Inhibition of Estradiol-Induced Pituitary Hypertrophy in Rats.* (22352)

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If adult rats are treated chronically with estrogens, their pituitary glands undergo changes in morphology and activity(1-4). Several natural and synthetic estrogens produce increases in weights of hypophyses and adrenals, along with other changes. Adrenal hyperplasia is apparently related to stimulation of ACTH production. This has been shown directly by bio-assay of circulating ACTH in blood of estrogen treated rats(5) and by indirect evidence. Increase in adrenal size and adrenal ascorbic acid depletion occur following estrogen treatment of intact rats(6, 7) but not in hypophysectomized rats given estrogens(8,9). Since stimulation of ACTH production and pituitary enlargement are usual consequences of prolonged estrogen treatment it seems of interest to determine if there is a causal relationship between morphologic and physiologic effects.

Synthetic estrogen tri-*p*-anisyl chloroethylene (TACE) has been reported as of no effect on pituitary size following administration of large doses for long periods of time(10). Including this compound, we compared several estrogens for their effects on pituitary weight and ACTH secretion in rats. Certain combinations were administered with total estrogenic dose adjusted so that comparisons could be made with groups receiving equi-potent doses of a single estrogen.

Methods. Dose groups of 16 male rats of Sprague-Dawley strain, weighing approximately 160 g at start of experiment, were employed. Estradiol-17β, diethylstilbestrol, and TACE were dissolved in olive oil so that each 0.1 cc contained 10 rat units. Estradiol-17β and diethylstilbestrol were administered subcutaneously. TACE was administered orally rather than subcutaneously because its mg potency is greater by this route. Estrogens were given daily for 30 days, alone and in combination in doses indicated in Table I. When combinations of estrogens were used, each was administered at a separate site. Control animals received olive oil orally and subcutaneously in volumes equal to those employed in experimental groups. Animals were sacrificed by decapitation on day following administration of last dose, and the pituitary, adrenals, thymus, and testes removed, blotted dry, and weighed on torsion balances.

Results. Estradiol or stilbestrol administration resulted in significant increase of pituitary weights, adrenal hyperplasia, and involution of the thymus glands. No pituitary hypertrophy occurred with TACE, but the effects on adrenal and thymus sizes were comparable to those obtained with other estrogens. Simultaneous administration of TACE with estradiol prevented pituitary enlargement, obtained when the same amount of estradiol is given alone. There was no significant inhibition of the estradiol-induced hypertrophy by concurrent injection of stilbestrol. However, contrary to the additive effect one might expect, a pituitary average weight resulted, lower than obtained when only estradiol is administered. The same inhibitory effect occurred when the two estrogens used in combination were stilbestrol and TACE. Combinations of 2 estrogens were neither additive nor inhibitory in regard to adrenal and thymus weight changes. Since estrogens produce adrenal hypertrophy and thymus involution only in rats with intact hypophyses, it is assumed that these estrogen effects are indirect, depending on stimulation of ACTH release from the hypophyses. Testicular atrophy, indicative of prolonged estrogenic suppression of pituitary gonadotrophin production, was approximately the same in all groups.

Discussion. That stilbestrol causes a significant, but lesser degree of pituitary hyperplasia than estradiol, and that TACE causes no increase in pituitary weight should first be considered on the basis of comparative

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TABLE I. Average Body and Organ Weights following 30 Days Treatment with Estrogens.

Groups*	Daily dose, R.U.†	Final body wt, g	Avg pituitary wt, mg	Avg adrenal wt, mg	Avg thymus wt, mg	Avg testis wt, mg
Control		230	7.4 ± .4‡	14.9 ± .6	351 ± 20	1367 ± 40
TACE	10	190	7.3 ± .4	23.2 ± 1.2	257 ± 14	645 ± 70
	20	183	7.7 ± .1	23.8 ± 1.7	232 ± 21	784 ± 95
Stilbestrol	10	203	9.1 ± .3	28.2 ± 1.0	291 ± 19	684 ± 62
	20	198	9.1 ± .4	27.1 ± 1.2	237 ± 11	730 ± 71
Estradiol	10	203	15.6 ± 1.1	28.0 ± 1.1	247 ± 18	805 ± 79
	20	209	20.6 ± 1.2	26.9 ± 1.7	225 ± 14	841 ± 74
Estradiol + TACE	10 + 10	185	9.9 ± .6	29.2 ± 4.3	264 ± 23	642 ± 53
Estradiol + Stilbestrol	10 + 10	198	12.5 ± 1.3	34.0 ± 1.1	215 ± 15	627 ± 55
Stilbestrol + TACE	10 + 10	196	8.2 ± .8	23.3 ± 1.2	254 ± 15	788 ± 76

* 16 animals/dose group.
† 10 R.U. = Estradiol, 3.2 μg; stilbestrol, 30 μg; TACE, 210 μg.
‡ ± stand. error.

doses of each estrogen used. In the design of our experiment equi-potent doses of each estrogen were approximated using a biologic criterion of activity (the rat unit), *i.e.*, the dose necessary to cause vaginal cornification within 72 hours in 50% of a group of castrated female rats. However, even this endpoint cannot serve to equate the doses precisely since duration of effect for each estrogen differs. Estradiol and stilbestrol could be termed short-acting estrogens, since vaginal cornification following a single dose rapidly disappears. On the other hand, TACE is long-acting, maintaining vaginal cornification for several days following a single administration. In this 30-day study, a difference of this nature would tend to "favor" the short-acting estrogens because the 30 daily injections would create a series of sharp peaks and drops in circulating estrogen level, whereas a more constant estrogen stimulus to the pituitary follows similar treatment with TACE. It cannot be supposed that the constancy of estrogen levels, *per se*, is the variable factor responsible for pituitary-sparing effect, since parabiosis experiments show that endogenous estradiol stimulation from enlarged ovaries of a parabiont united to a castrate partner brings about substantial pituitary enlargement(11).

Pituitary changes following estrogen combination treatments warrant special consideration. In every case, the average pituitary weight following administration of 2 estrogens fell between the averages obtained when each substance was given individually. The most striking inhibition, therefore, occurred when TACE, which causes no pituitary enlargement, was administered concurrently with estradiol, which creates the largest pituitaries. These results suggest a competitive action between the 2 estrogens, rather than an additive one as occurs when the dose of estradiol is doubled.

In addition to pituitary hypertrophy during estradiol or stilbestrol treatment, greater ACTH activity is indicated by adrenal weight increase and thymus involution. But TACE treated animals, with normal sized pituitaries, also appear to undergo some measure of adrenal stimulation. This observation suggests that estrogen-induced pituitary hyperplasia and increase of ACTH output are not necessarily related phenomena. Substantiating this view are the data showing that even though it was possible to inhibit pituitary weight increases by combining TACE with another estrogen, no inhibition of adrenal

stimulation occurred in this situation.

Final consideration should be given to the question of non-specific stress caused by estrogen treatment. When estrogens are administered to rats, starvation is a possible evocative agent for adrenal changes(12), since lowered food intake is a characteristic occurrence. Thus, adrenal effects, symptomatic of increased ACTH output may result from estrogen stimulus directly, or may be caused indirectly by hunger situation created. In the present experiment, interpretation of results does not hinge on this matter since the problem under consideration is the relationship between estrogen-induced pituitary hypertrophy and ACTH output, regardless of whether the latter is directly or indirectly stimulated. Furthermore, major comparisons of adrenal effects are made among groups which have all been treated with estrogens so that food deprivation factor is constant. Food consumption values are approximately the same for each group. However, this factor bears on the problem of expressing data as average absolute gland weights or gland weights per unit body weight, for the final body weights of experimental groups were less than controls. In our results, the relationships described are in the same direction on either basis but since group differences are somewhat altered by different treatment of data a choice between the two may be pertinent. It is our belief that the data expressed as average gland weight express more precisely the actual trend of events since body weight loss of estrogen treated groups, particularly the TACE groups, is due mainly to loss of body fat. It presents a somewhat artificial analysis to adjust glandular weights according to depletion of body fat stores.

Summary. Equi-potent doses of estradiol caused greater pituitary enlargement in rats than stilbestrol. A third estrogen, TACE, caused no enlargement whatsoever. When two estrogens were given concurrently there was a competitive, rather than an additive, action. Hence, with each combination the average pituitary weight was between averages obtained when each substance was administered individually. With all estrogens there was evidence for ACTH-stimulated adrenal hyperactivity, determined by increase in adrenal weight and thymus gland involution. The observations that (1) TACE causes adrenal stimulation without pituitary enlargement and (2) combinations of estrogens can bring about an inhibition of the usual pituitary enlargement without a corresponding inhibition of adrenal stimulation suggest that the estrogen-induced hyper-production of ACTH does not depend upon pituitary hypertrophy.

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