THE EFFECTS OF SEX STEROIDS ON THE PROLACTIN CONTENT OF HYPOPHyses AND SERUM IN RATS

By

O. L. Wolthuis

ABSTRACT

Prolactin determinations have been carried out on the hypophyses and serum of rats. It was found that:
1. Hypophyses of intact mature female rats contain almost twice as much prolactin as those of mature female rats spayed two months previously. The pituitary prolactin content in these spayed female rats is virtually identical with that of intact or castrated mature male rats.
2. Treatment of intact mature female rats with oestradiol benzoate (50 μg daily for one week) considerably increases the prolactin content in the hypophyses and serum.
3. Treatment of spayed mature female rats with sex steroids for two weeks shows that:
   a. oestradiol benzoate (50 μg daily) increases the prolactin content in the hypophyses and serum;
   b. testosterone propionate (2 mg daily) also increases the prolactin content in the hypophyses and serum, although the increases found were smaller than those obtained with the above-mentioned dose of oestradiol;
   c. progesterone (5 mg daily) did not significantly alter the pituitary prolactin content, whereas a highly suggestive increase was found in the serum content.

From the results it was concluded that:
1. Physiological amounts of androgens do not affect the prolactin function of the hypophysis, whereas physiological amounts of oestrogens do affect it.
2. All three sex steroids investigated increase prolactin production in and secretion from the hypophysis. A negative feedback seems to be absent.
In two previous articles we have described a new prolactin assay method and provided arguments for its specificity (Wolthuis 1963 a and b). The criterion in this test is the average number of corpus luteum cell nuclei (CLCN) per surface unit in histological preparations of the ovaries from HCG- and PMS-treated hypophysectomized immature female rats.

The aim of the experiments described here was twofold:

a. to test the usefulness of the method; we used it in some comparative assays and matched the results with those obtained by other investigators using different assay techniques;
b. to obtain new data on the effects of steroids; this was possible since not only the changes in the prolactin contents of hypophyseal material, but also those in the blood serum could be measured.

METHODS

All the rats used were of an inbred, but not pure laboratory strain. Donor rats were between 200 and 250 g body weight at the beginning of each experiment. When the effect of gonadectomy was studied, removal of the ovaries took place two months before autopsy; in experiments in which the effects of sex steroid administration were investigated, injections started one month after gonadectomy. All operations were performed under ether anaesthesia. Doses and injection period of each steroid are described under Results. Blood was obtained by heart puncture under ether anaesthesia, after which the animals were killed by a blow on the neck. At autopsy the hypophyses were taken out of the sellae after the posterior lobes had been removed. Blood and anterior lobes were pooled per group of similarly treated animals. After standing overnight in a cold room (0–4°C), blood was centrifuged at 5000 revolutions per minute for 15 minutes.

The sera obtained were kept frozen until assay. The hypophyses were homogenized in saline by means of a Potter Elvehjem apparatus. After the proper dilutions (0.5 hypophysis in 6 ml saline) had been made they were also kept frozen until used.

Treatment of the assay animals, checking completeness of hypophysectomy and preparation of the ovaries were exactly as described previously (Wolthuis 1963 a). In these experiments the total doses were equivalent to 0.5 hypophysis or to 12 ml serum per assay animal, divided over 6 days. Even if it is clear that a decrease in the number of CLCN is correlated to a higher dose of prolactin, the comparison between two counts does not give a direct quantitative value for the difference between the amounts of prolactin involved. It is for this reason that, for a very rough estimation of the absolute content, we give the prolactin equivalents in IU, calculated with the aid of a previously established regression equation (Wolthuis 1963 a). The numbers thus obtained make no pretence to being correct, but they approximately define the relation between the prolactin contents of the hypophyses or serum of two animal groups (e. g. treated or untreated). Their level itself is no doubt also determined by daily and seasonal variations. These variations, however, are small; this became evident by determining the pituitary prolactin content in male and female rats, which were spread over a period of nearly two years.

Degrees of significance are expressed as follows:

$$- = P > 0.05, + = 0.05 \geq P > 0.01, ++ = 0.01 \geq P > 0.001, +++ = 0.001 > P.$$  
They are computed from Student's $t$-test.
RESULTS

A. Pituitary prolactin contents in male and female rats were compared in two experiments. Each experiment consisted of two completely different series. In the first experiment the effects of hypophyseal material obtained from intact mature male and female rats were compared. As may be seen from Table 1 (groups A and B), hypophyses from males contain less prolactin than hypophyses from females. This was confirmed in the second experiment, where the effects of gonadectomy on the prolactin content of male and female hypophyses were studied. It can be seen from Table 1 (groups C–F) that two months after spaying the prolactin content of the female hypophysis is lowered to the level of the male hypophysis, whereas no changes are observed after castration of male rats. In this series of experiments the hypophyseal weights were also recorded: it may be calculated that the prolactin concentration per mg hypophysis was higher in intact females than in intact males, and also higher in spayed females than in castrated males.

B. The effects of sex steroids

Oestradiol benzoate (O.B.): As an introductory experiment the effects on the pituitary and serum prolactin content caused by a daily dose of 50 µg oestradiol benzoate during one week were studied in intact female rats. The controls received oil. From Table 2 it can be seen that treatment with oestrogens caused a significant increase in the prolactin content in the hypophyses as well as in serum. Pituitary prolactin concentration also increased.

After this experiment had shown that it was possible to estimate changes in hypophyseal material and even in serum, the following scheme (see also Discussion) was adopted to study these effects of sex steroids more systematically: mature female rats, spayed one month previously, were treated with sex steroids for two weeks. The hypophyses as well as the serum of the donor rats were assayed for their prolactin content:

1. Oestradiol benzoate (O.B.): a daily dose of 50 µg during two weeks causes significant rises in the prolactin content of the hypophyses as well as in the serum (see Table 3). Hypophyseal concentration increases.

2. Testosterone propionate (T.P.): after a two weeks’ treatment with a daily dose of 2 mg, a significant rise in pituitary and serum prolactin content is observed (see Table 3). The increases, however, are smaller than those obtained with oestrogen treatment. Hypophyseal prolactin concentration increases.

3. Progesterone: hypophyseal prolactin contents undergo no significant changes after treatment with 5 mg progesterone daily for two weeks (see Table 3).
Table 1.
The hypophyseal prolactin content of intact mature rats and of mature rats 2 months after gonadectomy.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Gonads</th>
<th>Pituitary weight (mg) (No. between brackets)</th>
<th>Additional treatment during last 6 days (total dose)</th>
<th>Number of CLCN** per 0.0972 mm²</th>
<th>Estimated amount of prolactin in IU</th>
<th>Number of assay animals</th>
<th>Group letter</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>present</td>
<td>—</td>
<td>0.5 hypoph. equiv.</td>
<td>329 ± 2.2</td>
<td>0.26</td>
<td>11</td>
<td>A</td>
</tr>
<tr>
<td>male</td>
<td>present</td>
<td>—</td>
<td>0.5 hypoph. equiv.</td>
<td>332 ± 2.6</td>
<td>0.16</td>
<td>11</td>
<td>B</td>
</tr>
<tr>
<td>female</td>
<td>present</td>
<td>12.8 ± 0.93*(11)</td>
<td>0.5 hypoph. equiv.</td>
<td>292 ± 4.2</td>
<td>0.29</td>
<td>8</td>
<td>C</td>
</tr>
<tr>
<td>male</td>
<td>present</td>
<td>7.6 ± 0.29 (10)</td>
<td>0.5 hypoph. equiv.</td>
<td>336 ± 4.1</td>
<td>0.15</td>
<td>7</td>
<td>D</td>
</tr>
<tr>
<td>female</td>
<td>removed</td>
<td>9.9 ± 0.35 (14)</td>
<td>0.5 hypoph. equiv.</td>
<td>333 ± 5.3</td>
<td>0.16</td>
<td>7</td>
<td>E</td>
</tr>
<tr>
<td>male</td>
<td>removed</td>
<td>10.7 ± 0.25 (13)</td>
<td>0.5 hypoph. equiv.</td>
<td>334 ± 4.3</td>
<td>0.16</td>
<td>7</td>
<td>F</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

** CLCN = corpus luteum cell nuclei.

Significance of differences in countings (for symbols, see Methods): A - B: + + +
C - D: + + +
C - E: + + +
C - F: + + +
Table 2.
The changes in the prolactin content in hypophyses and serum of intact mature female rats after treatment with oestradiol benzoate for one week.

<table>
<thead>
<tr>
<th>Daily treatment</th>
<th>Pituitary weight (mg) (No. between brackets)</th>
<th>Additional treatment during last 6 days (total dose)</th>
<th>Estimated amount of prolactin in IU</th>
<th>Number of assay animals</th>
<th>Group letter</th>
</tr>
</thead>
<tbody>
<tr>
<td>oil</td>
<td>12.3 ± 0.54* (20)</td>
<td>0.5 hypoph. equiv. 292 ± 4.7*</td>
<td>0.29</td>
<td>14</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 ml serum 405 ± 3.9</td>
<td>none detected</td>
<td>6</td>
<td>B</td>
</tr>
<tr>
<td>50 µg oestradiol benzoate</td>
<td>17.8 ± 0.67 (19)</td>
<td>0.5 hypoph. equiv. 252 ± 3.5</td>
<td>0.53</td>
<td>11</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 ml serum 322 ± 11.1</td>
<td>0.19</td>
<td>7</td>
<td>D</td>
</tr>
</tbody>
</table>

Significances of differences:  
A – C: + + +  
B – D: + + +  

* Standard error of the mean.  
** CLCN = corpus luteum cell nuclei.
Table 3.
The effect of administration for two weeks of oestradiol, testosterone and progesterone on the prolactin content of female mature rats spayed one month previously.

<table>
<thead>
<tr>
<th>Donor animals: Adult spayed female rats</th>
<th>Assay animals: Hypophysectomized immature female rats treated with 10 IU HCG and 10 IU PMS daily during 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily treatment</td>
<td>Pituitary weight (mg) (No. between brackets)</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>oil</td>
<td>11.9 ± 0.31* (28)</td>
</tr>
<tr>
<td>50 µg oestradiol benzoate</td>
<td>29.8 ± 1.20 (29)</td>
</tr>
<tr>
<td>oil</td>
<td>11.3 ± 0.31 (28)</td>
</tr>
<tr>
<td>2 mg testosterone propionate</td>
<td>11.7 ± 0.36 (30)</td>
</tr>
<tr>
<td>oil</td>
<td>11.6 ± 0.33 (29)</td>
</tr>
<tr>
<td>5 mg progesterone</td>
<td>11.7 ± 0.29 (29)</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
** CLCN = corpus luteum cell nuclei.
*** One animal discarded according to Chauvenet's criterion.
(ri Documenta Geigi, Wissenschaftliche Tabellen, 1955, p. 43).

Significance of differences: A – C: ++ ++
B – D: + ++
E – G: ++ +
F – H: ++ +
J – L: —
K – M: — p, K – M: +
The results of serum estimations, however, strongly suggest that the prolactin content of the serum has increased; the differences with serum obtained from oil-treated controls might be significant (see Discussion).

**DISCUSSION**

A. *Comparison of the pituitary prolactin content of mature intact and gonadectomized male or female rats*

The results indicate the existence of an effect of ovarian steroids on the prolactin function of the hypophysis, whereas there is no such effect with testicular steroids. In the light of further experiments described below, it is likely that ovarian oestrogens are already effective in physiological amounts, whereas this is not the case with physiological amounts of androgens. If this also occurs in other species, it might explain why the mammary gland is less developed in the male.

The effect of spaying in female animals (decrease in pituitary prolactin content) confirms the results of earlier investigations (*Reece & Turner* 1937; *Meites & Turner* 1948). The absence of such changes in males after castration and the finding that the hypophyseal prolactin content in males is lower than in females are also in accordance with previous results (*Reece & Turner* 1936).

One theoretical restriction must be made with regard to the conclusions on the effect of physiological amounts of oestrogens and androgens: if physiological amounts of androgen have an effect, and if after castration the prolactin content remains at the androgen-induced level (»fixation of a sex characteristic«), there would be no difference between normal and castrated males either. In such a case the prolactin content in the castrated male would not represent the theoretical baseline, *i.e.* the amount found in an animal that has matured in the complete absence of sex steroids. Moreover, in view of the fact that no difference has been observed between spayed females and castrated males, a *partial* fixation of the oestrogen effect would have to be assumed, since in spayed females, too, the baseline is not reached. It seems rather improbable that the identical amounts of prolactin found in intact males, castrated males and spayed females should have such a complicated background. An experimental approach to this problem might be through the estimation of hypophyseal prolactin contents of adult animals which have been gonadectomized at birth.

B. *Content and concentration*

Although we have also mentioned changes in hypophyseal prolactin *concentration* (see Results), we do not attach much value to these changes for two reasons:

1. As the hypophyseal body is not homogenous, variations in pituitary
weight may reflect volume changes in cells other than those producing prolactin.

2. Only the total amount of prolactin that can be made available seems important to us and not whether this amount is secreted from a large or a small hypophysis.

C. The effect of oestradiol benzoate

1. Intact mature female rats.

The increase in the prolactin content of both hypophyses and serum after treatment with 50 µg oestradiol benzoate daily for one week indicates an increased production of prolactin. Another explanation might be the inhibition of peripheral utilization of prolactin, but this seems far-fetched, especially so since it does not explain the high prolactin content of the hypophysis.

The increases in the hypophyseal prolactin content as found by us confirm previous reports with similar results (Reece & Turner 1937; Reece 1938; Grosvenor & Turner 1960) obtained with local pigeon-crop methods. Quantitatively, comparison with results found in the literature is difficult, as rat-strains, dosage and duration of treatment vary. The impression is obtained, however, that the greatest increases in intact animals may be produced by short-term oestrogen treatment (e.g. 5 days – see Grosvenor & Turner 1960). After a longer treatment the animals become pseudo-pregnant, and interference by progesterone may occur. Progesterone is known to diminish the increase in hypophyseal prolactin content induced by oestrogen treatment in guinea-pigs (Meites & Turner 1942) and in rats (Reece & Bivins 1942).

As far as we know the increase in the prolactin content of serum of oestrogen-treated rats is a new finding. Recently, Talwalker et al. (1962), using the Reece-Turner pigeon-crop sac method, were unable to demonstrate prolactin in the serum of oestrogen-treated rats. As we have explained before (Wolthuis 1963 a), this is probably due to the fact that with our method estimations can be carried out in 12 ml serum or perhaps even more; this is not possible in the local pigeon-crop methods.

2. Spayed mature female rats.

For a more systematic study of the effects of oestradiol, testosterone and progesterone on the prolactin content of hypophyses and serum, interference by endogenous gonadal steroids seemed undesirable, and hence spayed mature female rats were used. One month after gonadectomy they were treated with various sex steroids during two weeks; we preferred two weeks to one, so that more marked effects could be obtained. In these animals, too, we found that oestrogen treatment (50 µg daily during two weeks) increased the prolactin content in the hypophyses as well as in the serum. With regard to the increase in hypophyseal content, these findings confirm previous reports (Reece & Turner 1937; Reece 1938; Reece & Bivins 1942).
In view of recent findings, both in vivo (Desclin & Koulischer 1960) and in vitro (Nicoll & Meites 1962), it seems likely that the increased production of prolactin brought about by oestrogen treatment is at least partly due to a direct action of oestrogens on the hypophysis itself.

D. The effect of testosterone propionate

In this case too, we found an increase in prolactin content of the hypophyses and serum. Again, the increase in the hypophyseal content confirms previous findings (Reece & Mixner 1939; Meites & Turner 1942), and the increase in prolactin content in serum after testosterone treatment is, as far as we know, a new finding. As in the case of oestrogens, treatment with testosterone propionate increased production of prolactin. In spite of the high dose of testosterone propionate administered, the increases (in hypophyses and serum) were smaller than those obtained with oestrogen treatment. This is in accordance with the results mentioned under A and B, although it does not exclude the possibility that doses of testosterone between physiological amounts and 2 mg testosterone daily might cause even greater effects: in analogy, it is known that after treatment of male guinea-pigs with increasing doses of diethylstilboestrol the hypophyseal prolactin content reaches a peak with 0.2 mg in five days; higher doses cause smaller increases (Meites & Turner 1942).

The fact that testosterone in high doses causes prolactin secretion might account for some cases of gynaecomastia, occurring around the age of puberty in boys with a healthy liver.

E. The effect of progesterone

Applying the same experimental design we investigated the effect of a daily dose of 5 mg progesterone. In contrast to other investigators (Reece & Bivins 1942; Meites & Turner 1942), we did not find an increase in hypophyseal prolactin content after treatment with progesterone. As to serum, we could not demonstrate that an increase takes place, although our findings are highly suggestive of such an increase. The average number of nuclei in assay animals treated with control serum was 377, which was the lowest control value obtained in the two years in which these and other experiments took place. This means that the differences between the control values and those obtained with serum from progesterone-treated animals (359) is smaller than might be expected. Nevertheless, this difference was almost significant (Student's t = 2.00 with 16 degrees of freedom, whereas in this case a Student's t of 2.12 is necessary for a \( P = 0.05 \)).

Since we have not found any difference between the average number of corpus luteum cell nuclei in 37 saline-treated assay animals and those in 31 assay animals treated with control serum in these and other series (Wolthuis 1962), we may assume that, with our method, no prolactin is detectable in the
serum of mature spayed female rats. If this assumption is accepted, testing for unilateral deviation is permissible and the difference between the prolactin content of the control serum and serum from progesterone-treated animals becomes significant ($P < 0.05$).

These results strongly suggest that after progesterone treatment too, the production of prolactin is enhanced. In our experiments, this was not accompanied by a concomitant rise in hypophyseal content. In spite of the high dose of progesterone administered, the increase found in serum was smaller than that obtained with oestradiol- or testosterone-treatment.

F. General considerations

Looking back to the introductory remarks with regard to the aim of these experiments, the following may be said:

1. The results of assays of hypophyseal material with our method are generally in accordance with those obtained by other investigators who used a pigeon-crop method, except in those experiments where progesterone was administered.

2. The results of prolactin assays in serum provide new data, indicating that all three steroids tested increase prolactin production: no negative feedback was found. It seems to us that one of the most intriguing questions for the future will be the elucidation of the factor(s) responsible for the termination of corpus luteum function.

REFERENCES

Meites J. & Turner C. W.: Endocrinology 30 (1942) 726.

Received on September 26th, 1962.