NOTE

Demonstration of Activin in Normal Pituitary and in Various Human Pituitary Adenomas by Immunohistochemistry

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Abstract. Inhibins and activins have been known to modify the secretion of various pituitary hormones. To study whether inhibins and activins are present in human pituitary tissues, immunohistochemical studies with antisera to activin A and inhibin α subunit were performed on 9 human pituitary adenoma tissue specimens and one sample of normal pituitary tissue adjacent to one adenoma. Activin immunoreactivities were demonstrated in the cytoplasms of one GH and one PRL and two non-functioning adenomas and one normal pituitary tissue, but they were negative in one PRL, one ACTH, one FSH and two non-functioning adenomas. Thus, the presence and absence of activin in the same type of adenoma in regard to hormone production, suggested that the difference in immunostaining simply reflected the difference in the activin concentration. In contrast to this, inhibin α subunit immunoreactivity was not found in any of the tissues studied. These data suggested a local synthesis of activin in the normal pituitary as well as various kinds of pituitary adenoma tissues and its local role in the human pituitary gland.

Key words: Activin, Inhibin, Pituitary adenoma, Immunohistochemistry

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INHIBINS and activins have been known not only to regulate gonadotropin secretion in normal human subjects [1, 2], but also to modify FSH [3], GH and PRL [4, 5] secretion from hormone producing human pituitary adenomas. Although their endocrine, autocrine or paracrine roles have been suggested [6], the evidence has been scarce and inconsistent as to the presence or absence, and the subtype of inhibins and activins present in the normal human pituitary as well as in adenoma tissues [7-9]. So far, Alexander and Klibanski detected inhibin α subunit mRNA in human gonadotropinoma [7] and Haddard et al. reported the presence of βα subunit, but not βαA subunit mRNA, in gonadotropinomas [8]. On the other hand, we recently found that inhibin α and βα subunits and activin type 2 receptor mRNAs were expressed, with the ubiquitous presence of βαA subunit mRNA in all human pituitary adenoma tissues, regardless of their hormone production [9]. This prompted us to investigate the presence of inhibin or activin in various kinds of human pituitary adenoma tissues immunohistochemically with antisera to activin and inhibin α subunit, respectively.

Materials and Methods

The pituitary adenoma tissues were obtained from ten operated human pituitary adenomas. One normal pituitary tissue sample was obtained as tis-
sue adjacent to one of the non-functioning adenomas which happened not to be included in this study (Table 1). The diagnosis was made by clinical, pathological and endocrinological studies.

**Immunohistochemistry**

Pituitary tissue and tumor specimens obtained at surgery were fixed in 20% formalin and embedded in paraffin wax for hematoxylin-eosin staining and immunohistochemical staining. Sections of 4 μm thick were subjected to immunohistochemistry by incubating the tissues overnight in antisera diluted to 1:400. The avidin-biotin immunoperoxidase method [10] was carried out for immunohistochemistry by using Dako’s kits (Kyoto, Japan). Localization of the staining was evaluated by light microscopy.

**Antisera preparation**

Antiserum for activin was raised in rabbits against recombinant activin A. The antiserum reacted with the monomer and dimer of the βA subunit, but did not crossreact with intact inhibin A [11].

Antiserum for inhibin was raised in rabbits against synthetic human inhibin α subunit N-terminal fragment, cyclic [Cys6, Tyr7] human inhibin α (6–30) NH2. The antiserum reacted with recombinant human inhibin A, but did not react with activin A or TGF-β in RIA (unpublished data). Both of the antisera were used at final concentrations of 1:400. Normal rabbit serum was used for negative control studies.

**Results**

As shown in Table 1, activin immunostaining was positive in 5 out of 10 tissues studied. Positive staining was obtained in one GH adenoma (Fig. 1), one prolactinoma, two non-functioning adenomas (case 6 is shown in Fig. 2) and one normal pituitary tissue. In these tissues, the number of positively stained cells varied and all immunoreactivities showed cytoplasmic distribution. In contrast, one prolactinoma, one ACTH adenoma with Cushing’s disease, one FSH adenoma and two non-functioning adenomas showed negative staining for activin.

**Discussion**

The study demonstrated the presence of activin in a variety of human pituitary adenoma tissues and in one normal pituitary tissue immunohistochemically. The characteristics of anti-activin A used in this study were proven to be specific for the monomer and dimer of the βA subunit and did...
not crossreact with intact inhibin A, which shares $\beta_\alpha$ subunit with activin A, by Yasuda et al., who examined the specificity of the same antiserum and demonstrated the existence of activin A in A- and D-cells of rat pancreatic islets [11], but cross-reactivity with activin B or AB was not proven.

Immunoreactivities of activin were positive in one normal pituitary tissue, one GH adenoma, one prolactinoma and two non-functioning adenomas, while they were negative in one ACTH, one FSH adenoma, one prolactinoma and two non-functioning adenomas. The presence of activin immunoreactivity of normal pituitary could be possible in gonadotrophs when considering the role of activin in gonadotropin secretion. The modifying role of activin on GH and PRL secretion from human GH adenomas [4, 5], and gonadotropinomas [3], and its modifying effect on PRL and ACTH secretion in rat pituitary cells [12, 13] may confirm the present results. Our previous studies [9] demonstrating the ubiquitous presence of inhibin $\beta_\alpha$ subunit mRNA by a quantitative reverse transcription-polymerase chain reaction (RT-PCR) method in all of the human pituitary adenomas, regardless of their hormone production, was the strongest indicator of the presence of activin A in all human pituitary adenomas. The difference in activin immunostaining observed in the present study may therefore simply reflect the difference in the concentration of activin A present in the tissues studied.

The study demonstrated that the subcellular distribution of activin immunoreactivities was totally cytoplasmic. The subcellular localization of activin in the present study was in accord with those reported by Roberts et al. in the rat anterior pituitary [14, 15]. They found inhibin $\alpha$ and $\beta_\beta$ immunoreactivities in cytoplasm of gonadotropes and their co-storage with LH and FSH secretory granules. Cytoplasmic localization may not necessarily indicate the site of synthesis or site of action of activin, but it is highly suggestive that activin is involved in the secretory processes of pituitary hormone or in cell growth. Further histochemical or morphological studies are needed to clarify the significance of the presence of activin in the normal human pituitary and in pituitary adenoma tissues.

Inhibin could not be demonstrated in the present immunohistochemical study in human pituitary tissues, but it is possible that our inhibin antiserum was not potent or specific enough to react with the entire $\alpha$ subunit or intact inhibin, since an N-terminal fragment of the $\alpha$ subunit was used to raise the antibody. Our previous study demonstrated the presence of $\alpha$ mRNA in 85% of various human pituitary adenomas [7], but we have found that the concentration of $\alpha$ subunit mRNA was much lower than that of $\beta_\alpha$ subunit mRNA by means of a quantitative RT-PCR in human pituitary adenoma tissues [16]. The level of inhibin may not be high enough to be detected by the immunohistochemical method. Further studies are needed to demonstrate inhibin protein in human pituitary tissues.

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References