Effects of ergocryptine on prolactin secretion during concurrent pregnancy and lactation in the rat

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Summary. Ergocryptine (2 mg/kg) caused short- and long-term reduction of prolactin secretion in rats experiencing concurrent lactation and pregnancy. The long-term effects of the drug lasted at least 60 days and resulted in reduced milk secretion and termination of pregnancy. Prolactin replacement therapy at a low dose (5 i.u./day) was unsuccessful in overcoming these effects but a higher dose (up to 60 i.u./day) increased milk production and maintained pregnancy. One possible explanation of these results is that prolactin, rather than the suckling stimulus, was responsible for the suppression of oestrous cycles, because ergocryptine brought about a resumption of oestrous vaginal smears in all treated rats in spite of continued suckling.

Introduction

The ergot alkaloid, ergocornine, can inhibit prolactin release from cultured pituitary halves (Meites *et al.*, 1972). This inhibition of prolactin release is associated with a rise in the pituitary prolactin content (Lu, Koch & Meites, 1971), suggesting that ergot alkaloids, which are known to act as dopamine agonists, are probably acting at the pituitary level. Ergocryptine is a closely related ergot alkaloid and has been used extensively to terminate both pregnancy and lactation in the rat (Shelesnyak, 1957; Dohler & Wuttke, 1974; Lu *et al.*, 1976). Most studies which have been made of ergocryptine suppression of prolactin secretion have concentrated on the short-term effects, and the present study was carried out to investigate the long-term effects of ergocryptine on concurrent pregnancy and lactation, with particular reference to the role of prolactin in pregnancy.

Materials and Methods

Mature female inbred Wistar rats weighing 180–250 g were paired and allowed to complete a normal pregnancy (22 ± 0.16 days ($N = 20$) in our colony). The males were left with the females throughout pregnancy and impregnation occurred at parturition, the females then experiencing concurrent lactation and pregnancy. Vaginal smears were taken daily and the day of finding spermatozoa in the vaginal smear was designated Day 1. The males were removed the next day and female rats were then housed individually with their litters, which were adjusted to 6 young on Day 2, and allocated to a treatment group. Food and water were always available.

Group 1 contained the control animals which were given a subcutaneous injection of 0.2 ml 50% ethanol solution on Day 4 ($N = 10$).

Group 2 rats were treated with 2 mg ergocryptine (α -ergocryptine: Sigma Chemical, London)/kg body weight on Day 4. The ergocryptine was dissolved in absolute ethanol and injected as a 50% ethanol solution (0.2 ml/250 g body weight) ($N = 12$).

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Group 3 rats received ergocryptine treatment as in Group 2 and also 5 i.u. prolactin (ovine prolactin, 20–25 i.u./mg; Ferring, Malmö, Sweden) in 0.2 ml saline (0.154 M-NaCl) daily from Day 4 until Day 21 (N = 18).

Group 4 animals received ergocryptine as in Groups 2 and 3, but the prolactin was given twice daily in 0.5 ml saline in increasing doses and with the evening dose being twice that of the morning dose. The doses administered (i.u.) were 10 on Days 4 and 5, 20 on Days 6 and 7, 30 on Days 8 and 9, 40 on Days 10 and 11, 50 on Days 12 and 13 and 60 on Days 14 and 15 (N = 4).

For each group vaginal smears were taken daily and the young were weighed at the same time. The young were removed on Day 21. Litter growth rate was expressed in terms of individual growth rates and was calculated from the formula:

$$\text{Mean growth rate of young} = \frac{\text{litter wt on Day } x + 1}{\text{rat on Day } x} - \frac{\text{litter wt on Day } x}{\text{no. in litter}}$$

For the investigations on prolactin secretion 6 rats from Groups 1 and 2 were bled from the tail vein immediately before and then at 8 and 24 h and 3, 5, 7, 9 and 11 days after the first injection (Day 4). Blood was allowed to clot at room temperature for 30 min, centrifuged at 1000 g for 5 min and 100 µl aliquots were stored in duplicate at -20°C. The prolactin content of all samples was determined with a radioimmunoassay kit provided by NIAMDD. Serum prolactin was expressed in terms of ng NIAMDD-rat-PRL-RP1/ml. Sensitivity of the assay was 0.4 ng and inter- and intra-assay coefficients of variation were 11 and 4% respectively.

Rats from Groups 1 and 2 were also used to study the duration of the long-term effects produced by the drug. Group 1 rats were mated for a third time at the end of the concurrent lactation and pregnancy, thus entering a second concurrent lactation and pregnancy. Because the rats in Group 2 aborted during the first concurrent lactation and pregnancy, a direct parallel could not be obtained. However, after the young of the first litter had been removed on Day 21, the females were allowed to experience oestrous cycles for 10–20 days before being mated again and becoming pregnant for the third time. At parturition the females were re-impregnated to give a second lactation pregnancy (see Text-fig. 1). The number of young in the litters was again adjusted to 6 and the young were weighed daily. The females were untreated throughout this second lactation pregnancy and daily vaginal smears were taken.

Text-fig. 1. Experimental protocol used to observe growth rates of young rats during an untreated, second, concurrent lactation and pregnancy in Group 1 (N = 9) and Group 2 (N = 9) rats. P = pregnancy; L = lactation; C = control ethanol injection; E = ergocryptine injection; OC = oestrous cycles.

Results

The growth rates in the four groups of rats are shown in Text-fig. 2. In Group 1, the growth rate rose steadily throughout lactation and remained steady from about Day 10. In Group-2 rats there was a marked drop in litter growth rate immediately after injection, but the rate increased for about 3 days before falling again to reach a nadir 9 days after injection (Day 13). Prolactin

Text-fig. 2. Mean growth rates ($\pm$ s.e.m.) of young rats during concurrent lactation and pregnancy in rats of Group 1 (O, untreated, 10 litters), Group 2 (Δ , ergocryptine, 12 litters), Group 3 ($\times$, ergocryptine and low dose of prolactin, 18 litters) and Group 4 ($\bullet$, ergocryptine and high dose of prolactin, 4 litters).

replacement therapy (Group 3) overcame the initial drop in growth rate seen in Group 2 but did not prevent the second drop. The growth rates in Group 4 were better than those in Groups 2 and 3 but were not initially as great as those in Group 1 over the period Day 12 to Day 17. A growth spurt between Days 13 and 15 did result in growth rates exceeding those in the controls (Group 1) in the final stages of the experiment. The serum prolactin levels are illustrated in Text-fig. 3; they paralleled the changes in growth rate of the young ($r = 0.483$, 94 d.f., $P < 0.001$).

Associated with the secondary drop in serum prolactin levels was a return to oestrous smears in rats from Groups 2 and 3, indicating termination of pregnancy, whilst animals in Groups 1 and 4 maintained pregnancy. The time interval between injection and return to cyclicity is given in Table 1. The subsequent cycle consisted of a single epithelial and a single cornified smear followed by leucocytic smears until lactation ceased (21 days). These animals underwent subsequent oestrous cycles for 12–20 days; the 9 rats which had been treated with ergocryptine exhibited a total of 28 oestrous cycles, which consisted of 25 4-day cycles and 3 5-day cycles. These

Text-fig. 3. Mean ($\pm$ s.e.m., 6 rats/group) serum prolactin concentrations during concurrent lactation and pregnancy in rats of Group 1 (O) and Group 2 (Δ). * $P < 0.05$, ** $P < 0.01$ compared with Group 1.

Table 1. Effect of ergocryptine and prolactin on concurrent lactation and pregnancy

Group	No. of rats	No. of rats completing pregnancy	Days after injection before first oestrous smear*
1	10	9	23 $\pm$ 1
2	12	0	13 $\pm$ 2†
3	18	0	13 $\pm$ 1†
4	4	4	24 $\pm$ 2

* Days from injection (Day 4 of concurrent lactation and pregnancy). In rats maintaining pregnancy (Groups 1 + 4) the day of the first oestrous smear coincided with parturition.

† Significantly different from Group 1 value ($P < 0.001$, Student's *t* test).

9 rats then had a subsequent pregnancy (23 $\pm$ 1 day). The effects of ergocryptine on a second concurrent lactation and pregnancy occurring 50–60 days after injection of ergocryptine are shown in Text-fig. 4.

Text-fig. 4. Mean ($\pm$ s.e.m., 9 rats/group) growth rates of young rats during an untreated, second, concurrent lactation and pregnancy in Group 1 (O) and Group 2 (Δ) rats. * $P < 0.05$; ** $P < 0.01$ compared with Group 1.

In spite of regular oestrous cycles followed by a successful pregnancy after ergocryptine treatment, the concurrent lactation and pregnancy in Group 2 rats was abnormal in several respects: growth rate of young was significantly less on Days 5, 6, 7 and 8 of lactation, some deaths of young occurred in 5 litters, and only 4 out of the 9 rats maintained their pregnancy compared with all 9 in Group 1 ($P < 0.05$, χ^2 test). Spermatozoa were detected in the vaginal smears of all the rats after mating. A vaginal smear indicative of oestrus did not occur until Days 21–22 of lactation in those rats which failed to maintain pregnancy.

Discussion

Ergocryptine administered as a single subcutaneous injection produced its characteristic initial effect, reducing serum prolactin for approximately 24 h (Fluckiger, Marko, Doepfner & Niederer, 1976). It also produced embryonic resorption, as previously described for non-

lactating pregnant rats (Shelesnyak, 1957). However, the drug produced unusual long-term effects lasting up to 60 days in some rats.

The results reported in this study suggest that termination of pregnancy is brought about by means of this long-term effect on prolactin secretion rather than the immediate effect of the drug, since a low dose of prolactin overcame the initial effect, but was not able to prevent abortion or the long-term effect on growth rate of the young. A second possibility is that the secondary drop in prolactin levels might not be due to ergocryptine injection but to a reduction in suckling intensity. This may also explain discrepancies between the present work and that of Lu *et al.* (1976) who showed no return to oestrous cycles until 11 days after the start of continuous 2-bromo- α -ergocryptine treatment. There are a number of objections to this second possibility. Although the young did not receive much milk during the 24 h immediately after injection, they were able to recover well enough to maintain growth rates similar to those of controls for several days. This would suggest that a subsequent drop in growth rate would not occur unless there was a drop in milk production. In addition, those young which were growing fastest showed a similar drop in growth rate despite the fact that they still continued to gain considerable weight, and in some litters so many deaths occurred that the litter had to be replaced to maintain the suckling stimulus.

Detrimental effects of the drug on both pregnancy and lactation lasted up to 2 months and yet no long-term effects of the drug have been previously described at this dose (and higher) in the rat (Griffith, 1974), monkey (Griffith, 1976) or dog (Griffith & Richardson, 1975). All these studies were performed using a 2-bromo- α -ergocryptine (CB 154) and the drugs are known to have different effects (Fluckiger & Wagner, 1968). None of these studies has focussed their attention on the more demanding condition of concurrent lactation and pregnancy. The results reported here illustrate that sufficient prolactin secretion is permitted for normal oestrous cycles and pregnancy to occur and it is only when the extra demands of lactation are superimposed that deficiency in the prolactin secretion mechanisms becomes apparent.

The mechanism by which ergocryptine brings about the long-term effect on prolactin secretion is uncertain. The normal short-term suppression of prolactin secretion by ergocryptine is thought to act at the pituitary level, but this does not preclude the possibility that a secondary blockade by ergocryptine might occur at the hypothalamic level. Ergocryptine appears to act as a dopamine agonist, attaching to dopamine receptors and causing a prolonged dopamine-like action (Arai, Suzuki & Masuda, 1972; Macleod & Lehmyer, 1972; Sinha, Selby & Vanderlaan, 1974). It seems unlikely that this ergocryptine-receptor complex would persist for several days and this therefore suggests an inhibition of the synthetic process or long-term alteration of the eta cell membrane. Evidence suggests that, as the pituitary recovers from the initial effects of ergocryptine treatment, long-term alteration of the membrane does not occur.

There is some evidence, although not detailed, from other studies supporting the suggestion that ergocryptine suppresses prolactin production on a long-term basis. Yanai & Nagasawa (1970) have shown that ergocryptine can block the development of hyperplastic alveolar nodules in mice. Similarly Quadri & Meites (1971) have reported suppressed growth of spontaneous mammary tumours in old female rats. Smith, Beck, Convey & Tucker (1974) have shown that ergocryptine inhibition of prolactin can last up to 5 days following two consecutive injections.

Obviously the long-term effects of ergocryptine in female rats require further study.

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