Evidence for Separate Corticotrophin- and Luteinizing Hormone-Releasing Factors in Hypothalamic Extracts. (29601)

S. M. McCann,* A. V. Schally,† R. Nallar‡ and C. Y. Bowers

Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia; and Veterans Administration Hospital and Dept. of Medicine, Tulane University School of Medicine, New Orleans, La.

The hypothalamus exercises an important regulatory influence over the secretion of both adrenocorticotrophin (ACTH) and luteinizing hormone (LH) since increased release of these two trophic hormones is induced by appropriate stimulation of the hypothalamus, whereas their secretion can be markedly reduced by hypothalamic lesions (1). This hypothalamic influence on anterior pituitary function appears to be mediated by means of neurohumoral transmitters released into the hypophyseal portal system, for crude or purified extracts from the stalk-median eminence (SME) region can augment release of LH(2-6) and ACTH(7-10). It appears likely that separate LH-releasing and corticotrophin-releasing factors (CRF's) are secreted into the portal vessels to trigger release of the appropriate hormone, because the physiological conditions which govern the secretion of ACTH are different from those that affect LH secretion. For example, alterations in the titer of circulating corticoids have little or no influence on LH secretion in ovariectomized rats but markedly affect the secretion of ACTH(11). Similarly, the ovulatory surge of LH secretion is not accompanied by a detectable increase in ACTH secretion(12). In the following paper evidence is presented which indicates that the LH-releasing factor (LH-RF) can be separated from CRF and vice versa.

Methods. Preparation of extracts. Beef or sheep SME tissue was collected from freshly killed animals at the slaughter house and immediately frozen. After preliminary extraction and treatment, the crude extract was subjected to gel filtration on Sephadex by methods which have been previously described(13,14). Preparations Mc-1 and -2 (bovine) with LH-releasing activity were prepared in Philadelphia(14). Preparations CY-2-B-6 (ovine) with LH-releasing activity and fraction AVS-32-19 #60 (bovine) with ACTH-releasing activity were prepared in New Orleans(15).

Bioassays. LH-release was evaluated by the percentage ovarian ascorbic acid depletion (OAAD) evoked by intravenous (i.v.) administration of the fraction into immature rats which had been pretreated with gonadotrophins(2). The increment in plasma LH activity was also estimated by i.v. injection of several extracts into ovariectomized, estrogen progesterone-blocked rats(16). The increment was the difference between the OAAD evoked by plasma from control rats and that evoked by plasma from the experimentals.

ACTH release was evaluated in several test systems. The increment in plasma free corticosterone concentration which followed i.v. injection of extract was measured in Nembutal-morphine-treated rats(17), and, or in Dexamethasone-treated rats, which were also anesthetized with Nembutal(18). In addition, the adrenal ascorbic acid depletion (AAAD) from i.v. injection of the extracts was determined in rats with effective median eminence lesions(10). Possible ACTH-like activity of the extracts was measured by their effect on plasma corticosterone of the hypophysectomized rat(19). Pressor activity was determined by the method of Dekanski(20).

Results. Fractions with LH-releasing but no corticotrophin-releasing activity. Three fractions were tested which had significant LH-releasing activity (Table I). Two of these (McC-1 and CY-2-B-6) were also

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TABLE I. Effects of LH-RF Fractions on ACTH and LH Release.

Preparation	Type of assay	Dose	Response	P
	Assays for ACTH release			
MeC-1	M.E. lesions	.2 ml	−37 (3) *†	n.s.‡
"	" "	1.0 "	−13 ± 15 (4)	n.s.
MeC-2	" "	.1 "	− 4 ± 16 (6)	n.s.
"	" "	.3 "	35 ± 27 (5)	n.s.
CY-2-B-6	" "	500 μg	−16 ± 11 (5)	n.s.
"	" "	1000 "	− 6 (2)	n.s.
"	Dexamethasone-blocked	500 "	.6 ± .6§	n.s.
	Assays for LH release			
MeC-1	OAAD	.2 ml	12.4 ± 3 (8)‖	<.01
MeC-2	"	.1 "	12.0 ± 2 (11)	<.01
CY-2-B-6	"	100 μg	7.4 ± 2 (4)	>.1
"	"	500 "	16.2 ± 3 (4)	<.01

* Mean ± S.E.M. (No. of test rats). † mg % Adrenal ascorbic acid depletion. ‡ Not significant. § Increment in plasma corticosterone (μg %). ‖ Ovarian ascorbic acid depletion (OAAD).

evaluated in the ovariectomized, estrogen progesterone-blocked rat. McC-1 gave increments of plasma LH activity of 6.0, 8.5, 14.2, and 11.2% as determined by OAAD, which were highly significant ($P<0.001$). CY-2-B-6 also produced highly significant increments in plasma LH activity of 8.2 ± 1.4 and 13.7 ± 3.4% ($P<0.01$ in both instances) at a 100 μg dose.

All 3 of these preparations of LH-RF were devoid of significant corticotrophin-releasing activity at doses which were active in releasing LH. The doses of McC-2, McC-1 and CY-2-B-6 were increased by factors of 3, 5 and 5, respectively, without exhibiting significant CRF activity (Table I).

Pressor contamination of each of the samples was too little to have any effect on ovarian ascorbic acid. It ranged from 10 to 30 mU of vasopressin at the doses which were active in releasing LH.

A fraction with CRF activity and little or no LH-releasing activity. Fraction AVS-32-19 #60 exhibited significant ACTH-releasing activity in all 3 test systems used (Table II). It had no detectable ACTH-like activity when tested in hypophysectomized rats at a dose of 750 μg (*i.e.*, < 0.3 mU of ACTH). The dose injected into rats with lesions was 5 times higher than that used in the other tests, but even if this meant that 1.5 mU of ACTH was injected, this quantity would still be insufficient to account for the AAAD observed. Previous work has repeatedly shown that these rats with lesions are relatively insensitive to ACTH under the conditions employed here(10, unpublished observations). Approximately 6 mU of ACTH are required to produce the AAAD observed. AVS-32-19 #60 contained 14 mU of pressor activity at the 750 μg dose. This quantity of vasopressin is too little to account for the observed

TABLE II. Effects of CRF Fraction AVS-32-19 #60 on ACTH and LH Release.

Type of assay	Dose (μg)	Assay No.	Response	P
	Assays for ACTH release			
Nembutal-morphine	750	1	14.9 ± 4.8*†	<.05
" "	750	2	6.2 ± 2.2	<.05
" "	750	3	4.7 ± 2.2	<.1
Dexamethasone-blocked	750	1	3.8 ± .8	<.01
M.E. lesions	3,750	1	92 ±22‡ (5)	<.02
	Assays for LH release			
OAAD	750	1	5 ± 2.5 (4)§	n.s.‖
"	3,750	1	6 (3)	n.s.

* Increment in plasma corticosterone (μg %). † Mean ± S.E.M. (No. of test animals). ‡ mg % Adrenal ascorbic acid depletion (AAAD). § % Ovarian ascorbic acid depletion (OAAD). ‖ Not significant.

effects in any of the ACTH-releasing tests employed here. This fraction had little or no effect on ovarian ascorbic acid at either the 750 or 3750 μg doses.

Discussion. Crude acidic extracts of rat or beef SME are equipotent in their ability to release ACTH (AAAD in rats with lesions) and LH (OAAD in immature rats pretreated with gonadotrophins)(2,10,21). By this we mean that the minimal effective doses (MED) for LH and ACTH release were the same. The partially purified extracts tested here by contrast gave selective effects on the release of ACTH and LH. Several fractions were capable of releasing LH but were devoid of CRF activity even when the dose was increased by a factor of 3- to 5-fold. One fraction was examined which had CRF activity by all criteria used, yet it was inactive in releasing LH. All preparations tested contained too little pressor activity to account for the results obtained. No detectable ACTH was found in the preparation of CRF. Preparations of LH-RF were free of LH since they emerged from the Sephadex column in the molecular size range of 1000-1500. Furthermore, since these fractions were also active in the ovariectomized, estrogen progesterone-blocked rat where dilution of any administered LH would certainly lower its concentration below the detectable range(21), the results reported here represent LH release rather than contamination of the extract with LH.

Thus it appears that LH-RF and CRF represent separate chemical entities. Presumably, each is liberated into the hypophyseal portal vessels in response to specific stimuli to release the appropriate adenohypophyseal hormone.

Summary. 1. Several fractions obtained after gel filtration of beef (and sheep) stalk-median eminence tissue were active in releasing LH as judged by their ability to evoke OAAD in immature rats pretreated with gonadotrophins and to increase plasma LH in ovariectomized, estrogen progesterone-blocked rats. These same fractions failed to release ACTH even when the dose of extract was increased by a factor of five. 2. A fraction from beef stalk-median eminence was active in releasing ACTH as judged by its ability to increase plasma free corticosteroids in both Nembutal-morphine and Dexamethasone-blocked rats. The fraction also evoked AAAD in rats with effective hypothalamic lesions. This extract had little or no LH-releasing activity in immature rats pretreated with gonadotrophins. 3. Since equivalent doses of crude hypothalamic extracts are equally active in releasing ACTH and LH, whereas the purified fractions tested here gave selective release of either LH or ACTH, it is concluded that the LH-RF and CRF are distinct entities.

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Preparation of Human Chorionic "Growth Hormone-Prolactin."* (29602)

HERMAN COHEN, MELVIN M. GRUMBACH,† AND SELNA L. KAPLAN‡

Department of Pediatrics, Columbia University, College of Physicians and Surgeons and Babies Hospital, Presbyterian Hospital, New York City, and Princeton Laboratories, Princeton, N. J.

Evidence that the human and simian placenta secretes a hormone with growth hormone-like and prolactin-like activity, which differs in its immuno-chemical, physicochemical and biologic properties from pituitary growth hormone, has been reported from this laboratory(1,2). It was suggested that this hormone, which appears to be similar, if not identical, to human "placental lactogen" isolated by Josimovich and MacLaren (3,4) is an important metabolic hormone of pregnancy(1). In view of uncertainties concerning its major actions, the tentative designation chorionic "growth hormone-prolactin" (CGP) has been applied(1). The human placental hormone shows an incomplete cross reaction when reacted with rabbit antisera to(1-4) human pituitary growth hormone (HGH), and a lesser binding affinity than HGH for rabbit anti-HGH serum(1). Similarities in the relation of the chorionic hormone to pituitary growth hormone and that which exists between human chorionic gonadotropin and human pituitary luteinizing hormone have been pointed out(1). The present report describes a simple method for the partial purification of chorionic "growth hormone-prolactin" from human placentae utilizing immunochemical and zone electrophoretic techniques to detect the hormone and to assess homogeneity.

Materials and methods. Antisera were prepared in rabbits by injection of HGH or of human CGP and rendered specific by absorption with human serum proteins as described elsewhere(1,5). The immunochemical and electrophoretic methods have been reported previously(1,5,6).

In a typical experiment (Table I), 50 g of lyophilized term placental tissue were ground in a Waring blendor and extracted with 1500 ml of 0.3 M KCl at pH 10. All extractions were performed at cold room temperatures. The extract was centrifuged and the dark brown supernatant dialyzed against running tap water for 24 hours. A euglobulin fraction was removed by centrifugation and the clear supernatant lyophilized to yield 12.5 g of reddish powder.

Five grams of the 0.3 M KCl extract were dissolved in 200 ml distilled water, an equal volume of saturated ammonium sulfate added, and the mixture placed in the cold room for one hour. A precipitate was formed which was removed by centrifugation, suspended in distilled water, and dialyzed overnight against distilled water. After centrifugation to remove an inactive precipitate, the solution was lyophilized to yield 370 mg of tan powder, representing 220 g of fresh placenta.

Further purification was obtained by chromatography on a column of diethylaminoethyl (DEAE)-cellulose equilibrated with 0.01 M *tris* buffer, pH 9.0 (Fig. 1). Stepwise elution with 0.05 M NaCl, 0.1 M NaCl, and 0.33 M NaCl in 0.01 M *tris,* pH 9.0, yielded several components, only one of which (Frac. E) formed a strong precipitin band when reacted with rabbit anti-HGH serum or rabbit anti-human CGP serum by

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