Effect of a Hypothalamic Extract on Serum Prolactin Levels During the Estrous Cycle and Lactation[1] (34819)

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There is considerable evidence from both *in vitro* and *in vivo* studies that hypothalamic extracts from mammals can inhibit pituitary release of prolactin, indicating the presence of a prolactin-inhibiting factor (PIF) in the hypothalamus (1). Injection of a hypothalamic extract in postpartum lactating rats prior to permitting suckling by their litters for 30 min was reported to prevent depletion of pituitary prolactin content (2). Also, injection of a hypothalamic extract before glass rod stimulation of the uterine cervix of proestrous rats prevented a fall in pituitary prolactin content (3). Serum prolactin values as determined by radioimmunoassay recently were found to be significantly higher during the proestrous and estrous phases of the cycle in rats than during diestrus (4, 5). They were also shown to be increased by suckling during postpartum lactation (5). It was of interest, therefore, to determine whether an injection of hypothalamic extract during proestrus, estrus, or prior to suckling could reduce serum prolactin levels.

Materials and Methods. Mature female Sprague-Dawley rats (Spartan Animal Farms, Inc., Haslett, Mich.), weighing 200–250 g each, were housed in a temperature- (75 ± 1°F) and light-controlled (14 hr light daily) room. The rats were fed a diet of Wayne Lab Blox pellets (Allied Mills, Chicago, Ill.) *ad libitum.*

Daily vaginal smears were initially followed for at least 2 cycles on all rats to be given hypothalamic or cerebral extracts during the estrous cycle. At 8–8.30 AM on the day of proestrus or estrus, a pretreatment sample of about 0.5 ml of blood was removed for prolactin assay by heart puncture under light ether anesthesia. Light ether anesthesia has previously been found not to alter serum prolactin values (5). The rats were then injected with a neutralized acid extract of eight rat hypothalami from mature male rats or an equivalent amount of rat cerebral extract, half administered intraperitoneally and half through the femoral vein. Control rats were injected in a similar manner with physiological saline in volumes equivalent (0.2 ml) to that used for the brain extracts. The brain extracts were prepared according to methods described previously (6). At 1 and/or 4 hr after injection of the brain extracts, about 0.5 ml of blood was removed again for prolactin assay.

Other female rats were bred with males, and on Day 4, 5, or 6 postpartum the litters were removed from the mother rats for a period of 12 hr and a blood sample was taken from each rat. They were then injected with physiological saline, hypothalamic or cerebral extract, half given intraperitoneally and half intravenously. The litters were then replaced or not replaced with their mothers for 3 hr, at the end of which time a second blood sample was removed.

The blood serum of all rats was measured for prolactin by radioimmunoassay (5). Each sample was assayed at three dilutions, averaged and expressed in terms of a standard rat prolactin preparation (HIV-8-C, 23 IU/mg) obtained from Dr. S. Ellis, NASA Research Center, Ames, Calif. Significance of differences between groups was analyzed by

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TABLE I. Effects of Hypothalamic (HE) and Cerebral (CE) Extracts on Serum Prolactin Levels of Cycling Rats During Estrus or Proestrus.

Treatment	No. of rats	Serum prolactin concn (mμg/ml)		
		Pretreatment	1 hr later	4 hr later
Exp. 1: Estrus				
Saline controls	8	45.2 + 5.6[a]		45.8 + 20.1
8 CE/rat	7	68.9 + 9.54		47.3 + 15.5
8 HE/rat	8	43.2 + 6.0		13.0 + 1.1[b]
Exp. 2: Proestrus				
Saline controls	6	43.8 +13.8		39.5 + 5.2
8 HE/rat	10	42.1 + 9.3		16.6 + 2.8[b]
Exp. 3: Proestrus				
Saline controls	8	31.5 + 5.9	34.7 + 6.3	26.2 + 4.7
8 HE/rat	8	30.8 + 7.7	19.1 + 2.5[b]	8.8 + 1.0[b]

[a] Mean and standard error of mean.

[b] Significantly different from HE pretreatment values and from saline pretreatment and post-treatment values ($p < .01$).

Student's t test and the t test for paired observations.

Results. Table I. Expt. 1, shows the effects of injecting a cerebral or hypothalamic extract during the estrous cycle on the day of estrus. Administration of saline had no effect on serum prolactin 4 hr later, whereas injections of hypothalamic extract (HE) significantly reduced serum prolactin concentration. Cerebral extract (CE) produced no significant change in serum prolactin levels over pretreatment control values. In the proestrous rats in Expts. 2 and 3, HE again significantly reduced prolactin in the serum below pretreatment levels. In Expt. 3 the decrease in serum prolactin was significantly greater at 4 hr than at 1 hr after injection of HE.

Table II shows that serum prolactin concentration was very low in postpartum lactating rats 12 hr after litter removal. Administration of CE or HE produced no change in serum prolactin values in the nonsuckled rats when assayed 3 hr later, except in the rats given the equivalent of 8 HE each. In this latter group, serum prolactin was significantly lower than in the pretreatment saline controls and all other post-treatment groups. In rats suckled for 3 hr after 12 hr of no suck-

TABLE II. Effect of Hypothalamic (HE) and Cerebral (CE) Extracts on Serum Prolactin Levels in Postpartum Lactating Rats.

Treatment 12 hr after no suckling	Serum prolactin concentration (mμg/ml)		
	12 hr no suckling	15 hr no suckling	12 hr no suckling, 3 hr suckling
Saline controls	16.2 ± 1.3[a] (10)[b]	19.2 ± 3.6 (5)	117.1 ± 24.7 (5)
4 CE	—	15.5 ± 0.3 (3)	101.6 ± 14.5 (3)
8 CE	—	16.6 ± 2.7 (3)	133.0 ± 15.0 (3)
4 HE	—	26.1 ± 8.7 (3)	102.9 ± 10.0 (4)
8 HE	—	9.5 ± 0.8 (8)[c]	103.7 ± 6.3 (3)

[a] Mean and standard error of mean.

[b] Figures in parentheses indicate the number of rats.

[c] Significantly lower than 12-hr saline control value and 15-hr values of other groups ($p < .01$).

ling, neither CE nor HE inhibited the approximately 5-fold or greater increases in serum prolactin concentration evoked by the suckling stimulus.

Discussion. These results indicate that injection of the equivalent of eight rat hypothalami significantly reduced the serum prolactin levels in cycling rats on the day of proestrus or estrus, when values are normally higher than during diestrus (5). HE decreased serum prolactin values to those found during diestrus. No significant differences were found previously between proestrous and estrous serum prolactin levels in cycling rats, although estrous values tended to be somewhat higher (5). In Expt. 3, Table I, serum prolactin was reduced more at 4 hr than at 1 hr after HE injection, suggesting that 4 hr permitted HE to exert a greater inhibition on prolactin release by the pituitary. Experiments are now in progress to determine the duration of the inhibitory effects of an HE injection on serum prolactin levels, and to see whether any dose-response relationship can be established between the amount of hypothalamic extract given and serum prolactin concentrations.

In postpartum lactating rats, serum prolactin values are normally high and fall drastically when the suckling stimulus is removed by exclusion of the litters (5). In the present study, removal of litters for 12 hr during postpartum lactation reduced serum prolactin to diestrous values, in agreement with previous findings (5), whereas return of litters for 3 hr of suckling greatly increased serum prolactin values, also in agreement with previous results. Removal of litters was reported to be followed by an increase and suckling by a decrease in pituitary prolactin content (2, 5). In the present experiment, administration of HE did not inhibit the approximately 5-fold or greater increase in serum prolactin concentration induced by suckling for 3 hr. This suggests that the stimulus of suckling was greater than the inhibition by the HE on pituitary prolactin release. Previously Grosvenor *et al.* (2) reported that a single injection of a rat HE effectively blocked the fall in pituitary prolactin content which normally followed 30 min of suckling after 8 hr or more of nonsuckling. There is considerable evidence that the suckling-induced release of pituitary prolactin is related to the intensity of the suckling stimulus (7). It is possible, therefore, that if the litters in the present experiment had been permitted only 30 min instead of 3 hr of suckling, the HE would have inhibited the rise in serum prolactin values.

A dose of 4 or 8 HE given after 12 hr of no suckling had little or no effect on the low serum prolactin levels when sampled after an additional 3 hr of no suckling. The dose of 8 HE apparently reduced serum prolactin values below the pretreatment saline controls and all other 15-hr values. However, since no pretreatment blood samples were taken from the HE-treated rats, it can not be concluded that serum prolactin levels were actually decreased by the HE.

A prolactin-releasing factor (PRF) has been postulated by some investigators to be present in rat hypothalamic extracts, based mainly on *in vitro* experiments (8). If such a factor actually exists in rat hypothalamic extracts, its effects were not evident in the present investigation. In fact, only an inhibiting effect by the HE was observed in these animals. Further evidence for the inhibitory role of the hypothalamus is indicated by observations that serum prolactin levels rise significantly after transplantation of a pituitary to the kidney capsule of hypophysectomized rats (9), placement of bilateral lesions in the median eminence (9), or administration of reserpine or chlorpromazine (10). Recent work by Porter (personal communication) indicates that PIF activity can be detected in the hypothalamo–pituitary portal blood of untreated rats. These as well as related studies support the view that the predominant action of the rat hypothalamus on pituitary prolactin release is inhibitory in nature.

Summary. On the morning of proestrus or estrus, mature rats were injected with an extract of eight rat hypothalami or an equivalent amount of cerebral tissue. Blood was removed just prior to injection of hypothalamic extract and at 1 and/or 4 hr after injection, for assay of prolactin by radioimmunoassay. In each of the three experiments,

hypothalamic extract significantly depressed serum prolactin values, confirming the presence of prolactin-inhibiting activity in the hypothalamus. Cerebral extract had no effect. In another experiment, the litters of postpartum lactating rats were removed for 12 hr and the rats were injected with an extract of four or eight rat hypothalami or an equivalent amount of cerebral tissue. The litters were then returned for 3 hr of suckling. Blood samples taken before and after suckling, showed that neither brain extract prevented the suckling stimulus from producing a 5- to 6-fold increase in serum prolactin values. This indicates that 3 hr of suckling is a more powerful stimulus to prolactin release than a single injection of hypothalamic extract on inhibition of prolactin release.

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