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Comparison of Effects of Prolactin and Growth Hormone on Adrenal 5α-Reductase in Hypophysectomized Rats[1] (39287)

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(Introduced by R. Brandt)

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Previous studies from Kitay's laboratory have established that adrenal 5α-reductase regulates adrenal cortical hormone secretion in the rat by determining the balance between the secretion of corticosterone and its reduced metabolites, 5α-dihydrocorticosterone and 3β,5α-tetrahydrocorticosterone (1–4). In intact rats of either sex, adrenal 5α-reductase activity is low but is elevated by either gonadectomy, hypophysectomy, or suppression of endogenous ACTH release by cortisone (1, 2, 5). In castrate animals, this enzyme activity is completely suppressed with estradiol and testosterone replacement (1). Of the pituitary hormones, only prolactin inhibits 5α-reductase activity in the castrate (6). In hypophysectomized rats, inhibition of enzyme activity occurs after treatment with testosterone, ACTH, prolactin, and growth hormone (3, 5–7). Estradiol is not effective following pituitary ablation, and it has been suggested that the action of this steroid is mediated by its stimulation of endogenous prolactin secretion (5, 6). Positive interactions between various hormones which influence 5α-reductase have also been demonstrated (3, 7–9).

Prolactin and growth hormone, both inhibitors of adrenal 5α-reductase activity in hypophysectomized rats, have frequently been shown to exhibit overlapping actions (10). In the present study, the inhibitory effects of prolactin and growth hormone treatment *in vivo* on adrenal 5α-reductase activity are compared.

Materials and methods. Female Sprague–Dawley rats (101–150 g), obtained from Charles River Breeding Laboratories, were maintained under standardized conditions of light (12 hr/12 hr) and temperature (25 ± 1°) on a diet consisting of Wayne Laboratory Chow and water *ad libitum.* When the rats weighed approx 200 g, hypophysectomy was performed parapharyngeally under methohexital (Brevital) sodium (4.5 mg/100 g of body wt) anesthesia. Following surgery, 5 ml of isotonic saline was administered ip, and the animals were given 5% sucrose or glucose as drinking solution and housed in pairs in a warm (28°) room to minimize death. Bovine prolactin (NIH-P-B3) and growth hormone (NIH-GH-B16), obtained from NIAMD, were prepared for injection by suspension in saline and adjustment of the pH to 9.0–9.5 with 0.1 *N* NaOH to facilitate dissolution. The effects of varying doses of hormones (injected sc) were examined in 2- and 5-day hypophysectomized rats. In the 2-day experiment, the first daily dose of hormone was given as a single injection on the day of operation and as divided doses, half at 0900 and half at 1700 (b.i.d.), on the first postoperative day. In the 5-day experiment, the hormones were administered b.i.d. on the second through fourth postoperative day. The doses used in these experiments are indicated in the results section. In all experiments, saline-treated (pH 9.0–9.5) rats served as controls.

Rats were killed by decapitation on the morning following the last injection, and reductase activity was assayed in whole (10 or 20%, w/v) adrenal homogenates by the method of Tomkins (11) as modified by Kitay *et al.* (12). Completeness of hypophysectomy was determined by visual inspection at autopsy.

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Treatment means were compared statistically by the Duncan's Multiple Range Analysis performed with the aid of the IBM 1130 computer.

Results. Five α-reductase was measured in adrenal homogenates in intact female rats and at 2, 5, and 6 days after hypophysectomy (Fig. 1). In intact rats enzyme activity is low and increases progressively with time after pituitary ablation. Five α-reductase activity increases 35-fold 6 days after hypophysectomy.

The effects of varying doses of prolactin and growth hormone on adrenal 5α-reductase activity were examined in 2-day hypophysectomized rats (Table I). Prolactin at doses of 250 μg/100 g of body wt/day and 25 μg/100 g of body wt/day inhibited enzyme activity 53 and 33%, respectively. At a prolactin dose of 2.5 μg/100 g of body wt/day, enzyme activity was not inhibited (Table I, Experiment I). Growth hormone did not affect enzyme activity at either 250 or 25 μg/100 g of body wt/day dose levels (Table I, Experiment II).

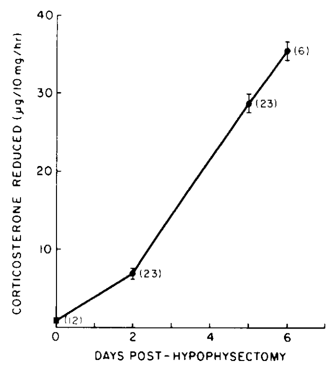

FIG. 1. Reductase activity in adrenal homogenates at various intervals after hypophysectomy. Each point represents mean ± SE of homogenates from individual animals. Numbers of observations are indicated in parentheses.

The effects of varying doses of prolactin and growth hormone were also examined in 5-day hypophysectomized rats. As shown in Table II, Experiment I, significant inhibition of enzyme activity was observed at all doses of prolactin. The amount of inhibition was comparable for both the high and intermediate dose levels (approximately 50%). At the lowest dose of prolactin, the degree of inhibition is significantly less (23%). Table II (Experiment II) shows that the highest dose of growth hormone (250 μg/100 g of body wt/day) produced a 29% depression in enzyme activity. The other doses of growth hormone were ineffective. It should also be noted that for a given dose of hormone, the amount of enzyme activity suppressed is greater in the 5-day hypophysectomized rat than in the 2-day hypophysectomized rat (compare Tables I and II).

In the final experiment, adrenal 5α-reductase activity was measured in 5-day hypophysectomized rats after treatment with growth hormone (250 μg/100 g of body wt/day) and/or prolactin (25 μg/100 g of body wt/day) (Table III). Individually, prolactin and growth hormone inhibited reductase activity 39 and 28%, respectively. The effects of these treatments did not differ significantly. In combination, the inhibitory effects of growth hormone and prolactin were

TABLE I. EFFECTS OF PROLACTIN AND GROWTH HORMONE ON ADRENAL 5α-REDUCTASE (5α-RASE) ACTIVITY IN 2-DAY HYPOPHYSECTOMIZED RATS.

Treatment	Dose of hormone (μg/100 g of body wt/day)	Number of rats	Corticosterone reduced	Relative 5α-Rase (%)
Experiment I				
Hypox + saline	–	9	6.0 ± 0.8[a]	100
Hypox + prolactin	250	9	2.8 ± 0.6[b]	47
Hypox + prolactin	25	7	4.0 ± 0.6[b]	67
Hypox + prolactin	2.5	9	6.2 ± 0.6	103
Experiment II				
Hypox + saline	–	6	5.2 ± 1.0	100
Hypox + growth hormone	250	9	4.8 ± 0.6	91
Hypox + growth hormone	25	7	6.4 ± 0.4	123

[a] Each entry represents mean ± SE of adrenal homogenates from individual animals expressed as μg/10 mg wet weight/hr.

[b] Differs from hypox + saline control at $P < 0.05$.

TABLE II. EFFECTS OF PROLACTIN AND GROWTH HORMONE ON ADRENAL 5α-REDUCTASE (5α-RASE) ACTIVITY IN 5-DAY HYPOPHYSECTOMIZED RATS.

Treatment	Dose of hormone (μg/100 g of body wt/day)	Number of rats	Corticosterone reduced	Relative 5α-Rase (%)
Experiment I				
Saline	–	3	31.6 ± 2.0[a]	100
Prolactin	250	4	14.8 ± 1.4[b]	47
Prolactin	25	5	17.4 ± 1.4[b]	55
Prolactin	2.5	3	24.4 ± 3.2[b, c]	77
Experiment II				
Saline	–	12	25.8 ± 1.6	100
Growth hormone	250	12	18.4 ± 1.6[d]	71
Growth hormone	25	4	24.8 ± 3.0	96
Growth hormone	2.5	6	27.2 ± 2.0	105

[a] Each entry represents mean ± SE of adrenal homogenates from individual animals expressed as μg/10 mg wet weight/hr.
[b] Differs from saline control at $P < 0.05$.
[c] Differs from high and intermediate doses of prolactin at $P < 0.05$.
[d] Differs from saline control at $P < 0.01$.

TABLE III. EFFECTS OF COMBINATION OF PROLACTIN AND GROWTH HORMONE ON ADRENAL 5α-REDUCTASE (5α-RASE) ACTIVITY IN 5-DAY HYPOPHYSECTOMIZED RATS.

Treatment	Dose of hormone (μg/100 g of body wt/day)	Number of rats	Corticosterone reduced	Relative 5α-Rase (%)
Saline	–	8	32.6 ± 1.8[a]	100
Prolactin	25	7	19.8 ± 1.2[b]	61
Growth hormone	250	9	23.4 ± 1.2[b]	72
Prolactin +	25	9	12.4 ± 1.6[b, c]	38
growth hormone	250		(10.6)[d]	(33)[d]

[a] Each entry represents mean ± SE of adrenal homogenates from individual animals expressed as μg/10 mg wet weight/hr.
[b] Differs from saline control at $P < 0.01$.
[c] Differs from individual hormone treatments at $P < 0.01$.
[d] Predicted value based upon sum of individual responses, computed as follows:
Saline − [(Saline-Prolactin) + (Saline-Growth Hormone)].

additive, producing a 62% inhibition which is comparable to that predicted by the sum of the individual treatments (values in parentheses, Table III).

Discussion. Kitay *et al.* demonstrated that adrenal 5α-reductase activity increases daily up to 3 days posthypophysectomy, after which no further increment is detected (5). As suggested by the authors of that study, the failure to observe a further elevation in enzyme activity was due to limitations of the assay system (insufficient substrate or too much enzyme). In the present study, these assay limitations were overcome by appropriate adjustment of substrate and homogenate concentration. Consequently, a progressive daily increase in enzyme activity is observed up to 6 days after hypophysectomy where 5α-reductase levels are 35 times that of intact controls which agrees with the observation of Colby *et al.* (13). The inflection in Fig. 1 suggests that the daily increase in enzyme activity doubles after 2 days posthypophysectomy. The progressive increase in adrenal 5α-reductase activity with time after hypophysectomy is in contrast to that seen after gonadectomy where enzyme activity remains at a constant level (equivalent in magnitude to that of 2-day hypophysectomized rats) (14). Furthermore, unlike the relatively rapid response to hypophysectomy, 5α-reductase activity is not detected until at least 2 weeks after postpuberal gonadectomy and at least 4 weeks after prepuberal gonadectomy (14).

In a previous report, treatment with large doses of bovine prolactin (250 μg/100 g of body wt/day) or growth hormone (500 μg/

100 g of body wt/day) from 2 to 4 days posthypophysectomy partially inhibited adrenal 5α-reductase activity in 5-day hypophysectomized rats (6). In the present study, the effects of prolactin and growth hormone were examined in 2-day hypophysectomized rats as well, since cholesterol sidechain desmolase activity decreases with time after pituitary ablation (15), and a physiologically relevant hormonal effect on 5α-reductase would most likely manifest itself during this period. With duration of pituitary absence, enzyme activity becomes increasingly sensitive and responsive to the inhibitory effects of either hormone. From 2 to 5 days the minimal effective dose for prolactin decreases and an effect of growth hormone emerges, confirming previous observations of an inhibitory effect of the latter hormone (6, 7). Furthermore, a given dose of prolactin produces a greater inhibition in enzyme activity in the 5-day hypophysectomized rat than in the 2-day experiment. It is conceivable, however, that the enhanced sensitivity and responsiveness to hormone treatment of 5-day hypophysectomized rats is an artifact of the design of the experiment whereby the absence of hormone from 0 to 2 days posthypophysectomy sensitizes the adrenal to hormonal influences.

A comparison of prolactin and growth hormone treatment groups 2 and 5 days after hypophysectomy reveals that the reductase pathway is 100-fold more sensitive to the inhibitory effects of the former. In the rat, the half-lives of natural prolactin and growth hormone are comparable (16, 17), suggesting that differing sensitivities demonstrated in this study are not due to different circulating concentrations of the two hormones. In the ovariectomized rat, no clear dose-response effect of prolactin over a 100-fold range (10 to 1000 μg/100 g of body wt/day) was observed (6). In the hypophysectomized rat, the inhibitory effects of the high and intermediate doses are comparable; however, a dose-response relationship is suggested between the intermediate and low doses of the hormone. The lowest effective dose of prolactin observed in this study is consistent with that reported to inhibit enzyme activity in ovariectomized rats and is comparable to or lower than current estimates of the daily secretion of the hormone in the cycling female rat (18–145 μg/100 g of body wt/day) (16). However, the minimal effective dose of growth hormone is considerably larger than its estimated daily secretion in the rat (30 μg/100 g of body wt) (17), and the effect of this hormone is evident only after the potential for steroidogenesis (cholesterol desmolase activity) has been markedly reduced. Since bovine hormone preparations were used in this study and the b.i.d. sc regimen does not adequately replicate normal secretory patterns, the question of physiological significance, although tempting, should be considered with reservation. Nevertheless, endocrine influences on adrenal 5α-reductase in turn affect the output of corticosteroids both *in vitro* and *in vivo* (1–8, 13). The stimulatory effects of prolactin on adrenal progesterone secretion in the ovariectomized rat (18) and of growth hormone on aldosterone secretion in the sodium-depleted hypophysectomized rat (19) may also involve the adrenal reductase mechanism.

The demonstration that prolactin and growth hormone exhibit additive inhibitory actions on reductase activity (i.e., a level predictable on the basis of addition of individual hormone responses) suggests distinct modes of action for both hormones. Different modes of action for the two hormones is also suggested by the delayed appearance of growth hormone responsive reductase activity and by the previous report that while prolactin inhibits enzyme activity in both the ovariectomized and hypophysectomized rats, growth hormone is effective only in the latter preparation (6). Similarly, the additive effects of ACTH and growth hormone on adrenal reductase activity in the hypophysectomized rat (7) may also be indicative of different modes of action for these two hormones.

Summary. Adrenal 5α-reductase activity was measured in female rats 0, 2, 5, and 6 days after hypophysectomy. Enzyme activity increased progressively exhibiting a 35-fold elevation at 6 days. The effects of high (250 μg/100 g of body wt), intermediate (25 μg/100 g of body wt), and low (2.5 μg/100 g of body wt) daily doses of bovine prolactin and bovine growth hormone were compared at 2

and 5 days posthypophysectomy. At 2 days, enzyme activity was partially inhibited by the high and intermediate doses of prolactin and not affected by growth hormone. At 5 days all doses of prolactin were inhibitory, whereas enzyme activity was suppressed only by the high dose of growth hormone. With a given dose of hormone, the amount of suppression of enzyme activity is greater at 5 days than at 2 days posthypophysectomy. In 5-day hypophysectomized rats the inhibitory effects of prolactin and growth hormone were additive. It is concluded that: (i) hormonal sensitivity and responsiveness of the adrenal reductase pathway increases with duration of pituitary ablation; (ii) the reductase pathway is more sensitive to the effects of prolactin than growth hormone; and (iii) the effects of growth hormone and prolactin on reductase activity are mediated via different mechanisms, as suggested by the additive effects of individual hormones.

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