Alteration by Neonatal Hypothyroidism of the Critical Period for the Induction of Persistent Estrus in the Rat

SAKAE KIKUYAMA
Department of Biology, School of Education,
Waseda University, Tokyo

Synopsis

The effects of hypothyroidism for a short period of postnatal life on the induction of persistent estrus by androgen were investigated in female rats of 10 and 13 days of age. Hypothyroidal newborn rats produced by feeding propyl thiouracil (PTU) to the mother rats, were more susceptible to the persistent-estrus inductive influence of androgen than normal and thyroxine-theraped hypothyroidal rats of the same age. It is concluded that the period during which androgen exerts a persistent-estrus inductive influence was prolonged in hypothyroidal rats, possibly as the result of the retardation of development of the hypothalamic region, the site affected primarily by androgen.

Female rats treated with sex steroids in early postnatal life become anovulatory and exhibit persistent cornification of the vaginal epithelium (persistent estrus) after maturity (see Takewaki, 1962).

The period during which the hormones induce persistent estrus seems to end at around 10 days after birth (Barraclough, 1961; Kikuyama, 1963; Kikuyama and Kawashima, 1966). There are several experiments suggesting that the steroids exert their persistent-estrus inductive influence upon the central nervous system regulating pituitary functions (Segal and Johnson, 1959; Barraclough and Gorski, 1961; Kikuyama, 1961). Accordingly, it is postulated that the sex steroids act on the central nervous system at a particular stage of development to inhibit the maturation of the hypothalamic-hypophysial gonadal system characteristic of the female, and that once that stage has been passed the hormones can no longer exert an organizational effect on the neural structures.

The present experiments were designed to ascertain whether experimentally-produced neonatal hypothyroidism prolongs the period during which androgen exerts a persistent-estrus inductive influence, since hypothyroidism in the early postnatal period brings about retardation of growth and maturation, particularly of central nervous system (see Eayers, 1964).
Table 1. Effects of PTU on growth, eye-opening and thyroid glands in androgenized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Eye opening age in days</th>
<th>Body weight at TP injection (g)</th>
<th>Body weight at sacrifice (g)</th>
<th>Thyroid glands at sacrifice (mg/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a) N</td>
<td>7</td>
<td>16 – 17</td>
<td>13.2 ± 0.3b)</td>
<td>281 ± 7</td>
<td>8.0 ± 1.0</td>
</tr>
<tr>
<td>10 N</td>
<td>16</td>
<td>16 – 17</td>
<td>18.0 ± 0.3</td>
<td>271 ± 6</td>
<td>8.4 ± 0.4</td>
</tr>
<tr>
<td>10 PTU</td>
<td>14</td>
<td>17 – 19</td>
<td>15.1 ± 0.5c)</td>
<td>267 ± 6</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>10 PTU+T4</td>
<td>13</td>
<td>13 – 15</td>
<td>16.7 ± 0.4</td>
<td>265 ± 9</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>13 N</td>
<td>14</td>
<td>16 – 17</td>
<td>21.0 ± 0.6</td>
<td>283 ± 9</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>13 PTU</td>
<td>15</td>
<td>17 – 20</td>
<td>18.0 ± 0.5d)</td>
<td>267 ± 6</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>13 PTU+T4</td>
<td>11</td>
<td>14 – 15</td>
<td>19.7 ± 0.9</td>
<td>264 ± 8</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>PTU</td>
<td>6</td>
<td>17 – 19</td>
<td>–</td>
<td>274 ± 8</td>
<td>8.2 ± 1.0</td>
</tr>
</tbody>
</table>

a) Days of age at TP injection.
b) Mean ± S.E.
c) Statistically significant against N and PTU+T4 groups at P < 0.05.
d) Statistically significant against N group at P < 0.05.

Androgen treatment

Single subcutaneous injections of testosterone propionate (TP) were administered. The dose of TP was 10 µg dissolved in 0.004 ml of sesame oil per gram body weight.

Three groups of seven, sixteen and fourteen normal rats each (N rats) reared by mothers given no PTU received TP injections at 7, 10 and 13 days of age, respectively. Two groups of fourteen and fifteen PTU rats each were injected with TP at 10 and 13 days respectively. The remaining six rats received no TP injection. Two groups of thirteen and eleven T4+PTU rats each were given injection of TP at 10 and 13 days, respectively.

In all the rats, the day of eye opening and vaginal opening were recorded. When the animals reached 60 days of age, vaginal smears were examined every day until sacrificed at 150 days of age. At sacrifice, ovaries and thyroid glands were taken out, weighed, fixed in Bouin’s solution, cut in paraffin and stained with Delafield’s hematoxylin and eosin for histological study.

Results

As shown in Table 1, the administration of PTU to mother rats resulted in the retardation of growth and eye opening in the young. Thyroxine injections to the young from the day of birth nullified the effects of the goitrogen. Although the body weights of PTU rats at the initiation of TP injections were significantly different from that of N rats, no differences were noticeable between them at the time of sacrifice. At the time of sacrifice there were no differences in either structure or weight of thyroid glands among normal, hypothyroidal and thyroxine-theraped hypothyroidal rats.

Four of seven N rats which were androgenized at 7 days of age, showed continued vaginal cornification from the beginning of smear examination. In the remaining three, estrus became persistent by 75 days of age. At sacrifice, their ovaries had follicles of various sizes but no corpora lutea. In eight of sixteen N rats which received TP at 10 days of age, normal estrous cycle took place throughout the period of smear examination. Ovaries of these animals contained both follicles and corpora lutea. In seven animals of this group estrus became persistent at 92 – 128 days of age. Their ovaries were lacking corpora lutea. In the remaining one, the estrous cycle became...
irregular at 136 days of age, follicles and small corpora lutea being present in the ovaries.

In only three of fourteen PTU rats androgenized at 10 days of age, the normal estrous cycle ran throughout the period of smear examination. Their ovaries had both follicles and corpora lutea. In eight animals of this group, estrus eventually became persistent at 84 – 130 days of age after they had shown normal or prolonged estrous cycles. Ovaries of these persistent-estrous rats had no corpora lutea. Ovaries of the remaining three rats which exhibited irregular estrous cycle contained a few degenerating corpora lutea in addition to follicles.

Seven of thirteen PTU+T4 rats androgenized at 10 days of age exhibited normal estrous cycles, having both follicles and corpora lutea in their ovaries. In one animal of this group, the normal estrous cycle was replaced with a prolonged-estrous cycle at 132 days of age. Ovaries of this animal contained follicles and small, old corpora lutea. The remaining five came to show continued cornification of the vaginal epithelium after normal or irregular estrous cycles ran for the first 25 – 63 days of the smear examination. Four of them had no corpora lutea in their ovaries. The ovaries of the remaining one contained small corpora lutea besides follicles.

Twelve of fourteen N rats which had received TP injection at 13 days of age, exhibited normal estrous cycle throughout the period of smear examination. Their ovaries had follicles and corpora lutea. The remaining two showed irregular estrous cycles for 14 – 20 days prior to sacrifice. Their ovaries contained follicles and small corpora lutea.

Ten of fifteen PTU rats androgenized at 13 days of age, exhibited normal estrous cycles, having both follicles and corpora lutea in their ovaries. However, in four animals of this group, estrus became persistent at 118 – 132 days of age. In three of them, ovaries contained no corpora lutea, and in the remaining one, a few degenerating corpora lutea were present. In one female of this group, cycles became irregular when the animal reached 130 days of age. At sacrifice, the ovaries contained both follicles and small corpora lutea.

Eleven PTU + T4 rats which had been subjected to androgen at 13 days of age invariably

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Table 2. Effects of TP on vagina, ovaries and estrous cycle in normal, hypothyroidal and thyroxine-therapied hypothyroidal rats

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>No.</th>
<th>Vaginal opening (age in days)</th>
<th>Ovarian weight (mg/100g body weight)</th>
<th>Corpora lutea</th>
<th>Estrous cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ ± -</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>7a) N</td>
<td>7</td>
<td>13 – 15</td>
<td>15.0 ± 1.0b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 N</td>
<td>16</td>
<td>15 – 16</td>
<td>22.5 ± 2.0</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>10 PTU</td>
<td>14</td>
<td>15 – 19</td>
<td>18.3 ± 1.0c)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>10 PTU+T4</td>
<td>13</td>
<td>15 – 16</td>
<td>22.9 ± 1.5</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>13 N</td>
<td>14</td>
<td>17 – 18</td>
<td>25.3 ± 1.0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>13 PTU</td>
<td>15</td>
<td>18 – 20</td>
<td>24.7 ± 1.7</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>13 PTU+T4</td>
<td>11</td>
<td>16 – 19</td>
<td>25.7 ± 1.0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>PTU</td>
<td>6</td>
<td>38 – 45</td>
<td>25.1 ± 0.6</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

a) Days of age at TP injection.
b) Mean ± S.E.
c) Statistically significant against N and PTU + T4 groups at P < 0.05.
d) +, Presence of corpora lutea; ±, Presence of atrophic corpora lutea; –, absence of corpora lutea.
e) R, regular cycle; I, irregular cycle; P, persistent estrus.
showed normal estrous cycle. Their ovaries had both follicles and corpora lutea.

In six PTU rats which had received no TP injection, the ovaries had many corpora lutea in addition to follicles of various sizes.

The state of ovaries and estrous cycle is shown in Table 2 and diagramatically in Fig. 1.

**Discussion**

The results obtained in the present experiment favor the view that the critical period for the induction of persistent estrus by androgen is variable. The hypothyroidal rats of 10 and 13 days of age were more susceptible to the persistent-estrus inductive influence of TP than normal and thyroxine-therapied hypothyroidal rats of the same age. The present results are in harmony with the results reported previously that in the female rats of the Wayne strain given thyroxine for a short period of postnatal life, a disturbance of estrous cycles by TP was less marked than in the females which had received no thyroxine injections (Kikuyama, 1966).

The site which is primarily affected by early treatment with androgen is proposed by Barraclough and Gorski (1961) to be the region of the anterior preoptic and suprachiasmatic nuclei. The presence of the critical period for the induction of persistent estrus by androgen seems to indicate that the steroid affects this part of the brain at a particular stage of development. And, it is highly probable that the prolongation by goitrogen of the period during which TP induces persistent estrus is the result of retardation of the maturation of this region, although overall effects of hypothyroidism may not be excluded.

Precocious canalization of the vaginal orifice in androgenized rats is attributable to the direct action of TP on the vagina (Kikuyama, 1965). Eayers and Holmes (1964) reported that hyperthyroidism early in life brings about chronic hypothyroidism later. It is revealed that hypothyroidism in early postnatal life does not exert permanent influence on the thyroid judging from the weight and histological charac-
teristics of the gland at 150 days of age.

References