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Serotonin Involvement in the Regulation of Gonadotropin Secretion During Lactation in the Rat (41104)

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Abstract. A role for serotonin (5-HT) in lactational anestrus was investigated in ovariectomized lactating rats nursing 0, 2, 4, 8, or 12 pups on Days 10–12 of lactation. Hypothalamic 5-HT concentrations did not vary with litter size. On the other hand, suckling of 8 or 12 pups was associated with increased levels of the major metabolite of 5HT, 5-hydroxyindole acetic acid (5-HIAA). Rats suckled for 30 min after a 6-hr period of separation from their six-pup litters on Day 12 of lactation exhibited decreased amounts of 5-HT and increased levels of 5-HIAA compared to nonsuckled controls. These experiments indicate an increased activity of hypothalamic 5-HT-containing neurons following suckling.

Separation from their pups for 12 hr produced a threefold increase in plasma LH and a twofold increase in plasma FSH in ovariectomized, lactating rats. Reinitiation of suckling returned both LH and FSH to preseparation levels by +12 hr. A similar pattern was observed in rats pretreated with ergocornine, a dopamine agonist which blocked prolactin release. This was interpreted to mean that elevated prolactin is not required for suckling-induced declines in gonadotropins. Pretreatment with 5-HT antagonists, metergoline and pizotifen, appeared to interfere partially with the LH decrease which followed reinitiation of suckling in control animals. Moreover, metergoline blocked the suckling-induced decrease in FSH. Thus, the increased activity of 5-HT-containing neurons in the hypothalamus which follows suckling may in part mediate the suppression of gonadotropins that occurs in lactating rats.

Numerous studies have indicated that gonadotropin secretion is inhibited during lactation in the rat. Ovarian steroid secretion during lactation cannot account for this inhibition (1) since gonadotropin levels are greatly reduced in ovariectomized lactating rats (2–4). This suggests that a modification occurs in the regulation of gonadotropin secretion by the hypothalamopituitary unit during lactation.

The mechanism(s) by which lactation modifies gonadotropin secretion are essentially unknown; however, several hypotheses have been proposed. Experimental elevations of plasma prolactin have been shown to inhibit gonadotropin release in the rat (5–9). This has led to the suggestion that the copious amounts of prolactin released in response to suckling during lactation may feed back on the hypothalamus and or pituitary to reduce release of gonadotropins in these animals. An alternate hypothesis, which has received less attention, would be that the neural stimulation associated with suckling, while facilitating prolactin release, may also depress release of gonadotropin-releasing factor(s) from the hypothalamus, thus accounting for the low plasma levels of these hormones observed in lactating animals (10, 11).

Serotonin (5-HT), a neurotransmitter found in hypothalamic tissue, has been implicated in suckling-induced prolactin release (12). Furthermore 5-HT has been shown to inhibit secretion of gonadotropins in nonlactating rats (13–16). The purpose of the present research was to investigate the involvement of 5-HT in the reduced gonadotropin secretion seen in lactating rats.

Materials and Methods. *General.* Three experiments were performed. Experiments I and II involved measurement of endogenous 5-HT levels in lactating rats to ascer-

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tain if suckling modified the activity of 5-HT neurons within the hypothalamus. The third experiment was an attempt to link hypothalamic 5-HT activity to the depressed LH levels observed in lactating rats. Sprague–Dawley rats (Simonsen-Labs, Gilroy, Calif.) were used throughout. Virgin females (250 g) were placed with experienced males on the afternoon of proestrus. The presence of sperm in the vaginal smear the following morning confirmed mating. All animals were ovariectomized and litter sizes were adjusted on the day of parturition (Day 0 of lactation).

Experiment I. Animals with litter sizes of 0, 2, 4, 8, or 12 pups (5 rats per group) were killed by decapitation on Days 10 to 12 of lactation when they appeared to be actively nursing their young. The brain of each animal was removed within 2 min of death and immediately placed on dry ice. A group of virgin female (VF) ovariectomized controls was also included in the experiment. Hypothalami were obtained by making the following cuts: anterior–rostral to the optic chiasm through the anterior commissure; posterior–posterior to the mammillary bodies; lateral, on each side of the tuber cinereum approximately 2 mm off midline; dorsal, at the level of the anterior commissure. The resulting block of tissue (about 45 mg) was homogenized in 4 ml of acidified butanol immediately after dissection on the day of assay. 5-HT and its major metabolite, 5-hydroxyindole acetic acid (5-HIAA), were determined fluorometrically with all samples being run in a single assay.

Experiment II. Rats nursing six pups were separated from their young on Day 12 of lactation. After 6 hr of separation some of the mothers were reunited with their litters for a period of 30 min after which they were decapitated and their brains removed and frozen on dry ice. The remaining animals were killed after an additional 30 min of separation. Additional controls included VF rats ovariectomized 12 days earlier and animals which had given birth 10–12 days previously but had not been suckled (zero pup controls). Hypothalamic 5-HT and 5-HIAA were assayed fluorometrically in a single assay.

Experiment III. Blood was sampled from the jugular vein of lightly etherized rats on Day 10 of lactation (−12-hr sample). The animals were then kept separated from their litters of six pups for a period of 12 hr. One-half hour before the rats were reunited with their litters they were injected with either saline, the dopamine agonist, ergocornine methanesulfonate—0.5 mg/kg (10), or one of two 5-HT receptor blockers, metergoline—5 mg/kg (17), or pizotifin—30 mg/kg (18). Following collection of another blood sample (0 hr) the animals were placed with their litters for 12 hr. Blood was sampled at +6 and +12 hr during this period. LH and prolactin concentrations were determined in all samples by RIA.

Assays. Hypothalamic 5-HT and 5-HIAA were assayed by the method of Curzon and Green (19) with minor modifications. LH was quantified by the RIA procedure of Niswender *et al.* (20) using RP-1 pituitary reference standard. Results were expressed in terms of NIH-LH-Sl. Prolactin and FSH were determined using NIAMDD assay kits and results expressed in terms of the RP-1 standards.

Statistical comparisons between litter size groups in Experiment I were performed by analysis of variance and Student–Newman–Keuls multiple-range tests. Student's *t*-test was used to evaluate differences in 5-HT and 5-HIAA levels in Experiment II and plasma LH concentrations in Experiment III.

Results. *Experiment I.* Hypothalamic 5-HT and 5-HIAA levels for rats nursing 0 to 12 pups are shown in Fig. 1. Overall, 5-HT concentrations averaged 1.320 ng/mg of hypothalamic tissue with no differences between VF controls and groups with different-sized litters. Control levels of 5-HIAA were 1.114 ng/mg in VF rats and 1.034 ng/mg in rats with 0 pups. Rats nursing 2 and 4 pups exhibited slight but nonsignificant increases in 5-HIAA levels compared to the 0-pup control group. Animals suckling 8 or 12 pups, however, displayed significant elevations in 5-HIAA (148 and 140% of 0-pup controls) indicative of enhanced 5-HT neuronal activity in the hypothalami of these rats.

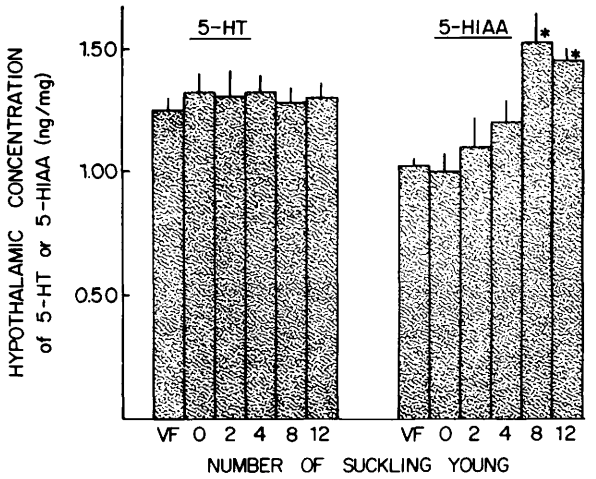

FIG. 1. Concentrations of 5-HT and 5-HIAA in the hypothalamus of rats killed while suckling from 0 to 12 pups on Days 10–12 of lactation. Compounds were determined by fluorometric assay. VF = virgin female controls. $N = 5$ for all groups. * = values were significantly different from zero-pup and VF control groups ($P < 0.05$).

Experiment II. As seen in Table I, hypothalamic 5-HT and 5-HIAA were not different in VF rats, zero-pup controls, or rats with six pups following 6.5 hr of separation from their young. However, rats reunited with their litters of six pups for 30 min prior to decapitation exhibited significantly decreased hypothalamic 5-HT and increased 5-HIAA levels compared to controls, providing additional evidence of an increase in hypothalamic 5-HT neuronal activity induced by the suckling stimulus.

Experiment III. Results of this experiment are summarized in Tables II and III. All rats had low LH levels on day 10 of lactation (approximately 1.0 ng/ml). Removal of young for a 12-hr period resulted in significant increases in plasma LH of about threefold. In lactating controls, reinitiation of suckling reduced circulating LH at +6 hr and by +12 hr LH levels had returned to the levels seen initially (−12 -hr sample) and were significantly lower than prior to the initiation of suckling. A similar pattern was observed in those animals given ergocornine prior to the reinitiation of suckling. However, in rats treated with the 5-HT antagonists, metergoline or pizotifen, suckling did not appear to reduce LH as severely as in saline or ergocornine-treated

TABLE I. HYPOTHALAMIC 5-HT AND 5-HIAA CONCENTRATIONS (ng/mg ± SEM) OF RATS ALLOWED TO NURSE THEIR YOUNG (SIX PUPS) FOR 30 min (+S) FOLLOWING A 6-hr PERIOD OF SEPARATION (SIX PUPS +S)

Type of rat	5-HT	5-HIAA	*N*
Virgin female	1.36 ± 0.06	1.00 ± 0.07	5
Zero pups	1.42 ± 0.04	1.14 ± 0.13	5
Six pups −S	1.48 ± 0.04	1.05 ± 0.02	5
Six pups +S	1.28 ± 0.04[a]	1.28 ± 0.04[a,b]	13

Note. Control rats were separated from their six-pup litters for 6.5 hr (six pups −S). Virgin female (VF) and zero-pup groups served as additional controls.

[a] Value significantly less than six-pup −S and zero-pup control groups ($P < 0.05$).

[b] Value significantly greater than six-pup −S and VF control groups ($P < 0.05$).

TABLE II. PLASMA LH LEVELS (ng/ml ± SEM) OF RATS SEPARATED FROM THEIR YOUNG (SIX PUPS) FOR 12 hr FOLLOWED BY REINITIATION OF SUCKLING FOR 12 hr

Treatment	Time relative to litter replacement (hr)			
	−12	0	+6	+12
Saline	0.78 ± 0.14 (100)[c]	2.36 ± 0.74[a] (303)	1.58 ± 0.70 (203)	0.88 ± 0.18[b] (113)
Ergocornine	0.81 ± 0.18 (100)	2.64 ± 0.67[a] (326)	1.54 ± 0.42 (190)	1.06 ± 0.40[b] (131)
Metergoline	1.02 ± 0.38 (100)	2.77 ± 0.45[a] (272)	1.97 ± 0.49 (193)	1.78 ± 0.45 (175)
Pizotifen	0.91 ± 0.34 (100)	2.40 ± 0.61[a] (264)	1.87 ± 0.38[a] (205)	1.60 ± 0.33 (176)

Note. Prior to pup replacement (0 hr) rats were injected with either saline, ergocornine, metergoline, or pizotifen.

[a] Value significantly greater than −12-hr mean ($P < 0.05$).

[b] Value significantly less than 0-hr mean ($P < 0.05$). $N = 6$ for all groups.

[c] Numbers in parentheses represent percentage of −12-hr LH value.

animals. LH means at +12 hr, while not significantly greater than the −12 values, were nonetheless not less than the 0-hr LH means. This suggests a partial interference in the mechanism by which suckling reduced LH secretion in these animals.

Plasma FSH also increased following 12 hr of pup separation (Table III) but to a lesser degree (twofold) than LH (threefold). Following replacement of the litters, FSH levels fell and the decrease was significant at 12 hr in the saline-injected group. A similar decline occurred in the ergocornine-injected animals. Metergoline treatment appeared to block the reduction in FSH brought about by pup replacement, plasma FSH at +12 hr being significantly greater than the −12-hr mean. Mean FSH at +12 hr in the pizotifen-treated rats while greater than that in any other group was not significantly larger than the −12-hr mean in this group.

Pup removal for 12 hr resulted in a highly significant decline in circulating prolactin (Table IV). Reinitiation of suckling was associated with elevated prolactin in all ani-

TABLE III. PLASMA FSH LEVELS (ng/ml) OF RATS SEPARATED FROM THEIR YOUNG (SIX PUPS) FOR 12 hr FOLLOWED BY REINITIATION OF SUCKLING FOR 12 hr

Treatment	Time relative to litter replacement (hr)			
	−12	0	+6	+12
Saline	201.8 ± 52.8 (100)[c]	449.8 ± 64.1[a] (223)	353.6 ± 100.9 (175)	261.4 ± 39.0[b] (130)
Ergocornine	355.0 ± 56.6[d] (100)	635.6 ± 54.8[a,d] (179)	545.0 ± 48.7[e] (154)	430.4 ± 43.8[b,d] (121)
Metergoline	217.3 ± 73.1 (100)	528.9 ± 44.4[a] (243)	433.6 ± 56.5[e] (200)	437.4 ± 54.0[d,e] (201)
Pizotifen	445.2 ± 65.8[f] (100)	613.8 ± 69.4[d,e] (138)	528.0 ± 65.7 (119)	496.2 ± 50.9[f] (111)

Note. Prior to pup replacement (0 hr) rats were injected with either saline, ergocornine, metergoline or pizotifen.

[a] Value significantly greater than −12-hr mean ($P < 0.01$).

[b] Value significantly less than 0-hr mean of same group ($P < 0.05$).

[c] Numbers in parentheses represent percentage of −12 means. $N = 6$ for all groups.

[d] Value significantly greater than mean of saline controls ($P < 0.05$).

[e] Value significantly greater than −12-hr mean ($P < 0.05$).

[f] Value significantly greater than mean of saline controls ($P < 0.01$).

TABLE IV. PLASMA PROLACTIN LEVELS (ng/ml) OF RATS SEPARATED FROM THEIR YOUNG (SIX PUPS) FOR 12-hr FOLLOWED BY REINITIATION OF SUCKLING FOR 12 hr

Treatment	Time relative to litter replacement (hr)			
	−12	0	+6	+12
Saline	240.4 ± 35.7 (100)[b]	26.6 ± 9.7[a] (11)	312.5 ± 36.8 (130)	324.9 ± 25.0 (135)
Ergocornine	275.0 ± 57.7 (100)	28.2 ± 2.5[a] (10)	17.2 ± 2.6[a] (6)	28.1 ± 10.2[a] (10)
Metergoline	197.5 ± 31.1 (100)	16.0 ± 2.5[a] (8)	260.8 ± 12.1 (132)	235.5 ± 32.9 (119)
Pizotifen	212.3 ± 49.3 (100)	21.9 ± 7.1[a] (10)	309.2 ± 23.7 (146)	268.9 ± 32.9 (127)

Note. Prior to pup replacement (0 hr) rats were injected with either saline, ergocornine, metergoline or pizotigen.

[a] Value significantly less than −12-hr mean ($P < 0.01$).

[b] Numbers in parentheses represent percentage of −12-hr means. $N = 6$ for all groups.

mals except those injected with ergocornine prior to pup replacement. Ergocornine treatment effectively suppressed plasma prolactin with mean levels not exceeding 30 ng/ml through the +12-hr sampling.

Discussion. Experiments I and II clearly demonstrate an increased activity of 5-HT neurons within the hypothalamus associated with suckling. While hypothalamic 5-HT levels did not vary with litter size in Experiment I, the increased metabolite (5-HIAA) levels in those rats nursing 8 and 12 pups indicate an enhanced neuronal activity associated with the increased strength of the suckling stimulus. A similar increase in 5-HIAA was observed when animals were intensely suckled by six pups for 30 min (Experiment II). Unlike Experiment I, 5-HT was reduced slightly but significantly in these animals. This difference is probably due to the acute nature of the suckling stimulus in Experiment II versus a more normal situation in Experiment I. These results are in substantial agreement with those of Mena *et al.* (21), who also observed decreased 5-HT and increased 5-HIAA in suckled rats. In addition, these authors reported a decrease in hypothalamic dopamine following the onset of suckling. Declines in median eminence and anterior pituitary dopamine have been reported by Chiocchio *et al.* (22) supporting a role for this monoamine in suckling-induced prolactin release in the rat.

Removal of suckling young results in a rise in plasma LH in ovariectomized lactating rats (11, 21). The current results indicate that plasma FSH also is elevated following removal of the suckling stimulus. Conversely, reinitiation of a strong suckling stimulus reduces circulating LH and FSH once again. Lactating control rats in Experiment III exhibited just such a pattern. Removal of six-pup litters for 12 hr resulted in a threefold increase in plasma LH and a twofold increase in plasma FSH. Replacement of the litters in turn brought plasma gonadotropin values back down to preseparation levels by +12 hr.

Ergocornine is a dopamine agonist which has been shown to block pituitary prolactin release (10). In the current study, animals were bled under light ether anesthesia, a condition known to increase prolactin release. Nonetheless, those rats in Experiment III treated with ergocornine did not show subsequent prolactin levels greater than 30 ng/ml (Table IV). Saline-treated controls and those rats injected with 5-HT antagonists, on the other hand, displayed plasma prolactin values of 200–300 ng/ml when in the presence of their pups. Since the reinitiation of suckling reduced plasma gonadotropins as effectively in ergocornine-treated animals as in saline-treated controls, it would appear that suckling may inhibit gonadotropin secretion by a prolactin-independent mechanism (10, 11). While this observation does not discount a role for prolactin in the inhibition of gonad-

otropin secretion in the lactating rat, it does indicate that lactation can inhibit gonadotropin release by other means as well.

The results obtained with metergoline and pizotifen were equivocal. Suckling led to a reduction in LH concentrations in these animals, however, plasma LH at +12 hr was intermediate between the depressed levels seen initially (−12 hr) and the elevated LH concentrations following 12 hr of pup separation (0-hr sample). This suggests a partial antagonism of the effect of suckling on LH. Plasma FSH at +12 hr in metergoline-treated animals was significantly greater than the −12-hr value suggesting a block by this compound of the suckling-induced decline in FSH. FSH was elevated at +12 hr in pizotifen-treated animals also; however, this observation is difficult to interpret due to the high initial (−12-hr) mean in this group. Thus, 5-HT, already implicated in the suckling-induced prolactin release (12), may also be involved in mediating the inhibitory effects of lactation on gonadotropin secretion.

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