

Immunohistochemical localization of hormones and peptides in the human pituitary cells in a case of hypercortisolism by ACTH secreting microadenoma

R. Reyes¹, F. Valladares², L. Díaz-Flores², L. Feria³, R. Alonso⁴, G. Tramu⁵ and A.R. Bello¹

¹Section of Cell Biology, School of Biology, University of La Laguna, Spain, ²Dept. of Pathological Anatomy and Histology, School of Medicine, University of La Laguna, Spain, ³Neurosurgery Service, University Hospital, University of La Laguna, Spain and ⁴Dept. of Physiology, School of Medicine, University of La Laguna, Spain and ⁵Laboratoire de Neurocytochimie Fonctionnelle, Talence, France

Summary. This study assesses the action of hypercortisolism on the hormone and peptide peradenoma region of removed ACTH-producing microadenoma. Our findings show that cortisol excess affects both ACTH and GH production, with no immunoreaction for these hormones. The remaining pituitary hormones (TSH, FSH and PRL) and POMC-derived peptides (β End, α MSH and β MSH) were not modified. Likewise, we observed pituitary immunoreactive cells for Neurotensin (NT), Intestinal vasoactive peptide (VIP), Substance P (SP) and Angiotensin-II (Ang-II). The colocalization demonstrated that NT was expressed in thyrotrope and gonadotrope cells, VIP in gonadotrope cells and SP in corticotrope cells. The results about Ang-II were inconclusive. On the other hand, immunoreaction for the NPY and Gal peptides were not present. In the adenomatous cells, the peptide NT is present in ACTH cells as well as SP. These results suggest a peptide regulation of pituitary cells in the pathological state that can differ between normal and tumoural cells of the same pituitary.

Key words: Cushing's syndrome, Microadenoma, ACTH, Peptides, Immunohistochemistry

Introduction

Numerous studies about hormone production in normal and tumoural pituitary tissue have been undertaken (Clerico et al., 1993; Smallbridge et al., 2003; Suzuki et al., 2003; Zangeneh et al., 2003); however, the effects of cortisol excess on hormone and

peptide production by the human pituitary tissue require further study, given the difficulties involved in obtaining adequate samples.

In Cushing's syndrome there is a negative corticosteroid feedback mechanism, in such a way that removal of the hyperplastic adrenal gland in patients with pituitary corticotroph adenoma can result in rapid growth of the pituitary neoplasm (Nelson et al., 1960). In this way, the majority of pituitary corticotroph microadenomas are responsible for pituitary-dependent Cushing's syndrome. Indeed, in a study of 260 cases of corticotroph adenoma, 66.9% presented Cushing's disease, while the rest were found to have Nelson's syndrome or were hormonally silent (Thapar et al., 1992). The non-tumoural tissue from the peradenoma region of removed ACTH-producing pituitary microadenoma may be used to study the action of hypercortisolism on hormone and peptide production by the normal pituitary tissue. Our objective was the study of this peradenomatous tissue as well as the expression of peptides in tumoural tissue.

Materials and methods

Pituitary sample

Adenomatous and normal peradenomatous tissue of human pituitary was obtained from a removed corticotroph microadenoma of a 38-year-old female patient presenting Cushing's syndrome. Previous to the surgical intervention, the increased basal ACTH production was 175.1 pg/ml (nv 9-52). The cortisol was 37 mcg/dl in blood (basal condition) (nv 9-52) and 514.3 mcg/24 h in urine (nv 25-120).

Tissue preparation

The sample was fixed with 4% paraformaldehyde

Offprint requests to: Dra. Aixa R. Bello, Department of Cell Biology, School of Biology, University of La Laguna. Avda. Astrofísico Fco. Sánchez s/n, 38206 La Laguna, Tenerife, Spain. e-mail: abello@ull.es.

and 0.2% picric acid in PBS containing 20% saccharose. Part of the specimen was processed for paraffin histology according to standard techniques. The sections (7 μ m thick) were stained with haematoxylin and eosin (H&E). The other part of the specimen was frozen in isopentane cooled with liquid nitrogen, cut into 5 to 8 μ m sections and mounted on gelatin-coated slides for immunohistochemistry.

Immunohistochemistry

The indirect immunohistochemical procedure was carried out by incubating the sections overnight at room temperature with antisera against derivatives from POMC (ACTH-1-24), ACTH-17-39 (dilution 1/1000), α MSH, β MSH, β End (dilution 1/800), FSH (1/1000), TSH, GH, PRL (1/800), and the regulatory peptides NT, VIP, SP and Ang-II (dilution 1/200). Sections were quenched of endogenous peroxidase activity with 0.1% hydrogen peroxide. Peroxidase activity was revealed in Tris-HCl buffer (pH 7.6; 0.05M) with 0.04% 4-chloro-1-naphthol (Sigma) and 0.001% hydrogen peroxide.

For colocalization, an elution-restaining procedure (Tramu et al., 1978) was used, which enabled a second immunohistochemical sequence using antibodies against FSH (1/1000), TSH (1/800), β MSH (1/800) and GH (1/800). Peroxidase activity was revealed through 0.005% 3,3-diaminobenzidine tetrahydrochloride (Sigma) and 0.004% hydrogen peroxide in Tris-HCl buffer.

Antibodies

As primary antibodies we used antisera developed in rabbit against adenohipophysary hormones and different regulatory peptides: ACTH (1-24), ACTH (17-39), α MSH, β MSH, β End, TSH, STH, FSH, PRL, NT, VIP, SP and Ang-II. Their immunological properties have been described in different research works (Dubois, 1972a,b; Tramu and Dubois, 1977; Hemming et al., 1986; Bello et al., 1991, 1992; Jamali and Tramu, 1999). The specificity of the immunostaining was assessed by replacing the specific antiserum by normal serum, omitting one step of the reaction, or following preabsorption of the antiserum with the corresponding antigen.

Results

Hormones

The pituitary tissue from the peradenoma region revealed a normal histological structure. Likewise, immunoreactions for TSH, FSH, Prolactin (Fig. 1b-d) and for POMC derived peptides, α MSH-ir, β MSH-ir and β End-ir (Fig. 2b-d), were positive and showed a normal distribution, although the response of β Endorphine was weaker and the immunoresponse for ACTH was

negative (Fig. 2a). Likewise, the immunoresponse for GH (Fig. 1a) was also negative.

Peptides

Neurotensin (NT) (Fig. 3a), vasoactive intestinal peptide (VIP) (Fig. 3b), substance P (SP) (Fig. 3c) and Angiotensin-II (Ang-II) (Fig. 3d) were present in some immunoreactive cells. No immunoreaction was found for Galanin (Gal) and Neuropeptide Y (NPY). The colocalization showed that NT was present in thyrotrope (TSH-ir) (Fig. 4a,b) and gonadotrope (FSH-ir) (Fig. 4c,d) cells, VIP in gonadotrope cells (FSH-ir) (Fig. 5a,b) and SP in corticotrope cells (β MSH-ir) (Fig. 5c,d). Ang-II-producing cells could not be determined. In the microadenoma of ACTH-ir cells (Fig. 6a), intense immunoreaction for NT was observed (Fig. 6b); The NT immunoreactive cells are also ACTH-ir (Fig. 6c,d). In addition, in adenomatous cells, intense SP immunoreaction (Fig. 6e) was observed.

Discussion

Our results show that in patients with Cushing's syndrome the normal pituitary tissue does not produce ACTH due to an inhibitory effect of cortisol on corticotrope cells. On the contrary, other hormones and peptides produced in the same cell type, such as α and β MSH, and β endorphine (Rosa et al., 1980), are present in a normal pattern, which suggests that they are regulated by different peripheral factors. The remaining hormones are expressed normally, except for GH, which was absent. In this regard, there is evidence relating corticotrophic and somatotrophic activity (Loli et al., 1998). These authors suggest a paracrine action of the ACTH released by the tumor, on the peradenomatous GH and PRL cells. Corticotrophin-releasing hormone may act as a growth hormone-releasing factor in some vertebrates (Rousseau et al., 1999). Likewise, corticoid receptors are present in somatotrope cells (Yokote et al., 1991). Given our results, a dual inhibitory action of cortisol is likely on both hypothalamic CRF and pituitary somatotrophic cell GH production. Other works have demonstrated that patients with Cushing's syndrome, present alterations on the GH axis (Wajchenberg et al., 1996; Leal-Cerro et al., 2002).

With regard to peptide production, our results reveal that there are immunoreactive cells for NT, VIP, SP and Ang-II in peradenomatous human pituitary tissue. These peptides are present in specific cell types: NT in thyrotrope and gonadotrope cells, VIP in gonadotrope cells and SP in corticotrope, β MSH-ir cells. Neurotensin has been proposed as a neurohormone involved in the regulation of anterior pituitary secretions after being released from nerve terminals to the median eminence (Vijayan et al., 1994); expression in the pituitary gland has been extensively studied in the rat (Goedert et al., 1984; O'Halloran et al., 1990; Bello et al., 1992, 1999)

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and its presence has also been demonstrated in thyrotroph and gonadotroph cells. These observations indicate that NT expression in anterior pituitary cells is sexually dimorphic in adult rats and that sex steroids are able to regulate the expression of this peptide in both sexes. Results shown during postnatal development in the pituitary rat (Bello et al., 2004), confirm the

suggestion that NT is involved in modulate reproductive function. In humans, the only data about presence of NT in pituitary cells have been demonstrated by Reyes et al. 1998, and Reyes et al.,work in progress). In these works, the authors show the presence of NT during the embryonic development until the moment of birth, finding a similar colocalization to the rat, with

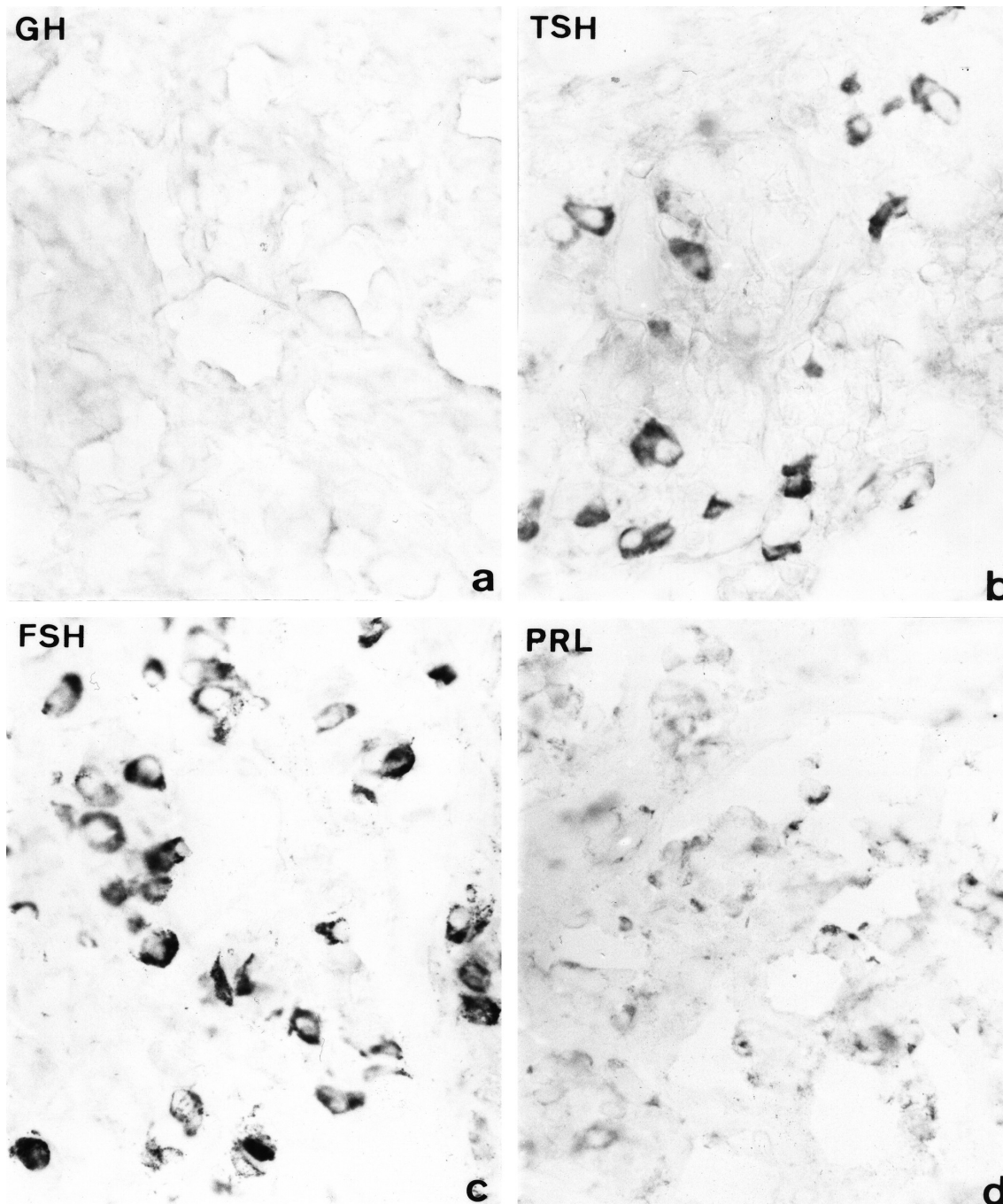


Fig. 1. Presence of pituitary hormones in periadenomatous cells in a female with Cushing's syndrome by ACTH secreting microadenoma. **a)** GH, **b)** TSH, **c)** FSH and **d)** PRL. x 600

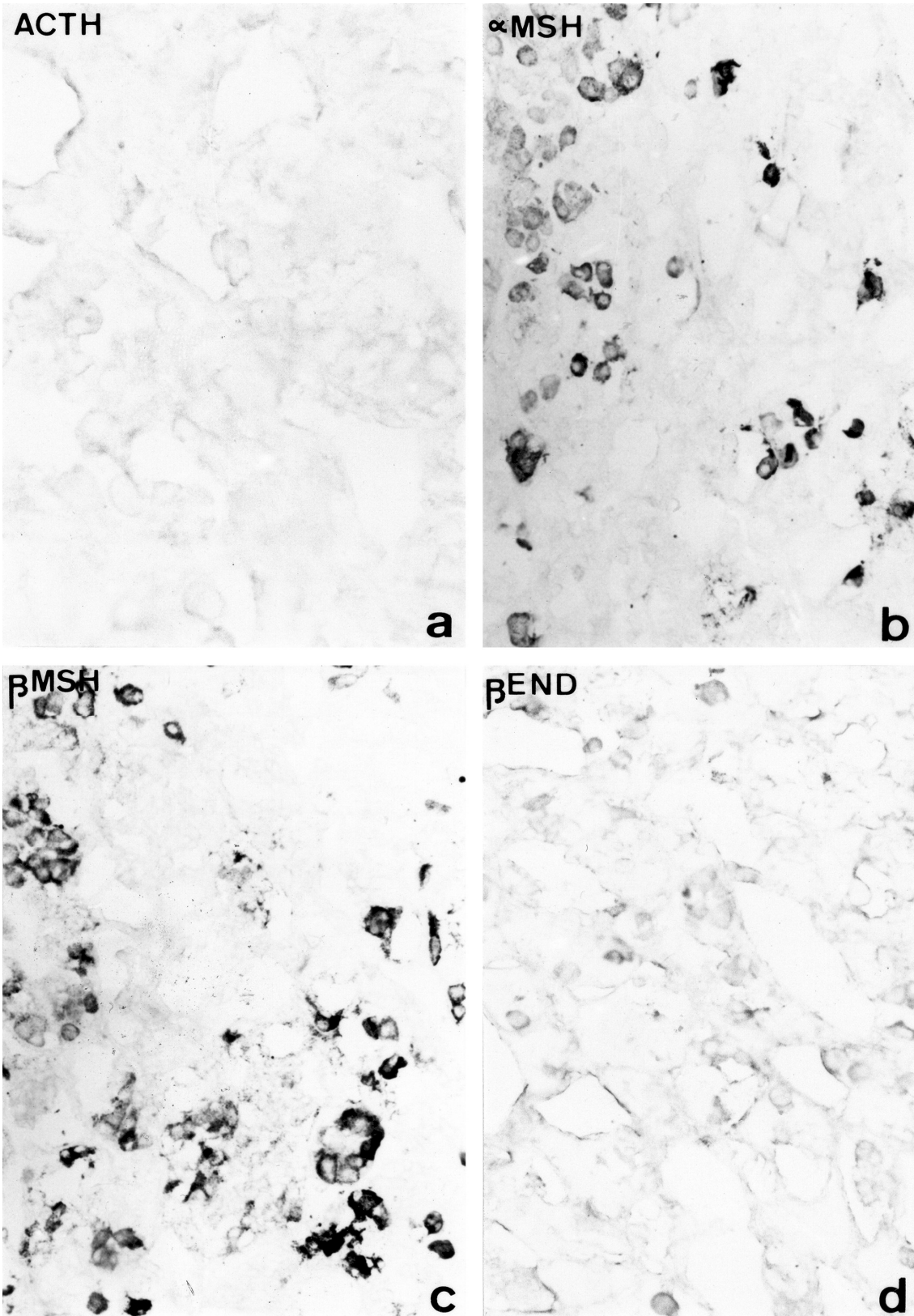


Fig 2. Presence of POMC derived hormones in pituitary cells in a female with Cushing's syndrome by microadenoma ACTH secreting; peradenomatous tissue. a) ACTH, b) α MSH, c) β MSH and d) β End. x 380

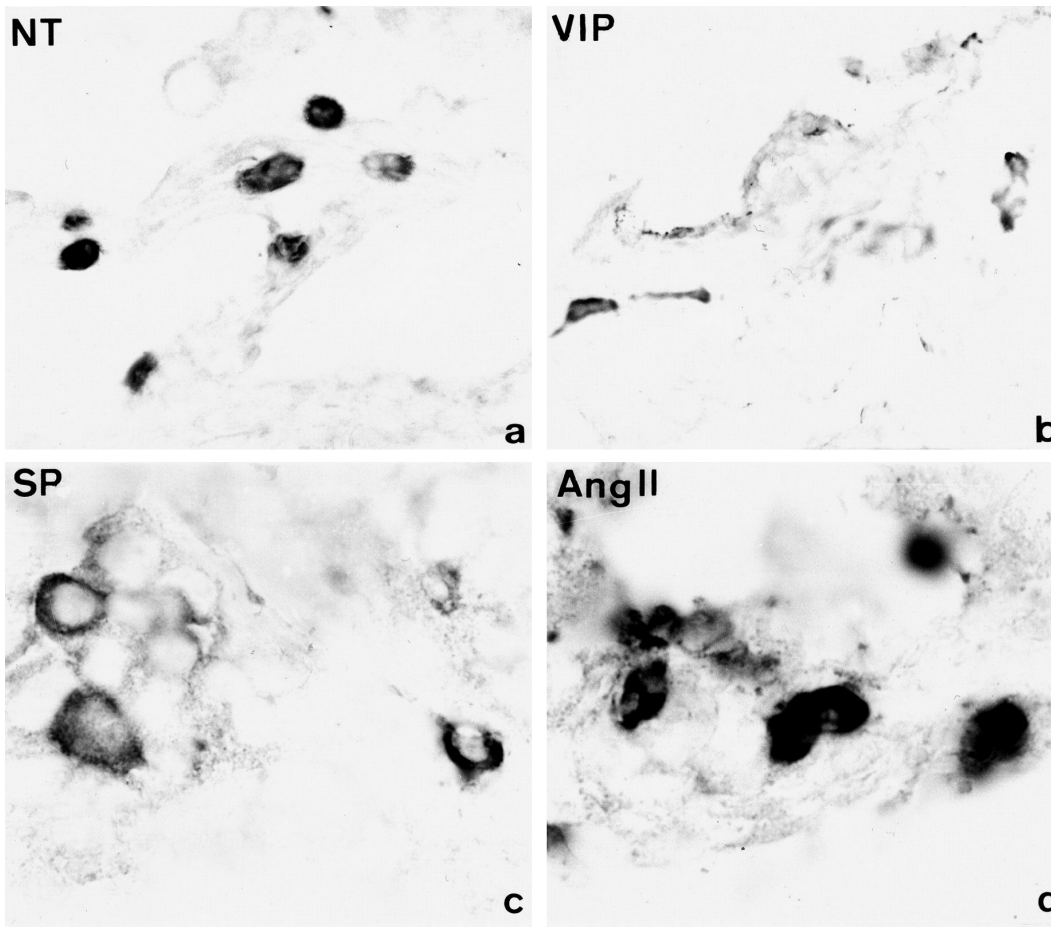


Fig. 3. Presence of regulatory peptides in pituitary cells in a female with Cushing's syndrome by microadenoma ACTH secreting; peradenomatous tissue. **a)** NT (x 1300), **b)** VIP (x1300), **c)** SP (x 1400), and **d)** Ang II (x 1400).

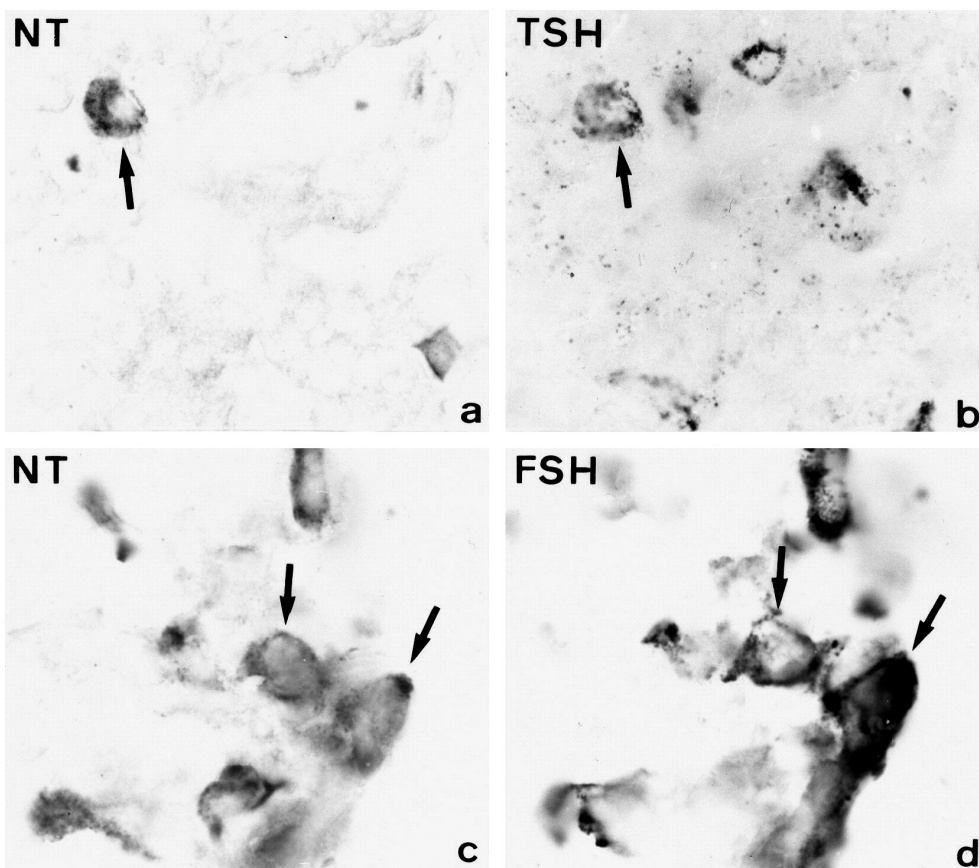


Fig. 4. Colocalization Peptides-Hormones in a female with Cushing's syndrome by microadenoma ACTH secreting; peradenomatous tissue. **a, b)** Colocalization of NT-ir in TSH-ir cells (x 1300). **c, d)** Colocalization of NT-ir in FSH-ir cells (x 1400).

gonadotroph and tyrotroph cells; at the moment of birth, other no identified cells apart from TSH and FSH cells were NT-ir. These cells could correspond to ACTH-ir cells; the ACTH-NT colocalization is very abundant in adenomatous cells, suggesting a role in the activity of the same. Although VIP has been shown in lactotrope cells in non human mammals and a relation with prolactin secretion has been suggested (Abe et al., 1985; Montagne et al., 1995), we demonstrate that VIP is present in human gonadotrope cells not being present in the adenomatous cells. Likewise, a direct and indirect relation with gonadotrope function (Hidalgo-Diaz et al., 1998) and lactotrope and somatotrope functions (Mau and Vilhardt, 1997) and SP in non human mammals have been suggested; also, in the rat SP seems to have an inhibitory effect on the response of adrenal axis to stress (Malendowicz et al., 1996); this inhibited role of the response to stress has been put to manifest in AtT20/D16v cells, a cellular model by product of a pituitary tumour (Melzig et al., 1998); about these cells, the SP seems to inhibit the answer to stress mediated by CRF. In this case, we have observed how the SP is

present in corticotrophs cells in peradenomatous tissue, and in more intense form in the adenomatous tissue, indicating a relation with the ACTH function. Likewise, a relation with lactotrope and corticotrope functions and Ang-II have been postulated (Diaz-Torga et al., 1998; Lenkei et al., 1999). Although in this work we have not concluded the Ang-II production by pituitary cells, recently we have shown the presence of Ang-II during human pituitary development in somatotrope cells (Reyes et al., 2001).

Only a few studies have investigated peptides relationship and pathological state. VIP-ir cells have been postulated in normal and tumoral pituitary cells, but the producing cell has not been identified (Wowra and Peiffer, 1984). Likewise, Bombesin and Somatostatin production by different tumors presenting ectopic Cushing's Syndrome has been described (Coates et al., 1986). Although no immunoreaction was found for Gal and NPY, galanin has been demonstrated in corticotrope cells in normal and adenomatous tissue (Leung et al. 2002). Likewise, in patients with pituitary adenomas, the galanin content was measured in tumours

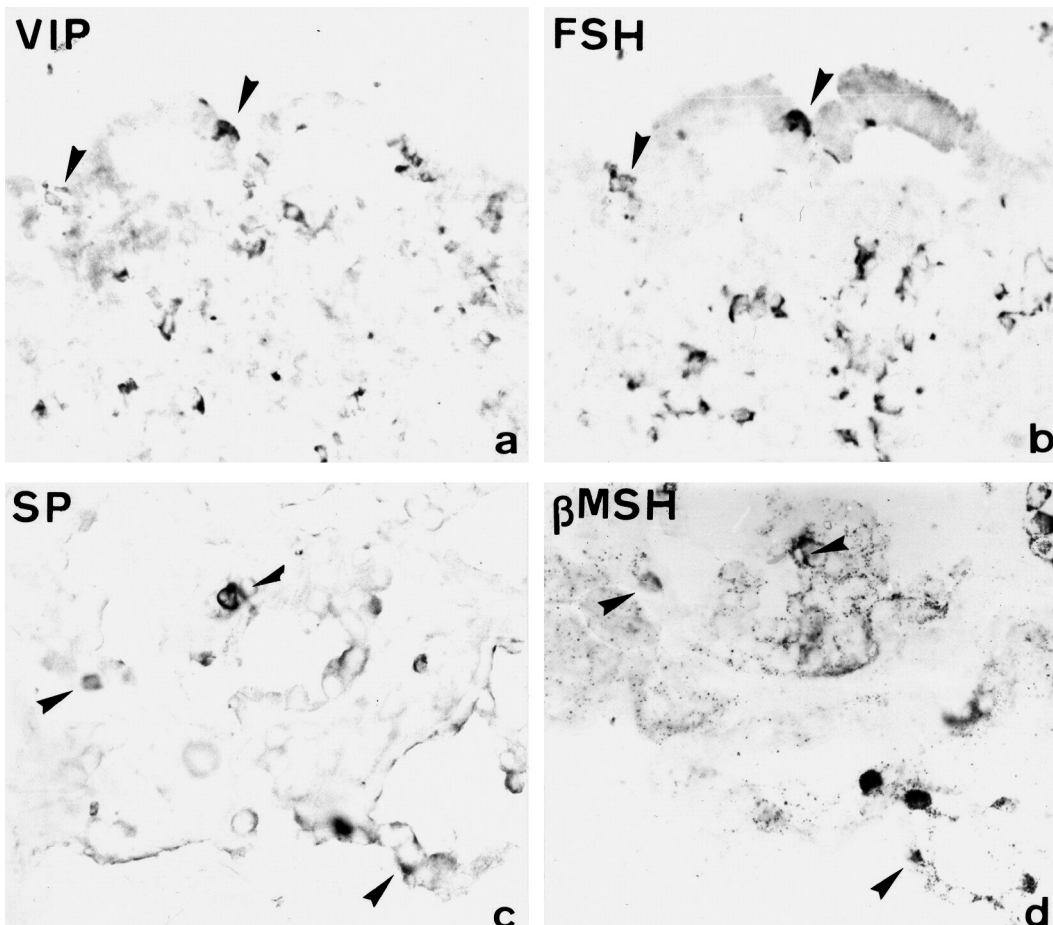


Fig. 5. Colocalization Peptides-Hormones in a female with Cushing's syndrome by microadenoma ACTH secreting; peradenomatous tissue. **a, b)** Colocalization of VIP-ir in FSH-ir cells. **c, d)** Colocalization of SP-ir in bMSH-ir cells. x 400

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and plasma, and the levels of plasma galanin were not related to tumour galanin concentration, suggesting an autocrine regulation mechanism for some pituitary tumours (Grenback et al. 2004).

In conclusion, a role as endocrine modulators in normal tissue have been suggested for these peptides, but their role in pathological tissue is not clear, probably they are acting as modulators in pathological state as

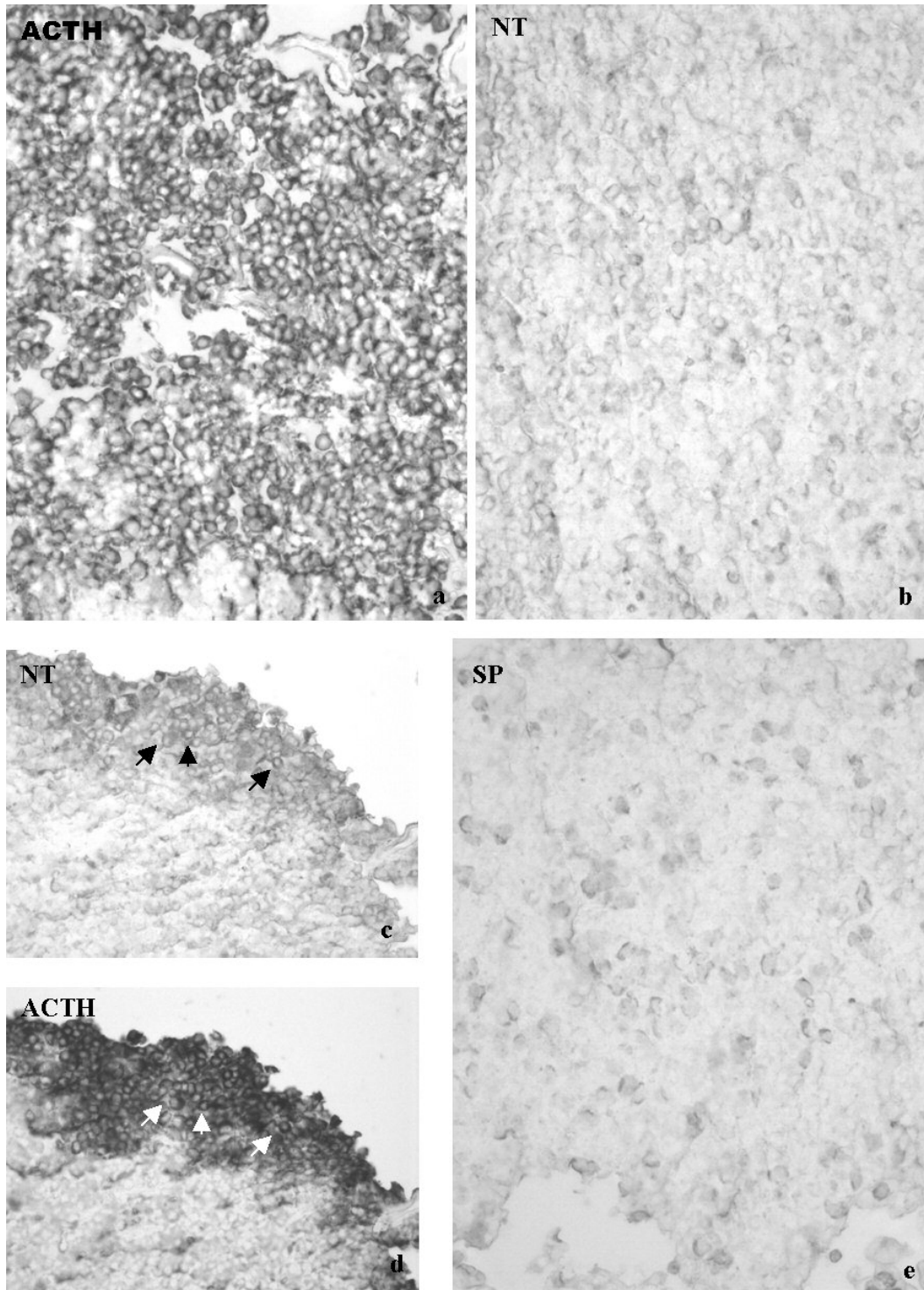


Fig. 6. Immunoreactivity in microadenoma cells. **a)** ACTH-ir (x 360). **b)** microadenoma cells NT immunoreactives (x 360). **c)** Cells ACTH-ir were also NT-ir. (**d.**) (x 200). **e)** Microadenoma cells SP-ir (x 400).

their production by different tumours suggest.

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