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Light and electron microscopic localization of the N-terminal fragment of human pro-opiomelanocortin in the human pituitary gland and in neoplasms*

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Summary. Immunohistochemical localization of the *N*-terminal fragment (1–76) (NTF) of human pro-opiomelanocortin (POMC) was studied in human adult and fetal pituitary glands, as well as in pituitary adenomas associated with Cushing's syndrome and in ectopic ACTH-producing tumors. Comparison of localization between NTF and ACTH was performed using mirror sections. Our results indicated concomitant localization of NTF and ACTH in the same cells, not only in normal adult and fetal pituitaries but also in pituitary adenomas and ectopic ACTH producing tumours. Specificity of the NTF staining was confirmed by immunoabsorption. Negative staining of the bovine pituitary gland indicated the immunohistochemical localization of *N*-terminal (1–45) of human POMC as there is a known species difference in the sequence 1–45 between human and the bovine *N*-terminal fragment. Presence of NTF in cisterna of rough endoplasmic reticulum indicates its production by small cell carcinoma. These findings, together with the previous studies, suggest that the complete form of POMC is produced in the tumours as well as in normal pituitaries.

Key words: Immunohistochemistry – Electron microscopy – ACTH – Pituitary gland – Neoplasm

Introduction

Peptides such as adrenocorticotropin (ACTH), β-lipotropin (β-LPH), β-endorphin, and γ-MSH are derived from a larger precursor molecule termed pro-opiomelanocortin (POMC). The 267-amino acid sequence of bovine

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POMC has been clarified by Nakanishi et al. (1979) by using cDNA recombinant techniques. Immunohistochemical studies on ACTH, the endorphins, and the various forms of MSHs have been extensive for the hypothalamus, pituitary, and tumours, but investigations of localization of the *N*-terminal fragment have been few. We, together with other inverstigators (Bloom et al. 1977; Mains and Eipper 1978; Mendelsohn et al. 1979; Moon et al. 1973; Osamura et al. 1981, 1981; Pelletier et al. 1976; Weber et al. 1978) have previously reported localization of ACTH, β-endorphin and γ-MSH in various tissues.

Recently, Seidah and Chrétien isolated the *N*-terminal 1–76 fragment (NTF) of human POMC and determined its amino acid sequence (Seidah and Chrétien 1981). Sequences (45–70) of NTF are the same as that of the analogous bovine fragment, except for the absence of Gly at 66.

This report describes immunohistochemical localization of human NTF in the human pituitary, as well as in neoplasms including pituitary adenomas and ectopic ACTH-producing tumours. Ultrastructural localization of NTF was also attempted in a case of small cell carcinoma of the lung.

Materials and methods

The immunohistochemical localization of human NTF was done on human fetal (11 weeks, 16 weeks old), adult (without endocrinologic problems) pituitaries, bovine pituitary, 3 pituitary adenomas associated with Cushing's syndrome and 3 ectopic ACTH-producing tumors (bronchial and thymic carcinoid, small cell carcinoma of the lung) following the methods previously described (Osamura et al. 1980, 1981, 1982, 1984).

The light microscopic immunohistochemical staining procedure was performed as follows. The tissue was fixed in 10% buffered formalin. 2–4 μm, paraffin sections were deparaffinized and hydrated in PBS. Endogenous peroxidase activity was inhibited by incubating the sections in 5 mM sodium periodate (in water) for 10 min. After washing in PBS, the sections were incubated with anti-human NTF rabbit serum (lot # 25.3.7 Diluted 1:1,000 in PBS) for 15 min. After thorough washing in PBS for 10 min, the sections were reacted with horseradish peroxidase-labeled goat IgG Fab raised against rabbit IgG (1:40 in PBS) for 15 min. Incubation with these antisera was done in a moist chamber. After thorough washing in PBS, the sections were stained in 0.02% 3,3′ diaminobenzidine 4HCl solution in 0.01 M Tris HCl, pH 7.6, containing 0.005% hydrogen peroxide, for 2 to 5 min. Subsequently, the sections were counterstained with methyl green, dehydrated in an alcohol gradient, and mounted.

Immunoelectron microscopic staining was performed by the method described previously (Osamura et al. 1982, 1984). In brief, small pieces of tissue from the serially transplanted small cell carcinoma in nude mouse were fixed in periodate lysin paraformaldehyde (PLP) solution (Mclean and Nakane 1974) overnight at 4° C. After thorough washing in PBS which contained graded concentration of sucrose up to 20%, the tissue blocks were quickly frozen in alcohol dry ice and 4 μ frozen sections were obtained by cryostat and attached to albuminized glass slides. The anti-NTF antibody and peroxidase conjugated second antibody were reacted on sections overnight respectively at 4° C. The sections were then fixed in 2% glutaraldehyde in SPB at pH 7.2 for 30 min and were incubated in DAB solution without hydrogen peroxide for 1 h. Subsequently, they were colorized in DAB solution with 0.005% hydrogen peroxide for 2 to 5 min. The sections were then postfixed by 2% osmic acid in 0.1 M SPB at pH 7.2 for 1 h, dehydrated in graded alcohol and embedded in Quetol by inverted gelatin capsule method (Osamura et al. 1982, 1984). Ultrathin sections were observed by JEOL 100C electron microscope.

Immunoabsorption test was performed immunohistochemically by 0.4 ml of anti-NTF (1:1000 in PBS) antiserum which had been preincubated with 40 μ g human NTF, γ1-MSH, γ3-MSH or ACTH 1–24 (Cortrosyn, Organon) respectively overnight at 4°C. Other immunohistochemical control studies included omitting the application of the primary antibody and/or peroxidase conjugated second antibody.

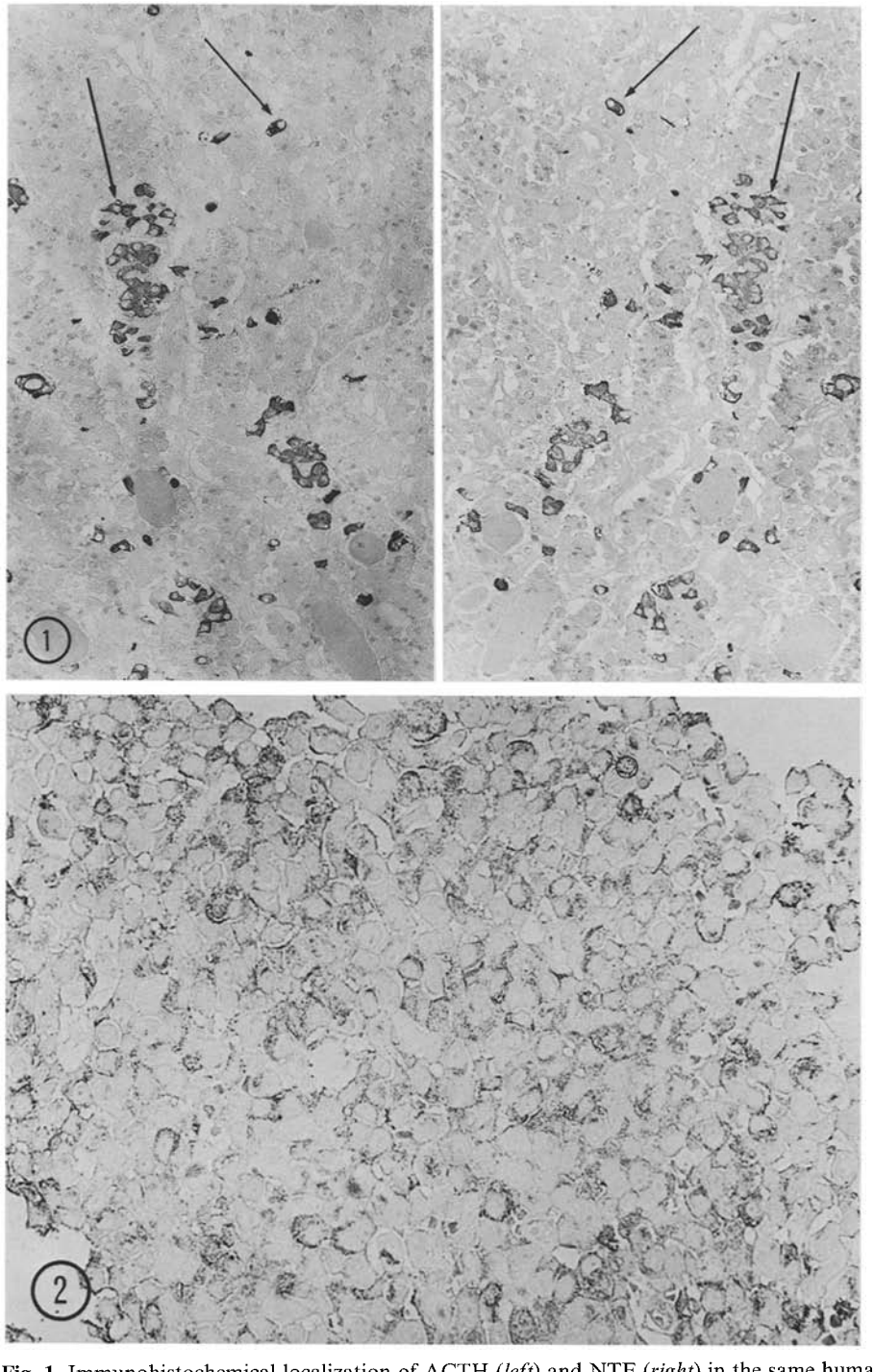

Fig. 1. Immunohistochemical localization of ACTH (*left*) and NTF (*right*) in the same human anterior pituitary cells. (Mirror sections. Corresponding *arrows* indicate the same cells ×180)

Fig. 2 Immunohistochemical staining of NTF in human pituitary adenoma. Most of the tumour cells are positive for NTF and also positive for ACTH. (×430)

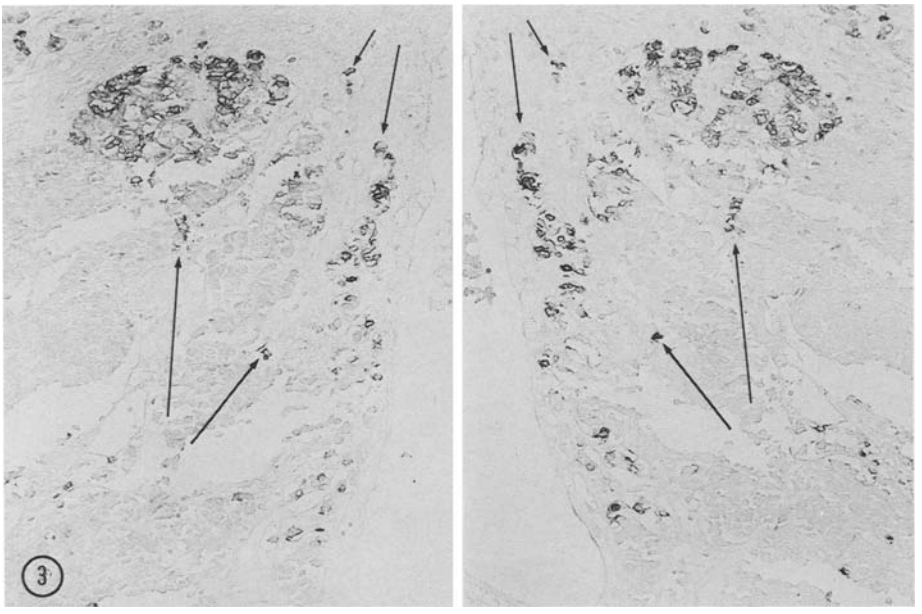

Fig. 3. Immunohistochemical co-localization of ACTH (*left*) and NTF (*right*) in the same cells of thymic carcinoid (ectopic ACTH producing tumour). (Mirror sections. Corresponding *arrows* indicate the same tumour cells ×300)

In order to confirm the co-localization of NTF with ACTH (17–39), the previously described mirror section technique was used (Osamura et al. 1980, 1981). In brief two consecutive paraffin sections were placed on a glass slide with their surfaces facing upward, so the sectioned surfaces of the same cells could be stained by two different antisera. The anti-ACTH 17–39 was prepared in our laboratory and its specificity has been previously reported (Osamura et al. 1977).

Results

The human adult anterior pituitary glands contained many NTF immunoreactive cells. The glial tissue of the posterior lobe, connective tissue or vascular endothelial cells were negative immunohistochemically. Most of the invading anterior lobe cells (Osamura and Watanabe 1978) were positive for NTF. By mirror section technique, NTF and ACTH(17–39) of POMC were localized in the same cells of anterior lobe (Fig. 1) as well as of the invading anterior cells. Those two antigens were also co-localized in the same cells of the human fetal pituitary glands.

In human pituitary adenomas associated with Cushing's syndrome, all three cases showed the co-localization of NTF (Fig.2) and ACTH 17–39 sequence in the same tumour cells. The cells containing these antigens were diffuse or rather selective in the tumour. Two cases of carcinoid tumours and a small cell carcinoma of the lung showed many NTF cells which were scattered or clustered. In these ectopic ACTH-producing tumours, NTF and ACTH (17–39) sequences were also present in same tumours cells (Fig. 3). Immunoelectron microscopically, in the small cell carcinoma of the lung, the tumours cells contained NTF in cisternae of predominant networks of rough

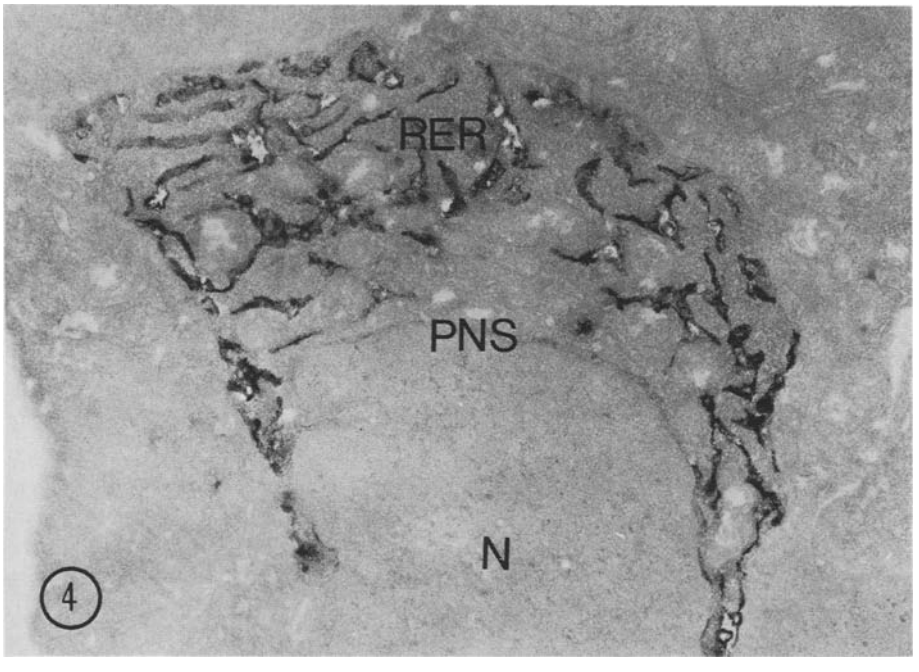

Fig. 4. Immunoelectron microscopy localization of NTF in human small cell carcinoma of the lung. NTF is localized in cisternae of networks of rough endoplasmic reticulum (RER) and in perinuclear space (PNS). (×11,000)

endoplasmic reticulum (RER). Secretory granules were rare and were positive for NTF.

By immunoabsorption test, immunohistochemical staining by anti-NTF antiserum disappeared completely after preincubation with NTF but was not affected by ACTH 1–24, γl-MSH or γ3-MSH. Omission of the application of the primary antibody and/or peroxidase conjugated second antibody gave negative immunohistochemical staining.

Discussion

Immunohistochemical localization of NTF in the rat has been previously described by Cantin et al. (1983), who demonstrated concomitant localization of NTF and ACTH in the same secretory granules of rat anterior pituitary, and who further suggested production of the precursor molecule in the rat pituitary.

We believe that this is the first report on immunohistochemical localization of NTF in the human fetal and adult pituitary, as well as in the neoplasms including pituitary adenomas associated with Cushing's syndrome and ectopic ACTH secreting carcinoids or small cell carcinoma of the lung. The previous biochemical report (Seidah and Chrétien 1981; Chan et al. 1983) described the specificity of the antibody as being against the 1–51

sequence of POMC as the sequence 45–76 of human NTF is structurally similar to that of bovine NTF. Our preabsorption test by immunohistochemical staining confirmed the specificity of staining for NTF. Negative staining for NTF in the bovine pituitary further suggested a specificity of staining for the 1–45 sequence of the *N*-terminal of POMC as the species difference lies in these sequences (Seidah and Chrétien 1981; Chan et al. 1983). Our immunohistochemical studies showed NTF and ACTH 17–39 co-localized in the same cells under the various conditions described above. On the basis of this and our previous observations concerning the localization of β-endorphin (Osamura et al. 1980) and γ-MSH (Osamura et al. 1981), it was suggested that anterior pituitary cells and invading anterior cells, as well as the fetal pituitary, contain many cells which produce the entire sequecne of POMC. Both pituitary adenomas and ectopic ACTH-producing tumours contain many cells that can be considered to be capable of producing the entire precursor. Immunoelectron microscopically, the presence of NTF in the cisternae of RER in small cell carcinoma of the lung indicates the actual production of NTF sequence in the tumour cells. This could be demonstrated only by the immunoelectron microscopic pre-embedding method (Osamura et al. 1982). Similar ultrastructural localization of ACTH, β-endorphin in small cell carcinoma and in human fetal pituitary gland has been reported from our laboratory (Osamura et al. 1984). These findings may further support the production of complete precursor in the lung carcinoma. However, it has been reported that glycosylation is different in the ectopic POMC from that of POMC produced in the pituitary gland (Nakai et al. 1981).

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