Abstract. Pentobarbital lengthens the estrous cycle when injected on diestrus 1 afternoon in the 4-day cyclic female rat. The present study was undertaken to elucidate the mechanisms involved. Bromocriptine (2.5 mg/kg sc) given 30 min before pentobarbital (30 mg/kg ip) prevented the effects of pentobarbital on both cycle lengthening and progesterone levels in diestrus 2. Serum prolactin concentrations rose 10 min after pentobarbital injection, peaked at 30 min, and returned to control values after 60 min. Our results suggest that pentobarbital-induced cycle lengthening is due to a high progesterone production on diestrus 2 morning related to the luteotropic action of prolactin.

In previous studies on the neuroendocrine mechanisms controlling estrous cycle rhythm in the rat, Chateau & Aron (1973) showed in female Wistar rats that pentobarbital, when injected on diestrus 1, lengthened the cycle from 4 to 5 days. It has been clearly demonstrated in our laboratory that estrous cycle duration in the rat is dependent on the pattern of progesterone secretion during the diestrous period (for review, see Aron 1979). Pentobarbital (PB) has also been shown to increase prolactin secretion in cyclic (Wakabayashi et al. 1971; Hoak & Schwartz 1978) and ovariectomized (Lawson & Gala 1974) rats. Since PRL is an important factor controlling corpus luteum function during estrous cycle (Boehm et al. 1984a) as well as during pregnancy and pseudopregnancy (Rothchild 1981), we raised the question whether PRL and progesterone secretions are implicated in the changes in estrous cycle rhythm induced by PB injection in the rat. Bromocriptine was used for studying a possible prolactin mechanism.

Material and Methods

Animals

Three to 4 months old female Wistar rats (strain WI in our colony) weighing 180–200 g were housed in a light and temperature controlled room. Illumination was provided from 05.00 to 19.00 h. Laboratory food and tap water were supplied ad libitum. Estrous rhythm was checked 5 days a week by vaginal lavages and only those females which had experienced 2 or 3 regular 4-day cycles were used. The 4-day sequence consisted of diestrus 1 (Di 1), diestrus 2 (Di 2), proestrus and estrus, ovulation taking place in the night following proestrus.

Experimental design

Experiment 1 was designed to determine whether bromocriptine (BRC) is able to prevent estrous cycle lengthening induced by PB injection. The first group of 30 females were given a single PB (Clin-Midy, Veterinary) injection (30 mg/kg ip) at different times of the estrous cycle, i.e. estrus at 18.00 h, Di 1 at 08.00, 15.00 or 18.00 h, Di 2 at 08.00 h, to determine the critical period for cycle lengthening. The second group of 41 females were treated on Di 1 as follows: saline at 15.00 h (controls); PB at 15.00 h; BRC (2.5 mg/kg dissolved with tartaric acid in saline sc) 30 min prior to PB given at 15.00 h; PB at 15.00 h followed by BRC at 15.30 h. All the females were killed on the day of estrus following treatment. The ovaries were removed for histological control of the occurrence of ovulation.

Experiment 2 was carried out in order to elucidate whether estrous cycle lengthening following PB treatment was related to a shift in progesterone secretion during the diestrous period. Progesterone concentrations in blood were measured in 3 groups of animals. Two groups of 12 animals each were treated either with PB or with saline in Di 1 at 15.00 h and blood was collected in Di 2 at 11.00 h (6 animals) or at 17.00 h (6
animals). One group of 6 animals received, in Di 1, BRC (2.5 mg/kg) 30 min prior to PB treatment and blood was taken in Di 2 at 11.00 h.

The aim of experiment 3 was to measure PRL concentrations in the afternoon of Di 1 in rats treated as in experiment 2. Blood samples were collected at 10, 30, 60 and 120 min after PB or saline injection.

**Blood sampling and hormone measurements**

Blood was collected by decapitation and serum was stored at −20°C until assayed. Progesterone and PRL were measured by radioimmunoassay as described in Bohm et al. (1982). The intra- and inter-assay coefficients of variation were 8 and 11% for PRL and 5 and 10% for progesterone. Prolactin concentrations have been expressed in µg/l of the RP1 NIADDK standard.

**Statistical analysis**

Changes in estrous cycle duration were studied using Fisher’s exact probability test (one-tailed test). The effect of PB with or without BRC on estrous cycle length was analysed by the Chi-Square method. One-way analysis of variance followed, when necessary, by contrast method of Scheffe for localization of differences was used for the comparison of progesterone and PRL values after logarithmic transformation of the data.

**Results**

**Cycle lengthening by PB (experiment 1)**

The results in Table 1 indicate that PB, when injected during the afternoon of Di 1 induces lengthening by 24 h in 4-day cyclic female rats, as previously shown by Chateau & Aron (1973). This effect was not observed when PB was injected in

<table>
<thead>
<tr>
<th>Pentobarbital administration</th>
<th>Estrus at 18.00 h</th>
<th>Diestrus 1 at 08.00 h</th>
<th>Diestrus 2 at 08.00 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of females with cycle lengthening of 1 day</td>
<td>0 / 6</td>
<td>1 / 6</td>
<td>5 / 6*</td>
</tr>
<tr>
<td>1 / 6</td>
<td>6 / 6**</td>
<td>1 / 6</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 vs 08.00 h diestrus 1 value. **P < 0.01 vs 08.00 h diestrus 2 value.

**Table 2.**

Effect of bromocriptine (BRC: 2.5 mg/kg sc) on pentobarbital (PB: 30 mg/kg ip) induced estrous cycle lengthening in 4-day cyclic female rats.

<table>
<thead>
<tr>
<th>Treatment on diestrus 1</th>
<th>Proportion of females with cycle lengthening of 1 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline at 15.00 h</td>
<td>2 / 10</td>
</tr>
<tr>
<td>PB at 15.00 h</td>
<td>8 / 10</td>
</tr>
<tr>
<td>BRC at 14.30 h followed by PB at 15.00 h</td>
<td>2 / 10*</td>
</tr>
<tr>
<td>PB at 15.00 h followed by BRC at 15.30 h</td>
<td>7 / 11**</td>
</tr>
</tbody>
</table>

*P < 0.01 vs PB at 15.00 h.
**Not significant vs PB at 15.00 h.

**Table 3.**

Effect of pentobarbital treatment (PB: 30 mg/kg ip) on diestrus 1, associated or not associated with bromocriptine injection (BRC: 2.5 mg/kg sc) on blood progesterone concentration on diestrus 2 in 4-day cyclic female rats (6 animals per group).

<table>
<thead>
<tr>
<th>Treatment on diestrus 1</th>
<th>Progesterone nmol/l (mean ± SEM) on diestrus 2 at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.00 h</td>
</tr>
<tr>
<td>PB at 15.00 h</td>
<td>43.2 ± 13.0*</td>
</tr>
<tr>
<td>BRC at 14.30 h</td>
<td>16.2 ± 2.2</td>
</tr>
<tr>
<td>PB at 15.00 h</td>
<td></td>
</tr>
<tr>
<td>Saline at 15.00 h</td>
<td>18.4 ± 3.8</td>
</tr>
</tbody>
</table>

*P < 0.05 vs saline and BRC + PB.
**P < 0.05 vs saline (controls).
Di 1 at 08.00 h (P < 0.05) or Di 2 at 08.00 h (P < 0.01). BRC prevented PB-induced cycle lengthening when injected 30 min before PB (P < 0.01), but not when injected 30 min after PB (not significant), as shown in Table 2.

**Progesterone concentration on Di 2 (experiment 2)**
As shown in Table 3, progesterone values were significantly higher in Di 2 at 11.00 h in PB-treated animals than in controls and BRC-treated females (P < 0.05). In Di 2 at 17.00 h, progesterone values also were significantly higher in PB-treated animals than in saline controls (P < 0.05). When injected with BRC prior to PB, the females showed low progesterone values at 11.00 h which did not differ from those noted in controls.

**Prolactin concentrations on Di 1 (experiment 3)**
As presented in Fig. 1, blood PRL concentration began to rise by 10 min after PB (P < 0.01), peaked by 30 min (P < 0.025) and returned to basal values by 60 min as compared with the controls. There was a tendency for prolactin further to decrease by 120 min after PB compared with the controls. BRC treatment before PB completely depressed PRL values.

**Discussion**
The present data are in agreement with previous reports of Okamoto et al. (1972) and Chateau & Aron (1973) showing that PB (30 mg/kg ip) was able to delay ovulation by one day when administered during Di 1. Dominguez & Smith (1974) observed cycle prolongation following PB treatment throughout a more prolonged period from estrus afternoon until Di 2 morning. This was likely due to the higher doses of barbiturates used. Such a delay in ovulation can also be induced by PB injection in the early afternoon of proestrus, before the 'critical period' for ovulation (Everett et al. 1949), but it is regulated by a distinct neuroendocrine mechanism. It has been clearly demonstrated that proestrus PB administration blocks the chain of neural events which initiate the discharge of ovulating hormones (Everett & Sawyer 1950; Kalra et al. 1971). The present results suggest that PB, when injected on Di 1 afternoon, interferes with the brain mechanisms controlling progesterone secretion thus causing a change in estrous rhythm.

Peripheral plasma progesterone values on Di 2 morning were higher in PB-treated females than in control and BRC-treated females; they were very similar to that usually observed in natural 5-day cyclic females from our colony (Boehm et al. 1984a). Previous findings demonstrated that estrous cycle duration was dependent on the pattern of progesterone secretion during the diestrous period of the cycle (for review see Aron 1979). Thus we can assume that estrous cycle lengthening in Di 1 PB-treated females is related to a shift in progesterone secretion on Di 2 morning. This results in a decrease of the rate of follicular growth and thus in delayed time of ovulation, as previously described by Buffler & Roser (1974) and Chateau et al. (1981).

Our results clearly show that PRL is involved in the action of PB on both cycle lengthening and progesterone secretion. On one hand, bromocriptine counteracted the effect of PB on both cycle lengthening and high progesterone levels on Di 2 morning. On the other hand, PRL concentration rose very rapidly after PB injection. This brief PRL rise is likely involved in PB action, since BRC when injected a short time after PB did not prevent PB-induced cycle lengthening. Such an effect of PB on PRL secretion is in accordance with previous reports showing a transient eleva-
tion (about 30 min) of PRL in ovariectomized (Lawson & Gala 1974) and cyclic (Wakabayashi et al. 1971; Hoak & Schwartz 1978) rats. PRL is a well documented luteotropic factor during pseudopregnancy and pregnancy (review in Rothchild 1981) as well as during the estrous cycle (Alloiteau & Vignal 1958; Van der Schoot & Uilenbroek 1983; Boehm et al. 1984a). The PRL rise may thus be considered as directly responsible for the maintenance of progesterone secretion and for changes in cycle duration.

An interesting point in our data is the existence of a ‘critical period’ for PB action with a diurnal pattern during Di 1. It is as if PB is capable of rising PRL secretion only during Di 1 afternoon but not on Di 1 morning. Such a rhythm in the central nervous activity controlling PRL secretion has been observed in some experimental and natural conditions. In ovariectomized or cyclic rats a single injection of testosterone or estradiol can induce a daily rhythm of PRL secretion with high levels in the afternoon (Caligaris & Taleisnik 1983; Boehm et al. 1986; Uchida et al. 1973). During acute ether stress, PRL rises on Di 2 afternoon but not on Di 2 morning (Boehm et al. 1982). Furthermore, PRL levels were observed to be higher in the afternoon than in the morning every day throughout the estrous cycle in normal female rats (Boehm et al. 1984b). However, other reports are not in keeping with the existence of a diurnal sensitivity rhythm of the central nervous control of PRL secretion. In the WI1 rats of our colony, PB was already effective in lengthening estrous cycle when injected on Di 1 morning (Chateau & Aron 1973). For their part, Wakabayashi et al. (1971), disregarding the stage of the estrous cycle and injecting PB in the morning, observed an increase in serum PRL values 30 min later. Therefore, further experiments are needed to confirm the existence of a diurnal sensitivity rhythm of the central nervous control of PRL secretion.

Acknowledgments

The authors express their thanks to Dr. S. Plas-Roser for her expert advice in prolactin radioimmunoassay and to Mrs. Lazarus for her excellent technical assistance. They are grateful to Mr. Dujol and Ms. Gangloff for the preparation of the manuscript. They are also deeply indebted to the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Rat Pituitary Hormone Program for the supply of rat prolactin RIA kit reagents and to Sandoz Laboratory (Basel) for the supply of bromocriptine.

References


Received September 18th, 1987.
Accepted February 9th, 1988.

Prof Claude Aron,
Institute of Histology,
4, Rue Kirschleger,
F-67085 Strasbourg Cédex,
France.