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Relation of the Thyroid Gland to the Effects of Estradiol on Adrenal Corticosterone Secretion in Rats (39483)

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Previous studies have demonstrated that estradiol affects various components of the pituitary–adrenocortical axis in rats (1). The net outcome of these actions is enhancement of adrenocortical secretion. Ovariectomy produces a fall in corticosterone secretion, an effect reversed by estradiol replacement. The decline in corticosterone output after castration is greater than can be explained by changes in plasma ACTH levels alone (2–4). The apparent discrepancy between ACTH and corticosterone output after gonadectomy is attributable to changes in the intra-adrenal metabolism or corticosteroids by castrates (4, 5). In the absence of the gonads, adrenal 5α-reductase activity increases, resulting in the intra-adrenal conversion of corticosterone (B) to 5α-dihydrocorticosterone (DHB) and 3β,5α-tetrahydrocorticosterone (THB). Secretion of these compounds accounts in part for the decline in corticosterone. Administration of estradiol to castrates lowers reductase activity and restores corticosterone secretion to control levels (4).

Labrie *et al.* (6) have proposed that the actions of estradiol on corticosterone secretion in rats are mediated by the thyroid gland. These investigators have shown that estradiol stimulates pituitary TSH secretion in rats, thereby increasing thyroid activity, which might in turn increase adrenal secretion. Previous studies had demonstrated an increase in adrenal steroid output after thyroid hormone administration. In addition, the effects of estradiol on adrenal function were found to be diminished in thyroidectomized male rats. However, indirect methods for the evaluation of adrenal secretion were employed and a physiologic role for estradiol in male animals seems unlikely. Furthermore, since estradiol acts on several components of the pituitary–adrenal regulatory system (1), it remains to be established whether its effects on adrenal secretion are fully or only in part mediated by the thyroid gland. For these reasons, further studies were conducted to determine the relation of the thyroid gland to estrogenic effects on adrenocortical secretion.

Materials and Methods. Female rats of the Sherman strain, obtained from Camm Research Institute, Wayne, New Jersey, were used in all experiments. Animals were maintained under standardized conditions of light (0600–1800) and temperature (22.0 ± 0.5°C) on diet consisting of Purina Laboratory Chow and water *ad lib.* Thyroidectomies, when indicated, were performed by the breeder on animals 25–30 days old. Ovariectomies were performed 1 week later with no further manipulation for 4 weeks. At that time a single subcutaneous injection of estradiol cyclopentyl-propionate (Estradiol Cypionate, Upjohn) 50 μg/100 g bw was administered. Four weeks later, adrenal vein blood was collected from each rat (7) following induction of anesthesia with sodium pentobarbital (4.5 mg/100 g body wt). Plasma was separated immediately and frozen for subsequent analysis. Corticosterone 1,2-^{3}H ($\sim 10^{-3}$ μCi, SA 40 Ci/mmole) was added to each sample for subsequent recovery calculations. Chloroform extracts were chromatographed on a Bush B1 system (8) and the zones corresponding to those of authentic corticosterone, 5α-dihydrocorticosterone (DHB) and 3β,5α-tetrahydrocorticosterone (THB) were eluted with methanol. Identification and homogeneity of these products has been established previously (4) by paper and thin-layer chromatography, infrared spectroscopy, and mass spectrometry. All three steroids were quantitated using a competitive protein binding technique (4, 9) with the appropriate standards. Reference steroids were obtained from Ikapharm, Ramat-Gan, Israel. The sensitivity

of the assay as employed was 0.5 ng for B, 5 ng for DHB, and 10 ng for THB. The differences in sensitivity reflect the relative affinities of each compound for canine plasma binding protein. Aliquot sizes were adjusted to accommodate the various standard curves. A portion of each corticosterone eluate was counted for ^3H content on a Packard Tri-Carb liquid scintillation spectrometer to determine steroid recoveries. The recoveries of corticosterone, DHB and THB in each sample were essentially identical and averaged about 70%. Adrenal 5α-reductase activity and corticosterone production by adrenal homogenates were assayed as previously described (5). Corticosterone was determined fluorometrically (10).

Results. As previously noted, estradiol administration to ovariectomized rats increased adrenal weight (Table I), presumably as a result of its effects on ACTH secretion (2). Removal of the thyroid gland markedly reduced adrenal weight and prevented the stimulatory response to estradiol. Corticosterone production by adrenal homogenates *in vitro* was increased equally by estradiol administration to castrated rats *in vivo* in the presence or absence of the thyroid gland. Similarly, the inhibitory effect of estradiol on adrenal 5α-reductase activity did not require the presence of the thyroid gland. Since we had previously demonstrated (4) that the effects of castration and gonadal hormone replacement on corticosterone secretion were mediated in part by changes in adrenal reductase activity, these observations suggested that the thyroid was not required for estrogenic stimulation of corticosterone output. Direct confirmation was obtained by evaluation of corticosterone and its 5α-reduced metabolites in adrenal venous blood.

Estradiol administration to ovariectomized rats increased *in vivo* corticosterone secretion either in the presence or absence of the thyroid gland (Table I). However, the increment was substantially smaller ($P < 0.01$) in thyroidectomized animals. In castrates with intact thyroids, the increase in corticosterone secretion produced by estradiol exceeded the decline in 5α-dihydrocorticosterone (DHB) and 3β,5α-tetrahydrocorticosterone (THB) output. As a result, total (corticosterone + DHB + THB) steroid secretion was significantly increased by estradiol.

In thyroidectomized castrates, on the other hand, the decline in DHB and THB secretion after estradiol treatment corresponded in magnitude to the increase in corticosterone. Thus, total (corticosterone + DHB + THB) steroid secretion, like adrenal weight, was unaffected by estradiol in

TABLE I. EFFECTS OF ESTRADIOL ADMINISTRATION ON ADRENAL REDUCTASE ACTIVITY AND STEROID SECRETION IN OVARIECTOMIZED AND THYROIDECTOMIZED FEMALE RATS.[a]

Group	Ovariectomized	Ovariectomized + estradiol	Ovariectomized + thyroidectomized	Ovariectomized + thyroidectomized + estradiol
Body weight (g)	285 ± 11	258 ± 7[b]	193 ± 13[b]	174 ± 9
Adrenal weight (mg)	72.2 ± 3.6	81.7 ± 3.0[b]	41.6 ± 4.8[b]	36.3 ± 3.6
Corticosterone production *In Vitro* (μg/100 mg/30 min)	12.6 ± 2.0	20.8 ± 2.6[b]	10.8 ± 1.2	21.8 ± 2.9[c]
Reductase activity (μg B reduced/10 mg/hr)	7.7 ± 2.0	0.4 ± 0.2[b]	8.7 ± 1.6	0.5 ± 0.2[c]
Steroid secretion *in vivo* (μg/kg/hr)				
Corticosterone (B)	72.3 ± 8.9	148.7 ± 12.9[b]	54.9 ± 6.3	86.3 ± 7.2[c]
DHB[d]	31.8 ± 3.6	12.3 ± 2.2[b]	20.1 ± 3.9[b]	4.9 ± 1.1[c]
THB[e]	21.4 ± 2.9	8.9 ± 1.9[b]	12.9 ± 2.9[b]	2.2 ± 0.7[c]
Total (B + DHB + THB)	125.6 ± 12.8	169.9 ± 13.8[b]	87.9 ± 8.9[b]	93.4 ± 8.8

[a] Values expressed as mean ± SE; 8–10 rats per group.
[b] $P < 0.05$ (vs ovariectomized group).
[c] $P < 0.05$ (vs ovariectomized + thyroidectomized group).
[d] 5α-dihydrocorticosterone.
[e] 3β, 5α-tetrahydrocorticosterone.

thyroidectomized castrates, indicating little or no change in ACTH secretion.

Discussion. Labrie, Fortier, and co-workers (6) have proposed that estradiol promotes adrenal steroid output in rats by increasing thyroid hormone secretion, which in turn increases corticosteroid binding globulin activity in plasma, thereby diminishing plasma "free" corticosteroid levels and stimulating ACTH secretion. Our studies support, only in part, the conclusions of Labrie *et al.* (6). When given to ovariectomized rats with intact thyroid glands, estradiol increases corticosterone secretion both by increasing tropic hormone (ACTH) stimulation of the adrenal cortex (2) and by decreasing the intra-adrenal conversion of corticosterone to 5α-dihydrocorticosterone (DHB) and 3β,5α-tetrahydrocorticosterone (THB) (4). The increase in plasma ACTH levels previously shown to be produced by estradiol administration to castrates (2), was manifested in the present studies as increases in adrenal size and in total secretion of corticosterone plus its principal adrenal metabolites (DHB + THB). Since neither of these changes was seen after estradiol administration to rats that were thyroidectomized as well as ovariectomized, the effects of estradiol (on adrenal secretion) resulting from changes in ACTH secretion do appear to be thyroid-mediated. However, estradiol, like testosterone (13) exerts direct effects on intra-adrenal steroid metabolism. Inhibition of adrenal 5α-reductase activity by estradiol is independent of the thyroid gland. Estradiol administration to thyroidectomized-castrated rats decreases the production and secretion of DHB and THB, thereby preserving corticosterone without affecting total steroidogenesis. Consequently, estradiol increases adrenal corticosterone secretion in ovariectomized rats even in the absence of the thyroid gland by altering the composition rather than quantity of steroid secreted. Thus, studies concerned with gonadal hormone effects on pituitary-adrenocortical function must take into account the multiple sites of action of estrogen and androgen. In addition, the data further emphasize the importance of hormonal interactions in the overall regulation of adrenal steroid secretion.

Summary. The relation of the thyroid gland to estrogenic effects on adrenal corticosterone secretion was examined in ovariectomized rats. Estradiol administration to castrates increased corticosterone output by increasing ACTH secretion and by decreasing adrenal 5α-reductase activity, diminishing the intra-adrenal conversion of corticosterone to 5α-reduced metabolites. When given to rats that were thyroidectomized as well as castrated, estradiol produced a far smaller increase in corticosterone secretion. The increment in corticosterone obtained in thyroidectomized rats was fully accounted for by the effects of estradiol on adrenal 5α-reductase activity. The results indicate that effects of estradiol on adrenal secretion resulting secondarily from changes in ACTH secretion are thyroid-dependent, whereas direct effects on intraadrenal steroid reductive pathways are not.

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1. Kitay, J. I., *In* "Functions of the Adrenal Cortex," (K. McKerns, ed.), Vol. 2. Appleton-Century-Crofts, New York (1968).
2. Coyne, M. D., and J. I. Kitay, Endocrinology **85,** 1097 (1969).
3. Kitay, J. I., M. D. Coyne, N. H. Swygert, and K. E. Gaines, Endocrinology **89,** 565 (1971).
4. Colby, H. D., and J. I. Kitay, Endocrinology **91,** 1523 (1972).
5. Kitay, J. I., M. D. Coyne, and N. H. Swygert, Endocrinology **87,** 1257 (1970).
6. Labrie, F., G. Pelletier, R. Labrie, M. A. H-Kim, A. Delgada, B. MacIntosh, and C. Fortier, Ann. d'Endocrinol. **29,** 29 (1968).
7. Lipscomb, H. S., and D. H. Nelson, Endocrinology **71,** 13, 1962.
8. Bush, I. E., *In* "The Chromatography of Steroids." Pergamon Press, New York (1961).
9. Murphy, B. E. P., J. Clin. Endocrinol. Metab. **27,** 973, 1967.
10. Guillemin, R., G. W. Clayton, H. S. Lipscomb, and J. D. Smith, J. Lab. Clin. Med. **53,** 830 (1959).
11. Fortier, C., Canad. Med. Assoc. J. **103,** 864, 1970.
12. Labrie, F., These de doctorat en Sciences, Universite Laval, Quebec, 1967.
13. Colby, H. D., and J. I. Kitay, Endocrinology **91,** 1247 (1972).

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