

INHIBIN IMMUNOREACTIVITY IN GONADAL AND NON-GONADAL TUMORS

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Summary—Inhibin immunoreactivity was estimated in a number of gonadal and non-gonadal tumors. Dog Sertoli cell tumors and human granulosa cell and Leydig cell tumors contained high concentrations of inhibin-like material. Levels, comparable with those in normal testes and ovaries were detected in human testicular non-seminomas and in ovarian cystadenomas, thecomas and adenofibromas. No activity was found in human testicular Sertoli/Leydig cell tumors and seminomas and in ovarian adenocarcinomas, teratomas and a dysgerminoma. Furthermore, human adrenal cortical tissue (tumor and hyperplastic adrenal) contained inhibin immunoreactivity. No activity was found in human tumors of the stomach, gut, liver, kidney, pancreas and mammary gland or in meningiomas. It is concluded that inhibin is not a good marker for specific gonadal tumors. Inhibin might have intratumor actions as a growth or differentiation factor.

INTRODUCTION

Inhibin has been defined as a gonadal glycoprotein hormone, which can suppress the pituitary production and secretion of gonadotropins, preferentially that of FSH [1]. Inhibin consists of two disulphide-linked subunits: the α -subunit is combined with either of two β -subunits, β -A or β -B. The primary structure of the inhibin subunits shows homology with that of a number of growth and differentiation factors, e.g. Müllerian inhibiting substance and transforming growth factor- β (see for reviews, De Jong [2], Ying [3], de Kretser and Robertson [4]). Combination of two inhibin β -subunits leads to the formation of activin, a protein which counteracts the action of inhibin on the pituitary secretion of FSH [5, 6].

The mRNA for the inhibin subunits is expressed in a large number of tissues apart from the gonads [7], and even in the gonads themselves inhibin subunits have been detected outside the cells which were traditionally believed to be the source of inhibin viz. the Sertoli cells in the testis [8] and the granulosa cells in the ovary [9]; Lee *et al.* [10] and Risbridger *et al.* [11] showed inhibin production in Leydig cells,

whereas a number of other authors showed the presence of inhibin subunits in theca or corpus luteum cells in the ovary (see for review, de Jong *et al.* [12]).

Recently, the presence of high peripheral levels of inhibin immunoreactivity in women with granulosa cell tumors was described [13]. In the present study, levels of inhibin immunoreactivity in human ovarian and testicular tumor tissue were estimated in order to ascertain the specificity of inhibin as a marker for granulosa and Sertoli cell tumors. Because of the fact that the incidence of Sertoli cell tumors in men is extremely low, this type of tumor was obtained from dogs. Finally, in order to substantiate the specificity of inhibin as a gonadal tumor marker, inhibin immunoreactivity was also estimated in a number of tumors of other organs.

MATERIALS AND METHODS

Tumor tissues

Sertoli cell tumors were obtained from dogs, whereas all other tumor material was of human origin. All tumor tissues were placed on ice immediately after excision. Part of the tissue was used for routine pathological procedures; the remaining tissue was stored at -80°C until processing. The frozen tissue was pulverized and homogenized as described earlier [14]. The

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homogenates were centrifuged for 30 min at 50,000 *g* at 4°C, and the supernatant was used for the estimation of inhibin (see below) and protein [15].

Inhibin assays

Inhibin immunoreactivity was assayed using the antiserum against bovine inhibin (No. 1989) described by Robertson *et al.* [16], using radioiodinated 32 kDa bovine inhibin as a label. The antiserum and the material for iodination were purchased from the Department of Anatomy, Monash University, Melbourne, Australia. Inhibin was labeled using [¹²⁵I]sodium iodide (Amersham, Amersham, U.K.) and Protag (Baker, Deventer, The Netherlands) as the oxidizing agent. Labeled protein was isolated after chromatography on prepacked Sephadex G-25 columns (PD-10, Pharmacia, Uppsala, Sweden). The standard used in the immunoassay was a bovine ovarian follicular fluid preparation with the arbitrary potency of 1 U/ μ g protein. The International Research Standard for inhibin (86/890, [17]), has a specific activity of 60 ± 10 U/ μ g, when expressed in units of this bovine follicular fluid standard.

Inhibin bioactivity was estimated as described by Grootenhuys *et al.* [18], using the same standard.

RESULTS

Testicular tumors

Dog Sertoli cell tumors. Inhibin bioactivity levels in the Sertoli cell tumors and in canine control testes are shown in Fig. 1, together with the results of the immunological esti-

mations of inhibin in plasma from these animals. Testicular inhibin bioactivity in the tumor tissue was significantly higher than that in the control testes ($P < 0.05$). Similarly, the peripheral levels of immunoreactive inhibin were significantly higher when compared with those in control dogs ($P < 0.01$ after logarithmic transformation). There was no relationship between testicular and peripheral concentrations of inhibin.

Human testis tumors. Results of estimations of immunoreactive inhibin in homogenates of human testicular tumors are summarized in Fig. 2. High concentrations were found in two out of three Leydig cell tumors, whereas non-seminomas contained inhibin levels comparable to those in normal human testes. In contrast, very low concentrations or non-detectable levels were found in mixed Sertoli cell–Leydig cell tumors and in seminomas.

Ovarian tumors

Levels of immunoreactive inhibin in human ovarian tumors are shown in Fig. 3. Significant concentrations were found in control ovaries, cystadenomas, thecomas and adenofibromas, whereas much higher levels were present in the supernatant of the granulosa cell tumor homogenates. The concentrations of inhibin immunoreactivity in adenocarcinomas, teratomas and a dysgerminoma were below the level of detection of the assay.

Other tumors

Finally, inhibin immunoreactivity was estimated in supernatants of homogenates of a

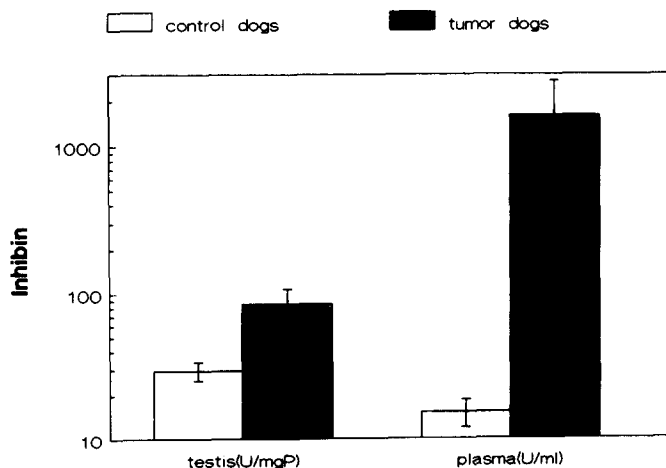


Fig. 1. Bioactive inhibin in control testes (open bar) or Sertoli cell tumors (hatched bar) from dogs and immunoreactive inhibin in plasma from the same animals (means \pm SEM, $n = 5$). Data from Grootenhuys *et al.* [20].

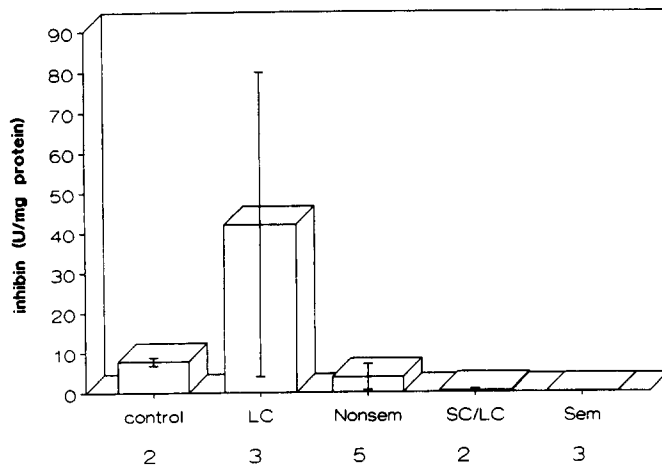


Fig. 2. Inhibin levels in human control testes and in testicular neoplasms: Leydig cell tumors (LC), nonseminomas (Nonsem), Sertoli cell/Leydig cell tumors (SC/LC) and seminomas (Sem). Figures indicate numbers of samples investigated.

number of other tumors. Inhibin was only detected in adrenal cortical tissue (hyperplastic adrenal: 3 U/mg protein; adrenal tumor: 1 U/mg protein), whereas no immunoreactivity was found in tumors of the stomach ($n = 3$), gut ($n = 4$), liver ($n = 3$), kidney ($n = 3$), pancreas ($n = 2$) and mammary gland ($n = 5$) or in meningiomas ($n = 5$).

DISCUSSION

Bioactive inhibin is produced in the granulosa cells of the ovary [9] and the Sertoli cells of the testis [8]. Furthermore, inhibin bio- and immunoactivity has been reported to be present in culture medium of Leydig cells [10, 11] and the presence of inhibin subunits has been reported in a number of other tissues including the adrenal gland, the kidney and the brain (see [12]).

The results reported here indicate the presence of inhibin immunoreactivity in Sertoli and granulosa cell tumors. It is not clear if this immunoreactivity should be ascribed to dimers of the inhibin α - and β -subunits or to the presence of an α -subunit, combined with part of its pro-sequence, which was shown to cross-react in the immunoassay used here [19]. Also, high circulating levels of immunoreactive inhibin were found in dogs with Sertoli cell tumors [20] and in women with granulosa cell tumors [13]. However, other types of tumors in the testis and the ovary also appear to contain inhibin immunoreactive material; in patients with these types of tumor increased peripheral levels of inhibin might also occur, thus rendering the specificity of inhibin as a marker for granulosa and Sertoli cell tumors questionable. Most of the non-gonadal tumours, however, did

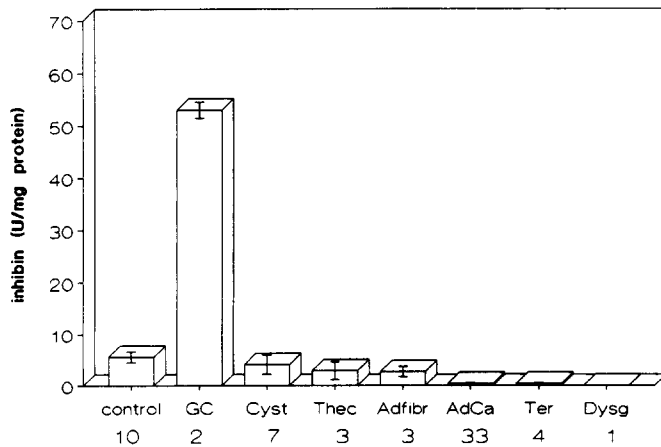


Fig. 3. Inhibin levels in human control ovaries and in ovarian neoplasms: granulosa cell tumors (GC), cystadenomas (Cyst), thecomas (Thec), adenofibromas (Adfibr), adenocarcinomas (AdCa), teratomas (Ter) and a dysgerminoma (Dysg). Figures indicate numbers of samples investigated.

not contain detectable amounts of inhibin immunoreactivity.

The presence of inhibin immunoreactivity in testicular non-seminomas is interesting, since this tumor may consist of a number of primitive cell types; so far, no clear correlation with a subtype of non-seminomas was observed. The finding that mixed Leydig–Sertoli cell tumors did not contain inhibin immunoreactivity is not understood, since both cell types contributing to this tumor apparently contain high concentrations of the immunoreactivity. Finally, the presence of high concentrations of inhibin-like material in tumor tissues and the large number of intra- and extra-gonadal effects of inhibin and related substances (see [12]), suggest that inhibin may affect cellular functions in the tumor, or in other cell types, such as erythropoietic cells [21] or thymocytes [22]. These possible effects of inhibin and related proteins as growth and differentiation factors should be envisaged when dealing with patients with inhibin-producing tumors.

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