Hyperprolactinaemia does not affect the development of the stimulatory mechanism of oestrogen-progesterone on LH secretion

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Abstract. Previous reports indicate that prolactin induces precocious puberty in the female rat. The present investigation was designed to determine if hyperprolactinaemia affects the development of the stimulatory effect of oestrogen-progesterone on LH secretion in prepubertal rats. Hyperprolactinaemia was induced by the administration of sulpiride at the dose of 7 mg/100 g body weight/day for 6 days. The rats were sacriﬁed 6 to 9 h after the last injection. Normal sulpiride-treated rats were injected 76 h before sacriﬁce with 10 μg of oestradiol benzoate and with 1 mg of progesterone on the day of sacriﬁce. The animals were killed at 14, 22, 24, 28 and 32 days of age.

The sulpiride treatment induced a signiﬁcant increase in serum prolactin levels (P < 0.01) at all ages studied. The treatment of oestrogen-progesterone induced prolactin release in controls and in the sulpiride-hyperprolactinaemic rats of 24, 28 and 32 day old but not in younger rats. The administration of oestrogen-progesterone alone in normal and in sulpiride hyperprolactinaemic rats produced a negative feed-back effect on LH secretion at 14 and 22 days of age and a signiﬁcant stimulatory action at 24, 28 and 32 days. No differences were observed in the basal LH serum concentration between control and hyperprolactinaemic rats at the different ages studied. Our ﬁndings suggest that the modiﬁcations in the onset of puberty induced by hyperprolactinaemia are not connected with alterations in the maturation of the hypothalamic-pituitary mechanism involved in the positive feed-back effect of ovarian steroids on LH secretion.

Oestrogen or progesterone injected into oestrogen-primed animals or women induces LH release (Odell & Swerdloff 1968; Caligaris et al. 1971a,b; Chang & Jaffe 1978). Previous reports (Caligaris et al. 1972; Scacchi & Moguilevsky 1973) have shown that this positive feed-back effect of oestrogen-progesterone on LH secretion in the female rat is associated with the development, during the prepubertal stage, of specific centres located in the anterior hypothalamic areas. The failure of ovarian steroids to activate the release of LH before 20—22 days of age appears to indicate that at this age the different structures involved in this mechanism reach maturation (Caligaris et al. 1972).

The administration of prolactin to female rats advances the onset of puberty (Clemens et al. 1969; Voogt et al. 1969; Wuttke et al. 1976). Prolactin implanted in the hypothalamus induced LH and FSH release (Voogt & Meites 1971; Voogt et al. 1969) and on this basis a direct stimulatory effect of the hormone on LlRl release has been suggested as one of the possible mechanisms of precocious puberty in hyperprolactinaemic rats. On the other hand an ovarian site of action of prolactin to induce

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Precocious puberty has also been suggested (Advis & Ojeda 1978).

Since the development of the positive feed-back mechanism of ovarian steroids on gonadotrophin is one of the events involved in the onset of puberty the present investigation was designed to ascertain whether sulpiride-induced hyperprolactinaemia may effect the development of the stimulatory effect of oestrogen-progesterone on LH secretion.

Material and Methods

Prepubertal rats of the Institute of Physiology of the Buenos Aires Medical School strain were used. They were housed in group cages under controlled conditions of lighting (12 h on, 12 h off) and temperature. Tap water and purine laboratory chow were available ad libitum.

Hyperprolactinaemia was induced by the administration of sulpiride, which has been shown to produce constantly elevated levels of endogenous prolactin in women (Delvoye et al. 1974) and animals (Horowski & Graff 1976). Sulpiride was administered subcutaneously at the dose of 7 mg/100 g body weight/day for 6 days dissolved in physiological solution. Controls were injected with the vehicle. The animals were sacrificed 6 to 9 h after the last injection. Normal and sulpiride treated rats were injected 76 h before sacrifice with 10 μg of oestradiol benzoate and with 1 mg of progesterone on the day of sacrifice. Steroid treatment was given simultaneously with sulpiride i.e. on the 3rd day of sulpiride administration the animals were injected with oestrogens and then on the 6th day with progesterone.

The animals were sacrificed by decapitation at 14, 22, 24, 28 and 32 days of age. The blood was collected from the trunk and allowed to clot at 4°C during 24 h. The samples were centrifuged for 10 min at 2500 r.p.m. and the serum was separated and stored frozen until estimation of prolactin and LH levels was carried out.

Serum prolactin and LH were measured in duplicate using the double antibody radioimmunoassay with kits supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Values are expressed in nanograms/milliliter on the basis of the NIAMDD reference preparation supplied with the Kit (NIAMDD-Rat-LH-RP-1 and Rat Prolactin RP-1). Results were statistically evaluated using variance and Tukey’s multiple range test (Tukey 1949).

Results

As can be seen in Table 1 the administration of 7 mg/100 g body weight of sulpiride for 6 days induced a significant increase in serum prolactin levels are as compared with control values (P < 0.01) at all ages studied. On the other hand the treatment of oestrogen-progesterone induced prolactin release in control and sulpiride hyperprolactinaemic rats of 24, 28 and 32 days of age but not in younger animals. Consequently prolactin levels in rats treated with sulpiride and oestrogen-progesterone at 24, 28 and 32 days old are signifi-

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Prolactin (ng/ml)</th>
<th>Saline (A)</th>
<th>Oe-P (B)</th>
<th>Sulpiride (C)</th>
<th>Sulpiride + Oe-P (D)</th>
<th>P valueb</th>
<th>&lt; 0.01</th>
<th>&lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>9.6 ± 0.8a</td>
<td>8.9 ± 0.9</td>
<td>40.0 ± 3.8</td>
<td>44.8 ± 5.9</td>
<td>A vs. C; A vs. D</td>
<td>B vs. C; B vs. D</td>
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<td>(6)</td>
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<tr>
<td>22</td>
<td>13.3 ± 1.8</td>
<td>21.3 ± 5.6</td>
<td>81.4 ± 3.7</td>
<td>138.0 ± 26.0</td>
<td>A vs. C; A vs. D</td>
<td>B vs. C; B vs. D</td>
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<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(7)</td>
<td>(8)</td>
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</tr>
<tr>
<td>24</td>
<td>16.4 ± 3.7</td>
<td>89.7 ± 9.8</td>
<td>75.5 ± 5.1</td>
<td>125.0 ± 13.5</td>
<td>A vs. B; A vs. C;</td>
<td>B vs. D;</td>
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<tr>
<td></td>
<td>(7)</td>
<td>(6)</td>
<td>(13)</td>
<td>(9)</td>
<td></td>
<td>C vs. D</td>
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</tr>
<tr>
<td>28</td>
<td>13.6 ± 2.8</td>
<td>138.0 ± 26.0</td>
<td>112.0 ± 14.0</td>
<td>236.0 ± 24.0</td>
<td>A vs. B; A vs. C;</td>
<td>B vs. D;</td>
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<td></td>
<td>(7)</td>
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<td>(8)</td>
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<td>A vs. D; C vs. D</td>
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<tr>
<td>32</td>
<td>15.7 ± 5.4</td>
<td>151.0 ± 18.3</td>
<td>108.2 ± 12.8</td>
<td>280.0 ± 54.2</td>
<td>A vs. B; A vs. C;</td>
<td>B vs. D;</td>
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<td></td>
<td>(8)</td>
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<td>(7)</td>
<td>(8)</td>
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<td>A vs. D; C vs. D</td>
<td></td>
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</tr>
</tbody>
</table>

a Mean ± se. The number of animals is in parenthesis.
b According to the Tukey’s multiple comparison «test»
c Doses and schedule of Oe-P and sulpiride treatment are described in the text.
Table 2.
Effect of oestrogen-progesterone (Oe-P) on LH release in prepubertal rats with sulpiride induced hyperprolactinaemia.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>LH (ng/ml serum)</th>
<th>$P^b$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (A)</td>
<td>Oe-P (B)</td>
</tr>
<tr>
<td>14</td>
<td>85.2 ± 7.3a</td>
<td>17.7 ± 3.4</td>
</tr>
<tr>
<td>22</td>
<td>68.0 ± 4.8</td>
<td>28.0 ± 8.3</td>
</tr>
<tr>
<td>24</td>
<td>35.0 ± 3.5</td>
<td>562.0 ± 80.0</td>
</tr>
<tr>
<td>28</td>
<td>25.2 ± 4.1</td>
<td>4400.3 ± 489.2</td>
</tr>
<tr>
<td>32</td>
<td>20.3 ± 3.2</td>
<td>3500.3 ± 320.1</td>
</tr>
</tbody>
</table>

a Mean ± se. The number of animals is in parenthesis.
b According to the Tukey’s multiple comparison «test»
c Doses and schedule of Oe-P and sulpiride treatment are described in text.

cantly higher than those in sulpiride treated rats and normal rats receiving oestrogen-progesterone.

The administration of oestrogen-progesterone induced in normal and in sulpiride-injected rats a similar pattern of LH variation at the different ages (Table 2). A significant decrease in LH levels was observed in both groups of rats at 14 and 22 days of age and a significant stimulatory action on LH release was seen at 24, 28 and 32 days.

No significant differences were observed in the basal LH serum concentration between control and hyperprolactinaemic rats at the different ages studied (Table 2).

Discussion

Previous studies demonstrated the failure of ovarian hormone to release LH before 22 days of age indicating that after this prepubertal stage the neural structures involved in this mechanism reach maturation (Caligaris et al. 1972; Scacchi & Moguilevsky 1973). The present results agree with previous reports, since a negative feed-back effect of oestrogen-progesterone on LH secretion was observed in prepubertal rats 14 and 22 days of age and a stimulatory effect at 24, 28 and 32 days. In addition our results demonstrated that hyperprolactinaemia does not modify the basal levels of LH and the age at which the positive feed-back effect of ovarian steroids on LH secretion appears in prepubertal rats. On the other hand, as has been reported (Döhler & Wuttke 1975; Moguilevsky et al. 1978) in normal prepubertal female rats, LH levels are higher in rats between 10 and 23 days of age than in older rats and this was also observed in the sulpiride induced hyperprolactinaemic rats (Table 2). This fact appears to indicate that hyperprolactinaemia does not affect the pattern of LH variation in prepubertal rats.

Several lines of experimental evidence show that hyperprolactinaemia can advance the onset of puberty in female rats (Clemens et al. 1969; Voogt et al. 1969; Wuttke et al. 1976; Voogt & Meites 1971; Advis & Ojeda 1978). A direct stimulatory effect of prolactin on the production of LH has been suggested as one possible mechanism by which the
hormone advances the onset of puberty (Clemens et al. 1969; Voogt et al. 1969). Nevertheless an inhibitory effect of prolactin on LH secretion was also proposed by Wuttke et al. (1976). The results presented here appear to indicate that the precocious puberty induced by hyperprolactinaemia is not related to changes in the maturation of events involved in the positive feed-back effect of ovarian steroids on LH secretion. Moreover it also appears that high levels of prolactin do not affect the LH pattern of secretion during the prepubertal stage. The possibility that hyperprolactinaemia advances the onset of puberty in female rats by affecting at hypothalamic levels other mechanisms than the maturation of the stimulatory effect of ovarian steroids on LH secretion remains to be studied. On the other hand Advis & Ojeda (1978) reported that sulpiride induced hyperprolactinaemic rats show an increase in the sensitivity of the ovaries to gonadotrophin and this fact could indicate an ovarian site of action of prolactin to induce precocious puberty.

A previous report indicates that oestrogens are unable to increase serum prolactin in rats younger than 24 days while dopaminergic antagonists like pimozide release prolactin a few days after birth (Ojeda & McCann 1974). The results observed in the present experiments confirm these data since oestrogen-progesterone did not induce hyperprolactinaemia in rats 14 and 22 days old while at this age sulpiride was effective in stimulating prolactin release.

It is interesting to note that the ovarian steroids further increase at 24, 28 and 32 days of age the high prolactin levels of the sulpiride treated rats. Both oestrogens and sulpiride appear to release prolactin acting at pituitary and central nervous system levels (Nicoll & Meites 1962; Labrie et al. 1978; McCleod & Robyn 1977; Laville 1972). Nevertheless we have demonstrated that the administration of oestrogen-progesterone significantly increases the high levels of serum prolactin in sulpiride treated rats, and this could be additional evidence for a different mechanism of action of these substances. On the other hand the fact that the maturation of the prolactin release mechanism of ovarian steroids and sulpiride occurs at different ages of the prepubertal stage (Ojeda & McCann 1974) further supports this point of view. Nevertheless, an additional stimulation of oestrogen-progesterone on the mechanism by which sulpiride induced hyperprolactinaemia cannot be ruled out.

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References


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