HYPOTHALAMIC-PITUITARY-TESTICULAR SYSTEM AND ADRENOCORTICAL FUNCTION

By

H. L. Verjans and K. B. Eik-Nes

ABSTRACT

Effect of intramuscular administration of ACTH or dexamethasone on blood serum levels of testosterone, LH and FSH was examined in intact and castrated, adult, male rats. Six IU ACTH or 1 mg dexamethasone were given daily for 7 days. Corticotrophin treatment had no influence on circulating testosterone, LH and FSH in intact or castrated male rats. Dexamethasone administration resulted in a slight elevation of serum FSH in intact animals but not in castrates. LH and testosterone remained normal in both intact and castrated animals injected with dexamethasone. Under our conditions of study the secretions from the adrenal gland appear to be insignificant for the regulation of pituitary secretion of gonadotrophins in the male rat.

Testosterone, 5α-dihydrotestosterone and oestradiol-17β are known secretory products of the testis having regulatory effects on the hypothalamic-pituitary axis in adult male rats. Gonadectomy in such animals results in very low circulating levels of testosterone and 5α-dihydrotestosterone (Coyotupa et al. 1973), while circulating LH and FSH will increase markedly (Gay & Bogdanove 1969; Gay & Dever 1971; Swerdloff et al. 1972, 1973; Dufy-Barbe & Franchimont 1972; Kalra et al. 1973). Administration of these testicular steroids (or their propionate and benzoate ester derivatives) can prevent the rise of serum gonadotrophins following castration. In this respect, oestradiol-17β and 5α-dihydrotestosterone are very potent (Swerdloff et al. 1973; Verjans et al. 1974). In addition to the testis, the adrenal cortex is a source of androgenic and oestrogenic steroids in various animal species (Dorfman & Ungar 1965). The contribution
of androgenic and oestrogenic steroids from the adrenal gland as potential regulators of circulating gonadotrophins has scarcely been investigated. Furthermore, the possible effect of 11-oxygenated and 11-deoxygenated corticosteroids is unknown in this respect. We have, therefore, studied effects of adrenal suppression and adrenal stimulation on the hypothalamic-pituitary-testicular system in intact and castrated, adult, male rats by determining circulating testosterone, LH and FSH levels in such animals by appropriate radioimmunoassay techniques.

MATERIAL AND METHODS

Animals

Adult, male, Wistar rats (3–4 months old) were used in the experiments. The animals were kept under controlled light (14 hours light and 10 hours darkness) and temperature (19–21°C) conditions. Rat chow and tap water were provided ad libitum.

Experiment I. – From a group of 18 animals with body weights varying from 225–250 g, 9 animals were gonadectomized under light ether anaesthesia and the rest was used as intact animals. Five gonadectomized and 5 intact animals were injected with 1 mg dexamethasone sodium phosphate (Decadron® phosphate, Merck Sharp & Dohme B. V., Haarlem, The Netherlands) in 0.25 ml buffer while 4 animals of each group were injected with 0.25 ml 0.9% sodium chloride solution only. Daily, intramuscular injections were given for a period of 7 days. Treatment of the gonadectomized animals started immediately following surgery.

Experiment II. – From a group of 16 animals with body weights ranging from 380–400 g, 8 animals were gonadectomized under light ether anaesthesia and the rest was employed as intact animals. Four gonadectomized and 4 intact animals were injected with 6 IU corticotrophin (Frederiksberg Chemiske Fabriker A/S, København, Denmark) in 0.1 ml aqua dest. while 4 animals of each group were injected with 0.1 ml water only. Daily, intramuscular injections were given for a period of 7 days. Treatment of the gonadectomized animals started immediately following operation. Twenty hours after the last injection blood samples from experiment I and II were collected following decapitation of the animals under light ether anaesthesia. The blood was allowed to clot overnight at 4°C. Resulting blood serum was then stored at –20°C until analysed for gonadotrophins and testosterone. Adrenal glands, testes, ventral prostate and seminal vesicles were dissected and weighed shortly after the animals were sacrificed.

Radioimmunoassays

Serum LH and FSH levels were measured using double antibody radioimmunoassays (Welschen et al. 1973). For both radioimmunoassays the procedures as described by Niswender et al. (1968) were followed. Iodination with 125I (Institut for Atomenergi, Kjeller, Norway) was performed according to the method of Greenwood et al. (1963). Serum samples from all experiments were assayed as duplicates in one assay and serum LH and FSH concentrations were expressed on the basis of reference preparations NIAMD RAT LH RP-1 and NIAMD RAT FSH RP-1 respectively. Radioimmunoassay of testosterone in serum was performed as described previously for plasma (Verjans et al. 1973). Statistical significance of the differences between data from the different animal groups was determined using Student’s t-test.
Table 1.
Mean (± sd) organ weights (g) of intact and castrated, adult, male rats treated with 1 mg Decadron® (Dex.) per day for 7 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Adrenals</th>
<th>Testis</th>
<th>Ventral Prostate</th>
<th>Seminal vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact, 1 mg Dex.</td>
<td>5</td>
<td>0.026 ± 0.003*</td>
<td>1.284 ± 0.070</td>
<td>0.139 ± 0.014</td>
<td>0.062 ± 0.009</td>
</tr>
<tr>
<td>Intact, saline</td>
<td>4</td>
<td>0.059 ± 0.008</td>
<td>1.394 ± 0.089</td>
<td>0.120 ± 0.010</td>
<td>0.075 ± 0.013</td>
</tr>
<tr>
<td>Castrate, 1 mg Dex.</td>
<td>5</td>
<td>0.026 ± 0.004**</td>
<td></td>
<td>0.040 ± 0.010</td>
<td>0.037 ± 0.004</td>
</tr>
<tr>
<td>Castrate, saline</td>
<td>4</td>
<td>0.056 ± 0.006</td>
<td></td>
<td>0.054 ± 0.008</td>
<td>0.035 ± 0.010</td>
</tr>
</tbody>
</table>

* P < 0.001, compared with intact controls.
** P < 0.001, compared with castrate controls.

RESULTS

Effects of treatment with ACTH or dexamethasone on organ weights

The effects of daily, intramuscular administration of 1 mg dexamethasone or 6 IU ACTH during 7 days on the weights of the adrenal glands, testis, ventral prostate and seminal vesicles in intact and castrated, adult, male rats have been summarized in Tables 1 and 2 respectively. Compared with control rats ACTH treatment during 7 days had no influence on body weight of either castrated or intact animals. Treatment with dexamethasone for 7 days caused, however, reduction of body weight (15–25%) in both intact and castrated animals. We have, therefore expressed the organ weights as absolute values rather than relative to total body weight. Following castration the weights of the

Table 2.
Mean (± sd) organ weights (g) of intact and castrated, adult, male rats treated with 6 IU ACTH per day for 7 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Adrenals</th>
<th>Testis</th>
<th>Ventral Prostate</th>
<th>Seminal Vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact, 6 IU ACTH</td>
<td>4</td>
<td>0.094 ± 0.010</td>
<td>1.640 ± 0.221</td>
<td>0.257 ± 0.026</td>
<td>0.232 ± 0.018</td>
</tr>
<tr>
<td>Intact, aqua dest.</td>
<td>4</td>
<td>0.069 ± 0.016</td>
<td>1.591 ± 0.109</td>
<td>0.246 ± 0.034</td>
<td>0.216 ± 0.018</td>
</tr>
<tr>
<td>Castrate, 6 IU ACTH</td>
<td>4</td>
<td>0.091 ± 0.012</td>
<td></td>
<td>0.091 ± 0.023</td>
<td>0.111 ± 0.014</td>
</tr>
<tr>
<td>Castrate, aqua dest.</td>
<td>4</td>
<td>0.081 ± 0.008</td>
<td></td>
<td>0.108 ± 0.029</td>
<td>0.131 ± 0.022</td>
</tr>
</tbody>
</table>

200
Accessory reproductive organs in the control animals fell, but no significant change was observed in the weights of the adrenal glands in these animals (Tables 1 and 2). Administration of 1 mg dexamethasone per day during 7 days resulted in a profound reduction in adrenal weight in intact \( (P < 0.001) \) and castrated \( (P < 0.001) \) animals (Table 1). Testicular weight, the weights of the ventral prostate and the seminal vesicles remained unchanged in intact animals following 7 days treatment with dexamethasone (Table 1). The weights of the latter two organs in castrated animals exhibited no change following dexamethasone treatment (Table 1). Corticotrophin treated intact and castrated, adult, male rats gained adrenal weight, these gains were, however, not statistically significant for intact \( (0.05 < P < 0.10) \) or castrated \( (0.20 < P < 0.25) \) animals compared with respective control rats (Table 2). Testicular weight and the weights of the accessory reproductive organs in both type of animals were not influenced by the treatment with ACTH (Table 2).

Effects of treatment with ACTH or dexamethasone on serum levels of testosterone, LH and FSH

The effects of daily, intramuscular administration of 6 IU ACTH or 1 mg dexamethasone for 7 days on serum levels of testosterone in intact and castrated, adult, male rats are depicted in Figs. 1 and 2 respectively. Seven days following castration circulating testosterone decreased to very low values, while circulat-

![Graph](image-url)

**Fig. 1.**

Effect of daily treatment with 6 IU ACTH for seven days on levels of serum testosterone in intact and castrated, adult, male rats. Mean data ± sp from four animals are shown.
Fig. 2.
Effect of daily treatment with 1 mg Decadron® (Dex.) for seven days on levels of serum testosterone in intact and castrated, adult, male rats. Mean data ± sd from four or more animals are shown.

Fig. 3.
Serum levels of LH and FSH in intact and castrated, adult, male rats treated with 6 IU ACTH per day for seven days. Mean data ± sd from four animals are shown.
Serum levels of LH and FSH in intact and castrated, adult, male rats treated with 1 mg Decadron® (Dex.) per day for seven days. Mean data ± SD from four or more animals are shown.

ing LH and FSH (Figs. 3 and 4) showed a remarkable increment. Daily, intramuscular corticotrophin treatment during 7 days did not bring about changes in serum levels of testosterone, LH and FSH in intact or castrated, adult, male rats in comparison with respective control animals receiving water only (Figs. 1 and 3). Incubation of 6 IU ACTH with diluted antiserum, however, revealed a slight cross-reaction of the ACTH preparation used with both the LH and the FSH antibodies. In experiment I serum FSH levels were somewhat higher than in experiment II (Figs. 3 and 4), which might be due to interassay-variations in the radioimmunoassay of serum FSH or to biological variation. Treatment with 1 mg dexamethasone per day for 7 days resulted in an increase in serum FSH levels in intact animals (P < 0.01) compared with the controls receiving saline only (Fig. 4). Such elevation was not observed in castrates following the same dose of dexamethasone (Fig. 4). The other serum parameters investigated were not influenced by dexamethasone treatment in either normal or castrated rats (Figs. 2 and 4).
DISCUSSION

Seven days following gonadectomy low testosterone (Figs. 1 and 2) and elevated gonadotrophin levels (Figs. 3 and 4) were found in blood serum of adult male rats. These data compare well with those of others (Coyotupa et al. 1973; Gay & Bogdanove 1969; Gay & Dever 1971; Swerdloff et al. 1971) and with data previously reported by our laboratory (Verjans et al. 1974, 1975). Daily, intramuscular administration of ACTH for 7 days was followed by adrenocortical hypertrophy in normal and castrated rats though this was not of significant nature (Table 2). Corticotrophin treatment for 7 days did not influence the hypothalamic-pituitary-testicular system investigated (Figs. 1 and 3). This is consistent with the observation that the weights of the androgen-sensitive, male, accessory organs remained unchanged following ACTH treatment in both intact and castrated animals compared with respective controls (Table 2). It has been reported (Hudson et al. 1965) that the administration of ACTH to normal men will not change plasma testosterone levels. Beitins et al. (1973) found, however, significantly lower testosterone and 5α-dihydrotestosterone concentrations in plasma of normal young men with no change in serum LH levels after exogenous ACTH. Gay & Dever (1971) showed that adrenalectomy in orchidectomized, mature, male rats produced no additional rise in serum levels of LH and FSH. Thus, the present data favour the suggestion that steroids of adrenocortical origin do not play a significant role as regulators for gonadotrophin secretion in male rats. Moreover, the amount of adrenocortical androgens and oestrogens secreted in castrated male rats cannot substitute for the testicular steroids in their feedback action on the hypothalamic-pituitary axis. Finally, if the major effect of a continuous “stress” situation is via pituitary secretion of ACTH, the possibility is remote that such a “stress” form will influence the testicular system.

Dexamethasone has a high glucocorticoid activity and will suppress ACTH secretion at a pituitary and/or hypothalamic level (Zimmermann & Critchlow 1969). Suppression of adrenocortical function in our investigation was evident by the decreased adrenal weights of dexamethasone treated normal and castrated rats (Table 1). In the castrates, 1 mg dexamethasone per day for 7 days had no measurable effects on circulating testosterone (Fig. 2), LH or FSH (Fig. 4), nor did this glucocorticoid affect the falling ventral prostate and seminal vesicles weights (Table 1). Thus, in the present study the endocrine changes induced by castration were not influenced by the use of a large dose of a potent glucocorticoid. It has been shown that administration of a daily dose of 3 mg cortisone for 15 days will cause a small weight decrease of the ventral prostate in castrated rats while a daily dose of 9 mg cortisone administered over the same period of time will not do so, both doses of cortisone were, however, found to induce growth of the seminal vesicles in such animals (Tisell 1970). Cortisone, having
a weak androgenic effect when administered alone, can, however, counteract partially the growth of the male, accessory, reproductive organs induced by testosterone propionate administration in castrated, adrenalectomized rats (Tisell 1972). Tveter & Aakvaag (1969) reported that corticosterone will reduce the uptake of $[^3]$H testosterone by the prostatic lobes and the seminal vesicles. Simultaneous administration of cortisol augments the androgenic activity of testosterone propionate as measured by ability to increase the weights of the seminal vesicles and the ventral prostate in immature castrates (Klaiber et al. 1968). These discrepancies could be due to differences in doses and duration of glucocorticoid treatment.

In the normal rat, dexamethasone treatment for 7 days did not influence circulating testosterone and LH (Figs. 2 and 4). This was also reflected by unchanged weights of the ventral prostate, seminal vesicles and testis in these animals (Table 1). Circulating FSH, however, exhibited a slight but significant elevation (Fig. 4) in the intact rat following dexamethasone treatment. It has been reported that cortisone exerts a deleterious effect on the germinal epithelium of rats and mice (Albert 1961). Thus, in the doses given, dexamethasone could have a direct effect on the testicular tissue and change the production in this tissue of factors responsible for regulated secretion of FSH. Setchell & Sirinathsinghji (1972) have published on FSH depressing activity in rete testis fluid and Van Thiel et al. (1972) have suggested that factors produced in the germinal epithelium are involved in FSH feedback control in men. Our data could indicate that dexamethasone will lower testicular production of an unknown factor controlling specifically FSH secretion in the male rat. Thus, a direct effect of large doses of dexamethasone on possible testicular factors, regulating serum FSH concentrations in normal rats, is possible. No data are, however, available on the effects of long-term treatment with dexamethasone on the pituitary-testicular system. Faiman & Winter (1971) have shown that administration of a total dose of 3 mg dexamethasone to normal men will elevate plasma LH levels but not those of plasma FSH and testosterone. Judd et al. (1973) reported, however, that a single dose of 2 mg dexamethasone did not block nocturnal rise of plasma testosterone in normal male subjects, a probable LH function. A total dose of 0.6 mg dexamethasone given on late dioestrus and early pro-oestrus will block ovulation in female rats, probably by inhibiting pituitary release of LH (Baldwin & Sawyer 1974).

The present work using stimulation or suppression of adrenocortical function in gonadectomized, adult, male rats with long-term administration of suprapharmacological doses of ACTH or dexamethasone and the work of Gay & Dever (1971) using adrenalectomized, orchidectomized, mature, male rats reveal that the steroids of the adrenal cortex do not significantly affect circulating gonadotrophin concentrations in such animals. The contribution of adrenal corticosteroids, androgens and oestrogens to the testicular steroids in regulating
circulating gonadotrophin concentrations is likely to be of minor importance. Thus, only secretory products of the testes reaching target organs in the brain via the general circulation seem to be responsible for control of the gonadotropic function of the hypophyseal-hypothalamic system in adult male rats.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Uilenbroek and Dullaart (Erasmus University, Rotterdam, The Netherlands) for the gifts of LH and FSH antiserum and the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Program, (Bethesda, Maryland, U.S.A.) for the gift of the preparations used in the radioimmunoassay of the gonadotrophins. We should also like to express our appreciation to Drs. J. Kvittingen and P. Halvorsen and Mr. N. Nesjan of the Regionsykehuset in Trondheim for all kind help.

REFERENCES


Setchell B. P. & Sirinathsinghji D. J.: J. Endocr. 53 (1972) IX.


Swerdloff R. S., Walsh P. C., Jacobs H. S. & Odell W. D.: Endocrinology 88 (1971) 120


Received on January 2nd, 1975.