Inhibitory Effect of Alcohol on the Established Suckling-Induced Prolactin Surge in Lactating Rats (43292)

MARAPPA G. SUBRAMANIAN[1]
Department of Obstetrics and Gynecology, Wayne State University, Detroit, Michigan 48201

Abstract. **The effect of acute alcohol infusion on the established suckling-induced prolactin surge in lactating rats was examined. Dams were implanted with an atrial catheter on Day 6 of lactation and blood sampling was done on Day 10. Following the separation of litters from dams for a 6-hr period, a baseline blood sample was removed via a catheter extension. Pups were weighed and returned to dams. Subsequent blood samples were obtained 10, 30, and 60 min after initiation of suckling. Dams were then infused with alcohol doses of 0, 0.5, 1.0, 2.0, or 2.5 g/kg body wt. Infusion (0.1 ml/min) was completed in approximately 30 min. Additional blood samples were obtained 10, 30, 60, and 120 min after the termination of infusion. In a separate group of rats, pups were removed from the dam after the first 60 min of suckling and additional blood samples were obtained 40, 70, 90, and 150 min after removal of pups (corresponding to 10-, 30-, 60-, and 120-min samples for rats infused with various alcohol doses).**
Alcohol, when administered after the establishment of suckling-induced prolactin surge and resulting in blood alcohol levels equal to or greater than legal human intoxication levels, inhibited prolactin release. However, continued suckling for an extended period (120 min in the present study) overcame this inhibitory effect, even when the blood alcohol level was comparable to (2.0 g/kg group) or greater than (2.5 g/kg group) the human legal intoxication level. Furthermore, in rats with established prolactin surges, the patterns of prolactin decline that followed alcohol administration or pup removal were comparable, indicating that similar mechanism(s) may be involved.
[P.S.E.B.M. 1991, Vol 198]

In mammals, suckling promotes prolactin secretion, and this suckling-induced prolactin is essential for the mammary glands to produce adequate milk (1–4). Following separation of pups, maternal plasma prolactin declines and subsequent vigorous suckling induces a pronounced increase in the dam's plasma prolactin level (5–10). Others have reported that separation of pups from the dam after the establishment of the suckling-induced prolactin surge results in a very rapid decline in plasma prolactin (11, 12).

Recently we have been studying the effect of acute alcohol administration on prolactin secretion in rats. Our studies have shown that alcohol given acutely does not affect basal prolactin in lactating (13, 14) and ovariectomized (15) rats; however, suckling-induced prolactin release is inhibited (14). In the present study, we report that alcohol administered after the establishment of a suckling-induced prolactin surge in lactating rats abruptly decreased maternal plasma prolactin levels. To gain some insight into the possible mechanisms involved in the alcohol-induced inhibition of prolactin secretion, we have compared this decline in prolactin to the decrease that occurs normally in dams following the removal of pups.

Materials and Methods

Animals. Virus-free Sprague-Dawley (Charles River Laboratories, Inc., Portage, MI) were purchased and kept under controlled temperature (71 ± 2°F), humidity (50%), and light (14:10-hr light:dark cycle). After 2 weeks of acclimation, female rats were housed individually with a male rat. Trays under the wire bottom cages were inspected every morning for sperm plugs as an indicator of mating. Pregnant rats were then

[1] To whom correspondence and requests for reprints should be addressed at Department of Obstetrics/Gynecology, Wayne State University, 275 East Hancock Avenue, Detroit, MI 48201.

Received February 19, 1991. [P.S.E.B.M. 1991, Vol 198]
Accepted May 9, 1991.

0037-9727/91/1981-0579$3.00/0
Copyright © 1991 by the Society for Experimental Biology and Medicine

individually housed. The day of parturition was designated as Day 1 of lactation and the litters were adjusted to 8 pups on Day 2. On Day 6, dams were implanted with an atrial catheter under ketamine hydrochloride (80 mg/kg body wt; Ketaset; Aveco Co., Inc., Fort Dodge, IA) and xylazine (4 mg/kg body wt; Rompun; Mobay Corp., Shawnee, KS) anesthesia. The catheter allowed us to withdraw blood samples and administer alcohol solutions in freely moving conscious animals. All experiments were done on Day 10 of lactation.

Blood Sampling. On Day 10 of lactation, pups were removed from the dam at 0700 hr and kept in a cage placed on a heated pad. At 1200 hr an extension was attached to the exteriorized portion of the catheter. The extension, consisting of a three-way stopcock (K75; Pharmaseal), polyethylene tubing (PE-50; Clay Adams), and a connector (22-gauge stainless steel tubing, 1.5 cm long), was filled with heparinized (10 IU/ml) 0.9% saline. After a stabilization period of 1 hr, a baseline blood sample (0.5 ml) was withdrawn and immediately transferred to a tube containing an equal volume of chilled phosphate-buffered saline (pH 7.0) with 10 IU heparin/ml. After each sample, fluid volume was replaced with warm (37°C) saline. Following removal of the baseline blood sample, pups were weighed and returned to the dam. Subsequent blood samples were obtained 10, 30, and 60 min after the initiation of suckling. Peak plasma prolactin is usually observed 60 min after suckling starts.

To study the effects of alcohol administration on the established prolactin surge, groups of rats were administered alcohol at 0-, 0.5-, 1.0-, 2.0-, and 2.5-g/kg body wt doses following the 60-min blood sample. Infusion of saline or alcohol in saline solutions was done using a Harvard infusion pump set to deliver 0.1 ml/min. During infusion, which lasted approximately 30 min and varied slightly depending on the body weight, pups were allowed to continue nursing. Dams given alcohol maintained the suckling posture. At the end of alcohol or saline infusion, 0.4 ml of saline was infused to ensure that no alcohol remained in the catheter. Alcohol infusions for the various groups were prepared as 7.5% (0.5 g/kg body wt group), 15% (1.0 g), 30% (2.0 g), and 37.5% (2.5 g) solutions in saline (v/v). Additional blood samples (0.6 ml) were obtained at 10, 30, 60, and 120 min after completion of infusion. Blood samples (0.5 ml) for prolactin analysis were mixed with an equal volume of phosphate-buffered saline, as described above, centrifuged, plasma separated, and stored at −20°C until assayed. For blood alcohol level (BAL) determination, 0.1 ml of blood was placed in a vial containing 1.0 ml *t*-butanol (8 mg%), crimp sealed, and kept frozen at −20°C until assayed by head space gas chromatography.

In a separate group of rats, pups were removed from the dam after the first 60 min of suckling and blood samples were obtained 40, 70, 90, and 150 min after the removal of pups. These sampling periods corresponded to 10-, 30-, 60-, and 120-min samples collected from the groups of rats infused with various doses of alcohol (infusion time was approximately 30 min).

Suckling Latency and Milk Consumption. Following the return of pups, the time taken for the majority of pups (five or more) to attach to the nipples and start vigorously suckling (suckling latency) was determined. The amount of milk consumed by the pups was estimated by subtracting the weight of pups obtained just prior to returning them to the dam from the weight at the end of the experimental period.

Prolactin and Alcohol Measurements. Prolactin in plasma was measured by double antibody radioimmunoassay using reagents provided by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, as described previously (13–16). Within- and between-assay variability expressed as coefficients of variation were less than 5% and 10%, respectively.

Blood alcohol levels were determined by head space gas chromatography using a Perkin Elmer (Sigma 2000) gas chromatograph, which has an automatic sampling turntable and an electropneumatic dosing system (14–16). Variation of both within- and between-assay measurements averaged less than 1%.

Data Analysis. Plasma prolactin levels were analyzed by repeated-measures multivariate analysis of variance (MANOVA). Values at individual time points were analyzed by one-way analysis of variance (ANOVA) with the Student-Newman-Keuls procedure applied post hoc. In the experiment comparing prolactin decline for the group of rats from which litters were removed to the two groups of rats administered with alcohol (2.0 and 2.5 g/kg body wt), prolactin levels following the first 60 min of suckling were taken as 100% and subsequent values were expressed as percentage of the 60-min values. BAL were analyzed by MANOVA. Milk consumption and suckling latency were analyzed by one-way ANOVA. All results are expressed as mean ± SE.

Results

Plasma Prolactin. Following a 6-hr separation of the litters from dams, suckling for 60 min profoundly increased plasma prolactin for all groups (Fig. 1). Analysis of prolactin for these periods (0, 10, 30, and 60 min of suckling and prior to infusion) showed only a time effect among groups ($P < 0.001$; MANOVA). Since there were no differences in the treatment of groups prior to infusion of various alcohol doses, prolactin values for all groups are combined and presented (Fig. 1; 0-, 10-, 30- and, 60-min values prior to infusion). Prolactin levels following infusion of saline (control) or alcohol in saline solutions (0.5, 1.0, 2.0, or 2.5 g/kg

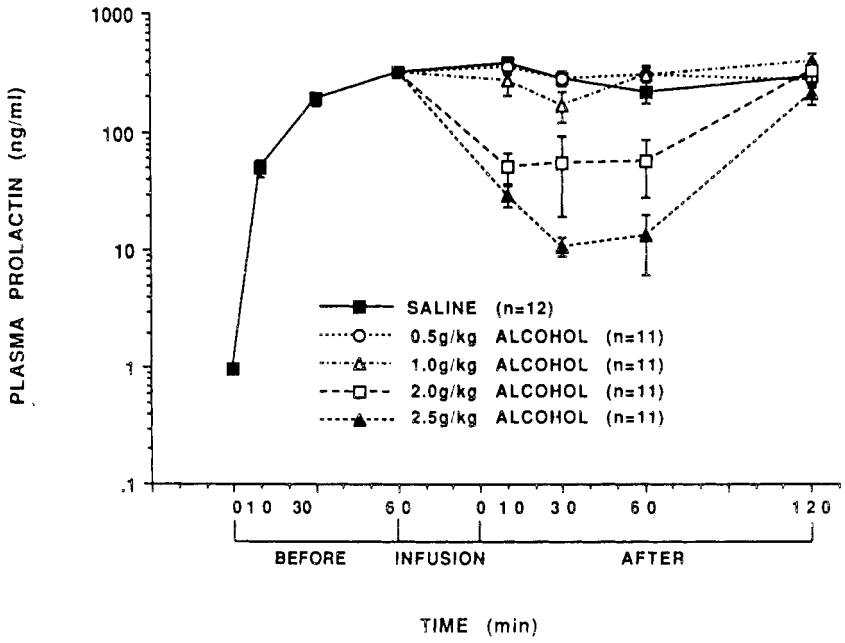

Figure 1. Plasma prolactin in lactating rats following separation of pups for 6 hr and 10, 30, and 60 min after suckling (0-, 10-, 30-, and 60-min levels before infusion). Following 60 min of suckling, rats were infused with saline or alcohol in saline. The infusion rate was 0.1 ml/min and was completed in approximately 30 min. Pups remained with their dam during infusion and subsequent sampling. Additional blood samples for prolactin measurement were obtained 10, 30, 60, and 120 min after completion of infusion. Values are expressed as mean ± SE.

body wt) showed group, time, and group × time interaction effects ($P < 0.001$; MANOVA). Subsequent evaluation by ANOVA and Student-Newman-Keuls multiple comparison test revealed that prolactin levels in the control were greater than 1.0, 2.0, and 2.5 g/kg body wt of alcohol groups after 10 min of suckling following infusion ($P < 0.05$). After 30 and 60 min of suckling, prolactin levels in the 2.0 and 2.5 g/kg body wt groups remained lower than the control ($P < 0.05$). By 120 min. prolactin values were comparable among the groups (Fig. 1).

The decline in prolactin observed following administration of the two highest alcohol doses (2.0 and 2.5 g/kg body wt) was compared to that observed following removal of pups (Fig. 2). All dams in these three groups were separated from their litters for 6 hr before being reunited. Plasma prolactin values after the initial 60 min of suckling were taken as 100% and subsequent values were calculated as percentages of the appropriate 60-min values. Data analysis revealed group, time, and group × time interaction effects ($P < 0.001$; Fig. 2). Subsequent analysis of individual times revealed differences among groups after 120 min of suckling following injection of alcohol solution or litter removal. At this time period, prolactin values for the alcohol-infused groups were greater than for the group from which the pups were removed ($P < 0.05$, Student-Newman-Keuls; Fig. 2).

Blood Alcohol Level. BAL for the groups infused with 1.0-, 2.0-, and 2.5-g/kg body wt alcohol doses are presented in Figure 3. For the group infused with the 0.5-g/kg dose, BAL after 10 min of suckling following infusion was 20 ± 2 mg/100 ml and was not detectable thereafter, so the data for this group were not plotted. Comparison of BAL for 1.0-, 2.0-, and 2.5-g/kg body wt groups revealed group, time, and group × time effects ($P < 0.001$; MANOVA).

Suckling Latency and Milk Consumption. Suckling latencies, defined as the time taken for the majority of the pups to attach to the nipple and start vigorously suckling, were: 4.2 ± 0.7 min (saline); 4.8 ± 0.8 min (0.5 g/kg body wt); 6.5 ± 0.8 min (1.0 g/kg); 4.6 ± 0.6 min (2.0 g/kg), and 4.4 ± 0.5 min (2.5 g/kg). There were no statistically significant differences among groups ($P > 0.05$; ANOVA). The quantities of milk consumed for the various groups were also similar: 6.8 ± 0.8 g (saline), 6.8 ± 0.5 g (0.5 alcohol g/kg body wt), 6.0 ± 0.6 g (1.0 g/kg), 5.1 ± 0.7 g (2.0 g/kg), and 6.2 ± 0.6 g (2.5 g/kg).

Discussion

Recent studies from our laboratory have shown that alcohol, when administered to dams prior to the onset of suckling, inhibited the suckling-induced prolactin surge in lactating rats (13, 14, 16). Furthermore, we have determined that the minimum BAL that would effectively inhibit the suckling-induced prolactin surge, when administered prior to the initiation of suckling, is comparable to or slightly lower than the human legal intoxication level (14, 16). The results from the present study demonstrate that alcohol, when administered after the suckling-induced prolactin surge is established,

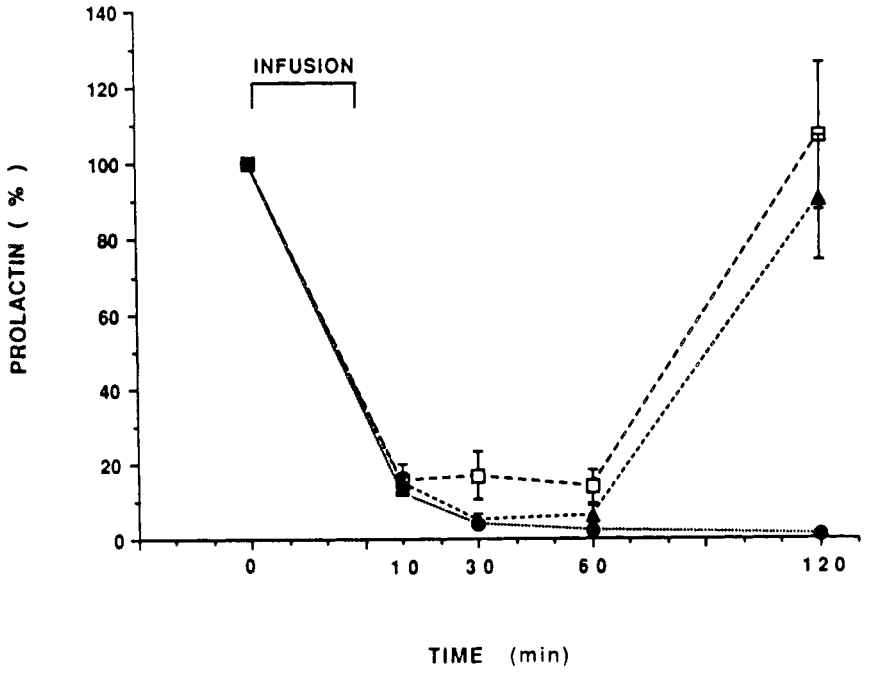

Figure 2. Pattern of plasma prolactin decline following removal of pups 60 min after the initial suckling compared with plasma prolactin levels in the groups of rats administered with alcohol at 2.0 and 2.5 g/kg body wt. Closed circles indicate pups removed; open boxes indicate alcohol 2.0 g/kg body wt; closed triangles indicate alcohol 2.5 g/kg body wt. Pups remained with the dams during and following infusion in alcohol administered groups. Prolactin values after the initial 60 min of suckling (before infusion) were set to 100% and subsequent values were expressed as percentages of the 60-min values for each group. Values are expressed as mean ± SE.

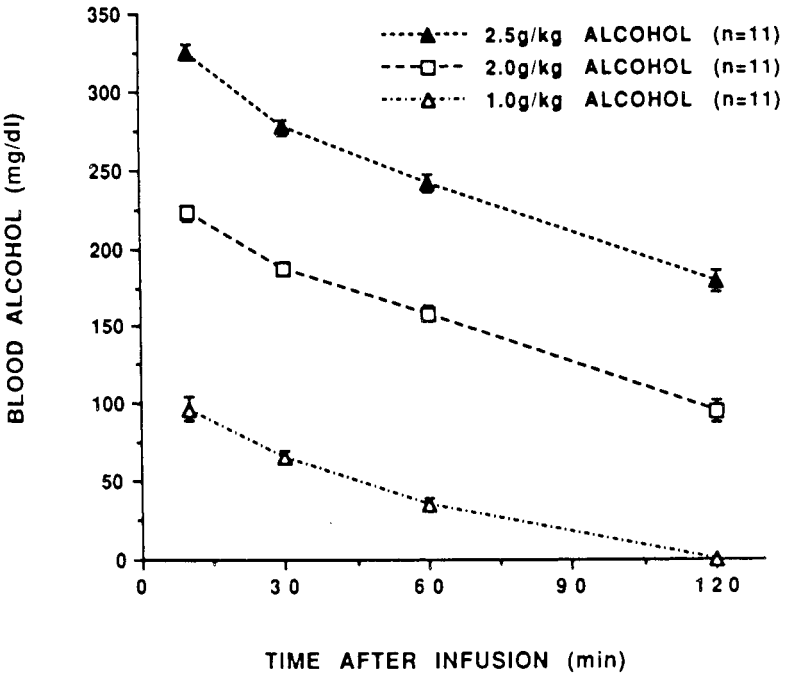

Figure 3. Blood alcohol levels following infusion of 1.0, 2.0, and 2.5 g/kg body wt alcohol. Blood samples were obtained 10, 30, 60, and 120 min after infusion. Values are expressed as mean ± SE.

also inhibits prolactin release. This inhibition was evident for the 1.0-, 2.0-, and 2.5-g/kg body wt groups 10 min after infusion (Fig. 1). BAL for the 1.0-g/kg group at this time period was 97 ± 8 mg/100 ml and the inhibitory effect of alcohol for 1.0- g/kg or greater dose levels corroborates our previous results (14, 16). Plasma prolactin levels for the 2.0- and 2.5- g/kg groups continued to be suppressed at 30 and 60 min of suckling

(Fig. 1). Correspondingly, BAL for these groups were greater than legal human intoxication levels at these time periods (Fig. 3).

Interestingly, although BAL were 95 ± 6 and 178 ± 7 mg/100 ml the 2.0- and 2.5-g/kg groups, respectively, after 120 min of suckling (Fig. 3), plasma prolactin was no longer inhibited (Fig. 1). A plausible explanation for this observation is that continued vigorous suckling by the pups for over 1 hr overcame the inhibitory effect of high BAL on prolactin secretion. Alternatively, the neural inhibitory effect of alcohol could become refractory after 1 hr.

The other noteworthy observation from the present study is the remarkable similarity in the pattern of prolactin decline observed following pup removal or infusion of 2.0- and 2.5-g/kg alcohol doses without litter separation (Fig. 2). The decrease in plasma prolactin in lactating rats following separation of mother and pups has been reported previously (11, 12, 17). Nagy and Halasz (11) suggested that a dopaminergic mechanism might play an important role in the inhibition of prolactin release in dams following litter separation. Whether a similar mechanism is involved in the prolactin decline following alcohol administration to dams with suckling pups needs to be elucidated. It is also possible that alcohol acts at a more peripheral level, perhaps by interfering with the generation and/or transmission of the neural stimulus induced by suckling.

Since all the dams were treated similarly up to the time they were reunited with pups (litter separation and no infusion of alcohol), we expected the suckling latencies among the groups to be similar. Furthermore, after 6 hr of separation, pups are likely to nurse more vigorously during the first hour and to obtain most of the milk during this period, which would explain the absence of difference in milk consumption among groups.

Utilizing a chronically catheterized lactating rat model, we have shown that alcohol administered after the establishment of a suckling-induced prolactin release inhibits plasma prolactin. However, continued suckling appears to overcome the inhibitory effect of alcohol, even when the BAL remains comparable to or greater than the human legal intoxication level. Comparable patterns of decline in plasma prolactin observed following removal of pups and following administration of 2.0- and 2.5-g/kg alcohol doses indicate that the same hypothalamic mechanism may be involved in these two instances of plasma prolactin decline in lactating rats.

This study was supported by National Institute of Alcoholism and Alcohol Abuse Grant AA07670.

The technical assistance of X. G. Chen and B. Bergeski is gratefully acknowledged. I am thankful to Dr. Ruth T. Savoy-Moore for her valuable suggestions and comments in preparation of the manuscript, Sue Corrion for blood alcohol analysis, Michael L. Kruger for data analysis, Dawn Cochrane for secretarial assistance, and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases for providing the prolactin radioimmunoassay materials.

1. Short RV. Breastfeeding. Scientific American **250**:35–41, 1984.
2. Martin CR (Ed). Endocrine Physiology. New York: Oxford University Press, pp620–680, 1985.
3. Yen SSC. Prolactin in human reproduction. In Yen SSC, Jaffe R, Eds. Reproductive Endocrinology. Philadelphia: Saunders, pp237–263, 1986.
4. Whitworth NS. Lactation in humans. Psychoneuroendocrinology **13**:171–188, 1988.
5. Amenomori Y, Chen CL, Meites J. Serum prolactin levels in rats during different reproductive states. Endocrinology **86**:506–511, 1970.
6. Blake CA, Sawyer CH. Nicotine blocks the suckling-induced rise in circulating prolactin in lactating rats. Science **177**:619–621, 1972.
7. Grosvenor CE, Whitworth NS. Evidence for a steady rate of secretion of prolactin following suckling in the rat. J Dairy Sci **57**:900–904, 1974.
8. Subramanian MG, Reece RP. Anterior pituitary and plasma prolactin in rats after 2 to 90 minutes of suckling. Proc Soc Exp Biol Med **149**:754–756, 1975.
9. Selmanoff M, Selmanoff C. Role of pup age, estradiol-17β and pituitary responsiveness in the differences in the suckling-induced prolactin response during early and late lactation. Biol Reprod **29**:400–411, 1983.
10. Mattheij JA, Swarts HJM. Evidence that exteroceptive stimuli other than suckling have no major effect on plasma prolactin in lactating rats. Acta Endocrinologica **114**:417–425, 1987.
11. Nagy G, Halasz B. Time course of the litter removal-induced depletion in plasma prolactin levels of lactating rats. Neuroendocrinology **37**:459–462, 1983.
12. Nagy GY, Banky ZS, Takacs L. Rapid decrease of plasma prolactin levels in lactating rats separated from their pups. Endocrinologia Experimentalis **17**:311–316, 1983.
13. Subramanian MG, Abel EL. Alcohol inhibits suckling-induced prolactin release and milk yield. Alcohol **5**:95–98, 1988.
14. Subramanian MG, Chen XG, Bergeski BA. Pattern and duration of the inhibitory effect of alcohol administered acutely on suckling-induced prolactin in lactating rats. Alcohol: Clin Exp Res **14**:771–775, 1990.
15. Subramanian MG, Savoy-Moore RT, Bergeski BA, Kruger ML, Abel EL. Acute alcohol infusion does not alter plasma gonadotropins or prolactin in ovariectomized rats. Alcohol: Clin Exp Res **14**:191–194, 1990.
16. Subramanian MG, Chen XG, Bergeski BA, Savoy-Moore RT. Alcohol inhibition of suckling-induced prolactin release in lactating rats: Threshold evaluation. Alcohol **8**:203–206, 1991.
17. Chiocchio SR, Cannata MA, Cordero Funes JR, Tramezzani JH. Involvement of adenohypophyseal dopamine in the regulation of prolactin release during suckling. Endocrinology **105**:544–547, 1979.