

Ascorbic Acid Depletion Activity of the Pituitary of the Female Anole (35376)

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Almost no search for pituitary LH activity has been conducted among lower amniotes. Parlow's ovarian ascorbic acid depletion (OAAD) test (1) has been employed in birds (2-5) and, in combination with radioimmunoassay, in the turtle, *Chelydra serpentina* (6). Its specificity as an assay for avian LH has been questioned (4, 5), and its validity as an assay of reptilian LH is open to question. However, a knowledge of whether or not the adenohypophysis of an oviparous lizard contains a component capable of eliciting OAAD in a conventional assay mammal is a step toward elucidating the gonadotropic complex of lower amniotes. A study of the OAAD activity of the adenohypophyses of female green anoles is reported here.

Materials and Methods. Adenohypophyses were obtained from 527 sexually mature female anoles (*Anolis carolinensis*) collected at LaPlace, Louisiana in April, May, and June. Two hundred and seventy-three anoles had an egg in one oviduct, 115 had an egg in each oviduct, and 149 had only heavily yolked, unovulated eggs. The adenohypophyses were grouped according to whether they were from anoles with or without oviducal eggs. The first 108 glands were collected in April and pooled in concentrations of 9 glands/0.9 ml of chilled saline (Ringer). The remaining glands were collected in May and June and pooled in concentrations of 18 glands/0.9 ml of saline. Following sonification of the pooled glands and centrifugation of the suspension at 0°, the supernatant fluid was stored at -7 or -20°. Thereafter, 0.5 ml of each pool of supernatant fluid was withdrawn for assay and the remainder was discarded.

Holtzman rats appropriately pretreated commencing at 25 days of age with PMS and

HCG (1) were used as assay animals. The right ovary was removed between 6:00 and 10:00 a.m. (CST), blotted, weighed, and stored in metaphosphoric acid (MPA) at -7°. The rat was then injected intravenously with 0.5 ml of one of the following: saline, saline containing NIH-LH-SII in one of three concentrations (Table I), saline containing adenohypophyseal extract in concentrations of 5 or 10 gland-equivalents¹ from anoles with oviducal ova, or saline containing adenohypophyseal extract in concentrations of 5 or 10 gland-equivalents from anoles lacking oviducal ova. Three hr later the left ovary was removed, weighed, and stored. In preparation for estimation of ascorbic acid content by colorimetric analysis (7), each ovary was homogenated individually in cold MPA and the homogenate was filtered. The ascorbic acid content of each ovary was estimated by reference to a standard absorbance curve for ascorbic acid plotted each time an analysis was performed. A dose-response curve for ascorbic acid depletion of the left ovary in response to known concentrations of LH was also plotted.

The ascorbic acid content of the right (nontreated) ovary was used to estimate the pretreatment content of the left ovary. The latter value was then adjusted by analysis of covariance using the concentration of the right ovary as the independent variable. Whenever the concentration in the right ovary was less than 70 µg/100 mg of ovarian tissue, the data from that rat were discarded to eliminate any bias in depletion of the left ovary.

Results. The results of the treatments are given in Table I. A least squares analysis of

¹ Defined in Table I, footnote c.

TABLE I. Ascorbic Acid Content of Left Ovaries of Assay Rats After Treatment.

Treatment	N ^a	Ascorbic acid ($\mu\text{g}/100$ mg of ovarian tissue) ^b	Depletion (%) compared with saline
1 Saline	10	111.37 \pm 2.74	—
2 LH, 0.31 μg	5	100.12 \pm 3.84	10.1
3 LH, 1.25 μg	6	72.80 \pm 3.51	34.6
4 LH, 5.00 μg	4	56.27 \pm 4.80	49.5
5 5 gland-equivalents ^c from April anoles with oviducal ova	1	110.66 \pm 8.86	0.6
6 10 gland-equivalents from May-June anoles with oviducal ova	11	104.88 \pm 2.61	5.8
7 5 gland-equivalents from April anoles lacking oviducal ova	7	100.36 \pm 3.41	9.9
8 10 gland-equivalents from May-June anoles lacking oviducal ova	2	107.48 \pm 6.10	3.5

^a Number of rats.

^b Mean \pm SE of mean, after adjustment by analysis of covariance, the nontreated right ovary being the independent variable.

^c One gland-equivalent = $1/X$, where X equals the number of glands pooled in 0.9 ml of saline.

variance of the adjusted left ovarian ascorbic acid concentrations revealed a significant difference between treatments ($p < .01$). The analysis also showed that adjustment of the left ovarian ascorbic acid concentrations by covariance was a significant means of reducing the error term in analysis of variance ($p < .01$). There was significant depletion of ovarian ascorbic acid following injection of adenohipophyses compared with saline ($p < .05$). Fine pooled April glands from anoles lacking oviducal ova elicited OAAD approximating that elicited by 0.31 μg of mammalian LH, and greater than that evoked by any other glands. There was no significant difference in depletion when adenohipophyses from all anoles containing oviducal ova were compared with those from all anoles lacking oviducal ova. The three dose levels of LH resulted in a typical dose-response curve after adjustment by analysis of covariance, attesting to the validity of the technical procedures.

Discussion. At the initiation of the study it was expected that any LH activity of the anole pituitary would be subject to estimation from a conventional dose-response curve for LH. In practice, only the maximal OAAD evoked by anole glands approached the depletion resulting from the smallest dose

of LH. Furthermore, orthogonal comparisons showed that the extract from 10 glands did not cause an OAAD significantly greater than that from five. A pool of as many as 10 anole glands at some seasons of the year might be insufficient to evoke a significant OAAD in rat ovaries.

When the study was designed, we hypothesized that pituitaries from anoles about to ovulate might contain more gonadotropin with LH activity than pituitaries from anoles not about to ovulate. Therefore, anole pituitaries were placed in groups according to whether or not they had oviducal ova. The rationale was that anole ovaries probably ovulate alternately about every 14 days and that there is an interval of about 18 days between ovulation and oviposition of an egg (8); therefore, an anole with an ovum in each oviduct should have ovulated recently; and in an anole lacking oviducal ova, ovulation should be imminent. The data suggest that any LH activity of female anoles may be higher in anoles about to ovulate in the early part of the breeding season than at certain other times.

A source of error in the OAAD assay is the possibility that anole adenohipophyses may contain substances capable of releasing the rat LH, thereby indirectly inducing OAAD

(9). Therefore, it has not been demonstrated unequivocally that the greater depletion evoked by glands from April anoles lacking oviducal ova was correlated with a preovulatory surge of some gonadotropin. Also, the criteria for determining that ovulation was imminent may have been invalid, and the ovulatory hormone of anoles may have no LH activity. It has been suggested by Licht (10) that the anole may have an FSH-like gonadotropin complex only.

Summary. Ovarian ascorbic acid depletion activity was detected in adenohipophyses of sexually mature female green anoles in April, May, and June. The equivalent of five April glands from anoles lacking oviducal eggs, but with heavily yolked, unovulated eggs, evoked depletion approaching that evoked by 0.31 μ g of LH.

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