CHAPTER VI

EFFECTS OF HYPO- AND HYPERPROLACTINEMIA ON GLYCOGEN CONCENTRATION AND GLUCOSAMINE 6-PHOSPHATE SYNTHASE ACTIVITY IN THE UTERUS OF PREPUBERTAL RATS
INTRODUCTION

Pituitary Prl level is lower in immature female rats than in the adult, serum Prl levels are also very low before puberty and increase at the time of vaginal opening (Voogt et al., 1970; Dohler and Wuttke, 1974; 1975). Prolactin is implicated in the timing of the onset of puberty in rats (Voogt et al., 1969; Wuttke et al., 1976; de Jong and Schoot, 1979). Induction of hyperprolactinemia in prepubertal female rats, either exogenously or endogenously, has been demonstrated to advance puberty and this is associated with increased steroidogenic responsiveness of the ovary to gonadotrophins. In addition it has been proposed that this sensitizing effect of Prl may be related to an increase in the ovarian LH receptor content and that by this mechanism Prl would accelerate the normal process of ovarian follicular maturation (Advis and Ojeda, 1978; Ojeda et al., 1980b). Hyperprolactinemia in prepubertal rats leads to increased LH receptors in granulosa cells and is associated with increased uterine weight and uterine fluid accumulation (Advis et al., 1979).

Lesions of the anterior hypothalamus led to increased Prl secretions and precocious puberty (Alvarel et al., 1977; Alvarez, 1979) and also increased the
sensitivity of the uterus to estradiol (Advis and Alvarez, 1977) resulting in precocious puberty. This precocious onset of puberty was suppressed by the simultaneous administration to the lesioned rats of 2-bromo a-ergocryptine (Alvarez et al, 1977). This chapter deals with the effect of induction of hypo-and hyperprolactinemia on the ovarian and uterine weights, uterine glycogen and glucosamine 6-phosphate synthase activity in prepubertal rats.

MATERIALS AND METHODS

Prepubertal female rats of 35 days age were divided into five groups. One group was treated with 100 \( \mu g \) Be (in 25 \( \mu l \) of 1:2:1 propylyene glycol:saline:ethanol), a second group of rats of the same age was treated with 0.63 mg/kg of Pirn in 0.1 M tartaric acid. Third and 4th group of rats were treated with 50 \( \mu g \) of baclofen and 50 \( \mu g \) of muscimol in 25 \( \mu l \) of saline respectively. Fifth group served as controls. All injections were made sc, once daily for 5 days and were sacrificed 24 hrs after the last injection.

In another set of experiments 30 day old rats were ovariectomized under light ether anesthesia. Five-days after the surgery, they were divided into 5 groups.
Group 1 served as controls. Group 2 received a single dose of 1 \( \mu \text{g} \) estradiol benzoate (EB) per rat 24 hrs prior to sacrifice on day 40. Group 3 were injected with Pim alone once daily for 5 days starting from day 35. Group 4 received Pim for 5 days plus one dose of 1 \( \mu \text{g} \) EB and group 5 received Be for 5 days followed by a single injection of EB 24 hr before sacrifice. Estradiol was dissolved in sesame oil. All animals were autopsied on day 40.

At autopsy ovaries and uteri were removed, freed of fat and weighed on a torsion balance. Uterine glycogen was estimated according to the method of Lo et al. (1970) and uterine glucosamine 6-phosphate synthase activity was measured as described in Chapter II.

RESULTS

The effects of various Prl inhibiting and releasing drugs on weights of ovary and uterus and uterine glycogen content in the intact prepubertal rats are shown in Table 1. Treatment with Be significantly (P<0.05) decreased the ovarian and uterine weights and also the uterine glycogen concentrations. Administration of Pim on the other hand produced a significant increase in the weights of both ovary and uterus and glycogen
concentration. Muscimol and baclofen, two GABA receptor agonists, had no significant effect on the weights of the ovary and uterus or the uterine glycogen concentration (Table 1).

The glucosamine 6-phosphate synthase activity in the uterus was significantly (P<0.05) suppressed by Be treatment in intact prepubertal rats. Administration of Pim caused significant stimulation in the enzyme activity. Muscimol and baclofen on the other hand did not modify the enzyme activity (Table 2).

Ovariectomy significantly lowered the uterine weight, glycogen concentration and the glucosamine 6-phosphate synthase activity. A single dose of 1 \text{\mu g} EB in OVX rats 24 hrs before autopsy increased the uterine weight, glycogen concentration and the enzyme activity comparable to those of intact controls. Injection of Pim alone in OVX rats once daily for 5 days, did not modify any of the above parameters when compared to OVX controls. Pimozide administration for 5 days followed by a single dose of EB 24 hrs before autopsy produced an increase in the uterine weight and significantly increased the glycogen concentration and the enzyme activity when compared to OVX-EB treated rats. Bromo- cryptine, however reduced the stimulatory action of
Pimozide treatment in intact rats advanced the onset of puberty by two days as more than 60% of the animals exhibited vaginal opening on day 40. Whereas vaginal opening occurs normally around day 42/43 in our animal facility.

**DISCUSSION**

The present results show that Be treatment in the prepubertal female rats has a direct effect on the uterine sensitivity to estradiol as reflected in the decrease in the ovarian and uterine weights, glycogen concentration and glucosamine 6-phosphate synthase enzyme activity. A similar decrease in uterine weight was observed in rats treated with Apomorphine, another DA agonist (Mistry and Vijayan, 1982). Decrease in the ovarian LH binding sites was observed in Be treated rats (Marta et al, 1984). However, Hernandez and Alvarez (1980) reported no change in the uterine wet weight of rats treated with Be. The histological examination of ovaries from either hyper- or hypoprolactinemic immature rats have shown enhanced follicular development in hyperprolactinemic rats and delayed development in rats
in which serum Prl levels were chronically decreased (Advis et al., 1980). Pimozide, a DA receptor antagonist, on the other hand produced an increase in the ovarian and uterine weights as well as stimulation of glycogen concentration and the glucosamine 6-phosphate synthase enzyme activity.

The vaginal opening which is a marker for sexual maturation was observed earlier i.e., on day 40 in more than 60% of the pim treated rats. Vaginal opening occurred normally on day 42 in our animal facility. Thus, if vaginal opening is taken as a criteria for the onset of puberty, induction of hyperprolactinemia with Pim, advanced the onset of puberty at least by 2 days in more than 60% of the rats. Induction of hyperprolactinemia has been reported earlier to induce precocious puberty in rats (Advis and Ojeda, 1978; Advis et al., 1981). Baclofen and muscimol, GABA receptor agonists known to stimulate Prl, had no effect.

Ovariectomy significantly decreased the uterine weight, glycogen concentration and the enzyme activity. A single dose of EB, injected 24 hrs prior to autopsy, in OVX rats increased the uterine weight, glycogen concentration and enzyme activity. Administration of Pim alone for 5 days to OVX rats did not have any effect
on the uterine weight, glycogen concentration and enzyme activity. However, im followed by a single injection of estradiol 24 hrs before autopsy increased uterine weight, glycogen concentration and enzyme activity. The increase in enzyme activity was greater than those seen in EB treated rats. Injection of Be however, significantly reduced EB induced stimulation in all the parameters studied. Estrogens are known to increase uterine growth, protein, DNA and RNA synthesis (Kaye et al, 1972; Stormshak et, al, 1976; Mistry and Vijayan, 1985) glycoproteins>glycogen and glycosaminoglycans (Endo and Yosizawa, 1973; Takata and Terayama, 1977). Glycogen is the storage form of carbohydrate and is located in the myometrium of the uterus (Greigoire et al, 1967; Cecil and Bitman, 1968). In the uterus of immature and OVX rats there is a low level of glycogen which is increased by the administration of a single dose of estradiol. Synthesis and utilization of amino sugars and sialic acid are stimulated by estradiol (Endo and Yosizawa, 1968; Rajalakshmi et al, 1969). Glucosamine 6-phosphate synthase activity is regulated by estradiol (Rukmini, 1981). Glucosamine 6-phosphate synthase catalyzes the transfer of the amide group of L-glutamine to D-fructose 6-phosphate to form glucosamine 6-phosphate. This is the first step in the synthesis of amino sugar unit of glycoproteins and glycosaminoglycans.
These results provide further support for the view that Prl participates in the developmental processes that leads to puberty by increasing the ovarian steroidogenic responsiveness to gonadotrophins (Advis and Ojeda, 1978; Advis et al, 1981). Their results suggest that Prl exerts at least part of its sensitizing effect by enhancing and/or maintaining the formation of LH receptors in the granulosa cells. This may be primarily the consequence of a facilitatory interactions between high Prl levels and low FSH titres present in these animals. The magnitude of the Prl effect on LH receptors may have been further enhanced by estradiol, the secretion of which has been shown to be augmented in hyperprolactinemic rats (Advis and Ojeda, 1978).

It has been reported that Prl increases the ovarian estradiol receptor content (Gibori et al, 1979) and that inturn, increases Prl binding to the ovary (Hammond and Krall, 1979).

Hyperprolactinemic animals undergo precocious puberty and exhibit an increase in in vivo release of estradiol which is manifested as augmented uterine weight (Advis and Ojeda, 1978) and also an increase in the uterine glycogen content and the enzyme activity as observed in the present study. These changes reflect the presence of
active estradiol secreting follicles. Their occurrence in hyperprolactinemic rats strongly suggest that the process of follicular maturation is accelerated by the high Prl levels so that at the same chronological age hyperprolactinemic rats show follicles in a more advanced phase of development than in the control animals. Recently Advis et al, (1984) have shown that the sensitizing effect of Prl does not result from a direct stimulation of Prl on aromatase activity but is rather related to the fact that in hyperprolactinemic rats more follicles have developed to a stage in which the estrogen output is significantly increased. Thus blockade of Prl surge before puberty with Be brought about a decrease in the ovarian and uterine weights, glycogen and enzyme activity which can be explained in view of the above reports showing that low levels of Prl brought about a reduction in the follicular development leading to less estrogen output which is essential for the uterine growth.

Many studies have reported that the number of estrogen receptors in a target organ is dependent both on estrogen and Prl. The concentration of estrogen receptors is significantly reduced after OVX or hypophysectomy, while it is completely or partially restored by the administration of estrogen or Prl (Leung and Sasaki, 1973; Chamses et al, 1975; Kelly et al, 1977). In the
The present study administration of EB to OVX rats increased the uterine weight as well as uterine glycogen concentration and glucosamine 6-phosphate synthase activity. However, Pim alone to OVX rats did not modify any of these parameters. Vignon and Rochefort (1976) have found that in castrated rats Prl had no effect on the estrogen receptors of the uterus. Pimozide administration to OVX rats followed by a single dose of EB enhanced the uterine weight, glycogen concentration and the enzyme activity significantly greater than those in OVX-EB primed animals. This may be due to a synergistic effect of Prl with estrogen on the uterus. On the other hand when animals were treated with EB after OVX and Be, treatment, the responsiveness of the uterus was markedly suppressed when compared to the OVX and EB treated rats. Considering that the changes in uterine responsiveness to estradiol is dependent on central mechanism, the above results can be interpreted to mean that the decrease in uterine sensitivity to estradiol is mediated by a diminished Prl concentration.

The present results provide additional support for Prl involvement in bringing about change in uterine responsiveness to EB and the onset of puberty.
Table - 1.
Effect of Prl inhibiting/stimulating drugs on weights of ovary and uterus and uterine glycogen concentration in prepubertal female rats.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment/Dose</th>
<th>Ovary (mg)</th>
<th>Uterus (mg)</th>
<th>Uterine glycogen concentration mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control (11)</td>
<td>28.5 ± 0.7</td>
<td>46.8 ± 3.0</td>
<td>160.5 ± 6.9</td>
</tr>
<tr>
<td>2.</td>
<td>Bc 100 µg (8)</td>
<td>22.0 ± 0.8*</td>
<td>29.7 ± 1.0*</td>
<td>103.2 ± 11.2*</td>
</tr>
<tr>
<td>3.</td>
<td>Pim 0.63 mg/kg (13)</td>
<td>35.9 ± 1.2*</td>
<td>68.1 ± 2.8*</td>
<td>225.0 ± 10.8*</td>
</tr>
<tr>
<td>4.</td>
<td>Baclofen 50 µg (9)</td>
<td>30.2 ± 0.3</td>
<td>52.2 ± 3.5</td>
<td>170.7 ± 8.3</td>
</tr>
<tr>
<td>5.</td>
<td>Muscimol 50 µg (9)</td>
<td>31.1 ± 1.4</td>
<td>55.7 ± 3.1</td>
<td>169.4 ± 9.6</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. Numbers in parenthesis indicate number of animals in each group.

*P<0.05. Vs controls.
Table - 2.

Effect of prolactin inhibiting/stimulating drugs on uterine glucosamine 6-phosphate synthase activity in prepubertal rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/Dose</th>
<th>Enzyme activity (n moles/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>39.34 ± 1.3 (4)</td>
</tr>
<tr>
<td>2.</td>
<td>Bc 100 µg</td>
<td>32.18 ± 1.2* (4)</td>
</tr>
<tr>
<td>3.</td>
<td>Pim 0.63 mg/kg</td>
<td>46.86 ± 1.7* (4)</td>
</tr>
<tr>
<td>4.</td>
<td>Baclofen 50 µg</td>
<td>44.58 ± 3.6 (4)</td>
</tr>
<tr>
<td>5.</td>
<td>Muscimol 50 µg</td>
<td>44.77 ± 2.7 (4)</td>
</tr>
</tbody>
</table>

The values represent mean ± SEM. The number in parenthesis indicates the number of determinations consisting of 4-10 animals in each group.

*P<0.05. Vs control.
Table - 3.

Uterine weight, glycogen concentration and uterine glucosamine 6-phosphate synthase activity in OVX prepubertal rats treated with Pim/Bc and/or EB.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Uterine weight (mg)</th>
<th>Glycogen Concentration mg/100 g</th>
<th>Enzyme activity n mol/mg protein/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Intact Controls (11)</td>
<td>46.8 ± 3.1</td>
<td>160.5 ± 6.9</td>
<td>39.34 ± 1.3 (4)</td>
</tr>
<tr>
<td>2.</td>
<td>OVX Controls (9)</td>
<td>20.8 ± 1.8**</td>
<td>95.2 ± 8.9*</td>
<td>20.37 ± 1.7** (3)</td>
</tr>
<tr>
<td>3.</td>
<td>OVX + EB (9)</td>
<td>41.1 ± 1.2</td>
<td>169.8 ± 5.2</td>
<td>55.57 ± 2.9 (3)</td>
</tr>
<tr>
<td>4.</td>
<td>OVX + Pim (9)</td>
<td>23.3 ± 1.6</td>
<td>103.0 ± 5.1</td>
<td>22.80 ± 2.1 (4)</td>
</tr>
<tr>
<td>5.</td>
<td>OVX + Pim + EB (9)</td>
<td>46.4 ± 2.6</td>
<td>218.2 ± 7.1b</td>
<td>68.22 ± 2.8a (3)</td>
</tr>
<tr>
<td>6.</td>
<td>OVX + Bc + EB (9)</td>
<td>32.2 ± 1.6a</td>
<td>131.0 ± 5.7a</td>
<td>41.47 ± 3.5a (3)</td>
</tr>
</tbody>
</table>

Bromocryptine (100 µg/rat), Pim (0.63 mg/kg/rat) once daily for 5 days and EB (1 µg/rat/24 hr prior to sacrifice). Values represent mean ± SEM. The number in parenthesis for the enzyme activity indicates the number of determinations consisting of 4-10 animals in each group.

* P<0.02    || Vs Intact Controls.
**P<0.01   ||
a P<0.05    || Vs OVX + EB treated rats.
b P<0.02    ||