Effects of Chronic Administration of Sheep Stalk-Median Eminence Extract on Organ Weights of Immature Hypophysectomized Rats with Pituitary Grafts (35118)

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The release of anterior pituitary hormones is under hypothalamic control by means of specific releasing and inhibiting factors secreted into the hypophyseal portal system of veins (1). The hypothalamic influence is stimulatory except in the case of prolactin, the secretion of which is tonically inhibited by the hypothalamus. When the pituitary gland is transplanted to an ectopic site its function is seriously impaired except for prolactin secretion which continues and may even be enhanced.

The residual function of the ectopic pituitary appears to be, at least in part, dependent on circulating releasing factors which reach the transplant via the systemic circulation. The evidence for this statement is the decreased function of these grafts in hypophysectomized grafted rats bearing median eminence lesions (2) and the finding of releasing factors in peripheral blood of hypophysectomized animals (1).

Evidence for the existence of hypothalamic releasing factors has come mainly from acute experiments in which injection of hypothalamic extract produced a rapid increase in release of pituitary hormones (1). Evans and Nikitovitch-Winer (3) and Zanisi *et al.* (4) have reported that chronic administration of median eminence extract can reactivate secretion by pituitary grafts beneath the kidney capsule of immature female rats either when administered directly into the renal artery or when injected intraperitoneally. The present results indicate that these grafts can be activated in immature rats of either sex by intraperitoneal injection of sheep stalk-median eminence extracts. The effects in females were more pronounced than those in males.

Materials and Methods. Male and female Sprague-Dawley rats (Hormone Assay Lab, Chicago) were hypophysectomized (parapharyngeal approach) at 21 days of age. A single whole pituitary gland from a donor of the same age, sex, and strain was implanted beneath the right renal capsule while the recipient was anesthetized with ether at 23 days. The pituitaries were collected immediately prior to implantation and temporarily placed in tissue culture medium 199 (Difco). Body weight and nose–tail length of the recipients were recorded at this time.

All animals were housed in large shoebox cages, five rats/cage, maintained on a diet of beef heart and laboratory chow *ad libitum*, given free access to 5% glucose as well as water, and subjected to 12 hr alternating light–dark cycles.

Preparation of extracts. Crude ovine stalk-median eminence (SME) and cerebral cortical extract was used. An acetone powder prepared from the frozen tissue was extracted with glacial acetic acid and lyophilized as previously described (5). These powdered extracts were reconstituted to 9 mg/ml with acetic acid to a final molarity of 0.02 *M*, pH 5.5–6.0.

Protocol. Three daily ip injections of 3 mg of the respective crude extract (total 9 mg/day of 9 mg/ml extract) were given to all animals beginning on day 28 and continuing for 14 days at which time all animals were sacrificed. The following organs were freshly dissected and weighed in males: testes, seminal vesicles, both with and after expression of their secretions, ventral prostate,

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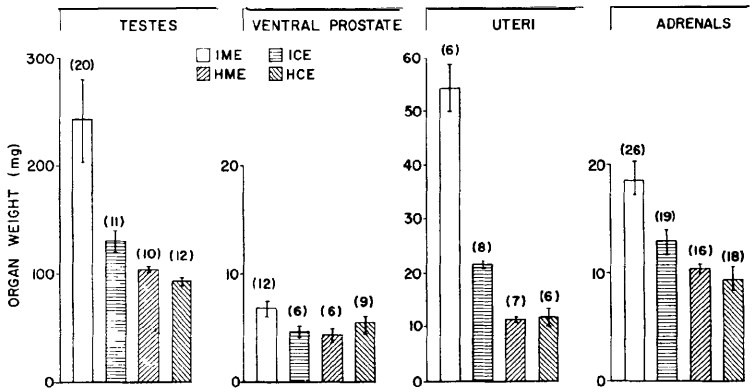

FIG. 1. Organ weights in hypophysectomized and hypophysectomized, grafted rats which were injected with stalk-median eminence (SME) or cerebral cortical extracts. Key to symbols: IME = hypophysectomized, implanted rats receiving SME extract; HME = hypophysectomized receiving SME extract; ICE = hypophysectomized, implanted receiving cerebral cortical extract; HCE = hypophysectomized receiving cortical extract. Vertical bars represent SEM; numbers in parentheses refer to the number of rats per group.

adrenal, and thyroid. In addition, nose–tail length and body weight were recorded. Histological sections of the testes were stained with hematoxylin and eosin. The following organs were weighed in females: ovaries, uterus, adrenal, and thyroid. Histological sections were made of the ovaries.

Completeness of hypophysectomy was assessed as previously described (2).

Four groups of both male and female rats were utilized: hypophysectomized + 1 pituitary implant + SME extract (IME); hypophysectomized + 1 pituitary implant + cerebral cortical extract (ICE); hypophysectomized + SME extract (HME); hypophysectomized + cerebral cortical extract (HCE).

All data were subjected to analysis of variance for two-way factorial design.

Results. Male organs. Animals with implants receiving crude hypothalamic extract (IME) had larger testes ($p < .05$) (Fig. 1) and ventral prostates ($p < .05$) (Fig. 1) than either those with implants receiving crude cerebral cortical extracts (ICE) or hypophysectomized animals receiving either extract (HME and HCE) ($p < .01$). Seminal vesicle size was not significantly altered (Table 1). Organ weights of hypophysectomized rats injected with either SME (HME) or cortical extract (HCE) were similar and atrophic.

Implantation alone (ICE) supported testes weights intermediate ($p < .01$) (Fig. 1) between those of hypophysectomized (HME and HCE) and implanted animals receiving median eminence extract (IME). All organ weights were considerably below those of intact rats. Histological inspection of various organs of the IME groups revealed well developed seminiferous tubules with germinal epithelium demonstrating progression to spermatids. Leydig cells in this group were more numerous than those present in the other groups. There were frequent mitotic figures. The ICE group had seminiferous tubules only one-half the diameter of the above group with fewer Leydig cells and mitotic figures, and with poor progression to spermatids. Both groups without implants (HCE and HME) had atrophic seminiferous tubules and only a few atrophic Leydig cells. There was only minimal germinal cell progression in these groups (HME and HCE).

Female organs. Female animals with implants receiving SME extract (IME) had larger uterine weights ($p < .01$) (Fig. 1) than either implanted animals receiving cerebral cortical extract (ICE) or hypophysectomized animals receiving either extract (HME and HCE). Simple implantation (ICE) maintained uterine weights above hypophysectomy levels ($p < .01$) (Fig. 1). Ovarian weights were larger ($p < .05$) (Table I) in the im-

TABLE I. Effect of Median Eminence Extract on Organ Weights and Growth in Hypophysectomized Rats with Pituitary Homografts.

Organ	IME	ICE	HME	HCE
Body wt (g)	82.0 ± 3.0 (20)[a]	81.0 ± 1.6 (11)	64.0 ± 2.2 (9)	62.0 ± 1.0 (12)
Seminal vesicles with secretions (mg)	8.35 ± 0.45 (20)	6.47 ± 0.67 (12)	7.26 ± 0.78 (11)	6.73 ± 0.76 (13)
Seminal vesicles without secretions (mg)	6.90 ± 0.54 (19)	5.60 ± 0.42 (11)	5.72 ± 1.33 (9)	5.50 ± 0.64 (12)
Thyroid (mg)	6.9 ± 0.8 (18)	4.3 ± 2.0 (14)	3.9 ± 0.22 (15)	4.6 ± 0.4 (14)
Nose–tail length (cm)	28.0 ± 0.3 (26)	27.9 ± 0.3 (19)	25.1 ± 0.4 (16)	24.5 ± 0.5 (18)
Ovaries (mg)	7.2 ± 1.5 (6)	3.2 ± 0.4 (8)	2.4 ± 0.2 (7)	3.6 ± 0.3 (6)

[a] Mean ± standard error (no. of animals/group).

planted group receiving hypothalamic extract (IME) than in the other groups (ICE, HME, and HCE). Organ weights were similar in hypophysectomized groups injected with either SME or cortical extract (HME and HCE).

Histological inspection of the ovaries of the IME group revealed multiple secondary follicles containing antra with three to four layers of granulosa cells. By contrast there was little evidence of follicular development in the ICE group, and the hypophysectomized groups receiving either extract (HME and HCE) had multiple primary but few secondary follicles.

Body weight and nose–tail length. Body weight ($p < .01$) and nose–tail length ($p < .01$) were increased by grafting (Table I). SME extract failed to increase body weight or nose–tail length above that seen in cortex-injected groups whether the animals were grafted or not. There was no difference between males and females in this respect; therefore these data are presented collectively.

Adrenals. Adrenal weight was greater ($p<0.01$), (Fig. 1) in the implanted group receiving SME extract (IME) than in all other groups (ICE, HME, HCE) for both males and females. In addition, implantation alone (ICE) supported adrenal weight above hypophysectomized levels ($p<.01$) (Fig. 1). SME extract failed to alter adrenal weight in hypophysectomized rats on comparison to the cortex-injected controls. As there was no difference between male and female animals, these data were also pooled.

Thyroid. Thyroid weight was not significantly different between the implanted groups (IME and ICE); and implantation alone (ICE) did not increase thyroid weights above hypophysectomy levels (Table 1) in either males or females.

Completeness of hypophysectomy. The completeness of hypophysectomy was assessed as previously described (2) and no pituitary tissue was observed in the pituitary capsule of any test animal.

Discussion. As observed previously by others pituitary function was seriously curtailed in hypophysectomized rats with pituitary grafts under the kidney capsule (6). In the males there was slight maintenance of testicular weight above the value observed in hypophysectomized controls. In females uterine weight was slightly elevated. The grafts produced some stimulation of growth as evidenced by increased body weight and length of the rats of both sexes and they also increased adrenal weight slightly. They had no other effects on organ weights measured.

The hypothalamic extract administered appeared to be free of significant contamination with anterior pituitary hormones since it failed to alter organ weights of the hypophysectomized rats. In hypophysectomized males with grafts the SME extract produced significant stimulation of testicular and prostatic weight. Although testicular weight doubled, it still was far below adult levels. In the testes there was histological evidence of tubular growth and of stimulation of early stages of spermatogenesis. The effect on prostate weight was quite modest and there was no significant stimulation of seminal vesicles.

In females the most striking effect of hypothalamic extract was on uterine weight which more than doubled. Although evidence of follicular development was observed histologically, there was only a slight increase in ovarian weight. Vaginal opening did not occur. Both males and females exhibited adrenal enlargement but there were no significant effects on thyroid weight or bodily growth.

The data thus indicate that the hypothalamic extract significantly stimulated gonadotropin secretion. Presumably both FSH and LH were released since spermatogenesis and follicular development were activated indicative of the presence of FSH; and prostatic and uterine weight increased indicative of gonadal hormone release induced by (ICSH) LH. Thus, the hypothalamic extract must have contained both FSH-releasing factor (FRF) and LH-releasing factor (LRF). Similar extracts are active both *in vivo* and *in vitro* to release FSH and LH in acute experiments (1). Since adrenal weight increased in both males and females, ACTH was released and the extract presumably contained corticotropin-releasing factor. At the dose injected there was no evidence for the presence of thyrotropin-releasing factor or growth hormone-releasing factor in the extract since there was no increase in thyroid weight or stimulation of growth.

Since these effects were obtained after injection of extract for a 2-week period, it is almost inconceivable that they could have been caused by release of preformed stores of hormones from the grafts. Rather, *de novo* synthesis of tropins and subsequent release must have taken place. It would appear that the hypothalamic-releasing factors must promote not only release but also synthesis of pituitary hormones. Whether the effects on synthesis are primary or secondary to release cannot be stated from these results (1).

Our results are in agreement with those obtained by Evans and Nikitovitch-Winer (3) and Zanisi *et al.* (4) and indicate, in addition, that chronic injection of hypothalamic extract can activate secretion from male as well as female pituitaries. The effects observed by Zanisi *et al.* (4) with ip injection of SME extract were more marked than those observed in the present study in that vaginal opening occurred and there was a larger increase in organ weights. These differences may have been caused by the use of a lower effective dose of hypothalamic extract in the present work.

Summary. Single pituitary grafts from donors of the same sex maintained some growth and led to slight maintenance of testes in male and uteri in female hypophysectomized rats. There was slight maintenance of adrenal weight in both sexes. Injection of crude sheep stalk-median eminence (SME) extracts resulted in a further significant increase in testes and ventral prostate weights in hypophysectomized, grafted males and in uterine and ovarian weights in grafted females on comparison to the weights of these organs in grafted animals injected with cerebral cortical extract. Adrenal weight was increased in rats of both sexes by SME extract. The results provide evidence that chronic injection of hypothalamic extract can increase the weight of pituitary target organs in both male and female rats presumably via stimulation of both release and synthesis of pituitary hormones.

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