The Influence of Coitus, Suckling, and Prolactin Injections on Pregnancy in Pelvic Neurectomized Rats[1] (35921)

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In the rat, corpora lutea formed during the 4–5 day estrous cycle secrete insufficient progesterone to support a decidual reaction (1). Activation of the corpora lutea, *i.e.*, increased progesterone secretion, can be invoked in a variety of ways (2), but the natural response depends upon stimulation of tactile receptors in the genitalia by the act of coitus. Bilateral pelvic neurectomy (PN) in rats prevents luteal activation normally induced following either mechanical or coital stimulation (3, 4). Neither pregnancy nor pseudopregnancy in PN rats occur unless exogenous progesterone is administered (3). Electrophysiological experiments in intact rats imply that neural pathways involve impulses which traverse the pelvic nerve and terminate on hypothalamic neurons, since hypothalamic unit recordings show changes in electrical activity following mechanical stimulation of the cervix (5, 6). Although no direct evidence exists demonstrating that neural stimuli from genital receptors can affect prolactin release, other indirect evidence supports such a concept. For example, electrical stimulation of the medial hypothalamus can invoke pseudopregnancy (7) and a number of reports (8–10) state prolactin is responsible for the functional activation of corpora lutea in the rat. In addition, a number of experiments provide evidence that the secretion of prolactin from the anterior pituitary is regulated by hypothalamic factors (11, 12). Others have reported that serum prolactin levels in intact rats are elevated for only the first 2–3 days of pregnancy, while during the remainder of pregnancy levels are comparable to those found during diestrus of the cycle (13, 14).

It is clear that afferent neural stimuli from the genitalia play an important role in the initiation of pregnancy or pseudopregnancy in the rat and indirect evidence imply that this response is dependent upon the release of prolactin from the pituitary. The purpose of this study was to examine the effect of neural inputs from perpherial sensory receptors on hypothalamic regulation of prolactin release and to show that in the absence of these neural stimuli, injections of this hormone could initiate luteal activation and maintenance of pregnancy.

Materials and Methods. Adult female rats of the CD strain (Willmington, MA) were housed under 14 hr light:10 hr dark with three rats per cage. Commercial pellets and water were provided *ad libitum*. Each female had at least two 4–5 day vaginal estrous cycles before assignment to a treatment. Bilateral pelvic neurectomy (PN) was performed under ether anesthesia using the anatomical approach described by Carlson and DeFeo (4). Complete PN was evidenced by inability to void the bladder without manual assistance and animals with incomplete PN were excluded.

Eight PN and 12 intact adult virgin females were adapted to a reversed light cycle (lights off 0500–1500 hr). On the day of vaginal estrus, females were placed with males. After mating was confirmed in each female by observations of eight or more intromissions and an ejaculation by the male, they were removed immediately.

The 12 non-PN females were divided into one group of eight and one of four. The group of eight were anesthetized and bilaterally pelvic neurectomized within 30 min. The other

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TABLE I. Effect of Dosage and Duration of Prolactin on Maintenance of Pregnancy in Pelvic Neurectomized Rats.

Treatment	Duration of injections	No. of rats pregn./total	No. of implant sites or fetuses/pregn. rat day: 8	16	Fetal wt (mg) 16
Sham neurectomy (SNe)	—	9/10	8.8 ± 1.7[a]	8.0 ± 1.6	790 ± 75
Bilateral neurectomy (PN)	—	0/10[b]	—	—	—
PN + prolactin, 28 IU[c]	1–16	4/4	10.3 ± 3.0	9.0 ± 1.8	988 ± 80
7.0 IU[c]	1–16	8/11	9.9 ± 2.1	8.5 ± 1.9	874 ± 48
3.5 IU[c]	1–16	2/4	8.0 ± 2.1	7.0 ± 1.8	912 ± 54
1.4 IU[c]	1–16	3/10[b]	10.3 ± 2.8	0[b]	—
280 IU	1	1/7[b]	9.0	0[b]	—
7.0 IU[c]	1–3	6/10	11.0 ± 2.2	11.0 ± 2.1	932 ± 52

[a] Mean ± standard error.

[b] $p < .05$; SNe vs appropriate group.

[c] Prolactin (NIH-S-8; 28 IU/mg) was injected twice daily sc in sterile saline.

group of four served as sham operated controls. These were anesthetized; the pelvic nerves were only exposed and handled. The eight previously pelvic neurectomized animals were subjected only to ether anesthesia 30 min after mating. Pregnancy was confirmed by laparotomy on days 8 and 16 following mating. Number of implantation sites or fetuses were recorded at these times.

Prolactin (NIH-S-8, 28 IU/mg) was injected twice daily (sc, in sterile saline) into PN females beginning on day 1 (vaginal plug or sperm) through day 15 at dosages of 0, 1.4, 3.5, 7.0, or 28.0 IU/injection. Additional groups were given 7.0 IU daily for the first 3 days or as a single 280 IU injection of prolactin on day 1. A control group consisted of sham neurectomized (anesthetized and pelvic nerve exposed), noninjected females. Vaginal smears were recorded daily. After mating, females which failed to return to vaginal estrus by day 4 were laparotomized on day 8 and their uteri were examined for implantation sites. Animals which were pregnant were sacrificed at day 16 and the number of fetuses and corpora lutea were counted. Evidence of bladder distention was noted and fetal weights were recorded in most instances.

Pregnant females were observed at 2–6 hr intervals on the day of expected parturition. When parturition was complete, females were PN under ether anesthesia; their litters were weighed and the number of pups were recorded. These PN females were paired overnight with males and after a positive postpartum mating (presence of sperm or plug, day 1) the litter was reduced either to zero on days 1, 3, or 6 or to 6 pups on day 3. An additional group consisted of intact-mated females whose litters were reduced to six pups on day 3. Females which failed to mate following parturition were excluded from the study. Mated females were laparotomized on day 15 and their uteri were examined for evidence of implantation sites and the corpora lutea were counted. In females, which possessed no visible implantation sites, the uteri were flushed and examined for blastocysts.

The data from all three experiments were analyzed by analysis of variance and appropriate subgroups were compared by t tests (15).

Results. Estrous cycles in 46 intact and in 22 pelvic neurectomized (PN) rats recorded over 3–6 consecutive cycles averaged 4.4 ± 0.3 and 4.3 ± 0.5 days, respectively. Following bilateral PN, mechanical stimulation of the cervix failed to initiate pseudopregnancy, cycles averaged 4.5 ± 0.4 days, and the mating of PN females to fertile males resulted in none of 10 females becoming pregnant (Table I). Neither sham neurectomy in nine out of 10 animals. (Table I) nor resection of the pelvic nerve within 30 min after mating in eight out of eight animals (not shown in

TABLE II. Effects of Various Intervals of Postpartum Suckling on Pregnancy in Pelvic Neurectomized Rats.

Treatment	No. of days 6 pups/female suckled	Day 15[a] No. pregn. females/total	Day 15[a] Av no. of implant sites or blastocyst/ pregn. female
Intact	15	9/10	13.0 ± 1.9
Bilateral pelvic neurectomy (PN)[b]	15	7/10	10.5 ± 1.1
	1	2/8[c]	11.5 ± 1.8
	3	3/9[c]	9.2 ± 0.9
	6	2/8[c]	9.3 ± 1.0

[a] All animals were sacrificed 15 days after mating.

[b] Pelvic neurectomy was performed under ether anesthesia 1–7 hr after parturition. A fertile male was placed with each female overnight and presence of sperm the following a.m. was considered evidence of postpartum mating (day 1).

[c] $p < .05$ (neurectomized group vs intact).

table) had an effect on conception rate or the number of young carried to term, but parturition was blocked in all of eight PN females. None of eight females, neurectomized prior to mating and anesthetized with ether 30 min after coitus, became pregnant and their postcoital vaginal cycles averaged only 4.4 ± 0.3 days over two consecutive postcoital cycles.

PN females, injected sc twice daily with dosages of prolactin between 28 IU and 7.0 IU beginning on the morning after mating (day 1), maintained pregnancy and normal numbers of young until sacrifice at day 16 (Table I). A low dose of 1.4 IU of prolactin injected twice daily was significantly less effective in both the initiation and maintenance of pregnancy than high doses (Table I). A single injection of 280 IU of prolactin on day 1 resulted in maintenance of pregnancy in only one of seven PN females (Table I). Estrous cycles in the six nonpregnant females of this group were not extended significantly ($p > .05$; 5.1 ± 1.2 days). Prolactin injections of 7.0 IU twice daily for 3 days (days 1–3) maintained pregnancy in six of 10 PN females. Numbers of implantation sites on day 8 and average number and weights of fetuses on day 16 per pregnant female were not significantly different ($p > .05$) among the various treatment groups (Table I).

If postpartum females, PN following parturition, were subsequently mated and allowed to continue suckling six young until sacrificed on day 15, pregnancy was maintained in seven of 10 rats. Nine of 10 intact, mated, continuously suckled females were pregnant when examined at this time (Table II). In contrast, if the entire litter was removed on day 1, 3, or 6 following PN and postpartum mating only two of eight, three of nine, and two of eight females, respectively, remained pregnant at day 15. Implantation, as evidenced by the size of the implantation sites, was usually delayed from 2 to 8 days in both intact and PN females which suckled six young throughout the 15 day experimental period. Nonimplanted blastocysts were recovered from two of these females upon flushing their uteri at day 15. Normal size 15 day embryos were present in all seven of the PN females that were pregnant following litter removal on days 1, 3, and 6.

Discussion. In female rats pregnancy was prevented when the pelvic nerve was bilaterally resectioned prior to mating with fertile males. These females continued to show a normal 4 to 5 day estrous cycle and repeated matings were unsuccessful for induction of pregnancy. These observations confirm the findings of others (3, 4). On the other hand, when bilateral pelvic neurectomy (PN) was performed 30 min after coitus, normal pregnancies resulted, although parturition was

blocked. The results clearly demonstrate the existence of a neuroendocrine reflex associated with activation of the corpora lutea of pregnancy and pseudopregnancy. Stimulation of tactile receptors in the genital region of sufficient magnitude (16, 17) results in impulses which are conveyed over neural pathways, involving in part the pelvic nerve, and terminating on hypothalamic neurons (5, 7, 18). The endocrine limb of this reflex probably involves hypothalamic humoral substances (11), regulated by a dopaminergic system (19), which control pituitary prolactin release. Spies and Niswender (20) have reported a marked increase in serum prolactin levels in intact rats 8–24 hr after mating; whereas serum levels of prolactin remain near base line values in PN females at similar postcoital time intervals. In intact females, prolactin levels decrease by 48 hr following mating and are no longer significantly different from serum levels of PN females. In the present study, blockade of pregnancy as a result of PN was prevented by injection of prolactin thus confirming the importance of this hormone during pregnancy. Moreover, twice daily injections of 7.0 IU of prolactin for 3 days beginning on the first day following mating proved as effective in maintenance of pregnancy as the same dose given twice daily for 15 days. Twice daily doses smaller than 7.0 IU of prolactin were less effective. Likewise, a single injection of 280 IU of prolactin administered in the a.m. on the day of vaginal sperm was not effective, probably due to inadequate endogenous levels for as long as 48 hr. Thus, both frequency of treatment and level of dose were important factors in the effectiveness of the response obtained.

In addition to tactile receptors in the genitalia which can influence prolactin secretion, it is well documented that stimulation of the mammary gland region can also influence pituitary content (21) and circulating levels of prolactin (11, 22). However, Grosvenor and Mena (23) have recently observed that a second 30 min period of suckling applied 2 hr after an initial 30 min suckling period failed to reduce prolactin concentration in the pituitary and they have postulated a refractory period in pituitary responsiveness to suckling which is influenced by a central neural component. This mechanism would result in intermittent bursts of prolactin rather than continuous elevated levels and the initiation of luteal function may be less responsive than the initiation of lactation to such fluctuating prolactin changes. Alloiteau (24) emphasized that postpartum rats allowed to suckle their litters for 3–5 days without vaginal smearing resulted in only 33% of these females becoming pseudopregnant. In the current studies when females were PN following parturition, but prior to the postpartum mating, pregnancy rates were only 25–33% if suckling was discontinued after 1, 3, or 6 days; whereas continuous suckling for 15 days resulted in 70–90% pregnancy rates in neurectomized and in intact females. Thus during lactation maintenance of pregnancy required the repeated stimulus of the mammary region when the neurogenic stimulus associated with coitus had been blocked by resection of the pelvic nerve.

The fact that 3–6 days of suckling was only about 50% as effective in maintenance of pregnancy as 3 days of exogenous prolactin (Tables I and II) may be related to level and mode of administration of hormone, intermittent versus continuous elevated prolactin secretion or to differences in neural versus hormonal feedback mechanisms on the hypothalamic pituitary axis regulating prolactin release. These alternative hypotheses might be related to background circulating levels of ovarian hormones since responsiveness to a variety of stimuli which can induce pseudopregnancy is affected by both estrogen and progesterone (25–27). Adler *et al.* (28) reported an increase in plasma progesterone 6 hr after mating and suggested this elevation in progesterone might be important in facilitating sustained prolactin release. Since the elevation of serum prolactin at 8–24 hr after mating is prevented by resection of the pelvic nerve (20), the elevation in progesterone at 6 hr would not occur. Whatever the underlying mechanism, the findings of this study add support to the work of others (12) implying that the source and nature of neural stimuli, *i.e.*, suckling or cervical, are capable of differentially modulating pituitary prolactin

secretion and possibly other gonadotropins.

Summary. Bilateral resection of the pelvic nerve (PN) in female rats had no effect on the estrous cycle, but prevented pregnancy when performed prior to mating. Pelvic neurectomy performed 30 min postmating did not affect the interim pregnancy, but blocked parturition. Twice daily sc injections of 7.0 to 28 IU of prolactin (NIH-S-8) into PN females beginning on the a.m. after mating (day 1) maintained normal pregnancy until sacrifice at day 16. Lower doses of 1.4 and 3.5 IU of prolactin twice daily were partially effective; whereas a single injection of 280 IU on day 1 was ineffective. Injections of 7.0 IU of prolactin on days 1–3 maintained pregnancy in 60% of the PN females. Following parturition, lactating females suckling six young were bilaterally pelvic neurectomized and mated to a fertile male. Pregnancy occurred and was maintained in 7 of 10 females, sacrificed at day 16 postpartum. Removal of the entire litter at day 1, 3, or 6 postpartum, resulted in maintenance of pregnancy in two of eight, three of nine, and two of eight PN females, respectively.

The results suggest that the source and nature of the neural stimuli which converge on the hypothalamus may differentially modulate the secretion of luteotropin(s) from the adenohypophysis.

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