* EtOH-treated females.

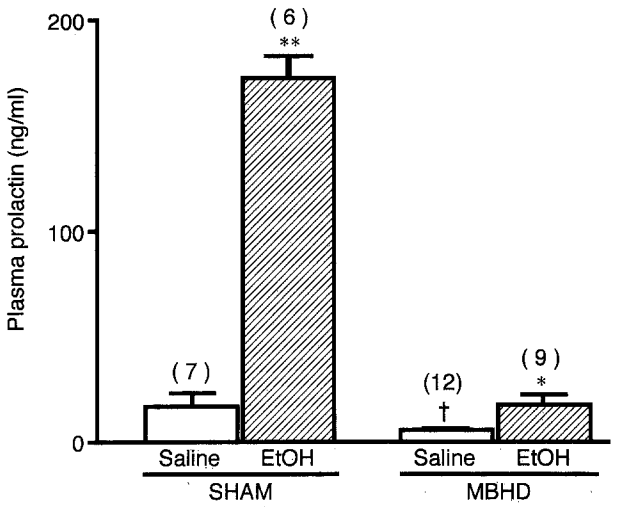

Fig 5. Plasma PRL concentration in response to IP administration of 500 mg/100 g BW EtOH or saline in sham-operated and MBHD male rats. Each bar and line represents the mean ± SEM plasma PRL for 6 to 12 rats; number of animals used is indicated in parentheses. *$P < .05$, **$P < .001$: *v* respective saline controls. †$P < .05$ *v* saline control in the sham group.

tated 30 minutes later. In the sham group, EtOH induced a highly significant ($P < .001$) elevation of plasma PRL relative to the saline treatment level. A significant ($P < .05$) response was also observed with the MBHD group (Fig 5). Although the absolute level of plasma PRL following EtOH administration was much higher in the sham group than in the MBHD group, precise comparison of the magnitude of the response to EtOH between the two groups was not possible, due to markedly different basal PRL levels. The basal PRL level was significantly ($P < .05$) lower in the MBHD group (5.3 ± 0.9 ng/mL) than in the sham group (17.2 ± 5.9 ng/mL).

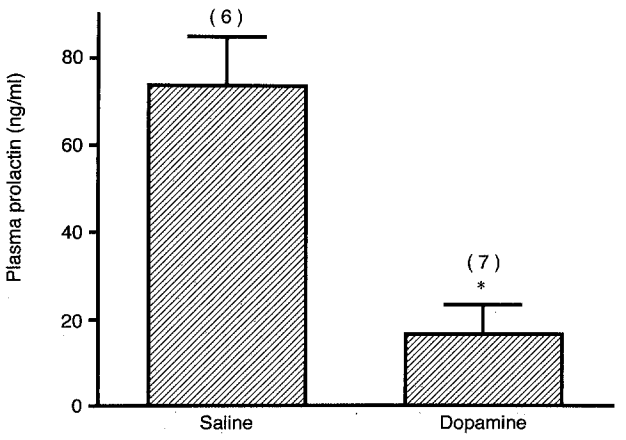

Fig 4. Plasma PRL concentration in response to IP administration of 500 mg/100 g BW EtOH with or without pretreatment with dopamine (1 mg per rat) in intact male rats. Each bar and line represents the mean ± SEM plasma PRL for 6 to 7 rats; number of animals used is indicated in parentheses. *$P < .01$: *v* saline-pretreatment control.

Effects of MBHA on EtOH-Induced PRL Release

Two groups of five MBHA rats each were administered EtOH (500 mg/100 g BW IP) or saline and decapitated 30 minutes later. In MBHA rats, EtOH induced a 3.1-fold elevation of PRL relative to the saline treatment level ($P < .001$). MBHA caused a marked elevation of basal plasma PRL concentration, and the plasma PRL value following saline treatment was as high as 101.1 ± 15.7 ng/mL (Fig 6).

DISCUSSION

The present data demonstrate a stimulatory effect of acute EtOH administration on PRL release in vivo, although the degree of contribution from some specific secretory mechanisms may not be readily determined by

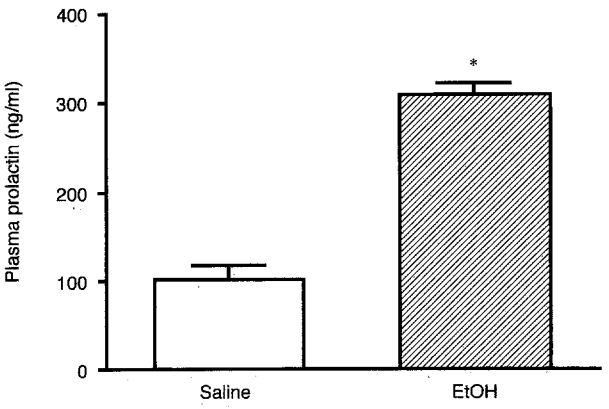

Fig 6. Plasma PRL concentration in response to IP administration of 500 mg/100 g BW EtOH or saline in MBHA male rats. Each bar and line represents the mean ± SEM plasma PRL for 5 rats. *$P < .001$ *v* saline control.

our experiments. These results are in accordance with some previous reports, except that there seems to be some discrepancy in the temporal course of the EtOH-induced PRL secretion.[5-7] In this study, the plasma PRL response reached a maximum plateau level at 15 minutes and returned to near-basal levels by 60 minutes. Seilicovich et al[5] reported a clearly elevated plasma PRL concentration 60 minutes after IP administration of EtOH in a dosage comparable to those used in our studies. In their study, it was not determined whether plasma PRL was elevated at earlier time points following administration of EtOH. In the study by Emanuele et al,[7] serum PRL level was significantly elevated at 90 and 180 minutes after but not 30 minutes after EtOH (3 g/kg BW IP) administration.

Generally, PRL secretory responses to various secretagogues or stimuli such as thyrotropin-releasing hormone or suckling are relatively rapid and reach the maximum level within 15 to 30 minutes. Therefore, there is some doubt as to whether the delayed responses of PRL following EtOH in some previous studies really represented a specific effect of EtOH. It is possible that at least part of the responses reported by various investigators, including our present communication, were somehow related to the experimental procedures rather than specifically to EtOH. In this connection, it is generally well known that a variety of nonspecific stresses are capable of producing an elevation of serum PRL.[12,13] Although many representative stress hormones, including cortisol and epinephrine, were reportedly not elevated after EtOH treatments,[14] there is also evidence to the contrary.[15] In our study, PRL responses in male rats were five times as great as in females. This may favor the specific nature of the EtOH-induced PRL response in our experiments, since PRL responses to some of the nonspecific stresses are known to be much higher in females than in males.[12,13] However, determining whether such sex difference holds true with all nonspecific stresses would require further study. Furthermore, pretreatment with dopamine completely suppressed the PRL response to EtOH, suggesting that this response involved some specific secretory mechanisms, since dopamine is deemed to be a physiological inhibitor of PRL secretion. Despite these indirect evidences that may favor a specific PRL secretion induced by EtOH in our studies, we cannot definitively rule out the possibility of a nonspecific stress effect associated with EtOH administration.

Our results showed that the effect of EtOH to induce PRL release was maintained after complete isolation of the medial basal hypothalamus from the rest of the brain by MBHD, although the apparent magnitude of the EtOH-induced PRL response was attenuated by this procedure. The retained responsiveness to EtOH in MBHD indicates that EtOH acts in the pituitary and/or hypothalamus to cause PRL secretion. However, the apparent attenuation of the EtOH effect in MBHD raises the possibility that EtOH has an additional extrahypothalamic site of action in stimulating PRL release. Previous studies indicated that MBHD completely abolished PRL release induced by ether and immobilization stress,[16,17] which apparently is not the case with EtOH in our studies. Thus, the pathways through which EtOH stimulates PRL release seem to be different from those involved in ether or immobilization stress.

Our results demonstrated that the responsiveness to EtOH was retained even after removal of the medial basal hypothalamus. In MBHA rats, EtOH induced a 3.1-fold PRL response relative to the PRL level in the saline control group. In MBHA, the connection between the pituitary and the brain is completely disrupted, and the lactotropes are generally free from any effects exerted via the brain, including nonspecific stress effects. Therefore, our results would suggest that EtOH has a direct stimulatory effect on lactotrophs in the anterior pituitary, causing secretion of PRL. This agrees with some previous in vitro studies.[18-20] Whether EtOH has an additional site of action within the hypothalamus cannot be determined from the present studies.

There was a remarkable difference between MBHD and MBHA rats with regard to basal plasma PRL concentrations. The former group exhibited significantly lower values compared with the controls, whereas several-fold increases above control values were observed with the latter. The low basal PRL in MBHD suggests that the extrahypothalamic brain area(s) normally sends tonic stimulatory neural input to maintain basal PRL secretory rates. The remarkably high level of basal PRL in MBHA can be explained by the absence of hypothalamic dopamine, a presumed physiological PRL-inhibiting factor, since MBHA completely ablates the dopamine-producing cells of the tuberoinfundibular system in the basal hypothalamus.

EtOH is metabolized primarily in the liver via acetaldehyde to acetic acid. Therefore, it is important to determine if the PRL response that occurred following administration of EtOH was caused by EtOH itself or by one of its metabolites. Our experiments were not designed to answer this question. Acetaldehyde, at least, could not be detected in the brain after administration of EtOH in the dose range we used.[21] This makes involvement of acetaldehyde in the possible extrahypothalamic action of EtOH unlikely.

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