LHRF CONTENTS OF HYPOTHALAMUS AND POSTERIOR LOBE OF HYPOPHYSIS IN HUMANS

(UDC 612.826.4 + 612.434]: 612.621.31)

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Translated from Byulletin' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 9,
pp. 3-7, September, 1965
Original article submitted May 9, 1964

It has already been established in this laboratory that extract of rat hypothalamus or posterior hypophysis in infantile female rats, previously given pregnant mare serum (33 I.U.†) to stimulate follicle development, will produce ovulation if the hypophysis is intact, but not if the animal has been hypophysectomized. From this it was concluded that rat hypothalamus and neurohypophysis contained a factor potentiating secretion of luteinizing hormone (LH) from the hypophysis [1, 2, 3]. By analogy with corticotropin releasing factor (CRF), this suggested transmitter of nerve influences on the secretion of LH may be termed LHRF [4] or LRF [6-9].

The idea of such a transmitter is in accordance with earlier publications by authors [4, 7, 9] who studied the effects of rat and sheep hypothalamus extracts by determining the reduction they caused in the ascorbic acid content of the ovaries. More recent studies [6, 11, 12] have shown that the agent acting in this way is present in the hypothalamus of both fully grown and immature animals, can be extracted with weak (0·1N) HCl, is thermostable, and is inactivated incompletely by pepsin and completely by trypsin. In some of these experiments [7, 9], however, the injection of extracts of hypothalamus or of vasopressin to hypophysectomized animals likewise reduced the ascorbic acid content of the ovaries. This indicates that the reaction by which investigators assessed the effect of LH is not specific for this hormone, that interpretation of the results obtained by means of it is difficult and that, in any case, they must be verified by more specific tests.

Some fresh investigations, proving the presence of LHRF in the hypothalamus, have been published recently. Of interest because of the method used are some experiments [10] in which extracts of bovine or rat hypothalamus have been injected directly into the hypophysis. This led to ovulation in adult female rats in which the development of spontaneous ovulation had been prevented with nembutal. Much larger doses of these extracts (10-48 times more) by intravenous injection were required to produce ovulation. The effect of acetate buffer extract of sheep hypothalamus has been studied in female rats with prolonged estrus following injection of androgen at the age of 5 days [5]. It was found that the formation of corpora lutea could be produced in such animals by injection of this extract in dosage equivalent to one quarter of the hypothalamus, or 1 g fresh tissue. The author observed a similar reaction, however, following injection of a similarly prepared extract of other parts of the brain, although dosage equivalent to 4 g fresh tissue had to be employed in these cases.

The results of an experimental investigation demonstrating the presence of LHRF in the hypothalamus and posterior hypophysis of man are now described.

The hypothalamus and hypophysis were removed from cadavers within 12-36 h after death and were placed in absolute acetone.

On the same day the hypothalamus was freed from meninges and was processed in one of two ways: it was ground with quartz sand and extracted for 1-2 h with 1·5-2·5 ml 0·1N HCl and the extract was centrifuged for 10-15

*Decreased.
†International units.

TABLE 1. Effect of Single Injection of 33 I.U. PMS on Ovulatory Power of Ovary in Infantile Female Rats of Various Weights

Weight group (g)	No. of animals	Ovulation reaction				
		negative	positive			
			total	weak	medium	strong
		no. of animals				
30—40	15	15	0	0	0	0
41—45	18	18	0	0	0	0
46—55	15	14	1	1	0	0
56—80	22	14	8	1	3	4

Note, In all tables: weak positive reaction 1-10 ova in recipient's ovaries; medium positive 11-19 ova; strong positive 20 or more ova in ovaries.

TABLE 2. Effect of Suspension of Posterior Lobe of Human Hypophysis on Ovulation Reactions in Infantile Rats of Different Weight Groups

Weight group (g)	No. of animals	Ovulation reaction				
		negative	positive			
			total	weak	medium	strong
		no. of examinations				
30—40	25	15	10	7	2	1
41—45	37	23	14	6	2	6
46—55	16	4	12	2	5	7

min at 3000 rpm or it was ground and then extracted for 2-3 h with 2 ml sodium-acetate buffer (pH 4.5), after which nine volumes of absolute acetone were added to the extract. After successive washing with acetone and then saline (twice), the precipitate was suspended in normal saline.

Under a dissecting microscope, the posterior lobe of the hypophysis was carefully separated from the anterior lobe (on the day of removal from the cadaver whenever possible); a suspension in distilled water was prepared and 0·5 ml of this was added to excipient.

The effects of the hypothalamus extracts and the posterior lobe suspensions were studied in infantile female rats, weighing 30-45 g (21-28 days old) by a method which had been described earlier [1]. Preovulation development of the follicles was first stimulated by subcutaneous injection of 33 I. U. pregnant mare serum (PMS). The preparation to be examined was injected 56 h later, hypothalamus extract intravenously and posterior lobe suspension intraperitoneally. After 22-24 h the recipients were autopsied and the number of individuals who had reacted was determined from the presence of a broad, opalescent segment in the upper third of the uterine tube; the number of ova present was determined.

In control experiments four groups of rats of different weights (30-40, 41-45, 46-55, and 56-80 g) were given only PMS (as in the main experiments, 33 I.U. once). These rats were autopsied 78-80 h after the injection of PMS.

RESULTS

Before evaluating the results of these experiments to determine the presence of LHRF in extracts of the hypothalamus and suspensions of the neurohypophysis, it was essential to ascertain whether the PMS, injected beforehand to stimulate preovulation development of the follicles, might not itself cause ovaulation.

Some authors [13, 14] state that a single injection of PMS in infantile rats does not lead to ovulation, for they observed the latter in only 1 of 200 individuals. It was thought important to verify this in view of the importance of the question in its bearing on the usefulness of the method. A special series of experiments was carried out for this purpose (Table 1).

The results indicated that a single subcutaneous injection of 33 I.U. PMS to infantile female rats did not cause ovulation in animals weighing 30-40 g nor in animals weighing 41-45 g. As the weight (and correspondingly age) increased, there were occasional cases of ovulation (1 in 15 rats weighing 46-55 g), and ovulation was produced in a considerable proportion of the animals weighing 56-80 g (8 times in 22 animals).

These results indicate that the production of ovulation is a very reliable test for the presence of exogenous or endogenous LH after preliminary injection of PMS, but only if used on animals weighing not more than 45 g,* and therefore, although the authors had previously used infantile rats of various weights and ages in their experiments, the present paper deals mainly (unless otherwise mentioned) with rats weighing between 30 and 45 g, i.e., animals in which the injection of PMS alone does not cause ovulation.

*In this connection we are doubtful about the interpretation given by Zarrow et al., for the results of their experiments [14], namely variation in the sensitivity of immature rats of different ages (up to 60 days) to LH. All the rats were previously given PMS. Our observations indicate that the age differences in the percentage of animals which reacted and in the number of ova, described by these authors, may depend on differences in sensitivity to PMS as well as to LH.

TABLE 3. Effects of Extracts of Human Hypothalamus on Ovulation Reactions in Infantile Female Rats

Type of extract	Dose (fraction of whole)	Weight group (g)	No. of animals	Ovulation				
				negative	positive			
					total	weak	medium	strong
				no. of animals				
HCl	1/2	30—40	4	3	1	1	0	0
	1/3		3	3	0	0	0	0
	1/6		2	1	1	0	1	0
	1/2	41—45	2	2	0	0	0	0
	1/3		7	3	4	2	1	1
	1/6		5	5	0	0	0	0
Na-acetate buffer, pH 4·5	1/3	30—40	2	1	1	1	0	0
	1/6		4	4	0	0	0	0
	1/3	41—45	4	3	1	1	0	0
	1/6		6	5	1	1	0	0

The hypophysis of 24 individuals of ages from 26 to 80 years were examined. Individual preparations were made from 20 of these (5 male, 7 female and 8 undetermined) and 2 mixed preparations from the remaining four. The results of these examinations are summarized in Table 2.

Ovulation developed after injection of suspension of posterior lobe of the hypophysis in 24 (about 40 percent) of 62 rats in the first two age groups, i.e., in rats in which the injection of PMS did not lead to ovulation. It is perhaps permissible to refer also to the results obtained with rats weighing 46-55 g, as ovulation was observed in 12 of 16 individuals in this group after injection of posterior lobe preparation, where in the control experiments in which PMS alone was injected, only 1 of 15 rats gave a positive reaction. The number of animals that could be used for assay of the individual samples, which formed the bulk of the material, was limited by the size of the dose, as each rat received the equivalent of 1/2, 1/4, or (only in occasional cases) 1/10 of the posterior lobe of the hypophysis. Generally, three of four rats were used for examination of each posterior lobe. Positive results were obtained with 16 posterior lobe preparations (14 individuals and 2 mixed), and strongly positive reactions were most frequent when large doses had been injected. It may be noted that, of the 6 posterior lobes giving negative results, one came from an individual who had died of cirrhosis of the liver, one from a woman of 80 who had been in a state of cachexia for a long period prior to death and a third from a woman who died in the sixth or seventh month of pregnancy. No details of the sources of the other three lobes giving negative results are known. No connection between reaction result and sex or age could be observed.

The results obtained with 16 individual extracts of hypothalamus (9 HCl and 7 Na-acetate buffer extracts with pH 4·5) were less constant. In these experiments, the entire hypothalamus was extracted and the animals were given 1/2, 1/3, or 1/6 of the whole. Most of the negative results were obtained with the smallest doses: ovulation was observed in only 2 of 17 rats given the equivalent of 1/6 of the hypothalamus. Ovulation developed in 7 (or about 30 percent) of 23 animals given the equivalent of 1/2 or 1/3 hypothalamus. Positive reactions (if ovulation in 1 of 2 or 3 individuals given the same material can so be regarded) were obtained with 5 of 9 HCl extracts and 3 of 7 Na-acetate buffer extracts.

These preliminary findings do not justify any conclusions as to which is the better method of extraction, but they do suggest that the agent concerned (LHRF) is incompletely extracted from the hypothalamus or is partially inactivated by either method, or is present in the hypothalamus in smaller quantity than in the posterior lobe of the hypophysis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
