Delayed pseudopregnancy in the mouse

S. R. Milligan, A. A. Khan and B. C. Thorne

Department of Physiology, University of London, King's College, Strand,
London WC2R 2LS, U.K.

Summary. Treatment of female mice with bromocriptine on Day 1 or 3 of pregnancy induced many of the females to return to oestrus. The incidence of extended cycles (7–10 days) and pseudopregnancies following this return to oestrus was significantly higher than in unmated control females. These observations are consistent with the existence of a 'mnemonic' component of the luteotrophic system in the mouse.

Introduction

In rodents like the rat and mouse, the development of luteal function is dependent on mating. Mating in the rat activates a mechanism responsible for providing continuing luteotrophic support during pseudopregnancy and the early part of pregnancy (Everett, 1967; Freeman, Smith, Nazian & Neill, 1974). Once activated, the continuation of this luteotrophic system is independent of the presence of either the corpora lutea or the ovaries (De Greef & Zeilmaker, 1976; Smith & Neill, 1976). The characteristics of the system are such that it appears as if the luteotrophic effect of mating is 'remembered' for some time after the actual act of mating and consequently the term 'mnemonic' has been used to describe it (Freeman *et al.*, 1974). The existence of such a mnemonic system provides a ready explanation for the phenomenon of 'delayed' pseudopregnancy in rats, in which the luteotrophic effect of cervical stimulation becomes evident even when the stimulation is given some days before the ovulatory stimulus that gives rise to the corpora lutea (Greep & Hisaw, 1938; Zeilmaker, 1965; Everett, 1967). Milligan, Charlton & Versi (1979) reported evidence for the existence of a similar mnemonic mechanism controlling luteal function in the vole (*Microtus agrestis*), but it is not known whether such a system exists in other species. Carlsen, Zeilmaker & Shelesnyak (1961) observed that after the termination of early pregnancy in mice following ergocornine methanesulphonate treatment, the next cycle in 4 out of 8 females was a pseudopregnancy. The occurrence of these 'delayed' pseudopregnancies is suggestive of the existence of a 'mnemonic' component of the luteotrophic system in the mouse. The present study was undertaken to confirm and extend the observations of Carlsen *et al.* (1961).

Materials and Methods

Adult Swiss mice (Schofield Ltd) were maintained under a lighting regimen of 12 h light (08:00–20:00 h)/24 h and fed on a pelleted diet (41 B Oxoid). Females were housed individually except at the time of mating. Vaginal smears were taken daily using a wire loop. Preliminary observations in 10 females smeared over 3 consecutive cycles indicated that the incidence of pseudopregnancies (≥ 11 days) or extended cycles (7–10 days) associated with this

smearing technique was low (0% and 6.7%, respectively). The day of oestrus was defined by the occurrence of a fully cornified vaginal smear which was followed by one containing leucocytes. Cycle lengths were defined by the interval between two consecutive periods of oestrus. For mating, a single male was placed in the cage of the female in pro-oestrus. When a copulatory plug was found, the male was removed; this day was designated Day 1 of pregnancy. Bromocriptine (2-bromo- α -ergocryptine mesylate: Sandoz Products Ltd, U.K.) was dissolved at a concentration of 5 mg/ml in a vehicle solution of 0.2 ml absolute alcohol, 0.8 ml 0.154 M-NaCl and 5 mg tartaric acid. All injections were given subcutaneously.

The lengths of oestrous cycles were recorded for 29 unmated mice over a combined total of 40 cycles (Group 1: 11 females for two cycles, 18 females for one cycle). The lengths of pseudopregnancies induced by sterile mating were recorded for 8 females (Group 2).

Mated females were injected with bromocriptine on Day 1 (Group 4) or Day 3 (Group 5) of pregnancy, or with the vehicle only on Day 3 of pregnancy (Group 6). An additional 21 unmated females were treated with bromocriptine on the day after oestrus (Group 3). The numbers of females returning to oestrus within 5 days of the injection and the lengths of the ensuing cycle were recorded for all treatments.

Results

The results are summarized in Table 1. Over 97% of cycles in unmated females (Group 1) were less than 7 days in length. The duration of pseudopregnancies induced by sterile mating (Group 2) was 12.0 ± 0.37 (s.e.m.) days ($n = 8$).

Table 1. The distribution of cycle lengths in mice after mating and/or treatment with bromocriptine

Group	Treatment	No. of females				
		Total	Returning to oestrus within 5 days	No. of cycles of:		
				≤ 6 days	7–10 days	≥ 11 days
1	Unmated	29	—	39	—	1
2	Mated by sterile male	8	—	—	—	8
3	Unmated + bromocriptine	11	11	10	1	—
4	Mated + bromocriptine on Day 1	26	20	5	7	8
5	Mated + bromocriptine on Day 3	34	21	14	2	5
6	Mated + vehicle on Day 3	16	0	13 females pregnant 3 females pseudopregnant		

All the mated females treated with the vehicle only (Group 6) became pregnant or pseudopregnant. However, the treatment of newly mated females with bromocriptine (Groups 4 and 5) terminated pregnancy in many cases. Females treated with bromocriptine on Day 1 of pregnancy (Group 4) returned to oestrus 4.52 ± 0.20 (s.e.m.) days after mating, while those treated on Day 3 (Group 5) returned after 6.52 ± 0.11 days. The number of these females that then displayed pseudopregnancy cycles (≥ 11 days) or extended cycles (7–10 days) was significantly higher than in the unmated females (Group 3) ($\chi^2 = 18.5$, $P < 0.001$ for Group 4, and $\chi^2 = 3.86$, $P < 0.05$ for Group 5). Group 4 females showed a significantly higher incidence of extended cycles and pseudopregnancies than did those in Group 5 ($\chi^2 = 5.61$; $P < 0.05$).

Discussion

Pregnancy was terminated in a high proportion of newly mated female mice following treatment with bromocriptine. This effect is presumably due to the inhibition of prolactin secretion and consequent regression of the corpora lutea (Charlton, Milligan & Versi 1978). The occurrence

of extended cycles and pseudopregnancies in many of the mice following their return to oestrus confirms the results of Carlsen *et al.* (1961) and is consistent with the existence of a 'mnemonic' component of the luteotrophic system. The effect of the initial mating would be to activate this component which would then continue, despite the bromocriptine-induced regression of the corpora lutea associated with the original ovulation, and support the new corpora lutea formed in association with the ovulation accompanying the return to oestrus.

Observations of rats and voles have suggested that the mnemonic component of the luteotrophic system may have a limited lifespan (Zeilmaker, 1965; Everett, 1967; Freeman *et al.*, 1974; Milligan *et al.*, 1979). The present observations of mice would be consistent with this idea. The interval between mating and the bromocriptine-induced return to oestrus was longer in those females treated on Day 3 of pregnancy (Group 5) than in those treated on Day 1 (Group 4). The lower incidence of extended cycles and pseudopregnancy in the Group 5 females may be a reflection of their later return to oestrus coupled with a limited duration of the mnemonic.

The demonstration of a mnemonic component of the luteotrophic system in the mouse raises the question of whether this is involved in termination of pregnancy induced by strange males (the 'Bruce effect'; Bruce, 1959). Milligan *et al.* (1979) have provided evidence in the vole that the effect of the strange male may be via an abolition of the 'mnemonic' system controlling the corpus luteum. Whether this is also true for the mouse remains to be established.

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