LH-Releasing Activity in Hypothalamic Extracts. (25864)

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(Introduced by John R. Brobeck)
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Release of luteinizing hormone (LH) from the pituitary appears to be under hypothalamic neurohumoral control(1,2). Since convincing evidence is now available indicating that a corticotrophin-releasing factor and vasopressin can release adrenocorticotrophin from the pituitary(3-6), it seems of interest to examine hypothalamic and median eminence extracts to determine if a substance originating in these structures may regulate release of LH. Ovarian ascorbic acid depletion (OAAD) from heavily luteinized ovaries of suitably prepared immature rats has been shown by Parlow(7) to be an extremely sensitive and specific assay for LH, a finding that we have confirmed(2). In the present study this assay has been used to assess LH-releasing activity of hypothalamic extracts.

Methods. Assay animals were prepared by the following technic. Female rats, 26 days old, of the Wistar or Sherman strain were injected subcutaneously (sc.), first, with 75 International Units (IU) of pregnant mare's serum gonadotrophin (Equinex),§ and 58 to 67 hours later with 30 IU of human chorionic gonadotrophin. Five to 7 days later, under ether anesthesia the left ovary was removed for analysis of its ascorbic acid concentration, and the substance to be assayed was injected intravenously (IV) during one minute. One hour after removal of the first ovary, the right ovary was similarly removed and analyzed for its ascorbic acid concentration by the method of Mindlin and Butler(8). Results were expressed as percentage depletion (decrease) in ovarian ascorbic acid of the second ovary as compared to the first gland. Extracts of the stalk-median eminence (SME) region from adult male rats, normal or hypophysectomized (hypox.),‖ were made by grinding this tissue and adjacent ventral hypothalamus in 0.5 ml of 0.1 N HCl with sand and a stirring rod. Ten or more SME's were pooled, and the volume adjusted with 0.1 N HCl so that quantity to be injected into each assay rat was contained in 1 ml of the mixture. The diluted extract was then centrifuged at 3000 rpm for 15 minutes, and the supernatant assayed as described above. Extracts of rat cerebral cortex were prepared and assayed similarly. Hypox. assay rats were operated upon by the parapharyngeal approach and immediately used for assay as described above.

Results. The results (Table I) indicate that unilateral ovariectomy alone failed to elicit a significant OAAD. When an extract prepared from the SME region of 2 hypox. or normal (Table II) rats was injected into each assay rat, a highly significant OAAD resulted ($P<0.001$). On the contrary, an extract of rat cerebral cortex equivalent in wet weight to that from SME failed to elicit a significant OAAD.

A number of pharmacologically active agents known to exist in hypothalamic tissue was assayed to determine if the OAAD induced by the hypothalamic extract could be accounted for by any of these compounds. Epinephrine and Substance P were both inactive in these assay rats. Histamine and serotonin, on the other hand, in doses estimated to be about 1000 times greater than the amount expected to be present in the extracts of SME(9,10), evoked small but significant OAAD's which were much smaller ($P<0.001$) than that obtained with SME extract. Seventy mU of a commercial extract of vasopressin (Pitressin), corresponding to the quantity of vasopressin previously shown

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‖ Obtained from Hormone Assay Labs., Chicago and used 24 hours post-hypox.

TABLE I. Ovarian Ascorbic Acid Depletion Induced by Hypothalamic Extracts and Various Pharmacological Agents.

Treatment	Ovarian ascorbic acid depletion (OAAD)	P *vs* control
Unilateral ovariectomy (control)	1 ± 1.8(12)*	
Unilateral ovariectomy plus:		
A. Extract stalk-median eminence, hypox. rat, 2 SME's	22 ± 1 (6)	<.001
B. Extract rat cerebral cortex	1 ± 3 (5)	ns†
C. Serotonin creatinine SO_4, 25 μg	8 ± 1.9 (5)	=.05
D. Histamine, .3 mg	9 ± 0.9 (5)	<.05
E. Epinephrine, 2 μg	4 ± 3 (5)	ns
F. Substance P, 1 mg	2 ± 2 (5)	"
G. Pitressin, 70 mU	8 ± 1 (4)	>.05
H. Pitocin, 70 mU	7 ± 3 (6)	ns
I. Pitressin, 70 mU + Pitocin, 15 mU	6 ± 1 (5)	"

* Mean ± S.E. of mean (No. of rats/group).
† ns = no significant difference from control.

to be present in 2 hypox. rat SME's(6), induced an OAAD of borderline significance that was much less than that obtained with SME extract ($P<0.001$). Seventy mU of oxytocin (Pitocin) did not produce a significant OAAD and was not able to potentiate the activity of Pitressin when injected at a dose equivalent to that expected to be present in the injected extract(11).

Because the extract of SME might have contained LH, it was important to assay it in hypox. rats. These were used immediately after hypophysectomy because we found that if we used the rats 18-24 hours post-hypox., there was a very low ovarian ascorbic acid and decreased sensitivity to LH. Even during the one hour interval following removal of the first ovary a small decrease in ovarian ascorbic acid occurred (Table II).

The data in Table II show that the OAAD induced by LH or Pitressin was the same in both normal and hypox. rats. Two or 4 SME's from normal donor rats induced a small OAAD in the hypox. rats, significantly greater than the control value at the higher dose of 4 SME's; however, a significantly greater OAAD was obtained in normal rats at both the 2 and 4 SME dose than occurred in hypox. rats.

Discussion. The results indicate that rat SME or adjacent hypothalamic tissue contains substances which can deplete ovarian ascorbic acid. This is not a non-specific effect, nor can the activity of SME be explained by its content of certain known hypothalamic constituents.

The results with vasopressin and oxytocin are of particular interest since Martini *et al.* (12) have reported a gonadotrophin-releasing action of Pitocin and Pitressin in the rabbit. In contrast in the present experiments all activity of Pitressin was present in the absence of the hypophysis suggesting a direct action of this substance on the ovary.

Since SME extract evoked a greater OAAD in the normal than in the hypox. rat, a release of LH from the hypophysis of the normal assay animals appears to have occurred. The nature of the substance(s) responsible for this action remains to be determined. Further experiments are also required to ascertain if this substance, tentatively designated

TABLE II. Comparison of Effect of LH, Pitressin, and Hypothalamic Extract in Normal and "Acutely" Hypophysectomized Rats.

Treatment	Ovarian ascorbic acid depletion (OAAD) Normal	Hypophysectomized	P (hypox. *vs* normal)
Control (unilateral ovariectomy)	1.3± 1.8 (12)*	8.2± 2.9 (10)	=.05
Unilateral ovariectomy plus:			
A. LH, .2 μg	20 ± 2.3 (10)	17 ± 2.7 (8)	ns†
1.0 "	30 ± 2.5 (10)	29 ± 2.1 (8)	"
B. Pitressin, 0.5 U	21 ± 3.2 (9)	24 ± 2.4 (10)	"
C. Rat SME extract—2 SME's	18 ± 1.5 (14)	11 ± 1.4 (14)	<.005
4 "	22 ± 2.0 (9)	16 ± 1.9 (11)‡	<.05

* Mean ± S.E. of mean (No. of rats/group).
† ns = no significant difference from control.
‡ P <.05 when compared to response to uni-ovariectomy alone in the hypox. rat.

LH-releasing factor, acts directly on the adenohypophysis.

The residual OAAD produced by SME extract in the hypox. rat is presumably due to the presence of LH or vasopressin or a combination of the 2 in the extracts.

Since the difference between the OAAD in normal and hypophysectomized assay rats injected with SME extract was not large, it would appear that the extracts caused release of only a small amount of LH from the hypophysis. As we have shown that content of LH in the pituitary of these immature assay animals is very low, the amount of LH released by the extract is probably a very significant percentage of this total. Their low hypophysial LH content may constitute a major disadvantage to use of these immature rats for assay of LH-releasing activity. Nevertheless, the data show that a significant release of LH was produced by SME extracts in these assay animals.

Summary. Acid extracts of rat SME tissue evoked OAAD in immature rats pre-treated with gonadotrophins. Part of the activity in the extracts could be accounted for by their content of LH or vasopressin or both, whereas the remaining activity appeared to be due to release of LH from pituitary of assay rats. The substance(s) responsible for LH-releasing activity of extracts has been called LH-releasing factor. The nature of this material is unknown, but it appears to differ from histamine, serotonin, substance P, epinephrine, and vasopressin or oxytocin.

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Crotalaria spectabilis Toxicity in Chickens.* (25865)

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Crotalaria spectabilis, commonly known as rattlebox, is a leguminous plant grown throughout the South as a cover crop on sandy soils. It grows wild along fence rows, in uncultivated fields and occasionally in row crops after cultivation has been completed. The plant, as well as its seed, is toxic to all farm animals. The toxic principle in *Crotalaria spectabilis* has been isolated and identified as monocrotaline(1,2). Neither lethal dose nor tissue changes associated with its consumption have been clearly defined in chickens(7). Toxicity studies of *Crotalaria spectabilis* for poultry were undertaken after numerous deaths in several large laying flocks that consumed a ration containing an unknown quantity of this legume. Examination of dead and morbid birds invariably revealed ascites and hemorrhage in the liver. It was

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