Prolactin and the control of cycle length in the female rat

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Abstract. Bromocriptine (BRC) was injected on oestrus afternoon at 16.00 h into 5-day cyclic female rats thus causing reduced cycle length to 4-days. When injected on dioestrus 1 at 13.00 h the drug remained without effects. Blood progesterone concentration on dioestrus 1 afternoon and on dioestrus 2 morning was shown to be lower in 5-day cyclic rats given BRC than in their untreated counterparts. No changes in the rate of follicular growth were observed on dioestrus 2 afternoon in BRC injected 5-day cyclic rats. The pattern of prolactin secretion on oestrus afternoon was compared in 4- and 5-day cyclic female rats. High prolactin values were observed on early afternoon in the former and on late afternoon in the latter. The decline in progesterone secretion during the dioestrous period of the cycle was supposed to be responsible for cycle shortening in 5-day cyclic rats given BRC. It was concluded that prolactin might play a critical role in the establishment of a 5-day rhythm in the rat by controlling progesterone secretion during the dioestrous period of the cycle.

Previous findings in our laboratory clearly demonstrated (for review, see Aron 1979) that oestrous cycle length was dependent on the pattern of progesterone secretion during the dioestrous period of the cycle. In 4-day cyclic rats, ovarian progesterone secretion declined from dioestrus 1 morning to dioestrus 2 morning (Roser & Bloch 1971) while only on dioestrus 2 afternoon in 5-day cyclic rats (Roser & Bloch 1969, 1971). Enhancing progesterone values on dioestrus 2 morning by injecting LH or progesterone (Buffler & Roser 1974) on dioestrus 1 lengthened a 4-day cycle to 5-days. Lowering progesterone values on dioestrus 2 morning by exposure to olfactory cues (Chateau et al. 1976) reduced a 5-day cycle to 4-days. Since prolactin is considered as the main luteotrophic factor in the rat, it was tempting to speculate that oestrous cycle duration was dependent on prolactin secretion. At first sight, this should not be the case since no difference in prolactin secretion was found between 4- and 5-day cyclic rats (Nequin et al. 1979). However, preliminary results (Boehm et al. 1980) showed that bromocriptine, when injected on oestrus afternoon, was able to reduce a 5-day cycle by 24 h. The present study was then undertaken to verify that bromocriptine induced cycle shortening in 5-day cyclic rats was related to a shift in progesterone secretion on dioestrus 2 morning. It seemed also of interest to reevaluate the pattern of prolactin secretion by comparing prolactin values during oestrus afternoon in 4- and 5-day cyclic rats, respectively.

Material and Methods

Animals
Adult virgin female Wistar rats (strain W1 of our colony) were used. They were 3 to 4 month old and weighed 180 to 220 g. Food and water were available ad libitum. The animals were kept under a rhythm of short day natural lighting during months November to January, at a temperature of 22–24°C. Cycle length was determined by daily vaginal smears. Only those females which had experienced 3 regular 4- or 5-day cycles were used.

Experimental design
In experiment 1, we studied in 5-day cyclic rats the effects on cycle length of a single sc injection of bromocriptine (BRC) (2 mg/animal) at 16.00 h on oestrus or at
13.00 h on dioestrus 1. The animals were killed at the first oestrus following BRC injection and the ovaries were removed to determine whether ovulation actually took place during the preceding night. Non-BRC injected females served as controls.

In experiment 2, 4- and 5-day cyclic rats were decapitated on dioestrus 1 and 2 at 11.00 and 17.00 h for measuring progesterone concentration following BRC injection on oestrus at 16.00 h. Non-BRC injected females served as controls. The ovaries of those females which were decapitated at 17.00 h on dioestrus 2 were removed for determination of the rate of follicular growth.

In experiment 3, non-BRC injected 4- and 5-day cyclic females were decapitated during oestrus afternoon with the aim to establish the pattern of prolactin secretion.

Hormone assays
Blood was allowed to clot overnight, centrifuged at 4°C and serum was stored at −20°C until assayed. Progesterone was determined using a radioimmunoassay procedure described previously (Boehm et al. 1982). The intra- and interassay variation coefficients were 5 and 10%, respectively.

Prolactin radioimmunoassay was performed as described by Boehm et al. (1982). The intra- and inter-assay variation coefficients were 8 and 11%, respectively.

Methods for the study of follicular growth
All follicles whose mean diameter exceeded 400 µm were counted and measured using an ocular micrometer. Previous work showed that the follicles committed to ovulate were recruited from a pool of follicles exceeding 500 µm (Aron et al. 1967) and that the number of follicles greater than 400 µm remained unchanged from the morning of dioestrus 2 to that of prooestrus (Buffler & Roser 1974). The follicular diameter was determined as follows. The section showing the largest dimension of each follicle was selected; the follicle was then measured in two directions at right angles and the mean values of the two measurements were calculated. The mean follicular diameter in each animal was estimated. From these data, the mean diameter of the follicles was calculated for each group of animals.

Statistical analysis
The effects of BRC on cycle length were analysed by the χ square method. Progesterone values after logarithmic transformation of the data were analysed by two-way analysis of variance for unequal number and by Scheffe’s t-test.

Two-way analysis of variance for equal numbers was used for the comparison of follicle diameters. One-way analysis of variance and Newmann Keuls method served to compare prolactin values after logarithmic transformation of the data.

<p>| Table 1. Effect of bromocriptine (BRC) treatment on cycle length in 5-day cyclic female rats. |
|-------------------------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of rats with 24 h cycle shortening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 16.00 h on oestrus</td>
</tr>
<tr>
<td>BRC (2 mg/rat)</td>
<td>15/18*</td>
</tr>
<tr>
<td>Controls</td>
<td>6/29</td>
</tr>
</tbody>
</table>

* P < 0.001 compared to corresponding value for controls.

Results

1) BRC induced cycle shortening in 5-day cyclic rats
Table 1 shows that BRC when injected on oestrus at 16.00 h reduced cycle length (χ² = 17.61; P < 0.001) but remained without effect when given on dioestrus 1 at 13.00 h. Cycle shortening did not affect the ovulatory processes. Table 2 indicates that the mean number of corpora lutea on oestrus did not differ in females with reduced cycle duration and in those which maintained a 5-day cyclicity.

<p>| Table 2. Effect of bromocriptine (BRC) treatment at 16.00 h on oestrus in 5-day cyclic female rats, on ovulation at the end of the next cycle. |
|-------------------------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of corpora lutea (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h cycle shortening</td>
</tr>
<tr>
<td>BRC (2 mg/rat)</td>
<td>9.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(15)*</td>
</tr>
<tr>
<td>Controls</td>
<td>9.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
</tr>
</tbody>
</table>

* Number of animals.
2) Blood progesterone concentration and follicular growth following BRC treatment

The data illustrated in Fig. 1 show that blood progesterone values on dioestrous 1 morning and dioestrous 2 afternoon did not differ in 4- and 5-day cyclic rats irrespective of whether they were given BRC. In contrast, blood progesterone concentration appeared to be lower on dioestrous 1 afternoon in 5-day cyclic rats given BRC than in untreated 5-day cyclic rats and in 4-day cyclic rats as well ($P < 0.05$). Blood progesterone concentration on dioestrous 2 morning was lower in 5-day cyclic rats given BRC than in their un.injected counterparts ($P < 0.05$) but did not differ from that in 4-day cyclic rats ($P > 0.05$).

The data presented in Table 3 show that BRC treatment did not affect the follicular size on dioestrous 2 afternoon in either 5- or 4-day cyclic rats ($P > 0.05$). Follicular size appeared to be higher in 4-day than in 5-day cyclic rats whether or

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**Fig. 1.**
Concentration of peripheral plasma progesterone during the dioestrous period in control (a) and bromocriptine-treated (b) 5-day cyclic rats, and in control (c) and bromocriptine-treated (d) 4-day cyclic rats. Bromocriptine (2 mg/animal) was injected sc on oestrus at 16.00 (* number of animals).

**Table 3.**
Comparison of the size of ovarian follicles exceeding 400 µm in 4-day and 5-day cyclic female rats given or not given bromocriptine (BRC) on oestrus afternoon.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Mean follicular diameter ± SEM on dioestrous 2 at 17.00 h in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-day cycles</td>
</tr>
<tr>
<td>Controls</td>
<td>490 ± 15</td>
</tr>
<tr>
<td></td>
<td>(12.9 ± 1.1)</td>
</tr>
<tr>
<td>2 mg BRC/animal at 16.00 h</td>
<td>458 ± 8</td>
</tr>
<tr>
<td></td>
<td>(13.7 ± 1.3)</td>
</tr>
</tbody>
</table>

The mean number of follicles in each group are in parentheses. *8 animals/group.

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not they were given BRC ($P < 0.01$). The number of follicles greater than 400 µm did not differ in the different experimental groups.

3) Comparison on oestrus afternoon of the pattern of prolactin secretion in 4- and 5-day cyclic rats

As shown in Fig. 2, very high prolactin values in 4-day cyclic rats were observed between 14.00 and 17.00 h, with a significant decrease between 18.00 and 19.00 h ($P < 0.05$). A reverse situation occurred in 5-day cyclic rats. Prolactin concentration appeared to be low between 14.00 and 17.00 h. A significant rise was noted at 18.00--19.00 h ($P < 0.05$).

Discussion

The data reported here indicate that a dose of 2 mg BRC, when given on oestrus afternoon at 16.00 h in 5-day cyclic rats, is capable of reducing cycle duration by 24 h. Recent work in our laboratory (Boehm et al. 1984) showed that treatment of 4-day cyclic rats with 1 mg BRC on either pro-oestrus or oestrus completely depressed acute and basal release of prolactin. The effects of BRC on the oestrous rhythm were certainly due to prolactin suppression during the period extending from oestrus at 16.00 h until dioestrus 1 at 13.00 h, since BRC treatment at this time no longer shortened oestrous cycle duration. Our observations are in keeping with those of Van der Schoot & Uilenbroek (1983) who, using daily BRC injections from either oestrus or metoestrus morning, observed that only the animals which were given BRC injections starting on oestrus displayed oestrous cycle shortening.

The depression of pituitary prolactin secretion in 5-day cyclic rats caused a significant decrease in blood progesterone concentration as soon as on dioestrus 1 afternoon and on the morning of dioestrus 2 as well. These changes in blood progesterone levels may account for the cycle shortening observed in BRC treated females. Previous findings in our laboratory emphasized the role played by progesterone in the regulation of oestrous cycle duration in the rat. An excess of endogenous (Buffler & Roser 1974; Plas-Roser et al. 1977) or exogenous progesterone (Buffler & Roser 1974) on dioestrus 2 morning was demonstrated to cause a 24 h cycle prolongation in 4-day cyclic rats. In contrast, blood progesterone levels on dioestrus 2 morning appeared to be decreased in 5-day cyclic rats whose cycle duration was reduced by exposing the females to pheromones (Chateau et al. 1976).

No changes in the rate of follicular growth on dioestrus 2 afternoon were observed in the females displaying oestrous cycle shortening after BRC treatment. This was rather surprising since an increase in follicular size on dioestrus 2 always occurred in 5-day cyclic rats with reduced cycle duration under pheromone exposure (Chateau et al. 1976). On the other hand, a decrease in the rate of follicular growth at this stage of the cycle was associated with 24 h cycle prolongation in 4-day cyclic rats exposed to high levels of circulating progesterone (Chateau et al. 1981). Therefore we are led to admit that the acceleration of growth of the ovarian follicles as soon as on dioestrus 2 is not a prerequisite for ovulation to be advanced by 24 h in 5-day cyclic rats. However, we cannot rule out the possibility that changes in the rate of follicular growth on the expected day of dioestrus 3 may account for cycle shortening in the present experimental conditions. Indeed this hypothesis needs verification. In any event we know that female rats given LH on dioestrus 2 afternoon without any preliminary stimulation of follicle development may ovulate during the ensuing hours (Chateau & Aron 1971).

Interestingly, prolactin release on oestrus afternoon appeared to be delayed in 5-day cyclic rats with respect to 4-day cyclic rats. In the former, high prolactin values were observed at 18.00--19.00 h. In the latter, the highest prolactin values occurred at 14.00--15.00 h. Since BRC was injected at 16.00 h in 5-day cyclic rats it would be tempting to speculate that the depression of prolactin on late afternoon was responsible for the impairment of the luteal function on dioestrus and consequently for cycle shortening. Recent observations in our laboratory provide support to this assumption.

It is commonly admitted that corpus luteum can function autonomously during the oestrous cycle in the rat. Previous work (Boehm et al. 1984) in our laboratory cast doubt on this conception. Some degree of hormone dependency of luteal function has been established in 4-day cyclic females. The present data collected in 5-day cyclic rats demonstrate that prolactin may play a critical role in the establishment of a 5-day rhythm in the female rat.
Acknowledgments

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References


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