The *in Vitro* Release of LH During Continuous Superfusion of Single Rat Anterior Pituitary Glands[1] (34692)

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Various investigators (1–10) have reported different methods to demonstrate *in vitro* the ability of rat pituitary gland to respond to different stimuli. Thus, it has been shown that presumed neuroendocrine factors of hypothalamic origin are capable of inducing the release of pituitary hormones in the absence of short feedback, long feedback, or other complicating regulatory mechanisms. These results appear to be important since they permit one to study the action of different substances on the release of pituitary hormones under conditions of complete anatomical and functional isolation. However, these methods have provided only a static view of the pattern by which the hormones are released *in vitro*. Thus it is not known whether or not these release patterns approximate those seen in the intact animal (11, 12).

In an attempt to elucidate this problem, the pattern of release of luteinizing hormone (LH) *in vitro* in response to hypothalamic extract has been studied by a technique of continuous superfusion. The results of these experiments, in which the LH content in the perfusate from single intact rat anterior pituitary glands has been determined at 1- or 3-min intervals by radioimmunoassay, are described below.

Materials and Methods. Hypothalamic extracts were prepared by homogenization of rat hypothalamic tissue in 0.1 *N* HCl (20 hypothalami/ml), followed by centrifugation. Prior to use, the supernatant fluid was neutralized with 1.0 *N* NaOH and diluted in incubation medium (1.0–7.5 hypothalami/ml). Extracts from rat cerebrum were similarly prepared, and an amount comparable to that used for a single hypothalamic extract will be referred to as one equivalent of cerebral cortex.

Incubation procedure. The design of the incubation procedure is shown in Fig. 1. The intact rat anterior pituitary gland was incubated in a small vial of approximately 350-μl capacity, attached to inlet and output tubing. All tubing was Tygon (Technicon Instruments Corp., Chauncey, N. Y.) with an internal diameter of 0.020 in. The incubation medium consisted of Krebs–Ringer–bicarbonate–glucose (KRBG) buffer, pH 7.4. The medium was continuously transferred by a peristaltic pump from its container, through the inlet tubing (capacity = 100 μl), and into the closed incubation chamber, thereby superfusing the intact rat pituitary gland while maintaining the level of fluid constant at 200 μl. The incubation medium was recovered in test tubes from the outlet tubing (90 cm, 100 μl) at a rate of 200 μl of fluid/min. Samples were recovered by changing tubes at 1- or 3-min intervals. The incubation was carried out at room temperature for a maximum of 200 min. Continuous oscillation of the incubation chamber was assured by the use of a vibrating mixer. Induction of different solutions into the system was made by simply transferring the end of the inlet tubing from the buffer container into the tube where the solution to be tested was kept.

[1] This investigation was supported by a research grant from the National Institute of Child Health and Human Development (NIH-HD-04105).

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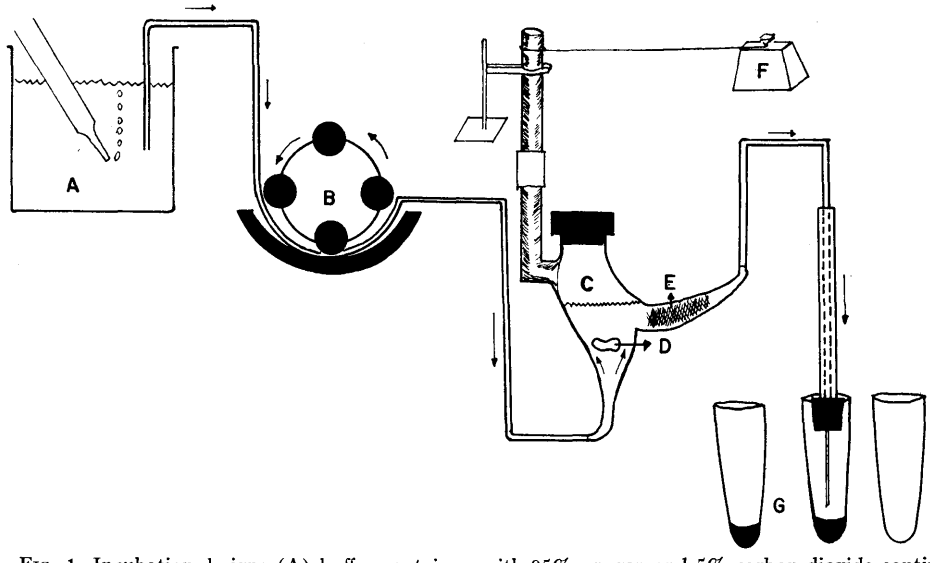

FIG. 1. Incubation design: (A) buffer container, with 95% oxygen and 5% carbon dioxide continuously bubbled; (B) peristaltic pump; (C) incubation chamber containing 200 μl of buffer; (D) intact rat anterior pituitary gland; (E) glass wool filter; (F) mixer producing vibrations of the incubation chamber; and (G) test tubes.

Both solutions were kept under continuous gas phase by bubbling with 95% oxygen and 5% carbon dioxide. Mature Holtzman female rats, approximately 300 g in weight and castrated at least 2 months earlier, were used as pituitary donors.

All samples were diluted fivefold in 0.01 *M* phosphate-buffered 0.14 *M* sodium chloride (pH 7.0)–1% egg white; and LH was measured in 10- or 20-μl aliquots of this solution by radioimmunoassay (13). 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 ovarian ascorbic acid depletion bioassay.

Results. In Fig. 2, typical profile of one incubation is presented. After 40 min of sam-

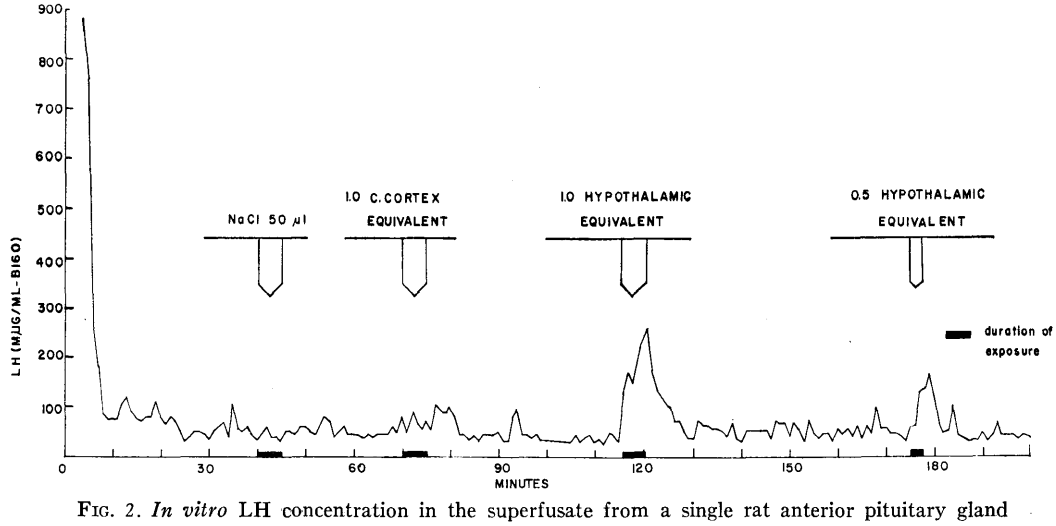

FIG. 2. *In vitro* LH concentration in the superfusate from a single rat anterior pituitary gland following exposure to: (a) NaCl (50 μl diluted in KRBG buffer) for 5 min; (b) one equivalent of cerebral cortex for 5 min; (c) one rat hypothalamic extract for 5 min, and (d) one half hypothalamic equivalent for 2.5 min. Samples were collected and measured every minute and the rate of superfusion was 200 μl/min.

ple collection, 50 μl of 0.1 *N* HCl, neutralized with 1 *N* NaOH and diluted 1:20 in KRBG buffer, were introduced via inlet tubing into the incubation chamber. No increased release of LH was found. After 70 min, 50 μl of cerebral cortex (1 cerebral cortex equivalent), diluted in 950 μl of buffer and introduced for a period of 5 min, produced a slight LH release. After 116 min, the introduction of 1 rat hypothalamic equivalent (50 μl) in 950 μl of buffer (5 min exposure) increased levels of LH in the superfusate from 4- to 6-fold over the basal LH release rate. After 175 min, the introduction for 2.5 min into the system of 0.5 hypothalamic equivalent, at the same concentrations, caused a release of LH approximately 2-fold greater than the basal values. The LH contamination in the hypothalamic extract used in this study, as determined by radioimmunoassay, was approximately 34 mμg of B160/hypothalamic equivalent. This quantity of contaminating LH activity introduced into the system for 5 min (200 μl/min) should have increased LH levels by no more than 7–12 mμg of B160/min, when 1 hypothalamic equivalent was used.

In Fig. 3 is shown the profile of an incubation in which a single pituitary gland was repeatedly stimulated for different periods of time. All doses were obtained from a single stock preparation of hypothalamic extract and variation in dosage was made by changing the duration over which the extract was introduced. LH contamination of this batch of hypothalamic extract did not exceed 20 mμg of B160/hypothalamic equivalent. This quantity of LH contamination, injected for 8 min (4 ml, 12 hypothalamic equivalents) should have increased the total amount of released LH (1450 mμg of B160) by only 240 mμg. In Fig. 4 the amount of released LH is related to the amount of hypothalamic extract introduced. The released LH has been calculated by subtracting from the total value the amount of hormone presumed to be present as a result of the basal release rate.

In all experiments to date, stimulation with hypothalamic extracts has been followed by a rapid release of LH. Once the peak of release has been reached, LH levels have declined until they reached the preceding basal release values. Introduction of NaCl did not elicit any response, while introduction into the system of equivalent amounts of cerebral cortex has consistently produced

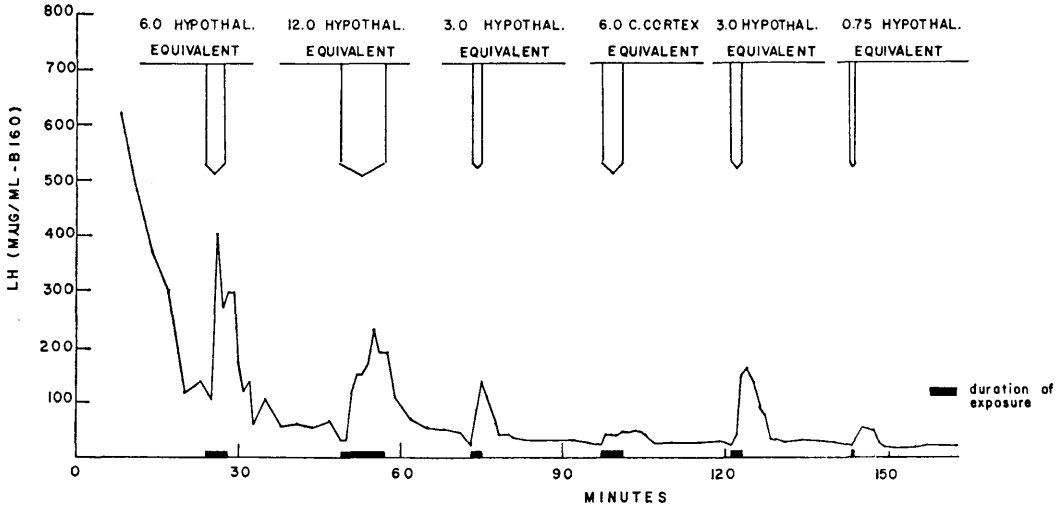

FIG. 3. Concentration of LH in the superfusate following exposure *in vitro* to hypothalamic extracts for 4, 8, 2, and 0.5 min. Exposure to 6 cerebral cortex equivalents was also done during a 4-min period. Samples were collected and measured at 1- or 3-min intervals. Superfusion rate was constant at 200 μl/min. Concentration of extracts in buffer was 7.5 hypothalamic or cerebral cortex equivalents/ml.

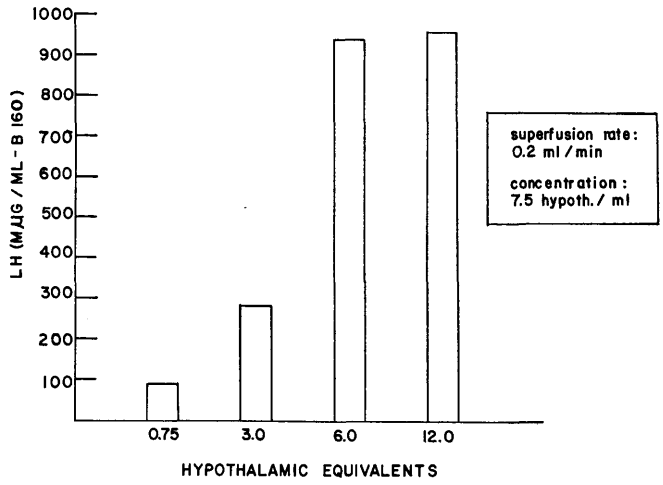

FIG. 4. Same data shown on Fig. 3 after subtraction of basal release values and LH contamination in hypothalamic extracts from the total apparent LH releases. All doses were derived from a single stock solution of hypothalamic or cerebral cortex equivalents (7.5 equiv/ml) and calculated in relation to the length of time over which they were introduced into the system.

small increases in LH release.

Discussion. Previous experiments have demonstrated the possibility of stimulating *in vitro* the release of LH from rat pituitary glands using hypothalamic extracts. These results, however, have only offered a static expression of the pattern involved in this release mechanism. To show significant responses a relatively large number of pituitary glands needs to be used in each experiment and an equal number of controls are required to evaluate the magnitude of the stimulated release. Further, this procedure necessitates the assumption that control and experimental flasks have equal responsiveness.

To our knowledge the observations described in this paper are the first showing: (i) that a single isolated pituitary gland can repeatedly respond to separate administrations of hypothalamic extracts by releasing measurable levels of hormones; (ii) that the response patterns consist of a brief release limited largely to the time in which hypothalamic extract is injected and are followed by a drop to a relatively constant basal release rate; and (iii) that the stimulated release of LH is dose-related.

From our results it is apparent that a constant basal release of LH occurs both before and after injection of hypothalamic extracts. The same finding has been shown by Gay *et al.* (12) *in vivo*. However, it is not possible to conclude whether or not these basal release patterns are due to the same mechanisms. The presence of a constant concentration of LH in the superfusate in this system furnishes an appropriate control to evaluate the release obtained in response to hypothalamic extracts.

The possibility of stimulating a single pituitary gland repeatedly permits one to investigate different facets of gonadotropin release. Although more observations are needed, the graded responses observed under our conditions indicate the potential use of this system to quantitate the releasing activity of hypothalamic extracts.

Since superfusion by continuously renewed medium avoids the accumulation of endogenous LH, while the rapid disappearance of hypothalamic extract permits only a limited stimulation time, it seems that a more physiological condition is reached as compared with precedent *in vitro* procedures. Continuous superfusion of a single pituitary gland might also permit new approaches to the study of mechanisms and dynamics of LH release and the study of the effects of inhibitory or stimulatory substances on this release.

Summary. Stimulation of single rat anterior pituitary glands has been obtained in a continuously superfused system as demonstrated by radioimmunoassay of LH in the superfusate. The results show the possibility of repeatedly stimulating a single pituitary gland, with approximately graded responses to hypothalamic extracts, in presence of a constant basal release rate. The data indicate that a relatively constant basal release rate is obtained under these conditions and that intermittent administration of hypothalamic extracts results in repeated acute release of LH. The period of release was largely limited to the time during which hypothalamic extract was administered. Equivalent amounts of cerebral cortex extract only produced slight increase of LH concentration in the superfusate.

The authors thank Dr. Vernon C. Gay and Dr. Gordon D. Niswender for constructive advice and guidance during the course of these investigations.

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Received Dec. 15, 1969. P.S.E.B.M., 1970, Vol. 133.