LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE IN THE MAMMALIAN HYPOTHALAMUS (1)

B. L. BAKER, W. C. DERMODY AND J. R. REEL
Department of Anatomy, The University of Michigan Medical School, and Endocrine Section, Parke Davis Research Laboratories, Ann Arbor, Michigan 48104

ABSTRACT Utilizing immunohistochemistry with rabbit antiserum to synthetic luteinizing hormone-releasing hormone (LRH), LRH was localized in the peripheral region of the median eminence in the mouse and rat, and more generally in the median eminence of the guinea pig.

Presently available evidence derived from physiological and anatomical studies indicates that luteinizing hormone-releasing hormone (LRH) is elaborated in the hypothalamus, transported to the median eminence in axons, and there secreted into the primary plexus of the hypothalamo-hypophyseal vascular system. Greatly needed at the moment is a clear demonstration of what nerve cells elaborate LRH and where this hormone is released into the blood stream. Recently Leonardelli et al. ('73) used immunofluorescence to describe the localization of LRH in the median eminence of the mouse and guinea pig. Subsequently, they (Barry et al., '73) reported the localization of LRH in nerve cell bodies scattered through the anterior hypothalamus from the preoptic and septal areas to the caudal portion of the tuber cinereum.

The objective of our preliminary paper is to report the localization of LRH in hypothalami of rat, mouse and guinea pig utilizing immunohistochemistry.

MATERIALS AND METHODS Four female rats of the Sprague-Dawley strain, one female and one male of strain 129 SvSl mouse, and one female guinea pig were

used. All were adults. The rats and mice were killed instantly by decapitation with a guillotine; the guinea pig was anesthetized by an intraperitoneal injection of sodium amytal and then decapitated. After exposure of the superior surface of the brain and removal of the cerebellum and medulla oblongata the ventral surface of the thalamus and the hypophysis were flooded with Bouin's fluid; subsequently fixation was continued by immersion after excision of the hypothalamo-hypophyseal region.

Following fixation the tissue was embedded in paraffin, sectioned at 3-5 μ and the sections stained with the peroxidase-conjugated antibody procedure of Nakane and Pierce ('67) and/or the immunoglobulin-enzyme bridge technique of Mason et al. ('69). Antiserum to LRH (anti-LRH) was prepared in mature male Dutch-belted rabbits. Synthetic LRH (500 μg) was emulsified in Freund's complete adjuvant containing 5.0 mg/ml of Mycobacterium tuberculosis and injected at multiple intradermal sites according to the method of Vaitukaitus et al. ('71). Specificity of the antiserum was determined as described previously (Dermody et al., '73). To verify specificity of the staining obtained two controls were used: (a) normal rabbit serum was substituted for anti-LRH, and (b) anti-LRH was absorbed with the synthetic LRH prior to use in the immunohistochemical procedure.

RESULTS In rats LRH appeared as a collection of granules in an area of nerve fibers at the periphery of the median eminence. The stain formed a crown over the peripheral portion of the pars tuberalis and the part lying between the infundibulum and the basal hypothalamus (fig. 1). Only occasionally in rats were colored deposits visible in other portions of the external layer of the median eminence. In mice (fig. 2) LRH occurred in the peripheral region of the median eminence and was located along the proximal border of the external layer.

The guinea pig exhibited a much more general distribution of LRH throughout

the external layer of the median eminence (fig. 3) than was true of the rat and mouse. Here rather dense accumulations of colored granules appeared in the region bordering the pars tuberalis. LRH was not detected in cell bodies of the suprachiasmatic, ventromedial, ventrolateral, arcuate, paraventricular, or supraoptic nuclei, or in the ependyma of any of the three species.

Since no staining occurred in the median eminence or contiguous tissue in any of the three species if anti-LRH was absorbed with synthetic LRH prior to the immunohistochemical procedure (fig. 4), or if normal rabbit serum was substituted for anti-LRH (fig. 5), one may conclude with reasonable assurance that LRH was specifically localized.

DISCUSSION Some tentative generalizations may be useful. First, there is probably a considerable difference among mammals in the ease with which LRH may be localized. Of the mammals studied to date, the guinea pig appears to be the species of choice; thus far we have failed with the gerbil. Second, since the hypothalamic concentration of LRH is exceedingly low (Schally et al., '73) and the hypothalamus contains peptidases capable of inactivating LRH (Griffiths and Hooper, '73), treatment of the animals and preparation of the tissues must be of critical importance. We can expect that with improvement of the technical procedures LRH will be demonstrable in areas other than those described herein. Indeed with the guinea pig Barry et al. ('73) showed the distribution of LRH-containing nerve cell bodies only after increasing hypothalamic LRH content by castration and blocking axoplasmic flow by administration of colchicine. If an immunohistochemical procedure for localization of LRH is to be of optimal utility in advancing research in neuroendocrinology its sensitivity must be increased sufficiently to make other forms of physiological intervention unnecessary.

LITERATURE CITED

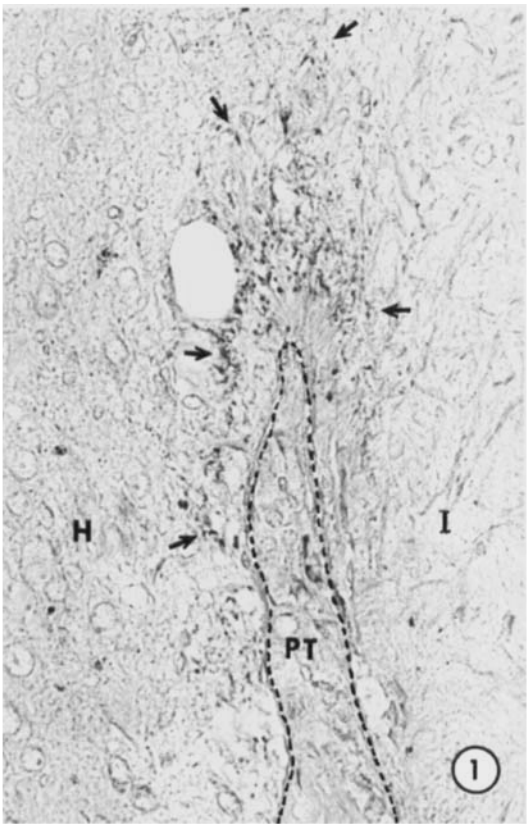
Barry, J., M. P. Dubois, P. Poulain and J. Leonardelli 1973 Caractérisation

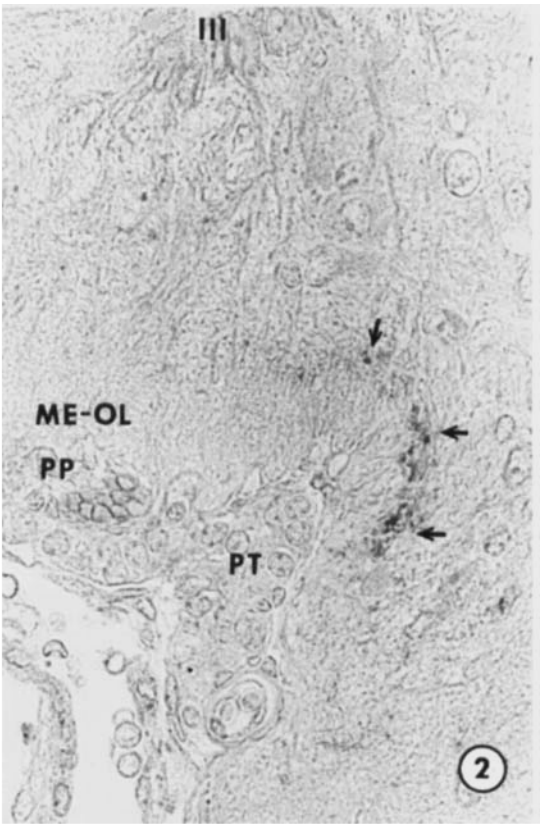
et topographie des neurones hypothalmiques immunoréactifs avec des anticorps ánti-LRF de synthèse. C. R. Acad. Sci. Paris, 276: 3191-3193.
Dermody, W. C., E. A. Becvar, B. L. Windsor and J. R. Reel 1973 A radioimmunoassay for luteinizing hormone releasing factor (LRF). Fed. Proc. 32: 239.
Leonardelli, J., J. Barry and M. P. Dubois 1973 Mise en évidence par immunofluorescence d'un constituant immunologiquement apparenté au LH-RF dans l'hypothalamus et l'éminence médiane chez les mammifères. C. R. Acad. Sci. Paris, 276: 2043-2046.
Mason, T. E., R. F. Phifer, S. S. Spicer, R. H. Swallow and R. B. Dreskin 1969 An immunoglobulin-enzyme bridge method for localizing tissue antigens. J. Histochem. Cytochem., 17: 563-569.
Nakane, P. K., and G. B. Pierce, Jr. 1967 Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens. J. Cell Biol., 33: 307-318.
Schally, A. V., A. Arimura and A. J. Kastin 1973 Hypothalamic regulatory hormones. Science, 179: 341-350.

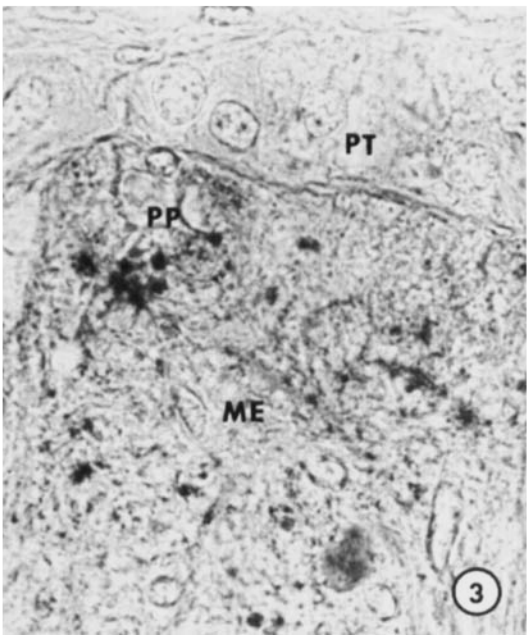
FIGURE LEGENDS

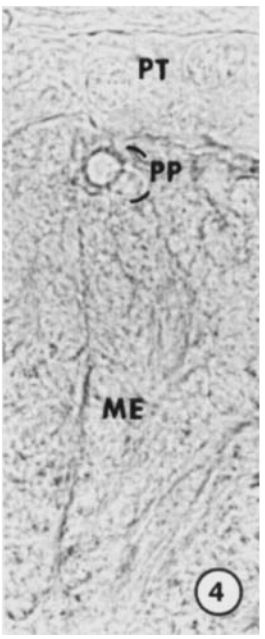
All preparations illustrated were stained with the Mason et al. procedure.

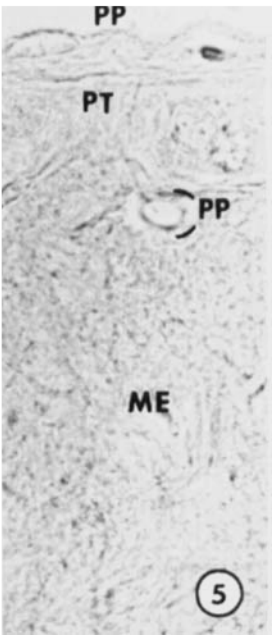
1 Parasagittal section through the median eminence (ME) and infundibular stem (I) of a female rat and including the pars tuberalis (PT) between the basal hypothalamus (H) and the stem. The section is oriented vertically with the lower side being caudal and the upper side cephalic. LRH, represented by granules (arrows), is in the nervous tissue encompassing the pars tuberalis. 3 μ. X 400.
2 Transverse section through one side of the median eminence (ME) of a female mouse. The lateral portion of the pars tuberalis (PT) lies beneath the median eminence. The primary plexus (PP) of blood vessels appears between the median eminence and the pars tuberalis. The third ventricle (III) is above. LRH (arrows) is localized in the peripheral portion of the external layer (OL) of the median eminence. 5 μ. X 400.
3 Transverse section through the dorsal portion of the guinea pig median eminence (ME). LRH is localized rather generally in the external layer that faces the pars tuberalis (PT). 5 μ. X 1000.
4 Control section adjacent to that shown in fig. 3. Staining was prevented by absorption of anti-LRH with synthetic LRH prior to the immunohistochemical procedure. 5 μ. X 1000.
5 Control section adjacent to that shown in fig. 3. Staining was prevented by substitution of normal rabbit serum for anti-LRH in the immunohistochemical procedure. 5 μ. X 1000.


H
I
PT
1


III
ME-OL
PP
PT
2


PT
PP
ME
3


PT
PP
ME
4


PP
PT
PP
ME
5

Vaitukaitus, J., J. B. Robbins, E. Nieschlag and G. T. Ross 1971 A method for producing specific antisera with small doses of immunogen. J. Clin. Endocr., 33: 988-991.

REFERENCE

1 Supported in part by NIH grant HD 03159-06.