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Effect of Orchiectomy on Pituitary Secretion of ACTH

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ABSTRACT

The effects of prepuberal gonadectomy on pituitary secretion of ACTH were studied in adult male rats. Plasma concentration of ACTH in unstressed adrenalectomized rats was increased following orchiectomy to 197% (95% confidence limits 134-289%) of the control level. Higher plasma ACTH concentrations were also observed in castrated animals with adrenals intact after the acute stress of ether anesthesia when compared to similarly stressed controls (159%; 121-215%). In both experiments, replacement with testosterone reversed the response to orchiectomy. Unstimulated release of ACTH in vitro by whole pituitary glands from orchiectomized donors, without or with testosterone replacement in vivo, did not differ from that of control glands. Addition of stalk-median eminence extract to the incubating glands, however, resulted in a greater R EMOVAL of the ovaries from rats results in decreased synthesis and release of ACTH (1). In addition, adrenal secretion of corticosterone is also significantly depressed, independent of changes in ACTH secretion (2) . These effects are reversed with estradiol administration. The data thus demonstrate stimulation of the pituitary-adrenal system by estrogen at two different sites, i.e., significant and separable enhancement of secretion of both pituitary ACTH and adrenal corticosterone.

Less information is available concerning changes in pituitary ACTH secretion in relation to testicular function. Kitay (3) has shown that adrenal corticosterone secretion is depressed following orchiectomy and that this defect is reversed by testosterone replacement. However, in contrast, pituitary ACTH content and adrenal size are increased in release of ACTH by pituitary glands from the orchiectomized group (162% of control; 124-211 %) and partial restoration toward the control level with testosterone replacement (130% of control; 110-153%). Parallel studies with pituitary glands from adrenalectomized animals yielded similar results. Direct addition of testosterone in vitro did not affect pituitary ACTH release. Hypothalamic content of corticotropin releasing activity was not altered by castration or testosterone replacement. The data indicate that orchiectomy results in elevated pituitary ACTH release independently of concomitant changes in adrenal function or conditions related to steroid feed-back. The increase seems related, at least in part, to pituitary hyper-responsiveness to corticotropin releasing factor. (Endocrinology 89: 1024, 1971)

REMOVAL of the ovaries from rats results in decreased synthesis and release of ACTH (1). In addition, adrenal secretion of corticosterone is also significantly depressed, independent of changes in ACTH secretion (2) . These effects are reversed with estradiol administration. The data thus demonstrate stimulation of the pituitary-adrenal system by estrogen at two different sites, i.e., significant and separable enhancement of secretion of both pituitary ACTH and adrenal corticosterone. Less information is available concerning changes in pituitary ACTH secretion in relation to testicular function. Kitay (3) has shown that adrenal corticosterone secretion is depressed following orchiectomy and that this defect is reversed by testosterone replacement. However, in contrast, pituitary ACTH content and adrenal size are increased in castrates suggesting increased synthesis and release of ACTH. The studies of Zizine suggested that testosterone administration inhibits ACTH release, as indicated by de-creased adrenal ascorbic acid depletion after stress (4), and decreased compensatory adrenal hypertrophy after unilateral

adrenalectomy (5). In the present study, blood levels of ACTH were measured directly in orchiectomized and orchiectomized, testosterone-treated rats to obtain direct evidence concerning the influence of testosterone on ACTH output by the pituitary gland. In vitro experiments were employed to assess the manner in which testosterone alters ACTH secretion.

Materials and Methods

Male rats of the Sherman strain were used as experimental animals; female rats of the Sprague-Dawley strain were used for in vitro bioassay procedures, while male animals were used for in vivo assays. The animals were given a diet of Purina Laboratory Chow and water ad lib. and maintained at a constant temperature of 22 C + 0.5. Gonadectomy was performed at 23 days of age with no further manipulation 1024 (except removal of wound clips) for the next 6 weeks. Control animals were sham-operated at the same age. Testosterone phenylacetate (Perandren, Ciba) was administered to gonadectomized male rats in the form of a single, sc injection of 5 mg/ lO0g body weight. Control rats were not injected, since preliminary experiments revealed no effects from a single injection of the vehicle (distilled water) alone. Determinations were made 14 days after testosterone injection. Plasma concentrations of ACTH in resting animals are at or below the lower levels of sensitivity of the assay method presently in use. Therefore, bilaterally adrenalectomized rats (absence of corticosteroid feedback raises blood ACTH to the range of assay) were used to test the effect of orchiectomy on plasma concentrations of ACTH. Depot injections of testosterone phenylacetate were given on the same day to one-half of the gonadectomized-adrenalectomized animals. Two weeks after adrenalectomy, blood was collected without stress by decapitation within 20 sec after handling. A second method of elevating blood levels of ACTH to detectable concentrations is the application of an acute stressful stimulus. This procedure tests the overall responsiveness of the hypothalamo-hypophyseal system since the magnitude of the increase in blood ACTH is a summation of any modifications in hypothalamic (CRF) and pituitary (ACTH) secretion. Rats

with intact adrenals were subjected to ether anesthesia as standardized by Wells et al. (6) . Blood was collected upon decapitation 2.5 min after the onset of anesthesia to obtain peak post-stress plasma concentrations of ACTH (7). All blood specimens were centrifuged immediately in the cold in silicone-coated glass tubes. Plasma from each experimental group of 5-6 animals was pooled, frozen at -5 C, and subsequently assayed for ACTH according to the in vivo bioassay method of Vernikos-Danellis et al. (7). Details of this method have been presented previously (1). Pituitary stalk-median eminence (SME) extracts for corticotropin-releasing activity (CRA) were prepared according to the method of Chan et al. (8). Pituitary incubation procedures and the bioassay for CRA are modifications of the original method of Saffran and Schally (1, 9). Pituitary glands from both intact and castrated male rats were also incubated with testosterone (10-6M or 10-5M) added to the incubation medium. Control incubates received equal volumes of diluent (10 ul double-distilled ethanol). In all bioassay procedures, the ACTH concentration of either the plasma or pituitary incubation media from control animals was used as the standard with an arbitrarily assigned potency of 100%. Corticotropin concentrations from both gonadectomized, and gonadectomized, testosterone-injected male rats were compared to the control animals in multiple experiments and the results are expressed as percent of control.

Results

Plasma concentrations of ACTH. Two weeks following adrenalectomy, plasma levels of ACTH were measurable in all three groups of animals. Orchiectomy doubled ($p < 0.05$) the resting levels of plasma ACTH compared to control animals (Table 1). Testosterone administration effectively lowered the plasma concentration of ACTH in gonadectomized rats so that the confidence limits overlapped with those of both the intact control and gonadectomized groups. Similar results were observed in animals subjected to the stress of ether anesthesia. Blood concentrations of ACTH were again raised to measurable levels in all treatment groups,

TABLE 1. Effect of orchiectomy and testosterone replacement on plasma concentration of ACTH measured by an in vivo bioassay procedure

Each potency is expressed as a percentage of ACTH released from pituitary glands of control rats. 95% confidence limits are indicated in parentheses. Each value is based on the pooled data from 2-4 individual experiments (pool of 4 pituitary glands/experiment; where indicated 3/5 SME added per pool of 4 pituitaries). but the level in orchiectomized animals was more than 1,½ times greater than that in control animals ($p < 0.05$). Administration of testosterone limited the response of the hypothalamo-hypophyseal system in the gonadectomized rats so that the plasma concentration of ACTH was comparable to that of control animals. When direct comparisons were made between castrated and castrated, testosterone-injected animals, a significant depressant effect of testosterone was evident in either case ($p < 0.05$ with response of castrated animals defined as 100%). Pituitary release of ACTH in vitro. Limitations of sensitivity of the bioassay procedure precluded further study of ACTH secretion in vivo. Additional experiments were then designed to extend evaluation of the effects of orchiectomy to measurement of ACTH release by incubating pituitary glands in vitro. Both intact and adrenalectomized animals were used to permit correlations with the results of the preceding experiment. Release of ACTH by unstimulated pituitary glands is not altered by orchiectomy (Table 2), nor is any effect observed after testosterone replacement in vivo. Addition of hypothalamic extract (equal to 3 / 5 SME) to the incubating glands consistently resulted in a 2- to 3-fold increase in ACTH output. Direct quantitative assays of the potency of the uniform SME used in these experiments were not made. Compared to the response of control glands stimulated with SME, the release of ACTH by pituitaries from orchiectomized rats is enhanced (162 % ; $p < 0.05$). / n vivo replacement with testosterone resulted in lowering of release toward but not to the control level. Bilateral adrenalectomy did not alter the pattern of response associated with removal of the testes (Table 2). ACTH release by

unstimulated pituitary glands from orchiectomized, adrenalectomized rats was unchanged. Again, in the presence of SME, a significant hyperresponse (134% ; $p < 0.05$) was obtained in the castrate group compared to the control.

Each potency is expressed as a percentage of ACTH released from pituitary glands receiving diluent (10 µl ethanol) added in vitro. 95% confidence limits are indicated in parentheses. Pool of 4 pituitary glands per experiment; where indicated 3/5 SME added per pool of 4 pituitaries. In an additional experiment (Table 3), testosterone was added directly in vitro to incubating pituitary glands from both intact and orchiectomized rats. The responses were variable, but in no instance was a significant change in ACTH output obtained compared to control (diluent added) performance. Hypothalamic content of corticotropin-releasing activity. To determine whether changes in hypothalamic storage of CRA might be a factor in increased secretion of ACTH in vivo after prepuberal orchiectomy, the hypothalamic content of CRA was determined. Comparable aliquots of SME extract from the three groups of experimental animals were assayed in vitro. Extracts from intact, orchiectomized (90% ; 73-112 %) and orchiectomized, testosterone-injected (114%; 62- 208%) rats contained similar quantities of corticotropin-releasing material.

Discussion

The data indicate that pituitary secretion of ACTH is increased following prepuberal orchiectomy in rats subjected to either chronic bilateral adrenalectomy or an acute stressful stimulus when compared to the response of intact controls. Moreover, testosterone administered as replacement prevents the increment in plasma ACTH levels attributable to orchiectomy. The response to testosterone is effective in adrenalectomized rats and those with adrenals intact. These results substantiate earlier studies based on measurements of pituitary ACTH content and/ or adrenal weight (3, 10) indicating that testosterone suppresses pituitary secretion of ACTH. The inhibitory effects of testosterone on ACTH secretion are opposite to the stimulatory effects of estradiol on pituitary ACTH

release reported previously (1) . Urquhart et al. (11) introduced the hypothesis that ACTH secretion is controlled by the rate of hepatic metabolism of corticosterone. Since prepuberal orchiectomy enhanced hepatic reduction of the A-ring in corticosterone (3), a secondary effect of orchiectomy would then be an increased demand for ACTH stimulation of the adrenal cortex to maintain a present level of blood corticosterone. The present data demonstrate that ACTH secretion is modified by orchiectomy in the absence of the adrenal glands. Under such conditions neither the adrenal nor the hepatic component of steroid feedback can play a role in the changes observed. Roy et al. (12) have proposed that high doses of testosterone stimulate ACTH release as indicated by increases in adrenal weight. However, it has been shown that large amounts of testosterone increase adrenal weight (13) and adrenal corticosterone production (14) directly in the absence of ACTH. In vitro experiments were used to explore a possible mechanism whereby testosterone influences the secretion of ACTH. In the unstimulated state neither orchiectomy nor replacement with testosterone alters the total release of ACTH from incubating pituitary glands. Similar results were obtained in female rats subjected to ovariectomy with or without estradiol replacement (1). However, the addition of SME extracts containing corticotropin releasing activity changes the pattern of ACTH release in vitro. The capacity of pituitary glands from orchiectomized rats to release ACTH in vitro is more than 1.5 times that of control glands. This effect parallels the difference in plasma ACTH seen in vivo in orchiectomized and control male animals subjected to a stressful stimulus. The direction of the response of the pituitary gland to secrete ACTH following gonadectomy in male animals is opposite that found in female rats. In the latter, ovariectomy produces a drop of close to SO% in ACTH release in vitro and in vivo (1). Testosterone administration in castrated male rats did not fully suppress pituitary release of ACTH in vitro in response to stimulation with hypothalamic extract. A more definitive effect may have been obtained if androgen treatment had been continued beyond the 2-week replacement time arbitrarily selected for study. The in vitro data suggest that one of the changes in pituitary function seen after prepuberal

orchiectomy is increased responsiveness to CRF stimulation. This would allow a greater release of ACTH from the pituitary gland without an obligatory increment in output of the hypothalamic releasing factor. Pituitary glands from adrenalectomized animals incubated comparably showed similar responses. Unstimulated glands from control and gonadectomized donors did not differ significantly. Upon addition of SME extract, increased ACTH was released by pituitaries from adrenalectomized-orchiectomized rats compared to adrenalectomized controls. These additional data suggest that the enhanced pituitary response to CRA shown in vitro is not secondary to changes in corticosterone feedback induced by castration in vivo. Further attempts to define the site of testosterone action on the hypothalamo-hypophyseal system were unsuccessful. Hypothalamic concentrations of CRA were similar in all three treatment groups. In addition, testosterone added directly in vitro to incubating pituitary glands did not alter the release of ACTH. This observation does not completely exclude the pituitary as a site of action since the changes produced by testosterone may not be evident in a short incubation time. The data do permit the conclusion that testosterone inhibits pituitary secretion of ACTH independently of its effects on adrenal function. Furthermore, one action of testosterone is to decrease the responsiveness of the pituitary gland to maximal CRA stimulation. The testis appears to exert complex and apparently opposing effects on various components of the hypothalamic-hypophyseal-adrenal axis, in contradistinction to the consistently stimulatory, albeit multiple, effects of estradiol replacement in female rats (1, 3). Orchiectomy enhances pituitary ACTH secretion in vivo and pituitary responsiveness to hypothalamic stimulation in vitro, as shown by the present work. However, adrenal corticosterone secretion in vivo and adrenal responsiveness to ACTH in vitro are decreased simultaneously (3). Plasma corticosterone-binding-globulin activity is increased (15), but biological half-life is shortened (3) and the rate of hepatic metabolism of corticosterone is accelerated (3). The outcome is that castration results in no net change in plasma concentrations of corticosterone (3). This applies to animals at rest, after acute stress, or after stimulation with ACTH. Therefore, if plasma concentrations of corticosterone were

taken as the indicator of activity of the pituitary-adrenal axis, the testes would seem to be of little consequence. The data presented in this report contribute to the concept that testosterone exerts complex, balanced effects on the hypothalamo-hypophyseal-adrenal system.

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