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ADRENOCORTICOTROPIN AND ß-ENDORPHIN ARE COLOCALIZED IN THE NERVOUS SYSTEM OF RAT DUODENUM

Hermann J. Wolter

Universität Ulm, Abteilung Anatomie, 7900 Ulm, Oberer Eselsberg, FRG

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SUMMARY: Adrenocorticotropin and ß-endorphin-like immunoreactivities were visualized by an immunohistochemical method on adjacent serial sections of the nervous system of the rat duodenum. Perikarya lying in the myenteric plexus and stained alternately for ACTH or ß-endorphin, showed on two adjacent serial sections a co-existence of these two peptides within one and the same perikarya. A colocalization of ß-endorphin and ACTH is not demonstrated with certainty in the submucous plexus. These results may be evidence for a common occurence of the two peptides within perikarya of the rat duodenum.

Adrenocorticotropin (ACTH) and ß-endorphin are cleaved enzymatically from its common precursor molecule, the pro-opiomelanocortin (POMC) (1), and were demonstrated within neurosecretory pituitary cells as well as neuronal cell bodies and nerve fibres of the brain of various mammalian species by using biochemical and immunohistochemical methods. An ACTH- and ß-endorphin-like immunoreactivity have also been demonstrated by chromatographic methods in nerve tissue extracts of the mammalian gastrointestinal tract (15-19).

In the rat brain, ACTH and ß-endorphin were acertained by light- and electronmicroscopic studies predominantly in hypothalamic magnocellular neurons, and, in considerable amounts, also in extra-hypothalamic regions (2, 3-6). Both antisera stained on adjacent serial sections the same perikarya in rat hypothalamic magnocellular nuclei (4),

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the arcuate nucleus (7), and the human infundibular nucleus (14).

Studies on ultrastructural level demonstrated that the two peptides were colocalized within the same granulas of anterior pituitary cells (8).

In previously conducted experiments an ACTH as well as ß-endorphin-like immunoreactivity was revealed within neuronal cell bodies (9,10), and nerve fibres (20,21) of both the myenteric and submucous plexus of the rat duodenum by using specific antisera directed against synthetic ACTH - and ß-endorphin, respectively.

This study was conducted to reveal an immunohistochemical colocalization of the two peptides in neuronal structures of the rat duodenum and to discuss these findings in regard of its well-known existence in perikarya and nerve fibres of both the rat brain and pituitary.

METHODS

ACTH- and ß-endorphin antisera were raised in rabbits by immunization with its corresponding peptide (Immuno Nucl.Corp, Stillwater, MN). The two antisera were tested for staining specificities by immunohistochemical blocking tests. The preabsorption of the ACTH antiserum with synthetic ACTH prevented all the staining patterns for ACTH (1-10 µM). The same procedure was accomplished with the ß-endorphin antiserum and resulted in the same findings. Preincubation of the ACTH antiserum with ACTH-related (ACTH 1-24, ACTH 1-10, and α-MSH) (10 µM) and unrelated peptides (ß-endorphin and Met-enkephalin) (10 µM) revealed no change of the ACTH staining patterns. Synthetic Met-enkephalin and ACTH (1-39) (10 µM) blocked not the ß-endorphin immunofluorescence. Therefore, the antigenic site of the two antbody molecules seems to be located rather on its C-terminal ends. However, it is possible that subpopulations of the two antibodies recognize in the enteric nervous system also still uncharacterized peptides with amino acid sequences similar to ACTH and ß-endorphin. Control sections were also incubated with normal rabbit IgG (10 %). In this experiment no fluorescence for ACTH or ß-endorphin was visualized.

Male, colchicine-untreated rats (200-300 g) were deeply anaesthetized with Nembutal (50 mg/kg), the duodenum dissected and subsequently cut in consecutive

sections (2,5 µM) on a cryostat microtome (-25°C). The sections were fixed by immersion in fresh depolimerized paraformaldehyd (4 %, in 100mM phosphate-buffered saline, PBS; pH 7.4) for 90 min. After several rinsings the sections were stored in the same puffer,containing 5,0 % sucrose, overnight, at 4°C, followed by the incubation with a specific antiserum for ACTH or ß-endorphin (1:200 in PBS, containing 0.3 % Triton X-100) as well as normal swine IgG (10 %) for 16 hr. at 4°C. The sections were rinsed several times again and incubated with fluorescence iso-thiocyanate (FITC-) conjungated rabbit anti-swine IgG (90 min., 4°C), coverslipped and examined under a Zeiss epifluorescence microsocope.

RESULTS AND DISCUSSION

Adjacent serial sections through the rat duodenum showed a completely identical and nearly superimposable staining pattern for ACTH and ß-endorphin within neuronal cell bodies of the myenteric plexus. Five strong ACTH-stained perikarya are visualized in the rat myenteric plexus (Fig. 1a). The ACTH staining demonstrated in the submucous plexus seems to be localized on small nerve tissue, but the intensity of the ACTH-immunofluorescence corresponds with the staining within the perikarya of the myenteric plexus. The same perikarya, which are ACTH-positive stained, are also stained by the ß-endorphin antiserum on adjacent serial sections (Fig. 1b). The nervous tissue of the submucous plexus is only weak stained for ß-endorphin as it was localized outside the focus region. The co-existence of the ACTH or ß-endorphin immunofluorescence was rarely successful in the submucous plexus as the nerve tissue there is considerable smaller than in the myenteric plexus and therefore scarcely identical structures are obtainable on adjacent serial sections.

The evidence of an immunohistochemical colocalization of opioid peptides, originating from a common precursor

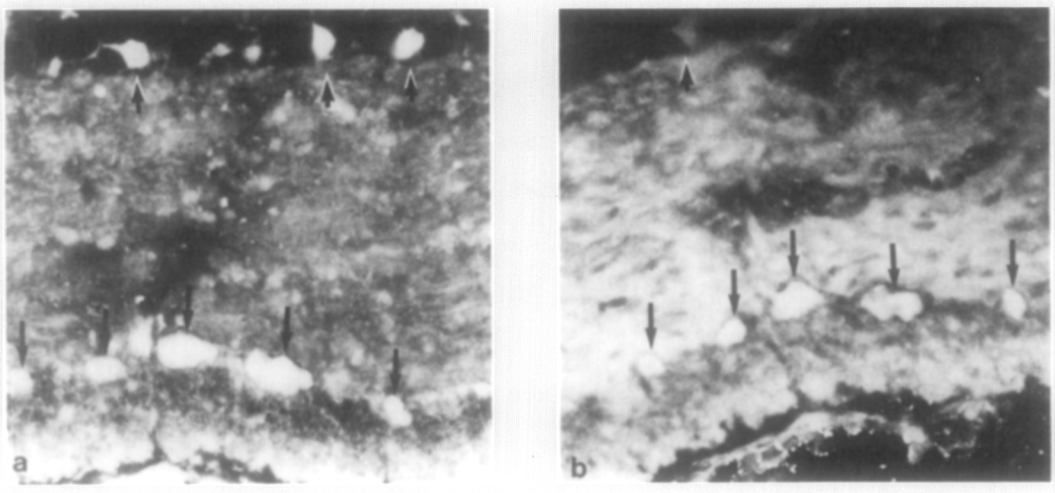

Fig. 1.

Two consecutive serial sections (2,5 µm) through the myenteric plexus of the rat duodenum are alternately stained with ACTH- or ß-endorphin antiserum. The two antisera have stained five neuronal cell bodies (long arrows), which are located side by side in virtually identical staining patterns. The same perikarya of the myenteric plexus are brightly immunofluorescent by the ACTH antiserum (a) and, but some faintly, also by the ß-endorphin antibodies (b). The nerve tissue of the submucous plexus (small arrows and small double-arrow) shows an ACTH-immunofluorescence. But only a small part of this nerve tissue can be visualized by staining with ß-endorphin antiserum (small double arrow). Technical reasons prevented the preparation of a photomicrograph, which shows identical staining patterns in the myenteric as well as submucous plexus of the rat duodenum (x 375).

peptide, succeeded within neuronal cell bodies of the rat brain. However, the staining patterns for these peptides was partially restricted to cell subpopulations (11).

The common appearance of peptides in one and the same neuronal cell body and its fibres may reflect a combined biosynthetic pathway for the two peptides. Indeed, simultaneous ACTH- and ß-endorphin secretion has been demonstrated *in vitro* and *in vivo* by the use of RIA- and immunohistochemistry techniques in the rat pituitary. Stimulation tests with the synthetic ovine corticotropin-

releasing factor (CRF) (12) showed a significant increase of the ACTH and ß-endorphin as well as corticosterone level in the plasma, hypophyseal portal blood, and cell culture supernatant (11-13). Pre-treatment of cultured anterior pituitary cells with dexamethasone resulted in a dose-related inhibition of the CRF-mediated ACTH- and ß-endorphin release.

Possible interactions between the duodenal ACTH/ß-endorphin system on the one site and the ACTH/ß-endorphin system of the hypothalamo-hypophyseal-adrenal axis on the other site must be elucidated in further investigations.

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