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Constant Monitoring of Plasma Luteinizing Hormone by Radioimmunoassay in Individual Rats Following Injection of Hypothalamic Extract* (33788)

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The presence of an LH releasing factor (LRF) in hypothalamic extracts from several species has been demonstrated primarily by means of a bioassay based on the phenomenon of ovarian ascorbic acid depletion (OAAD) in response to LH (1–3). Both the specificity of the assay and the inability of LRF to act directly upon the ovary of the assay animal have been questioned (4–6). Results are further complicated since LRF may act directly on the pituitary of the OAAD assay animals when they are used to measure LH in serum from experimental rats. Finally, the experimental approach to studies of LRF activity have been limited by the sensitivity of bioassays, which are not adequate to measure serum LH concentrations in individual rats.

The present results were obtained with radioimmunoassay for rat LH (7) which does not appear to measure LRF. The collection of blood through a carotid–aortic cannula eliminated both the problems of stress and the need for anesthesia. By utilizing whole blood in the radioimmunoassay, more than 30 sequential measurements of serum LH concentrations were obtained from each rat.

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Materials and Methods. Hypothalamic extracts were prepared by homogenization of rat hypothalamic tissue in 0.1 *N* HCl (5 hypothalami/ml) followed by centrifugation. The supernatant fluid was lyophilized and dissolved in phosphate buffered saline (PBS) just prior to use. Extracts of brain tissue from the cerebral hemispheres, including cerebral cortex, were similarly prepared and will be referred to as cortical extracts.

Mature Holtzman female rats, approximately 300 g in weight and castrated at least 1 month earlier, were anesthetized with ether and cannulated via the left carotid artery. The structure of the cannula is shown in Fig. 1. Following the operation, the rats were injected subcutaneously with estrogen (50 μg of estradiol valerate) and progesterone (25 mg) in peanut oil. Three days later the animals were heparinized and connected, via the cannula, to a peristaltic pump set to remove approximately 12 μl of blood/min. Blood was pumped directly into 100 μl Hamilton syringes (with the plunger removed) until approximately 70 μl of blood had been collected. The desired volume of blood, usually 60 μl, was added to the appropriate volume of PBS with 1% lyophilized egg white (Sigma) in an assay tube. The final volume of serum plus PBS-1% egg white equaled 500 μl. In most cases hematocrits were not measured, but were assumed to approximate 50%. The diluted blood samples were stored at 4° until

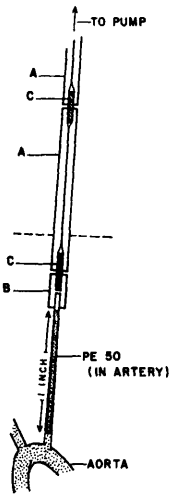

FIG. 1. A diagram of the cannula and connections used for continuous sampling of blood in the rat. With the exception of the polyethylene tubing inserted into the carotid artery (PE 50) all tubing is Tygon (Technicon Instruments Corp., Chauncey, New York), with an i.d. of 0.010 in. (A) or 0.040 in. (B). Connectors (C) were made from 20 guage stainless steel needles. To permit the use of such large connectors, the ends of the Tygon tubes were expanded by heat after being forced over a piece of wire. Thirty in. of Tygon tubing (0.010 in. i.d.) were used between the animal and the pump, resulting in a volume for the entire system of approximately 90 μl. The broken line indicates the level at which the cannula exits through the skin.

assayed, a period which did not exceed 1 week.

Following the collection of several control blood samples, cortical extract or hypothalamic extract was injected into the aorta through the cannula in 0.5 ml or less of PBS. Blood samples were then collected from the pump at approximately 5-min intervals over the next 1–4 hr. Samples of each hypothalamic extract were included in the radioimmunoassay to determine the extent to which the material was contaminated with LH or LH-like activity.

To determine whether or not whole blood could be utilized directly in the radioimmunoassay to obtain an estimate of serum concentration, three separate experiments were conducted in which blood from individual rats was divided into aliquots of serum, heparinized plasma and whole blood diluted in PBS. Aliquots of diluted whole blood were stored at room temperature or at 4° for 24 hr while aliquots of serum, plasma, and diluted whole blood were stored frozen for the same period of time. All aliquots were then radioimmunoassayed.

The radioimmunoassay procedures were identical to those reported for serum (7) except that PBS with 1% lyophilized egg white was used as the diluent throughout. All results have been expressed in terms of B160, a partially purified rat anterior pituitary gland extract, 1 mg of which is equivalent to 0.17 mg of NIH-LH-S1, as determined by OAAD bioassay.

Results. Representative results from experiments to determine whether or not whole blood stored in the manner described could be utilized directly in the radioimmunoassay are shown in Table I.

Following the injection of hypothalamic extract into ovariectomized, estrogen–progesterone-treated rats, plasma LH concentrations increased four to sixfold over preinjection values. In some cases, plasma LH concentrations did not reach maximum values until approximately 10 min after the injection of hypothalamic extract. Following this period plasma LH levels declined at a rate which suggested very little, if any, additional LH release. Plasma LH concentrations continued to decline until they had reached preinjection values and continued at that level for the remainder of the experiment (Fig. 2).

The LH contamination in the hypothalamic extracts used in this study, as determined by radioimmunoassay, was approximately 10 mμg of B160/hypothalamic

TABLE I. Concentrations of LH in Serum, Plasma, and Whole Blood of Castrated Rats.

Rat no.	Serum[a]	Plasma[a]	Blood[a]		
	−20°	−20°	−20°	4°	24°
1	156	165	146	159	148
2	157	164	148	145	148
3	125	116	125	115	122

[a] All samples were stored at the temperatures indicated for 24 hr prior to radioimmunoassay. All values are expressed as mμg B160/ml of serum or plasma, adjusted for hematocrit.

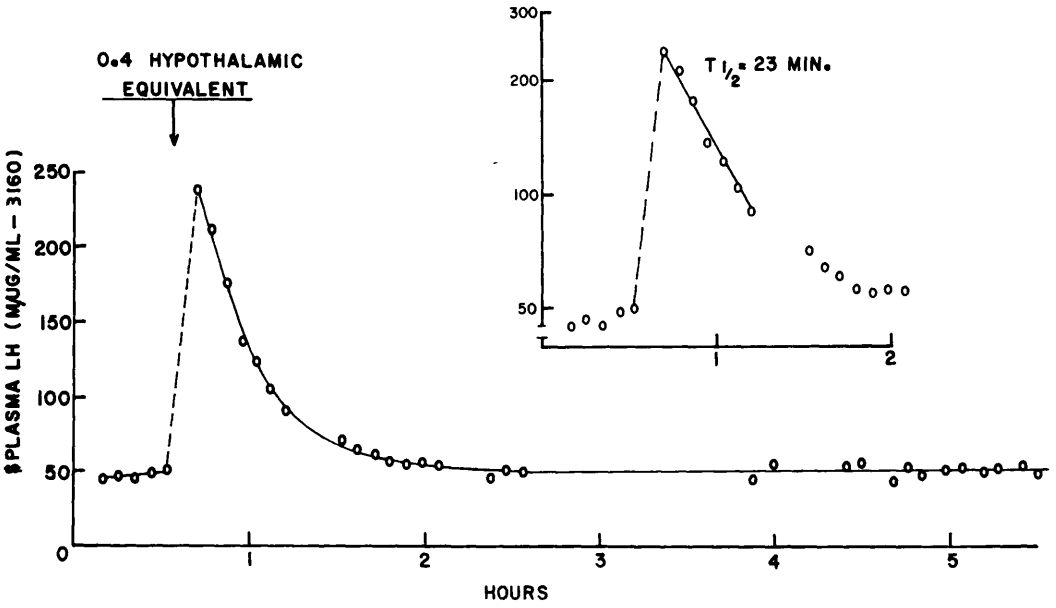

FIG. 2. Changes in plasma LH following the injection of hypothalamic extract into an ovariectomized, estrogen–progesterone-treated rat. Eight similar rats (sampled by cardiac puncture) exhibited no increase in serum LH at 15, 60, or 120 min after injection with an equal volume of PBS or cortical extract. The plasma LH values have been plotted on a semilog scale (inset). It is apparent that the early phase of the decline in plasma concentration may be characterized as an exponential decay curve with half time for LH disappearance (T ½), in this instance, of 23 min.

equivalent and did not appear to be decreased by boiling of the extract. This quantity of contaminating LH activity injected into 300 g rats should have increased plasma LH levels by no more than 2 mμg of B160/ml.

Discussion. While radioimmunoassays for rat LH have permitted the analysis of sequential blood samples from individual animals (8), relatively large volumes of blood have been required at each sampling to compensate for losses occurring in the process of obtaining serum. In addition, the stress associated with blood collection may have an effect on LH release which cannot be discounted in interpreting the results. In order to analyze thoroughly changes in circulating LH in individual rats over a period of several hours, both before and after the injection of hypothalamic extract, these problems were minimized by collecting whole blood via a carotid–aortic cannula from a nonanesthetized and unrestrained rat. The use of whole blood in the radioimmunoassay is an important development in an attempt to obtain numerous samples from individual rats. It is apparent from the data presented in Table I that the LH concentrations in whole blood can be used to calculate plasma and serum LH concentrations. In addition, these data indicate that whole blood can be frozen and thawed, or stored at room temperature, without altering radioimmunoassayable LH activity.

As has been reported elsewhere (9) the 3 days of estrogen–progesterone treatment used in these studies does not completely inhibit the release of LH in ovariectomized rats. The serum LH concentrations observed prior to the injection of hypothalamic extract were more than threefold greater than levels observed in the intact diestrous rat and nearly 50% of the concentrations found in the serum of castrated, nontreated female rats (9). It is thus apparent that release of LH was occurring in the rats used in this study both before and after the injection of hypothalamic extract, but it is not yet clear if this basal

LH secretion is controlled by the same mechanisms which release LH in response to exogenous hypothalamic extract.

One limitation of the technique employed is that the samples cannot be assayed in duplicate without increasing the required blood volume and correspondingly decreasing the time over which physiologic responses can be assured. Some protection against the acceptance of erroneous values is provided by an understanding of the disappearance rate for LH in the animal being studied. It is apparent that plasma LH concentration can be greatly increased between any two samples. However, if the half time for LH disappearance ("half-life") remains constant at about 25 min[3], each 5-min sample must be at least 88% of the previous value. In practice we found that, in our laboratory, such errors are not a problem. Figure 2, for example, includes every value obtained in the course of the experiment. When the range of the serum LH concentration exceeds the range of the assay, an accurate measurement may be obtained by alternating collection volumes (i.e., 10 and 40 μl of blood) to assure a measurement in the optimal portion of the dose–response curve (20–80% of antibody-bound radioactivity associated with PBS-control tubes).

Summary. Samples of whole blood were collected continuously over 5-min intervals for a period of several hours from individual, nonanesthetized, cannulated, ovariectomized, estrogen–progesterone-treated rats. Plasma LH concentrations were determined by radioimmunoassay of the whole blood after adjustment for hematocrit. The red blood cells influenced the resulting measurements only to the extent to which serum volume was displaced. After the injection of hypothalamic extract, plasma LH concentrations increased 4- to 6-fold within 10 min. Plasma LH concentrations then returned to, but did not fall below, preinjection values. The rate of hormone disappearance from the plasma suggests that, under these conditions, LH release in response to hypothalamic extract is limited to the first few minutes after injection.

[3] Results from a study now being prepared for publication suggest that this is true for intact rats during proestrus, following delivery, and at various intervals following castration.

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