Effect of Hypothalamic Extract on Hypophyseal LH in Immature Male Rats.* (29523)

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Control of anterior pituitary tropic hormones by factors extractable from hypothalamic or neurohypophyseal tissues is now well established. Release of hypophyseal LH following administration of such extracts has been reported for females of several species (1,2,3). The reaction, which is an acute release of considerable quantities of LH, proceeds even though the hypothalamus of the test animal is destroyed by lesions(4) or suppressed via administration of steroids (estrogen or estrogen plus progesterone)(1,5).

The experiments reported here attempt to evaluate the role of hypothalamic gonadotropin controlling factors in production or release of LH in chronically treated intact immature male rats. The action of these extracts was also tested in animals simultaneously receiving steroids. The results indicate that ovine hypothalamic extract does not release LH from the pituitary in amounts detectable via androgen production in the testes, unless exogenous steroid is also administered.

Materials and methods. Male rats (Holtzman strain) maintained in air-conditioned, light controlled (12-12 hr) quarters with free access to food and water were used. Normal males, 30 days of age, received 0.1 ml hypothalamic extract (equal to one hypothalamus), saline, or 20 mU vasopressin (Parke-Davis Pitressin) via the tail vein, under light ether anesthesia, twice a day for 7 days. In some groups, steroids (estradiol 17β 0.1 μg plus 1 mg progesterone; or testosterone propionate 100 μg) dissolved in 0.25 ml sesame oil were also given, subcutaneously once a day for 7 days. Autopsy was performed 24 hours after the last injection and organ weights obtained. Hypophyses were crushed in saline (0.5 ml/gland) and stored frozen until assayed.

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The hypothalamic material (HE) was prepared by fractional acetone precipitations of buffered acetate extracts of sheep hypothalamic tissue(3). The final product, which has been shown to be free of LH activity, but will cause the release of LH in properly prepared immature and mature female rats, and female weaver finches, was dissolved in physiological saline.

Pituitaries were assayed by the ovarian ascorbic acid depletion (OAAD) method of Parlow(6). The gonadotropin pre-treated Holtzman female rats were given one pituitary in 0.5 ml saline intravenously over a period of about 30 seconds. Four hours later, one ovary was removed and the quantity of ascorbic acid determined. The results were expressed in terms of the percent ascorbic acid depleted compared with saline injected controls. Control groups were given NIH-LH† also, but absolute equivalence to sheep LH was not determined.

Results. Organ and body weights of males receiving various treatments are given in Table I. Only males treated with 20 mU vasopressin showed a significant change in body weight. Administration of steroid resulted in a decline in testicular weight, testosterone propionate (TP) being more effective in this action than the estradiol plus progesterone (EP) combination. Hypothalamic extract (HE) intravenously twice a day was able to prevent the testicular weight loss associated with EP, but, at least with the dose used, could only partially overcome the inhibition produced by TP, and the testes were significantly smaller than controls in this group (Group VI).

Ventral prostates were not increased in size following treatment with either HE, vasopressin or EP alone. In combination

† LH was the gift of the Endocrinology Study Section.

however, HE plus EP produced a very significant enlargement (70.4%—Group IV). As expected, androgen stimulated prostatic growth (101% above oil controls) and combination with HE brought about a further increase of 25.4% (Group VI). Group VIII serves as a control for comparing the action of vasopressin plus oxytocin (Parke-Davis Pitocin) in the EP treated male. This group actually is made up of 4 sub-groups, each of which received EP plus vasopressin and oxytocin. The V/O ratios, in mU, used were: 10/10; 10/2; 10/1; 5/50. Body and organ weights were identical in all groups and therefore were combined and given as a single group for Table I. Prostates in these animals were significantly smaller than controls, which is in contrast to the HE + EP

TABLE I. Organ Weights of Males Treated with Hypothalamic Extract and Steroids.

Group No.	Treatment	No. of animals	Body wt (g)	Organ wt (mg/100 g body wt)			Ascorbic acid depletion (%)	No. of assay females
				Testes	Ventral prostate	Hypophysis		
I	Saline + oil	27	121 ± 1.7	1028 ± 40‡	52.7 ± 1.7	4.17 ± .07 (5.08 ± .09) *	30.1 ± 1.4	11
II	Hypothalamic extract (HE)	19	116 ± 3.2	1078 ± 27	53.1 ± 3.1	4.35 ± .09 (5.05 ± .14)	35.9 ± 1.3 P <.01	10
III	Estradiol + progest. (EP)	15	116 ± 2.6	814 ± 46 P <.01	52.4 ± 3.1	4.59 ± .12 P <.01 (5.35 ± .19) P <.05	34.2 ± 1.3 P <.01	10
IV	HE + EP	6	115 ± 2.1	972 ± 55	89.8 ± 7.2 P <.01	4.77 ± .11 P <.01 (5.48 ± .16) P <.05	8.9 ± 2.2 P <.01	5
V	Test. prop. (TP)	12	130 ±11.6	649 ± 36 P <.01	106 ± 6.3	3.61 ± .11 P <.01 (4.65 ± .15) P <.02	33.8 ± 1.8	6
VI	TP + HE	11	117 ± 3.6	867 ± 32 P <.01	133 ± 8.1 P <.02†	4.04 ± .08 (4.73 ± .14) P <.05	22.1 ± 1.6 P <.01	9
VII	Vasopressin, 20 mU	5	105 ± 4.6 P <.01	978 ± 109	48.9 ± 9.6	4.29 ± .21 (4.49 ± .21) P <.02	34.3 ± 2.3	5
VIII	Vasopressin + oxytocin + EP	27	128 ± 2.0	825 ± 39 P <.01	44.0 ± 1.6 P <.01	4.86 ± .08 P <.01 (6.21 ± .13) P <.01	34.0 ± 2.5	5

* Actual weight of pituitary. P values compared with saline controls. 8 μg NIH-LH- 46.9 ± 1.5% depletion in 27 animals.

† P value compared with TP alone. ‡ ± S.E.

males, and indicates that these 2 octapeptides cannot duplicate the action of the extract in steroid treated animals.

The statistical significance of the weight changes, both actual and relative, of the hypophyses in the various groups is indicated in Table I. EP alone and in combination with HE, or vasopressin + oxytocin brought about an increase in pituitary weight. In contrast, TP treatment resulted in reduced pituitary size, which was counteracted by simultaneous administration of HE.

Relative potency of the hypophyses in stored LH is indicated by the percent ovarian ascorbic acid depleted in an assay female following administration of the equivalent of one gland. Males treated with HE or EP alone have more hypophyseal LH (Groups II and III) than saline injected controls. When these two treatments are combined however, the opposite effect is seen, and pituitary stores of LH are greatly depleted (72% reduction). The reduction is even more pronounced when considering that the HE + EP animals have larger pituitaries than the saline controls.

Increase in pituitary LH in animals receiving 100 μg TP per day is not significant. However, as with EP, the extract caused a significant drop in LH in the presence of exogenous androgen.

Various doses of vasopressin + oxytocin were ineffective in causing a fall in LH potency of the pituitary. The amount of this gonadotropin in pituitaries of the different sub-groups of Group VIII varied and the value listed in Table I is the least amount of ascorbic acid depleted by a pituitary from the treated animals (vasopressin 10 mU + oxytocin 1 mU). The hypophyses of the animals in the other sub-groups were richer in LH, and a complete study of the effects of these neurohormones on both LH and FSH will be reported elsewhere. In all animals, however, LH release, and concomitant prostate enlargement, were not found, indicating a fundamental difference in the activities of HE and the posterior lobe hormones.

Discussion. Previous studies(7) have shown that in males of this strain, ventral prostates (mg/100 g body weight) do not change in weight from the 30th to the 45th day of age, suggesting that the pituitary is relatively inactive in releasing LH during this period. In contrast, during the next 15 days, (45-60th days) the sex accessories undergo a very active growth phase and more than double in size. Repeated administration of sheep hypothalamic extract during the stable period (30-37 days) does not result in an increase in prostate weight. Therefore, the extract does not cause release of LH in quantities sufficient to raise significantly the circulating level of androgen from the testes. The amount of LH in the hypophysis does increase, however, suggesting a stimulus for synthesis by the extract. Treatment with estradiol plus progesterone for 7 days also causes an increase in pituitary LH, but this can be explained on the basis of an inhibited release without a concomitant inhibition of synthesis(8).

In distinct contrast to its effects alone, the extract causes a phenomenal depletion of hypophyseal LH when given to males simultaneously receiving estradiol plus progesterone. The concept of a synergistic action of estradiol and progesterone in bringing about LH release, particularly at the time of ovulation in females, has received considerable attention(9), and is supported by the present results. Also, in this connection, Ramirez and McCann(5) reported that ovariectomized females, in which LH release was blocked by a large dose of estradiol plus progesterone, are extremely sensitive to the LH releasing action of rat median eminence extract. Here again, the steroids may be facilitating the action of the extract. Testosterone propionate also enhanced LH release by the extract in males, and decreased the quantity in the pituitary, but this steroid appears to be much less effective than EP in this facilitatory action.

The amount of LH released can be accounted for by the androgen produced and consequently by the prostatic enlargement, which is an important point in considering the LH releasing action of the hypothalamic extract. In Group IV, for example, which

received extract and EP, pituitary LH was reduced and prostates increased by 70.4%, while Group VI, treated with androgen and extract, had prostates enlarged by 25.4% and pituitary LH down by 26.6%. On the other hand, various combinations of vasopressin and oxytocin, even in EP treated males, could not cause pituitary LH output.

Recently, Igarashi and McCann(10) reported the release of hypophyseal FSH in female rats following administration of extracts of median eminence tissue. Assays for pituitary FSH were not performed in the present study, but the results suggest that this gonadotropin may be involved in the groups receiving steroid. The extract prevented the testicular weight reduction associated with EP, and prevented a great deal of the reduction accompanying the androgen treatment. Whether this involves the release of FSH by the extract cannot now be determined, but the possibility that the weight reduction seen in the testes is due to inhibition of only LH cannot be overlooked, particularly since testosterone propionate does not influence the output of FSH from the pituitary in castrate males(11).

In conclusion, the present study emphasizes the necessity of considering the steroid endocrine status of the animal in evaluating the effects of hypothalamic gonadotropin controlling factors. Certainly the results indicate that the quantitative and perhaps the qualitative actions of the extracts are altered by the presence of various steroids.

Summary. Immature male rats were given, intravenously twice a day, either sheep hypothalamic extract (HE), saline, vasopressin, or various combinations of vasopressin and oxytocin, *or* these materials plus testosterone propionate (TP) 100 μg or estradiol 0.1 μg plus progesterone 1 mg (EP) once a day, for 7 days. At autopsy, organ weights were obtained and pituitaries subjected to assay for LH potency.

HE alone did not cause a depletion in hypophyseal LH or any change in testicular or sex accessory weights. In combination with EP, and to a lesser degree with TP, however, HE caused a fall in pituitary LH potency and a concomitant and proportional rise in ventral prostate weight, via testicular androgen production. Vasopressin alone and vasopressin plus oxytocin with EP, were ineffective in causing release of LH.

The testicular weight reduction associated with EP treatment could be completely inhibited by the extract, but the reduction found with TP treatment could only partially be prevented by HE.

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