Dense plexus of substance P immunoreactive nerve terminals in eminentia medialis of the primate hypothalamus

(central anterior pituitary control/releasing/inhibitory hormone/local regulator)

T. HÖKFELT^{a,b}, B. PERNOW^c, G. NILSSON^d, L. WETTERBERG^e, M. GOLDSTEIN^f, AND S. L. JEFFCOATE^g

Departments of ^a Histology and ^d Pharmacology, Karolinska Institute, Stockholm, Sweden; ^c Department of Clinical Physiology, Karolinska Hospital, Stockholm, Sweden; ^e Department of Psychiatry, St. Görans Hospital, Stockholm, Sweden; ^f Department of Psychiatry, New York University Medical Center, New York, New York; and ^g Queen Charlotte's Hospital for Women, London, England

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ABSTRACT The indirect immunofluorescence technique of Coons and collaborators was used to study the distribution of substance P-like and luteinizing hormone-releasing hormone (LHRH)-like immunoreactivity in the eminentia mediana (eminencia medialis or infundibulum) of rats, monkeys, and human beings. In rats, abundant LHRH-positive fibers were present in the external layer (mainly lateral parts) whereas almost no substance P-immunoreactive nerves were observed in this region. In contrast, in the external layer of the primate eminentia medialis a dense plexus of substance P-positive nerve terminals was observed close to the blood vessels supplying the anterior pituitary gland. LHRH-immunoreactive fibers were also present, but with a more even distribution all over the eminentia medialis and in lower numbers. The present findings indicate that substance P may play a role in the control of hormone secretion from the anterior pituitary, either by being released into the portal vessels, i.e., acting as a releasing or inhibitory hormone, or by an action as local regulator (modulator or transmitter) at the level of the eminentia medialis. Thus, in agreement with many earlier studies, substances other than the "classical" releasing and inhibitory hormones may be important for the regulation of the pituitary gland. Furthermore, there may exist marked species differences with regard to the type of substances involved in the central control of the pituitary.

According to the classical concept of Harris (1), hormone secretion from the anterior pituitary is controlled by so-called hypothalamic releasing or inhibitory factors. These substances have been assumed to be produced in neurons in the so-called hypophysiotrophic area (2) of the hypothalamus projecting to the infundibulum (eminencia medialis). Here they are released into the hypophyseal portal blood vessels and transported to the anterior pituitary to influence events related to hormone synthesis, storage, and/or release. Up till now three factors have successfully been isolated and structurally characterized from basal hypothalamus: thyrotropin-releasing hormone (3, 4), LHRH (luteinizing hormone-releasing hormone; LRH) (5, 6), and somatostatin (growth hormone release-inhibiting hormone) (7). There is evidence for several other factors to control, e.g., prolactin, growth hormone, and adrenocorticotropin (ACTH) secretion (for a recent review, see ref. 8).

It is obvious from biochemical and histochemical studies that in the infundibulum there are present several substances, the roles of which are only incompletely understood. These include "classical" transmitters, such as acetylcholine, γ -aminobutyric acid, and monoamines, especially dopamine, and also several peptides previously not associated with a direct involvement in the pituitary control (9, 10). Thus, e.g., in the rat, immunoreactive enkephalin (11) and angiotensin II (12) have been identified. On the other hand, substance P, a peptide originally discovered by von Euler and Gaddum (13) and structurally

characterized by Leeman and collaborators (14), is found both biochemically (15-18) and immunohistochemically (19) in very high concentrations in the hypothalamus in general, but is present only to a very small extent in the rat median eminence, especially in the external layer. In contrast, we report in this study the presence of an extremely dense plexus of nerve endings containing substance P-like immunoreactivity in the external layer of the eminentia medialis of monkeys and human beings. The localization suggests that, in primates, substance P may be involved in the control of the anterior pituitary secretion.

MATERIALS AND METHODS

Antisera to synthetic substance P and LRH were raised in rabbits as described (20, 21). Male and female albino rats (Sprague-Dawley, body weight 150-200 g), African green monkeys (*Ceropithecus sabaeus*, 3-5 years old), and human postmortem brains were studied. The human brains were from two women (age 40 and 77 years) and one man (age 89 years) and the postmortem time was 4-6 hr. The rats and monkeys were anaesthetized with Nembutal (40 mg/kg, intraperitoneally and 25 mg/kg intravenously, respectively) and perfused for 30 min with ice-cold 4% formalin prepared according to Pease (22). After dissection the brains were immersed for 90 min in the same fixative. Slices of the human brains were immersed in the same fixative for 4-6 hr. After the slices were rinsed in 5% sucrose in 0.1 M phosphate buffer for at least 24 hr, the hypothalamus were cut on a cryostat (Dittes, Heidelberg, German Federal Republic). The sections (10 μ m thick) were fixed on chrome alum gelatin-coated object slides and processed for the indirect immunofluorescence technique of Coons and collaborators (23). Briefly, the sections were incubated in a humid atmosphere for 30 min at +37° with antiserum against substance P (dilution 1:40), rinsed in phosphate-buffered saline, incubated under the same conditions with fluorescein isothiocyanate-conjugated sheep anti-rabbit antibodies (Statens Bakteriologiska Laboratorium, Stockholm, Sweden), rinsed in phosphate-buffered saline, mounted in phosphate-buffered saline/glycerol (1:3), and examined in a Zeiss Junior Fluorescence microscope. Substance P and LRH antiserum blocked with an excess of substance P and LRH (50 μ g/ml of antiserum diluted 1:10), respectively, served as control serum.

RESULTS

In rats only few substance P-positive fibers were found at all levels of the eminentia mediana of the hypothalamus (Fig. 1A). They are located in the internal layer, but occasionally single

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Abbreviation: LRH, luteinizing hormone-releasing hormone).

^b To whom reprint requests should be addressed.

FIG. 1. Immunofluorescence micrographs of the eminentia mediana of rats after incubation with antiserum against substance P (A) and against LRH (B) and of the eminentia medialis of green monkeys after incubation with antiserum against substance P (C). A and B show consecutive sections. In rats only a few substance P-positive fibers are seen in the median eminence (arrows in A), mainly in the internal layer (A), whereas a dense plexus of LRH immunoreactive fibers is present (B), especially in the lateral, external zone (double arrowhead in B). Note lack of substance P fibers in this part (double arrowhead in A), whereas the arcuate nucleus (ar) contains a medium dense network of substance P-positive fibers (A). Arrowheads in A point to unspecific, weakly fluorescent processes probably of glial origin (tanocytes). Asterisks in A and B indicate 3rd ventricle. In monkeys (C), a very dense plexus of substance P-positive fibers is seen in the external layer (arrowheads) but also around blood vessels (asterisks) in deeper parts of the eminentia medialis (double arrowheads). Large arrowhead in C points to 3rd ventricle. Bar indicates 20 μ m.

fibers may penetrate into the external layer and reach the surface vessels, particularly in the medial parts. In contrast, staining of consecutive sections with antiserum against LRH revealed a very dense plexus of fluorescent fibers in the median eminence (Fig. 1B). At a rostral level they are present both in the medial and lateral parts mainly of the external layer; more caudally, the LRH-positive fibers are preferentially found in the lateral parts of the external layer. This distribution agrees

well with the results of Barry *et al.* (24) and of many other laboratories.

In *monkeys* and *human beings* a very dense plexus of substance P-positive fibers was observed in the eminentia medialis (Fig. 1C). The highest concentrations were observed in the most superficial zone of the external layer close to blood vessels. The fibers also surround blood vessels located at some distance from the surface. After parallel sections were stained with antiserum

against LRH, a considerably less dense network of fluorescent fibers was observed with a more even distribution over most of the eminentia medialis. Several LRH-positive cell bodies were observed in the adjacent infundibular nucleus. These findings confirm earlier studies, e.g., by Barry and Carette (25).

DISCUSSION

The present findings give evidence for dense networks of nerve terminals containing substance P, or a similar peptide, in the eminentia medialis of monkeys and human beings. Quantitatively, the substance P-immunoreactive plexus in this area is clearly of a higher density than that of LRH and thyroliberin and approximately equal to the somatostatin-positive network. Also, the number of tyrosine hydroxylase-positive fibers, mainly representing dopamine nerve endings, is lower than that of the substance P-immunoreactive ones. This has been established in parallel experiments by staining consecutive sections with antibodies to the latter two peptides and the catecholamine-synthesizing enzyme (T. Hökfelt, R. Elde, S. L. Jeffcoate, and M. Goldstein, unpublished data). In rats a different situation exists. Dense networks of LRH-, thyroliberin-, tyrosine hydroxylase-, and particularly somatostatin-positive nerves are seen in the external layer of the median eminence, whereas hardly any nerve endings react with antiserum against substance P (see ref. 10). On the other hand, the rat median eminence contains numerous enkephalin-immunoreactive nerve endings (10), whereas so far we have not observed this peptide in the primate eminentia medialis. These findings indicate that important species differences exist with regard to the type of systems projecting to the infundibulum. Thus, distinctly different mechanisms may operate in the hypothalamic control of the pituitary function in primates as compared to some rodents.

The function of substance P in the eminentia medialis of primates is not known. The high concentrations of substance P-immunoreactive terminals in the external layer close to the portal vessels could suggest that it may be released into the blood and transported to the anterior pituitary. Should this be the case, substance P represents a new hypothalamic releasing or inhibitory hormone. Alternatively, it may act at the hypothalamic level as a transmitter or modulator. Such a role has been proposed for dopamine in the rat median eminence (26). Possibly via an axo-axonic influence, dopamine inhibits (26, 27) or, according to other groups, stimulates (28) the LRH release. Dopamine may, in addition, be released into the portal vessels and may inhibit prolactin release at the pituitary level (29). We cannot exclude the possibility that substance P also has such a dual function.

The origin of the substance P-containing nerve endings in the external layer of the median eminence is not known. In rats, numerous hypothalamic substance P-immunoreactive cell bodies have been observed, including the ventromedial and the dorsomedial nucleus (30). Substance P-positive cell bodies have been observed in the basal hypothalamus of human beings (unpublished data), but more detailed studies are necessary to trace the origin of the substance projections to the external layer of the median eminence.

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