Effects of prolactin and the suckling stimulus on implantation and LH secretion in rats

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Summary. Litter removal during lactation led to an increase in serum LH concentration, an effect which was completely reversed by prolactin injections. Lactating rats receiving ergocryptine also showed increased serum LH but not to the extent found during litter removal, suggesting that both prolactin and suckling are important factors in the maintenance of lactational anoestrus. The early implantation induced by removal of the litter from rats concurrently lactating and pregnant was not prevented by prolactin. Implantation occurred early in similar rats with serum prolactin levels reduced by ergocryptine treatment and the effect was reversed by prolactin. However, early implantation led to failure of the pregnancy to reach term. These results show that, although prolactin is involved, the suckling stimulus is also essential to prevent implantation in rats with concurrent lactation and pregnancy.

Introduction

Suppression of oestrous cycles and delayed blastocyst implantation are both characteristic of lactation in the rat and are both considered to be the result of insufficient gonadotrophin secretion (MacDonald, Armstrong & Greep, 1967; Bindon, 1971; Lu, Chen, Grandison, Huang & Meites, 1976). The relative importance of the effects of increased prolactin secretion and the suckling stimulus on gonadotrophin secretion during lactation is still uncertain. Early studies (Desclin, 1947; Rothchild, 1960) supported the hypothesis that the suckling stimulus was the major factor responsible for lactational anoestrus but Maneckjee & Moudgal (1975a, b) suggested that prolactin plays a role in preventing the onset of oestrus and delaying implantation only in the presence of the suckling stimulus.

This study was undertaken to examine the role of prolactin in reducing gonadotrophin secretion during lactation and in delaying implantation in the lactating rat undergoing a concurrent pregnancy.

Materials and Methods

Experiment I: studies on the suppression of oestrous cycles

Nulliparous Wistar rats weighing 180–270 g were mated and allowed to undergo a normal pregnancy. Females were housed individually throughout pregnancy and at parturition (Day 0) the litter was adjusted to 6 young. Litters were weighed daily, except Sundays, and vaginal smears were taken at the same time. On Day 14 of lactation the parent rats were treated as shown in Table 1. Injections were given subcutaneously in 0.2 ml 50% ethanol for ergocryptine

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(Sigma Chemical Co., London) or 0.2 ml saline (9 g NaCl/l, pH 9) for prolactin (Sigma Chemical Co., London; stated by manufacturers to be essentially free of all other pituitary hormones except minor contamination with STH). Females in Groups 1 and 2 received injections of both vehicles.

On Day 16 all rats were killed by cervical dislocation, blood was collected from the heart and allowed to clot for 20–30 min at 4°C. Serum, obtained by centrifugation (1000 g for 20 min) was stored at –20°C for determination of serum LH and prolactin by radioimmunoassay using NIAMDD reagents. Serum prolactin was expressed in terms of ng NIAMDD-rat-PRL-RP1/ml and LH as ng NIAMDD rat LH-RP1/ml. The sensitivities of the assays were 0.4 ng prolactin/tube and 1.0 ng LH/tube and inter- and intra-assay coefficients of variation were 11 and 4% respectively for prolactin and 12 and 6% respectively for LH.

Experiment II: studies on implantation

Non-lactating rats. Nulliparous female rats weighing 180–220 g were mated and when spermatozoa were detected in the vaginal smear (Day 1 of pregnancy) each female was housed alone. On Days 3, 4 and 5, 3 rats were treated subcutaneously with ovine prolactin in 0.5 ml saline, 20 i.u. at 10:00 h and 40 i.u. at 17:00 h. Three rats received ovine prolactin as above but the vehicle was 0.5 ml 10% gelatin (warmed to 37°C). For the controls, 3 rats received saline injections and 3 gelatin injections. All animals were laparotomized under ether anaesthesia on Day 8 for inspection of the uteri for implantation sites. All animals were then allowed to recover and complete the pregnancy.

Rats with concurrent lactation and pregnancy. After a normal pregnancy females were allowed to mate at parturition (Day 0). Vaginal smears were taken to confirm mating by the presence of spermatozoa. The male was then removed and the females were housed individually with an adjusted litter size of 6 young. On Day 5 the females were allocated to one of the treatments shown in Table 2. Females in Group 5 received an increasing dose of prolactin throughout lactation since the initial dose was previously found to be inadequate in maintaining litter growth rate at a value similar to that of control rats (Flint & Ensor, 1979). All animals not treated with prolactin received saline vehicle and all animals not treated with ergocryptine received 50% ethanol.

Laparotomy, when performed, was carried out between 10:00 and 11:00 h on Days 8, 10, 12 or 14 as described in 'Results' and animals were then allowed to continue nursing the first litter until Day 21 when the young were weaned. The duration of pregnancy was noted in those animals that gave birth to young. Vaginal smears were taken and litters weighed daily except Sundays for 24 days. Any young which died during the experiment were replaced to maintain litter size.

Statistical analysis

Results were analysed statistically using Student's *t* test for unpaired observations.

Results

Experiment I: suppression of oestrous cycles

A leucocytic smear, characteristic of lactational anoestrus, was found in all rats on Days 14–16, except those in Group 2 which showed smears on Day 16 typical of pro-oestrus. As shown in Table 1, removal of the litter (Group 2) led to a significant reduction ($P < 0.05$) in serum prolactin and an increase in serum LH ($P < 0.01$). Prolactin treatment in Group 3 prevented the LH change when litters were removed. Treatment with ergocryptine (Group 4)

Table 1. Treatments of rats to study the suppression of oestrous cycles after parturition and mean $\pm$ s.e.m. serum prolactin and LH concentrations on Day 16

Group	No. of rats	Treatment				Day 16	
		Day 14		Day 15		Prolactin (ng/ml)	LH (ng/ml)
		10:00	17:00	10:00	17:00		
1	5	—*	—*	—*	—*	239 $\pm$ 60 ^a	5 $\pm$ 2 ^a
2	4	Litter removed	—*	—*	—*	59 $\pm$ 19 ^b	70 $\pm$ 11 ^b
3	4	Litter removed				53 $\pm$ 5 ^b	11 $\pm$ 3 ^a
		+ 10 i.u. prolactin	10 i.u. prolactin	10 i.u. prolactin	10 i.u. prolactin		
4 (a)	3	0.5 mg EC	0.5 mg EC	0.5 mg EC	0.5 mg EC	96 $\pm$ 3 ^b	6 $\pm$ 3 ^a
(b)	3	0.5 mg EC	1.5 mg EC	0.5 mg EC	1.5 mg EC	24 $\pm$ 4 ^b	15 $\pm$ 2 ^a
(c)	6	1 mg EC	3 mg EC	1 mg EC	3 mg EC	21 $\pm$ 3 ^b	24 $\pm$ 3 ^b
(d)	3	2 mg EC	6 mg EC	2 mg EC	6 mg EC	17 $\pm$ 3 ^b	33 $\pm$ 4 ^b

* Vehicle injected; EC = ergocryptine.

Values with different superscripts are significantly different, $P < 0.05$.

significantly reduced serum prolactin levels and also led to a dose-dependent increase in serum LH. Although serum prolactin levels were similar in Groups 4(b–d), litter growths in Groups 4(c) and 4(d) were more markedly reduced during the first 24 h of treatment (Text-fig. 1).

Text-fig. 1. Growth rates of litters of 6 young kept with primiparous lactating rats injected with 90% ethanol (Group 1) or ergocryptine at doses of 1 (Group 4a), 2 (Group 4b), 4 (Group 4c) or 8 (Group 4d) mg/day. See Table 1 for treatment schedules and numbers of litters. Values are mean $\pm$ s.e.m.

Experiment II: implantation

When prolactin was administered in saline or 10% gelatin (acting as a delay vehicle) on Days 3, 4 and 5 of pregnancy in primigravid rats, the timing of implantation was unaltered as judged by observations at laparotomy on Day 8 or by the duration of pregnancy (23 ± 0 days in 6 rats receiving vehicle alone, 23 ± 0 days in 3 rats receiving prolactin in saline and 23 ± 1 days in 3 rats treated with prolactin in gelatin).

Table 2. Treatments of rats undergoing a concurrent pregnancy and lactation to study implantation

Group	No. of rats	Treatment								Duration of pregnancy (days)
		Day 5		Day 6		Days 7-8	Days 9-10	Days 11-12	Days 13-20	
		10:00 h	17:00 h	10:00 h	17:00 h					
1	14	—*	—*	—*	—*	—*	—*	—*	—*	28 ± 1
2	6	Litter removed*	—*	Litter returned*	—*	—*	—*	—*	—*	—†
3	3	Litter removed + 20 i.u. prolactin	40 i.u. prolactin	Litter returned + 20 i.u. prolactin	—*	—*	—*	—*	—*	—†
4	32	0.5 mg EC	—*	—*	—*	—*	—*	—*	—*	—†
5	4	0.5 mg EC + 10 i.u. pro-lactin	10 i.u. pro-lactin	10 i.u. pro-lactin	10 i.u. pro-lactin	30 i.u. pro-lactin‡	40 i.u. pro-lactin‡	50 i.u. pro-lactin‡	60 i.u. pro-lactin‡	28 ± 2

EC = ergocryptine.

* Vehicle injected.

† Did not litter.

‡ Daily dose divided into equal morning and evening doses.

Of the rats with concurrent pregnancy and lactation, only 1 out of 11 in Group 1 (controls) showed visible implantation sites on Day 12 and parturition was also delayed about 4–5 days (Table 2). Removal of young on Day 5 for 24 h (Group 2) resulted in implantation by Day 10, an effect which high doses of prolactin (Group 3) could not prevent. Treatment with ergocryptine (Group 4) also effected early implantation in some rats (3/7 on Day 8, 4/6 on Day 12, 0/5 on Day 14), despite continued suckling. Prolactin injections (Group 5) prevented this, implantation in this group being seen on Day 14 with blastocysts similar in size to control (Group 1) rats and parturition being similarly delayed 4–5 days. When implantation occurred early, however, the pregnancy was not continued successfully to term (Table 2).

Discussion

Serum LH levels in this study were reduced to almost undetectable levels in lactating rats suckling 6 young, as found by McCann, Graves & Taleisnik (1961) and Hammons, Velasco & Rothchild (1973). Removal of the litter on Day 14 of lactation led to increased serum LH values, an effect which could be reversed by administration of prolactin. These studies were performed using rats on Day 14 of lactation because removal of the litter before this time results in a resumption of oestrous cycles in about 50% of animals only, probably due to a pseudo-pregnancy-like effect similar to that described by Rothchild (1960). Twice-daily injections of ergocryptine reduced both milk production (as judged by litter growth rates) and serum pro-

lactin, as previously described by Dohler & Wuttke (1974). Ergocryptine, administered at a dose which significantly reduced serum prolactin and reduced the growth-rate of the litter within 24 h led to increased serum LH values, although not to the same extent as when the litter was removed. These results illustrate the importance of both prolactin and the suckling stimulus in maintaining lactational anoestrus, in agreement with the findings of Maneckjee & Moudgal (1975b). Our results also suggest that prolactin plays the major role because it was almost completely effective in suppressing serum LH when acting alone, whilst the suckling stimulus was only about 60% as effective acting alone, compared with the combined effect of suckling and high serum prolactin levels. The suppression of oestrous cycles during lactation in the rat is essential for successful milk production since the rise in serum oestradiol-17 β concentrations which occurs during the follicular phase of the oestrous cycle would almost certainly inhibit milk production (Bruce, Cofré & Ramirez, 1973; Zamora, Mendoza & Ramirez, 1975).

Prolactin failed to delay implantation in the non-lactating pregnant rat, even with doses higher than those used by Maneckjee & Moudgal (1975a). In the pregnant and lactating rat, implantation could be induced by removal of the litter for 24 h on Day 5 of lactation as previously described by McLaren (1968). The delay in implantation which normally occurs has been shown to be due to inhibition of gonadotrophin secretion (Brumley & De Feo, 1964). Despite the fact that prolactin reduced serum LH in the lactating rat, as shown in this study, prolactin injections could not delay implantation in pregnant and lactating rats from which the litters had been removed. This might be expected, since serum prolactin levels are elevated during the first half of gestation in non-lactating rats (Butcher, Fugo and Collins, 1972) in which no delay in implantation is evident. Instead, a specific indicator of lactation, i.e. the suckling stimulus, acts with prolactin to produce the delay in implantation. Paradoxically, reduction of serum prolactin levels by ergocryptine initiated implantation, while concomitant prolactin treatment prevented this effect. These results again suggest that neither the suckling stimulus nor prolactin is effective in delaying implantation when acting alone and that both are required to produce this effect, findings in agreement with those of Maneckjee & Moudgal (1975a).

The failure of the pregnancy when implantation was induced by temporary removal of the litter or reduced serum prolactin concentrations may be due to the reduced serum LH levels present in the lactating rat, since LH has been shown to be important during the early post-implantation stages of pregnancy (Madhwa Raj & Moudgal, 1970). The larger the litter, the greater is the reduction in serum LH, the later is the eventual rise in serum LH concentration (Ford & Melampy, 1973) and the greater is the delay in implantation (Weichert, 1940).

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