Gonadotrophic Hormone Function in Persistent Estrous Rats with Hypothalamic Lesions.* (25753)

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Hypothalamic regulation of gonadotrophic hormone function in the rodent is well established but selective control over separate gonadotrophins has been difficult to assess. Destruction of the periventricular grey area and median eminence in the rat induces profound atrophy of female reproductive tract, a result which strongly suggests curtailment of both FSH and LH secretion by the adenohypophysis(1). In contrast, lesions in pre-optic and anterior hypothalamic areas produce persistent estrus, hypertrophied uteri and inhibition of corpus luteum formation in the ovary. Most experimenters consider these effects to reflect primarily a deficiency in LH secretion (2-5), with FSH-estrogen component of pituitary-ovarian interplay presumably spared. Others believe that destruction of the hypothalamic site governing reproductive rhythmicity abolishes an estrogen-sensitive area through which pituitary FSH secretion is cyclically restrained(6,7). Failure of feedback mechanism thus permits continued estrogen secretion from follicular ovaries. The present study attempts to resolve the basic problem of hormonal balance in persistent estrous lesioned rat by determining capacity of adenohypophysis to augment gonadotrophin secretion after unilateral oophorectomy. Degree of compensatory ovarian hypertrophy will be related to direct measurement of FSH in both blood and anterior pituitary.

Materials and methods. Young adult female albino rats (Wistar strain; initial weights 200-225 g) displaying 4-5 day cycles were used. Bilateral electrolytic lesions were made in pre-optic and anterior hypothalamic areas of the brain with Krieg-Johnson stereotaxic apparatus. Lesions were placed 7.0 mm anterior to ear plugs, .5 mm on either side of sagittal suture and .5-1 mm from base of skull. A direct current of 2 mA for 20 seconds was used. Lesion localization was checked on toluidine blue or chrome-alum-hematoxylin stained serial sections through the diencephalic area of formalin fixed, paraffin embedded brains. Persistent (frequent) estrus, determined from vaginal smears taken daily, was produced in approximately 75% of operated rats. Once well established (2-3 weeks after lesioning), periods of frequently occurring vaginal estrus, occasionally interspersed with 1 or 2 days diestrus, continued in some rats 4-10 months post-operatively. In the first experiment, 2 groups of estrous rats were unilaterally ovariectomized (left gonad) 25 and 60 days respectively after hypothalamic electrocautery. These rats, together with ovariectomized and intact controls, were sacrificed 19-21 days later. In the second experiment, vaginal cycles were studied for 120-300 days in a group of persistent estrous lesioned rats with gonads intact, to determine duration of effect and morphologic consequences to reproductive and endocrine systems. Just before autopsy, rats were lightly anesthetized with ether, and blood drawn by direct cardiac puncture. Sera obtained from pooled blood were frozen until time of assay. Right ovary, uterus (stripped of fluid), thyroids and adrenals were quickly dissected, weighed and prepared for histologic study. The hypophyses were rapidly removed. The anterior pituitaries, separated from posterior lobes by blunt dissection, were weighed to .1 mg on microtorsion balance and then pooled. Pituitary extracts for FSH assay were made by homogenization and extraction with acidified saline as routinely used for TSH(8). Sera and pituitary extract were assayed for FSH in 21 day old female rat using human chorionic gonadotrophin (HCG) augmentation method of Steelman and Pohley(9). The NIH-FSH-S1 preparation was used as standard.‡ Each

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TABLE I. Inhibition of Unilateral Compensatory Hypertrophy of Right Ovary in Hypothalamic Lesioned Persistent Estrous Rats.

Group	No. of rats	Mean body wt at sacrifice (g)	No. days lesioned	Mean ovarian wt (mg) Left	Right	% ovarian hypertrophy‡	Mean uterine wt (mg)
1st exp.							
Normal, intact	25	233 ± 3†	0	36.1 ± 2.4	40.4 ± 2.2	11.9 ± 5.7	491 ± 34
Normal, unilateral oophorectomy	25	250 ± 6	0	35.7 ± 1.5	57.4 ± 2.9	60.1 ± 8.0*	565 ± 41
Lesioned, unilateral oophorectomy	13	241 ± 7	44	30.2 ± 3.0	36.2 ± 4.2	19.9 ± 6.8	758 ± 46*
Idem	11	265 ± 10	81	33.3 ± 3.8	42.3 ± 5.9	27.0 ±10.0	611 ± 39*
2nd exp.							
Normal, intact	11	308 ± 12	0	42.7 ± 4.2	35.7 ± 2.8		659 ± 40
Lesioned, intact	9	312 ± 20	120–300	24.3 ± 1.9*	24.6 ± 2.0*		887 ± 63*

* Values considered significant ($P < .05$).
† Refers to mean ± stand. error of mean.
‡ Based on left ovarian wt (100%).

test animal was injected subcutaneously thrice daily for 3 days and received a total of 9 cc of serum or extract equivalent of 16 mg of anterior pituitary.

Results. Examination of the lesion in persistent estrous rats indicated a confluent area of necrosis basally placed and in the midline, extending rostro-caudally from pre-optic nuclear zone to supra-optic portion of the hypothalamus. Mid-regions of optic chiasm were frequently damaged, but supra-optic and paraventricular nuclei were usually spared. The caudal extreme of the lesion rarely impinged on the median eminence. Neighboring fiber tracts such as fornix and stria medullaris thalami were inconsistently affected. The tractus infundibularis was invariably damaged.

Three major findings are evident (Table I) namely; 1) left ovarian weight in lesion induced, persistent estrous rat remained within normal limits for at least 60 days post-operatively but eventually decreased. Histologically, the ovary appeared follicular in nature without evidence of fresh corpus luteum formation. 2) Despite eventual ovarian atrophy, significant uterine enlargement occurred in operated rats. 3) Compensatory hypertrophy of remaining gonad in unilaterally oophorectomized, lesioned animals was substantially inhibited. The degree of right ovarian hypertrophy (based on left gonad as 100%) in both groups of electro-cauterized rats was about 20-27%, values not significantly different from the normal right-left ratio (12%). Compensatory ovarian enlargement in rats not bearing hypothalamic lesions was 60%. There was no indication that lesions inducing estrus interfered with normal growth. Obesity rarely occurred. Although adenohypophyseal weight was not appreciably altered in short term operated animals, pituitary hypertrophy was found in rats still showing persistent estrus 4-10 months after hypothalamic destruction.

FSH assay of pituitary extract from unilaterally ovariectomized, lesioned rats indicated that ovarian response in test animals was slightly but consistently greater than that achieved with identical amounts of pituitary material from ovariectomized or intact controls (Table II). It thus appears that concentration of FSH in the adenohypophyses of lesioned rats following unilateral castration was at least equal to, if not greater, than in corresponding controls. Contrariwise, FSH was not detected in pooled sera from these animals but could be demonstrated in relatively high titer in blood of non-lesioned, castrates. (Detection of FSH in serum from intact control rats was questionable.) Although FSH content of blood and pituitary was not measured in persistent estrous animals with intact gonads, the results suggested that under conditions of enhanced demand, provoked by unilateral castration, FSH release from the pituitary was suppressed.

Discussion. Most investigators who have produced persistent vaginal cornification in rodents with hypothalamic lesions have also

TABLE II. FSH Assay of Adenohypophyses and Sera from Operated Rats.

Total dose FSH (μg)*	Mean ovarian wt (mg)	Donor group	Mean ovarian wt (mg) Adenohypophysis‡	Mean ovarian wt (mg) Serum‡
0	24.1 ± 1.7†	Normal, intact	36.4 ± 3.7	31.2 ± 3.7
25	30.5 ± 5.1	Normal, unilateral oophorectomy	37.6 ± 4.6	39.4 ± 3.8
100	37.9 ± 3.8	Lesioned (44 days) unilateral oophorectomy	44.3 ± 2.2	27.2 ± 3.4§
250	84.6 ± 9.6	*Idem* (81 days)	43.2 ± 5.7	

* All bioassay test rats received 20 units of human chorionic gonadotrophin (courtesy of Dr. D. McGinty, Parke, Davis & Co., Detroit, Mich.).
† Refers to mean ± stand. error of mean based on 4-5 test animals.
‡ Test animals received extract equivalent of 16 mg of pituitary or 9 cc of serum.
§ Represents response with pooled sera of both lesioned groups.

noted inhibition of corpus luteum formation in the ovary(2-6,10,11). Uterine hypertrophy can occur however without change in ovarian weight(2-5) and small ovaries may exist with or without enlarged uteri(11,10). These inconsistencies probably reflect 2 experimental variables; namely, degree to which hypothalamic lesion damages the arcuate nucleus-median eminence complex, and duration of persistent estrous condition. The high proportion of estrous animals produced was well correlated with placement of lesions in an area limited anteriorly to the pre-optic region, terminating posteriorly just short of the anterior tip of median eminence. Inasmuch as severe damage to median eminence and the associated peri-ventricular grey area usually results in profound atrophy of the reproductive tract and obesity(1,12), it is conceivable that the spectrum of results obtained, ranging from persistent vaginal cornification through varying degrees of genital tract involvement, actually reflects gradations in quantitative rather than qualitative damage to components entering in or comprising the median eminence. With time, the hormonal balance in persistent estrous rats may change so that some atrophy of the ovary co-exists with hypertrophied uteri. Change in target organ sensitivity also cannot be precluded. The precariousness of the pituitary-ovarian hormonal balance in such rats is easily demonstrated by exposing them to cold (5°C; 12-52 days) whereupon both ovary and uterus involute and the persistent vaginal cornification is replaced by anestrous condition(13).

The existence of discrete FSH and LH centers in the hypothalamus regulating cyclicity and other phases of reproductive activity does not appear to gain support from this study. The results, however do support the interpretation given by Flerko(6,7) namely that anatomic integrity of the rostral hypothalamic area in question is necessary for normal estrogen-FSH interaction. Inhibition of compensatory ovarian hypertrophy in persistent estrous rats, considered "pari passu" with bioassay data, indicates that hypothalamic lesions interfered not only with cyclic release of LH(2-5,12) but with pituitary FSH function as well. It is evident that FSH secretion, though probably constant, is not proceeding at optimal levels. The gonads are not enlarged, as would be expected if FSH secretion were maximal; moreover, compensatory hypertrophy of the gonad, a phenomenon known to require augmented FSH secretion, is inhibited. The demonstration that the FSH blood level in unilaterally castrated lesioned rats is significantly lowered is consonant with this interpretation. It cannot be conclusively stated as to whether curtailment of FSH secretion involved synthesis as well as release inasmuch as hormonal stores in pituitaries of ovariectomized, lesioned rats were at least equal to those of control glands. Estimation of both FSH and LH content in pituitaries of persistent estrous rats with intact gonads would be of great interest in view of eventual pituitary hypertrophy.

Summary. Electrolytic lesions in pre-optic and anterior hypothalamic areas of the brain induced persistent (frequent) vaginal estrus and uterine hypertrophy in rats examined 25-300 days post-operatively. Gonad weight eventually decreased. Compensatory hyper-

trophy of ovary after unilateral castration was inhibited in persistent estrous lesioned rat. FSH stores in adenohypophysis were maintained but titers in serum decreased. The results signify that adenohypophysis of persistent estrous lesioned rat loses its capacity to augment secretion of FSH under conditions of enhanced demand (unilateral oophorectomy).

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Comparison of Cholesterol and Estrogen-Induced Atherosclerosis in Cockerels.* (25754)

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Atherosclerosis can be induced in cockerels by administering estrogen(1,2) and by feeding a diet high in cholesterol(3). A comparison of results obtained by these procedures, separately and combined, has been made.

Material and methods. Method used in this laboratory for studying quantitatively the atherogenic response of cockerels to exogenous estrogen has been described(4). Cholesterol induced atherosclerosis was produced in White Leghorn cockerels, 4-5 wks old, by feeding chick growing mash containing cholesterol deposited on the diet from ethyl ether solution. Atherosclerosis was evaluated as % area of the aorta, brachiocephalic, and iliac arteries involved in plaque formation. Other procedures were similar to those described previously(4).

Results. Cholesterol-induced atherosclerosis. Graded levels of cholesterol fed to cockerels for 8 wks resulted in progressively larger values for degree of atherosclerosis, serum cholesterol, and phospholipid (Table I). Feeding .5% cholesterol diet longer than 8 wks did not elevate these values (Table II). Increasing dietary cholesterol to 1% resulted in lower serum cholesterol and phospholipid values in 16 and 20 wks than either 8 or 12 wks. Degree of atherosclerosis reached a peak at 12 wks which was twice as great as either dietary cholesterol level in 8 wks. Although increasing the amount of dietary cholesterol to 1% resulted in elevated serum cholesterol and phospholipid values, sensitivity to atherosclerotic vascular involvement was decreased. These data indicate that feeding young cockerels a .5% cholesterol diet for 8 wks is optimal for inducing hypercholesterolemia and plaque formation in the major arteries. This method for producing cholesterol-induced atherosclerosis in cockerels has been adopted for studying the disease in this laboratory.

Cholesterol-estrogen induced atherosclerosis. Comparison of results obtained with cholesterol and estrogen as atherogenic agents, separately and combined, during 8 wks was made (Table III). Although serum cholesterol, phospholipid, and polysaccharides in-

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