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Immunohistochemical staining of normal, hyperplastic, and neoplastic adrenal cortex with a monoclonal antibody against α inhibin

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Abstract

***Aims*—To investigate the immunohistochemical staining of normal, hyperplastic, and neoplastic adrenal cortex with a monoclonal antibody against α inhibin. Also, to determine whether immunostaining with this antibody is useful in differentiating between adrenal cortical neoplasms and other tumours involving the adrenal gland that might mimic them.**

***Methods*—Normal adrenal tissue (n = 20) and specimens from cases of adrenal hyperplasia (n = 13), adrenal cortical adenoma (n = 15), adrenal cortical carcinoma (n = 4), phaeochromocytoma (n = 8), and adrenal metastatic tumour (n = 7) were stained with a monoclonal antibody against the α subunit of human inhibin.**

***Results*—Positive staining with the anti-α inhibin monoclonal antibody was seen in all normal adrenal glands. Immunoreactivity was largely confined to the inner cell layers of the adrenal cortex, with no staining of the adrenal medulla. All hyperplastic adrenal glands and adrenal cortical adenomas and carcinomas were also immunoreactive. The other tumours studied were negative.**

***Conclusions*—There is consistent immunoreactivity with the anti-α inhibin monoclonal antibody in normal adrenal cortex and in hyperplastic and neoplastic adrenal cortical lesions. In the normal adrenal cortex, positive staining is mainly confined to the zona reticularis. Other neoplasms involving the adrenal gland are negative. Immunohistochemical staining with anti-α inhibin monoclonal antibody, performed as part of a panel, may prove to be of value in the distinction between adrenal cortical carcinoma and phaeochromocytoma or metastatic tumour.**

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Inhibin is a dimeric 32 kDa peptide hormone that is composed of an α subunit and a β subunit.[1] The hormone is normally produced by ovarian granulosa cells and testicular Sertoli cells and inhibits the release of follicle stimulating hormone from the pituitary gland, thus acting as a modulator of folliculogenesis.[2]

Inhibin is also produced by ovarian granulosa cell tumours and serum measurements can be used to detect recurrent or metastatic disease before it becomes clinically apparent.[3 4] Recently, immunohistochemical staining of tissue sections with anti-inhibin monoclonal antibodies has been performed. These antibodies are now of established value in the distinction between ovarian sex cord stromal tumours and other neoplasms that might mimic them.[1 5–8]

Extragonadal inhibin expression also occurs and inhibin subunits have been detected in the placenta, pituitary gland, adrenal gland, and liver.[9–12] The aims of the present study were to assess the immunohistochemical staining of normal adrenal tissue, and hyperplastic and neoplastic adrenal cortical lesions with a monoclonal antibody against α inhibin. We also wished to determine whether immunostaining with this antibody could be of value in the distinction between adrenal cortical neoplasms and other tumours involving the adrenal gland.

Materials and methods

Specimens included in the study were retrieved from the files of the department of pathology, Royal Group of Hospitals Trust, Belfast. Most of the specimens were surgical biopsy material, although eight normal adrenal glands and two adrenal cortical adenomas were derived from postmortem material. Table 1 shows the cases studied. The 13 cases of adrenal cortical hyperplasia comprised seven associated with Cushing's syndrome and six with Conn's syndrome. The 15 adenomas comprised 10 associated with Cushing's syndrome, three with Conn's syndrome, and two that were clinically non-functional. All adrenal cortical carcinomas were clinically non-functional. In these four cases, a diagnosis of malignancy was based on a

Figure 1 Immunohistochemical staining of normal adrenal gland with anti-α inhibin monoclonal antibody. Positivity is largely confined to the inner layers of the cortex, with no staining of the medulla.

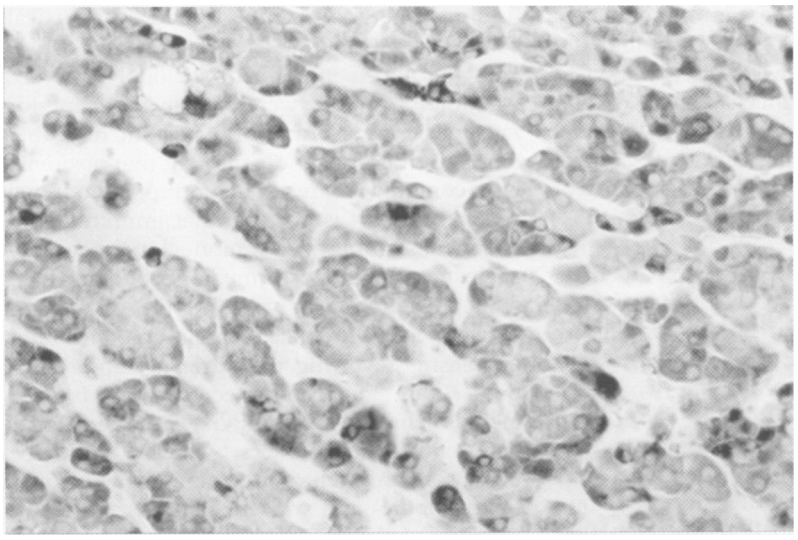

Figure 2 Immunohistochemical staining of adrenal cortical adenoma with anti-α inhibin monoclonal antibody.

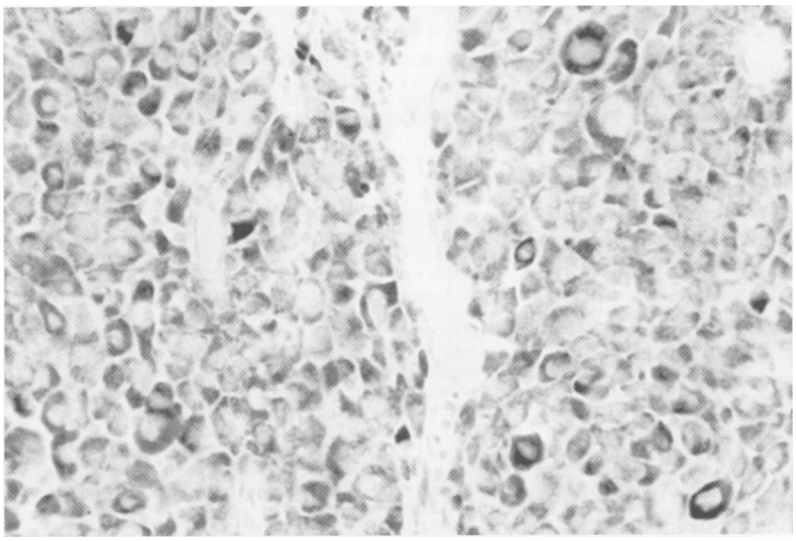

Figure 3 Immunohistochemical staining of adrenal cortical carcinoma with anti-α inhibin monoclonal antibody.

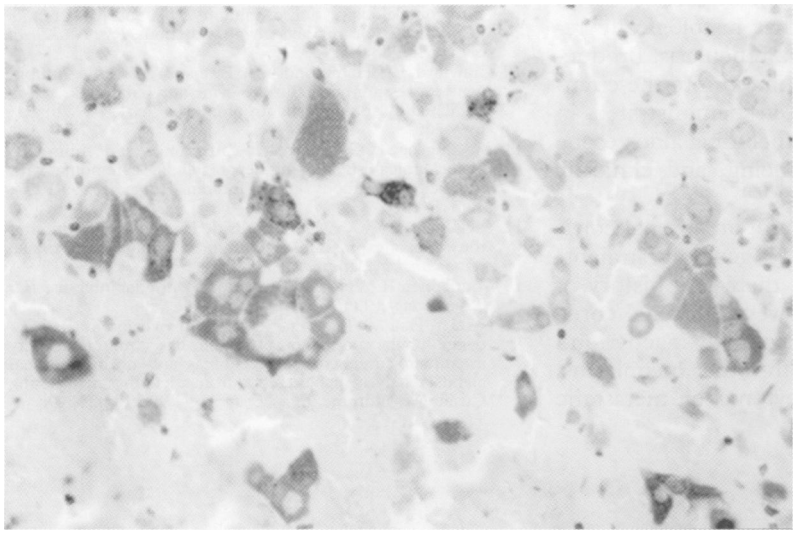

Figure 4 Immunohistochemical staining of adrenal cortical carcinoma containing bizarre cells with anti-α inhibin monoclonal antibody.

combination of clinical and pathological parameters including tumour size, tumour weight, the presence of necrosis, the presence of vascular invasion, nuclear pleomorphism, and mitotic activity. The primary tumours in the cases of adrenal metastases were lung carcinoma (n = 4), and single cases of breast carcinoma, renal cell carcinoma, and pleural mesothelioma. All specimens had been fixed in formalin, routinely processed in paraffin wax, and stained with haematoxylin and eosin.

Table 1 Cases included in study

	Number of cases
Normal adrenal	20
Adrenal cortical hyperplasia	13
Adrenal cortical adenoma	15
Adrenal cortical carcinoma	4
Phaeochromocytoma	8
Adrenal metastases	7

IMMUNOHISTOCHEMICAL STAINING

Sections from paraffin wax embedded blocks were cut on to aminopropyltriethoxysilane treated slides (Sigma, Poole, Dorset, UK) and dried overnight at 37°C. Endogenous peroxidase activity was blocked in 3% alcoholic hydrogen peroxide for 10 minutes. Sections were pretreated in an 850 W domestic microwave oven in citrate buffer pH 6.0 for 20 minutes and allowed to cool for 20 minutes. Sections were incubated for 30 minutes with a mouse monoclonal antibody to human inhibin 32 kDa α subunit (1/50 dilution; Serotec, Oxford, UK). Localisation was performed using biotinylated antimouse immunoglobulin (1/200 dilution, Dako, Copenhagen, Denmark) and peroxidase streptavidin biotin complex (Dako). Diaminobenzidine (Dako) was used as the chromagen. Sections were counterstained using Harris's haematoxylin. Negative controls, where the primary antiserum was omitted and replaced with mouse immunoglobulin (Dako), were performed in all cases. Positive controls were also run; these comprised ovaries containing follicular cysts or corpora lutea.

Results

There was positive staining with the anti-α inhibin monoclonal antibody in all normal adrenal glands. No difference in staining was noted between surgical biopsy and postmortem material. Immunoreactivity was cytoplasmic and granular and was most intense in the inner layers of the adrenal cortex (fig 1), representing the zona reticularis. In some cases, there was weaker staining of the outer layers of the adrenal cortex. There was no staining of the adrenal medulla.

All cases of adrenal cortical hyperplasia and adenoma (fig 2) were also immunoreactive. Positivity ranged from focal to diffuse and was most intense in compact cells with eosinophilic cytoplasm, which resembled the cells of the normal zona reticularis. There was little or no staining of cells with clear cytoplasm. There was no correlation between the pattern of staining and the hormonal profile. All cases of adrenal cortical carcinoma exhibited positive staining. Staining was diffuse in two cases (fig 3) and focal in two. Two cases of adrenal cortical carcinoma were composed of pleomorphic cells and both were positive with the anti-α inhibin monoclonal antibody (fig 4).

Small numbers of cells were immunoreactive in the case of metastatic mesothelioma. On closer examination, positivity was confined to entrapped adrenal cortical cells. There was no staining of the other tumours studied.

There was strong staining of positive control material in all cases. There was no staining of negative control material.

Discussion

Ovarian granulosa cells and testicular Sertoli cells are the main sources of circulating inhibin.[13] However, extragonadal expression also occurs and inhibin α subunits (which are needed for inhibin synthesis) have been detected in the placenta, pituitary gland, adrenal gland, and liver.[9–12] There is evidence that the adrenals may contribute to circulating inhibin levels. It has been demonstrated that adrenal veins have a higher concentration of inhibin-like immunoreactivity than the vena cava or peripheral veins.[14]

In the present study, we found consistent immunoreactivity in the normal adrenal cortex using an antibody against the α subunit of human inhibin. Immunoreactivity was most intense in the inner cell layers of the normal adrenal cortex, representing the zona reticularis. In some cases, there was weaker staining of the outer layers of the adrenal cortex and it is not possible using immunohistochemical studies alone to ascertain the exact site of inhibin production. This would require the use of alternative methods for the demonstration and localisation of mRNA sequences.

Consistent immunoreactivity with the anti-α inhibin monoclonal antibody was also seen in hyperplastic adrenal glands, in adrenal cortical adenomas, and in the small number of adrenal cortical carcinomas studied. These included both cortisol and aldosterone producing and hormonally non-functional lesions. Immunoreactivity was most intense in compact cells with eosinophilic cytoplasm that histologically resembled those of the zona reticularis. Production of and secretion of inhibin into the circulation has previously been demonstrated in human adrenal tumours, including aldosterone producing, cortisol producing, and clinically non-functional adenomas.[14]

Histological recognition of an adrenal cortical adenoma or carcinoma is generally straightforward, especially when there is a history of hormone production. However, adrenal cortical carcinoma is often hormonally inactive and confusion may arise with other neoplasms, especially phaeochromocytoma or metastatic tumour. The small numbers of adrenal cortical carcinomas in the present study were positive with the anti-α inhibin monoclonal antibody. All other tumours were negative, except for focal positivity in a case of metastatic mesothelioma. In this case it was thought that positivity was confined to residual entrapped adrenal cortical cells. It is possible that immunohistochemical staining with a monoclonal antibody against α inhibin, performed as part of a panel, may prove to be of value in the distinction between adrenal cortical carcinoma and other neoplasms involving the adrenal gland. Antibodies that might be used in such a panel include chromogranin, PGP 9.5, and other neuroendocrine markers, which should be positive in phaeochromocytoma. Anticytokeratin antibodies and antisera against carcinoembryonic antigen may also be utilised depending on the differential diagnosis considered.

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