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Immunohistochemical localization of α- and βA-subunits of inhibin/activin in human normal endocrine cells and related tumors of the digestive system

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Abstract Activin A and inhibin A, first isolated from the ovary, are dimeric proteins able to modulate pituitary FSH secretion. Inhibin A is a heterodimer composed of one α-subunit and one βA-subunit (α-βA), while activin A is a homodimer of the βA-subunit (βA-βA). Their identification in several tissues has suggested that they have numerous physiological functions, acting as either paracrine or autocrine factors. The aim of this study was to evaluate the expression of activin A and inhibin A in normal endocrine cells and in 70 endocrine tumours from different sites in the gastro-entero-pancreatic system, using specific monoclonal antibodies directed against the α- and βA-subunits of inhibin/activin. Immunoreactivity for the βA-subunit, but not for the α-subunit, was observed in normal G, EC, and GIP cells of the antrum and duodenum, and in pancreatic A cells. βA-subunit expression was observed in G cell and A cell tumours, and in a few insulinomas and ileal EC cell carcinoids. The α-subunit was found in rare cells in 7 of the 70 tumours and was colocalized with the βA-subunit in only 1 tumor. Specific types of endocrine cells from the gut and pancreas appear to produce only activin A, a possible paracrine or autocrine modulator. Activin A is mainly produced by tumours derived from endocrine cells that normally express it.

Key words Activin A · Inhibin A · Endocrine tumors · Digestive system · Immunohistochemistry

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Introduction

Activin and inhibin are both dimeric proteins first isolated from the ovary and able to modulate FSH release from the pituitary in a long-loop endocrine fashion [13, 20, 41]. Both inhibins and activins are sulphydryl-linked dimers comprising two of three distinct inhibin protein subunits (α, βA, βB). Inhibins are heterodimers composed of one α-subunit and one β-subunit (either βA or βB), producing inhibin A (α-βA) or inhibin B (α-βB) [22, 39]. Activins consist of homodimers of any combination of the β-subunits, resulting in activin A (βA-βA), activin AB (βA-βB), and activin B (βB-βB) [20, 41]. These proteins are members of the transforming growth factor-β (TGFβ) superfamily, which includes multiple forms of TGFβ, müllerian inhibiting substance (MIS), the decapentaplegic gene complex of *Drosophila*, bone morphogenic proteins and the amphibian protein VG-1. Many of these growth factors have been shown to have growth-promoting, growth-inhibiting, or both activities, depending on the current status of a particular cell [7, 23, 44, 45]. The identification of inhibin subunits in a wide variety of reproductive and non-reproductive tissues [25] has suggested that these peptides, in addition to regulating FSH biosynthesis and secretion in the pituitary, may have a greatly expanded biological role. These functions include the regulation of steroid production in the gonads and adrenal glands, and the modulation of cell growth and maturation in several tissues, both in the fetus and in the adult [13]. It has also been shown that many of these regulatory roles are exerted locally by a paracrine or autocrine mechanism [2, 5]. Activin signalling occurs via binding to heterotrimeric receptor complex with trensmembrane serine/threonine kinase activity [24]. A receptor with specific affinity for inhibin has not been identified; however, inhibin has been shown to bind to activin type II receptors, although with lower affinity than activin [13]. Activin action can also be regulated by two binding proteins. Follistatin binds activin with high-affinity, nearly irreversible kinetics and neutralizes activin when in complex form [34]. α2-Macroglobulin binds to activin or inhibin with its high capacity and low affinity, without

Table 1 Clinico-pathological and immunohistochemical profile of the 70 endocrine tumours (*nk* not known)

No.	Sex	Age	Site	Type	Size (cm)	Metastases or invasion	βA-Subunit	α-Subunit
1	F	65	Stomach	°type 1[a,b]	nk	No	5	0
2	F	90	Stomach	type 1	nk	No	0	0
3	M	76	Stomach	type 1	2	No	0	0
4	M	43	Stomach	type 3[a]	2	Node	0	0
5	M	26	Stomach	type 3	2	Node/liver	0	0
6	F	56	Stomach	type 3	8.3	Node	0	0
7	M	68	Stomach	NEC[a]	3	Node/liver	0	0
8	M	52	Stomach	NEC	5	Node	0	0
9	F	52	Duodenum	D-cell	1.5	Node	0	0
10	F	38	Duodenum	D-cell	1	Node	0	0
11	F	46	Duodenum	D-cell	2.5	Node/liver	0	0
12	M	52	Duodenum	D-cell	4	Node	0	0
13	F	42	Duodenum	G-cell	1	No	5	0
14	F	55	Duodenum	G-cell	0.5	No	70	0
15	M	36	Duodenum	gastrinoma	1.3	No	15	5
16	F	71	Pancreas	gastrinoma	nk	Liver/node	15	5
17	F	40	Pancreas	gastrinoma	2	Liver/node	60	0
18	F	52	Pancreas	somatostatinoma	5	Duodenum	0	0
19	F	33	Pancreas	insulinoma[c]	2.5	No	0	0
20	M	48	Pancreas	insulinoma[c]	2	No	60	0
21	M	50	Pancreas	insulinoma[c]	4	Liver	15	0
22	F	54	Pancreas	insulinoma	1	No	0	0
23	M	37	Pancreas	insulinoma	2.7	No	20	0
24	M	51	Pancreas	insulinoma	1.1	No	0	0
25	F	nk	Pancreas	insulinoma	1.3	No	0	0
26	F	28	Pancreas	insulinoma	1.1	No	0	0
27	M	49	Pancreas	insulinoma	0.9	No	0	0
28	F	50	Pancreas	insulinoma	1.5	No	0	0
29	F	25	Pancreas	insulinoma	1.1	No	0	0
30	F	57	Pancreas	VIPoma	5	Liver/node	5	0
31	M	26	Pancreas	VIPoma	5	No	0	2
32	M	nk	Pancreas	VIPoma	nk	No	0	3
33	F	66	Pancreas	A-cell	1.2	No	100	0
34	F	62	Pancreas	A-cell	1.7	No	80	0
35	F	63	Pancreas	A-cell	2.1	No	100	0
36	F	50	Pancreas	EC-cell	2	No	5	0
37	F	37	Pancreas	A/D-cell	6	Liver/node	30	30
38	M	63	Pancreas	undefined-cell	3	Duodenum	5	3
39	F	38	jejunum	undefined-cell	1.2	Node/liver	3	10
40	F	67	Ileum	EC-cell	2	Node/liver	0	0
41	M	52	Ileum	EC-cell	2	Node/liver	5	0
42	M	nk	Ileum	EC-cell	2.5	No	0	0
43	F	46	Ileum	EC-cell	1.5	Node	0	0
44	M	59	Ileum	EC-cell	2.5	nk	0	0
45	M	58	Ileum	EC-cell	2.5	Node/liver	20	0
46	M	39	Ileum	EC-cell	3	Node	0	0
47	F	75	Ileum	EC-cell	1.5	Liver	30	0
48	F	69	Ileum	EC-cell	2	Node	3	0
49	M	72	Ileum	EC-cell	1	Omentum	0	0
50	M	53	Appendix	EC-cell	2.5	No	0	0
51	M	25	Appendix	EC-cell	0.5	No	0	0
52	F	95	Appendix	EC-cell	2	No	0	0
53	F	14	Appendix	EC-cell	0.6	No	0	0
54	F	17	Appendix	EC-cell	0.5	No	0	0
55	F	24	Appendix	EC-cell	0.2	No	0	0
56	M	27	Appendix	EC-cell	0.5	No	0	0
57	F	21	Appendix	EC-cell	0.3	No	0	0
58	M	27	Appendix	EC-cell	1	No	0	0
59	F	15	Appendix	EC-cell	0.2	No	0	0
60	F	42	Appendix	EC-cell	0.9	No	0	0
61	F	24	Appendix	L-cell	1.5	No	0	0
62	nk	nk	Right colon	EC-cell	nk	nk	0	0
63	F	50	Right colon	EC-cell	nk	Omentum	0	0
64	M	46	Right colon	EC-cell	8	Liver	30	0
65	F	51	Rectum	L-cell	0.7	No	0	0
66	F	66	Rectum	L-cell	0.2	No	0	0
67	M	39	Rectum	L-cell	0.3	No	0	0
68	M	54	Rectum	EC-cell	2	No	0	0
69	M	66	Rectum	L-cell	1	No	0	0
70	M	70	Rectum	EC/L-cell	2	Liver	0	0

[a] Gastric ECL-cell tumors classified according to Rindi et al. [31]

[b] This tumor showed about 2% of EC-cells

[c] MEN-1 syndrome, in addition to the insulinoma, patients presented multiple glucagon-producing A-cell microadenomas intensely positive for βA-subunit of activin A, but lacked α-subunit immunoreactivity

altering their bioactivity, in contrast to the effects of follistatin [17].

Many of the studies localizing inhibin subunits in extragonadal tissues are based on analysis of expression of mRNA encoding α, βA and βB subunits. These studies have shown that, in addition to the ovary and testis, inhibin-α, βA and βB mRNAs are present in the placenta, pituitary, adrenal, bone marrow, kidney, spinal cord, brain and pancreas [25, 28]. As far as we know there is only one study localizing activin and inhibin α-subunit immunohistochemically in various human tissues [43]. In this study specific immunostaining for activin A was detected in Leydig and Sertoli cells of the testis, granulosa and luteal cells of the ovary, somatotrophs of the pituitary gland, thyroid follicular cells, neuronal cells of the brain, monocytoid cells of the bone marrow, cortical cells of the adrenal gland, and insulin-β cells of the pancreatic islets. In the same study inhibin α-subunit was detected in Leydig, granulosa and thecal cells, and in pituitary somatotrophs, but not in pancreatic islet cells. The finding of activin only in β cells in human islets reported by Wada et al. [43] is partly at odds with a previous study of Ogawa et al. [28], which demonstrated the presence of activin in both β cells and α cells of the rat pancreas.

Recently, inhibin has been reported as a sensitive marker for sex cord stromal differentiation in tumours of the ovary, including granulosa cell tumours, fibromas/thecomas, other sex cord-stromal proliferations, steroid cell tumours, and Sertoli-Leydig cell tumours [11, 14, 32]. Moreover, inhibin and activin have been demonstrated immunohistochemically in various types of pituitary adenomas [4, 30], but, as far as we know, there are no reports regarding the identification of these peptides in tumours of the gastro-entero-pancreatic endocrine system.

In the present study we investigated the presence and distribution of α and βA subunits of inhibin or activin in normal cells of the gastro-entero-pancreatic endocrine system and in a series of 70 related tumours.

Materials and methods

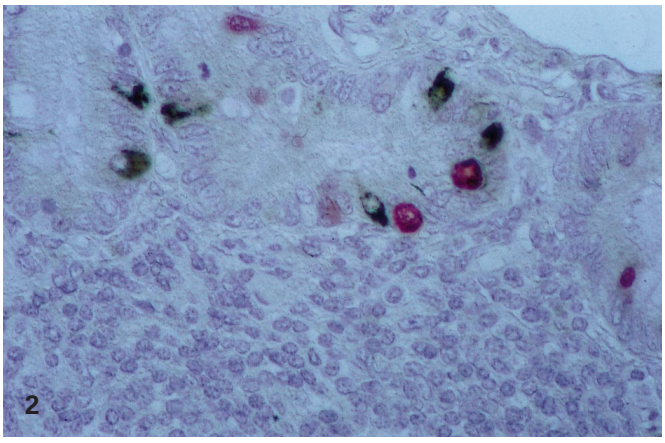
Samples of normal pancreases and normal mucosae of the whole gastrointestinal tract and of 70 functioning and nonfunctioning gastro-entero-pancreatic endocrine tumours (Table 1) were collected at surgery. For all histological, histochemical and immunohistochemical studies, tissues were fixed in buffered formalin (formaldehyde 4% w/v and acetate buffer 0.05 M), routinely processed and paraffin embedded. Sections (5 µm thick) were stained with haematoxylin-eosin (H&E) and Grimelius' silver stain.

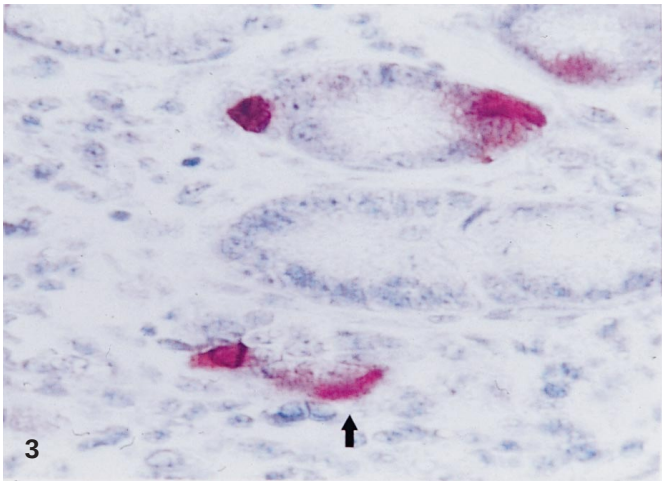
Fig. 1 βA-Subunit immunoreactivity in the cytoplasm of several endocrine cells of normal duodenal mucosa. ×250

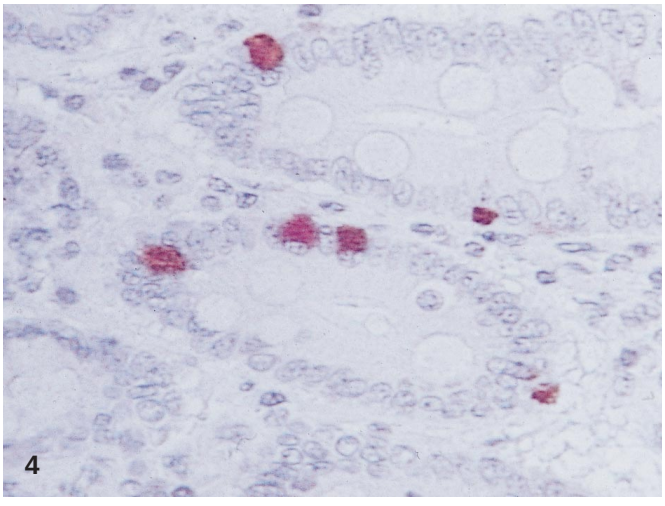
Immunohistochemical stainings was performed on serial sections using the antibodies and antisera listed in Table 2. Sections 3 µm thick were mounted on poly-L-lysine coated slides, deparaffinized and hydrated through graded alcohol to water. After endogenous peroxidase activity inhibition by treatment with 3% hydrogen peroxide for 10 min, primary antibody incubation was done at 4°C for 18–20 h, followed by the avidin–biotin complex (ABC) proce-

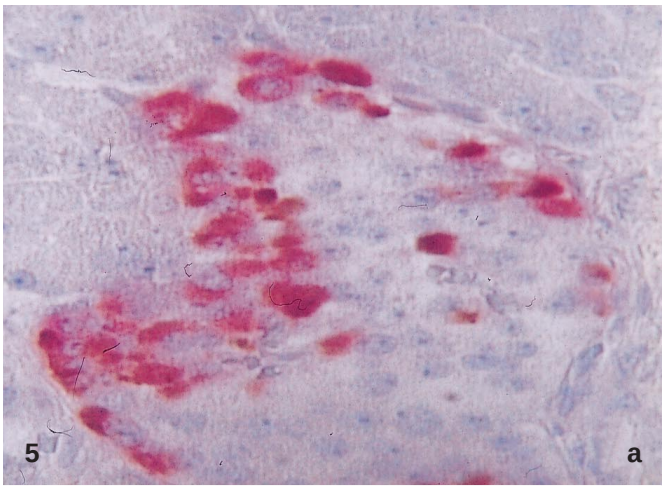
Table 2 Antibodies and antisera employed (*P/M* polyclonal/monoclonal)

Antibodies/antisera	P/M (Clone)	Dilution	Source
Insulin	M (AE9D6)	1:200	BioGenex Laboratories, San Ramon, USA
Glucagon	P	1:1250	Milab, Malmo, Sweden
Glucagon/glicentin	P	1:2500	Milab
Pancreatic polypeptide	P	1:4000	Cambridge Research Biochemicals, Cambridge, UK
Somatostatin	P	1:500	Dako, Copenhagen, Denmark
Serotonin	M (YC5)	1:50	Biogenesis, Bournemouth, UK
C-terminus gastrin-CCK-cerulein	M (B4)	1:10000	Farmitalia, Milan, Italy
Gastric inhibitory peptide	P	1:10	Milab
Secretin	P	1:500	Milab
α-Subunit	M (R1)	1:100	Serotec, Oxford, UK
βA-Subunit	M (E4)	1:100	Serotec









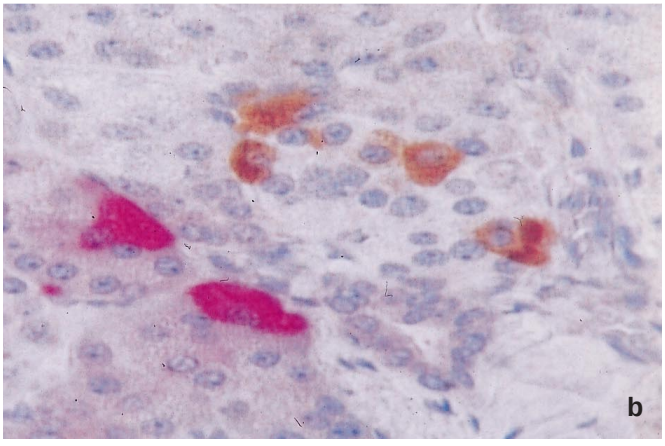


Fig. 2 Double immunostaining of normal duodenal mucosa showing colocalization of gastrin (using alkaline phosphatase developed to give a red end-product) and βA-subunit (using peroxidase developed to give a brown end-product) in some endocrine cells. ×250

Fig. 3 Normal duodenal mucosa: colocalization of serotonin (*red*) and βA-subunit (*brown*) in all enterochromaffin cells. ×250 but one (*arrow*)

Fig. 4 Double label immunostaining of normal duodenal mucosa demonstrating colocalization of GIP (*red*) and βA-subunit (*brown*) in some cells. ×250

Fig. 5 Double label immunostainings of normal pancreas showing that **a** glucagon immunoreactive cells (*red*) coexpress βA-subunit (*brown*), whereas **b** βA-chain (*brown*) does not colocalize with pancreatic polypeptide (*red*). ×250

dure [15]. The sections were then immersed in 0.03% 3,3' diaminobenzidine tetrahydrochloride and counterstained with Harris' haematoxylin. Colocalization studies were performed with double-label immunostains according to Mason et al. [21] or Lan et al. [18]. Sections incubated with antibodies directed against glucagon and somatostatin were pretreated for 10 min with 0.003% subtilisin (Sigma,P4789; protease type XXVII or Nagarse protease) in 0.05 M TRIS-buffered saline pH 7.4, while sections stained for the α-subunit of inhibins and βA-subunits of inhibins or activins were pretreated with 0.01 M citrate buffer pH 6 for 10 min in a microwave oven at 650 W. Specificity controls consisted of absorption of antibodies and antisera with their related antigens, omission of the first layer, and use of control tissues with or without the pertinent antigen.

Statistical analyses were performed using the Fisher exact test to evaluate the expression of α- and βA-chains in relation to clinico-pathological features.

Results

In the normal tissues investigated, intense cytoplasmic immunoreactivity for the βA-subunit was found in scattered endocrine cells of the stomach, duodenum (Fig. 1), and pancreas (Table 3), but was lacking in cells of the jeju-

Table 3 Distribution of α- and βA-subunits in normal endocrine cells of gut and pancreas

	βA-Subunit	α-Subunit
Fundus	rare	No
Antrum	Yes[a]	No
Duodenum	Yes[b]	No
Pancreas	Yes[c]	No
Jejunum	No	No
Ileum	No	No
Appendix	No	No
Rectum	No	No

[a] G- and EC cells
[b] EC, G, GIP cells
[c] A cells

num, ileum, appendix, and colon. βA-Subunit-immunoreactive (IR) cells were more numerous in the duodenal than in the gastric mucosa. In the stomach, the positive cells were more abundant in the antral than in the oxyntic mucosa and they were mostly located in the lower third of the glands. Double-label immunostains showed that, in the antrum, the βA-subunit-positive endocrine cells corresponded to about 60% of serotonin-IR cells and 10% of gastrin-IR cells. Owing to the rarity of βA-subunit-IR cells in the oxyntic mucosa it was not possible to establish which type of endocrine cell was expressing the βA-chain. In the duodenal mucosa βA-subunit-IR cells were numerous and mostly located in lower part of the crypts. Colocalization studies demonstrated that βA-IR cells corresponded to about 20% of gastrin-IR cells (Fig. 2), 70% of serotonin-IR cells (Fig. 3), and 90% of gastric inhibitory polypeptide (GIP)-IR cells (Fig. 4).

In the pancreas, βA-subunit immunoreactivity was found in cells located at the periphery of the islets, and also in endocrine cells dispersed throughout the exocrine parenchyma. Double label immunostains revealed that all βA-subunit-positive cells corresponded to glucagon-IR cells (Fig. 5).

No positive staining for the α-subunit of inhibin was found in any gastrointestinal or pancreatic endocrine cell (Table 3).

The clinico-pathological profile of each tumour studied is reported in Table 1. With the exception of cases 19, 20, and 21, which were associated with a MEN-1 syndrome, all tumours, both functioning and nonfunctioning, were sporadic. Clinically, the majority (52/70) of endocrine tumours investigated were nonfunctioning, with the exception of 1 duodenal gastrinoma with a typical Zollinger–Ellison syndrome (ZES) and 17 pancreatic neoplasms (2 gastrinomas with ZES, 1 somatostatinoma, 11 insulinomas, and 3 VIPomas with watery diarrhoea and hypokalaemia).

As shown in Table 4, βA-subunit immunoreactivity was mostly found in G cell (5/5 cases) and A cell (3/3 cases) tumours (Fig. 6) of the duodenum and pancreas, with a mean percentage of positive cells of 33% (range 5–70%) and 93% (range 80–100%), respectively. In addition, 3 of the 11 insulinomas, 1 nonfunctioning EC cell tumour (case 36) and 1 nonfunctioning A+D cell neoplasm

Table 4 Distribution of α and βA-subunits in endocrine tumours of the digestive system (*MP* mean percentage of immunoreactive cells in positive tumours)

Types	Syndrome	βA-subunit		α-subunit	
		No.[a]	MP	No.[a]	MP
Stomach					
ECL cell	No	1/6[b]	5	0/6	
NEC	No	0/2		0/2	
Duodenum					
D cell	No	0/4		0/4	
G cell	No	2/2	37.5	0/2	
	Yes	1/1	15	1/1	5
Pancreas					
G cell	Yes	2/2	37.5	1/2	5
A cell	No	3/3	93.3	0/3	
EC cell	No	1/1	5	0/1	
A/D cell	No	1/1	30	1/1	30
B cell	Yes	3/11	31.6	0/11	
D cell	Yes	0/1		0/1	
VIPomas	Yes	1/3	5	2/3	2.5
Undefined cell	No	1/1	5	1/1	3
Jejunum					
Undefined cell	No	1/1	3	1/1	10
Ileum					
EC cell	No	4/10	14.5	0/10	
Appendix					
EC cell	No	0/11		0/11	
L cell	No	0/1		0/1	
Right colon					
EC cell	No	1/3	30	0/3	
Rectum					
EC cell	No	0/2		0/2	
L cell	No	0/4		0/4	

[a] Number of positive tumors
[b] This ECL-cell tumor showed about 2% of EC-cells

(case 37) of the pancreas showed a consistent number of βA-subunit-positive cells, while there were few of these in 1 ECL cell gastric carcinoid (which also showed about 2% of EC cells, case 1) and in 2 tumours of undefined cell type (cases 38 and 39). Among the 25 ileal, appendiceal, and right colon EC cell tumours (carcinoids), βA-subunit-IR was only found in 4 out of 10 tumours of the ileum and in 1 of the 3 tumours of the right colon. The rectal neoplasms, including 4 L cell and 2 EC cell tumours, were all negative. Interestingly, in addition to a single insulinoma, the patients with the MEN-1 syndrome (cases 19–21) presented multiple glucagon-producing A cell microadenomas that showed βA-subunit-IR in the majority of cells.

The immunoreactivity for α-subunit of inhibin was only found in 7of the 70 (10%) neoplasms investigated, with a mean percentage of positive cells of 8%. Two tumours were gastrinomas, two VIPomas, and two other tumours were malignant neoplasms of undefined cell type. The βA- and α-subunit immunoreactivities in these neoplasms were not found in the same cells, suggesting

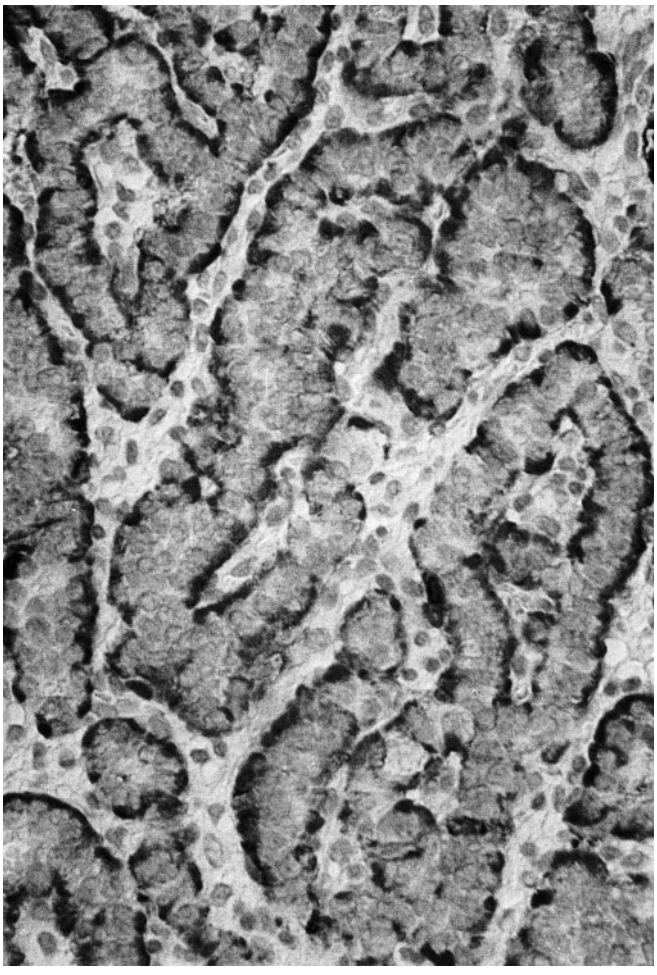


Fig. 6 βA-Subunit immunoreactivity in a pancreatic glucagon-producing A-cell tumour. ×250

that there were different cells producing activin A or inhibin. In only 1 neoplasm showing α-subunit positive cells (case 37) was colocalization of βA- and α-subunit detected in some tumour cells, suggesting that, in this case, there was a simultaneous production of both activin A and inhibin A in some tumour cells.

There were no statistically significant correlations between α- and βA-chain expression and the following clinico-pathological variables: malignancy of tumours, sex, age, and presence or absence of endocrine symptoms. The only statistically significant correlation found was between expression of the βA-subunit and malignancy of EC cell tumours. Malignant EC cell neoplasms (mostly located in the ileum and colon) expressed βA chain with a significantly higher frequency (P=0.01) than did benign EC cell tumours (mainly located in the appendix).

Discussion

We have investigated the immunohistochemical distribution of α-subunit of inhibin and βA-subunit of inhibin/activin in normal endocrine cells of gut and pancreas and in related endocrine tumours. Since the specific anti-βA-subunit antibody used recognizes the βA-chain, which is shared by both activin A and inhibin A, we have also examined the distribution of the α-subunit, with the aim of distinguishing the site of production of activin A from that of inhibin A. Cytoplasmic immunoreactivity for βA-subunit in the absence of α-subunit expression was indicative of the presence of activin A. In contrast, the simultaneous expression of both βA- and α-subunits was consistent with the presence of inhibin A, without excluding the possibility of the concomitant presence of activin A. Although the anti-βA-subunit antibody used was specific for activin A, the presence of activin AB (βA-βB dimer) could not be excluded.

In all the normal tissues that we examined the βA-chain was detected but not the α-subunit, indicating that only activin A was expressed. The βA-chain was found in foregut derivates (stomach, duodenum, and pancreas), but was lacking in midgut (jejunum, ileum, appendix)- and hindgut (rectum)-derived viscera. In the pancreas, we localized the βA-subunit (activin A) only in glucagon-producing A-cells. This is in agreement with several immunohistochemical studies showing that, in adult and fetal rat pancreases, activin A is localized in A cells [12, 28, 29, 46]. In addition to A-cells, activin A was also localized in D cells and B cells of the same species [28, 46]. The presence of activin A in B cells has also been demonstrated by Wada et al. [43], who examined the distribution of activin A in several human tissues immunohistochemically. These conflicting data may be due to the different types of antibodies used against inhibin and activin. In particular, with the combined use of anti-follistatin antibodies and of antibodies directed against follistatin-bound activin A [43] or against follistatin-bound βA-subunit [28] it was possible to demonstrate that activin A- or βA-positive pancreatic B cells were costained with anti-follistatin antibodies, suggesting that the activin–follistatin complex is present in these cells. Interestingly, follistatin, which binds to activin A and suppresses its biological actions in various biological systems [1, 16, 26, 27, 37], is confined to B cells in pancreatic islets. This has been elegantly demonstrated by Ogawa et al. [28], who also showed that, at least in rat pancreas, A cells, unlike B cells, contain inhibin/activin free from follistatin. Since some authors [40, 46] have demonstrated that activin A stimulates insulin secretion in rat pancreatic islets, it may be postulated that activin A, produced by A cells, may act as a paracrine factor regulating insulin secretion. Moreover, follistatin may be produced by B cells to counteract activin A actions. In this context it is interesting to recall that follistatin has many structural homologies with some growth factor receptors, such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor. Unlike these receptors, however, follistatin lacks a transmembrane domain [35, 36], representing, as a consequence, only a soluble receptor for activin A.

Progesterone and oestrogen have an important physiological role in the complex regulation of pituitary FSH

secretion, in which both activin A and inhibin are also involved [47]. This is in agreement with the finding that gonadotroph cells of the anterior pituitary express receptors for both progesterone (PgRs) and oestrogen (ERs) [38], and that they produce both α- and βB-subunits of inhibin-activin [25, 33]. Regulatory mechanisms including activin-inhibin and steroid hormones, similar to those operating in gonadotroph cells, might also be hypothesized for pancreatic A cells. In this context, it is of interest to recall that pancreatic glucagon-producing A cells and A cell tumours, besides producing activin A (as we have demonstrated in the present study) express PgRs [8, 19, 42]. Although a progesterone influence on glucagon secretion has not as yet been demonstrated and the significance of the presence of PgRs in A cells is not clear, the hypothesis that steroid hormones may influence pancreatic activin A secretion remains suggestive. Of course, more detailed biological studies are needed before we can hope to understand a possible interaction between progesterone and activin production in A cells.

Our findings show that endocrine cells of the normal gut mucosa, like A cells of the pancreas, express only the βA-chain of inhibin-activin, suggesting production of activin A but not of inhibin. In addition, colocalization studies clearly demonstrate that βA-subunit-IR is strictly confined to endocrine cells of the stomach and duodenum, and particularly to G, EC, and GIP cells. As far as we know, this is the first report demonstrating activin A expression in normal endocrine cells of the gut. Our findings suggest the hypothesis that activin A regulates some physiological functions of the gut; in particular, it is interesting to note that activin A is present in gastric inhibitory peptide (GIP)-producing cells and that GIP has an insulinotropic action potentiating the insulin release after oral administration of glucose [3, 9, 10]. Duodenal GIP cells thus represent a further type of endocrine cell that produces activin A and is involved in the regulation of glucose metabolism.

This is the first study that has examined the presence of the βA- and α-chains of inhibin and activin in human gastroenteropancreatic endocrine tumours. The βA-chain has been detected in all A cell and G cell tumours, reflecting the localization of the βA-subunit found in endocrine cells of the normal mucosa and pancreas. In addition, the βA-chain has been found in some EC cell tumours and in a few tumours of different type. In contrast, the α-chain of inhibin-activin has been detected less frequently in the neoplasms examined and has not been associated with specific functional types of tumours.

The specific part played by βA-chain and α-chain in the tumours examined is not known. The absence of a significant correlation with the functional or nonfunctional character of the neoplasms makes a role of α- and βA-subunits in the pathogenesis of endocrine symptoms unlikely.

Inhibin and activin are prime candidates as regulators of cell proliferation during morphological change in the the ovary, as well as being factors in abnormal proliferation and transformation of these tissues. Moreover, activin has been found to play an important part in autocrine growth regulation in human ovarian cell lines [6]. Whether such mechanisms are also involved in the growth of gastroenteropancreatic tumours remains to be determined. The de novo expression of βA-subunit in some ileal EC cell tumours, while absent in normal ileal EC cells, suggests a possible role for this subunit in the process of tumour growth control. Further studies are needed to clarify the role of activin-inhibin in the various endocrine tumours of the gastro-entero-pancreatic system. Demonstration of coexpression of activin and its receptor would provide important information. In situ hybridization studies to detect activin and inhibin mRNA would be useful to assess active synthesis of these factors.

The results of our study suggest that activin A, produced by specific types of pancreatic (A cell) and gut (G, GIP, EC cells) endocrine cells, may act locally as a paracrine factor in the regulation of some physiological functions, including the regulation of insulin secretion. In addition, endocrine tumours expressing activin A are mainly A cell and G cell tumours. Finally, activin A may be involved in the process of tumorigenesis of some ileal EC cell tumours.

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References

1. Asashima M, Nakano H, Uchiyama H, Sugino H, Nakamura T, Eto Y, Ejima D, Davids M, Plesso WS, Cichocka I, Kinoshita K (1991) Follistatin inhibits the mesoderm-inducing activity of activin A and the vegitalizing factor from chicken embryo. Rouxs Arch Dev Biol 200:4–7
2. Bilezikjian LM, Vale WW (1992) Local extragonadal roles of activins. Trends Endocrinol Metab 3:218–223
3. Creutzfeldt W, Ebert R (1985) New development in the incretin concept. Diabetologia 28:565–573
4. Demura R, Kubo D, Suzuki T, Yajima R, Tajima S, Takakura K, Demura H, Aiba M, Eto Y (1996) Demonstration of activin in normal pituitary and in various human pituitary adenomas by immunohistochemistry. Endocr J 43:429–423
5. DePaolo LV, Bicsak TA, Erickson GF, Shimasaki S, Ling N (1991) Follistatin and activin: a potential intrinsic regulatory system within diverse tissues. Proc Soc Exp Biol Med 198: 500–512
6. Di Simone N, Crowley WF, Wang QI-FA Jr, Sluss PM, Schneyer AL (1996) Characterization of inhibin/activin subunit, follistatin, and activin type II receptors in human ovarian cancer cell lines: a potential role in autocrine growth regulation. Endocrinology 137:486–494
7. Doctor JS, Jackson PD, Rashka KE, Visalli M, Hoffman FM (1992) Sequence, biochemical characterization, and developmental expression of a new member of TGFβ superfamily in *Drosophila melanogaster*. Dev Biol 151:491–505
8. Doglioni C, Gambacorta M, Zamboni G, Coggi G, Viale G (1990) Immunocytochemical localization of progesterone receptors in endocrine cells of the human pancreas. Am J Pathol 137:999–1005
9. Dupré J, Ross SA, Watson D, Brown JC (1973) Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37:826–828
10. Elhai D, Andersen DK, Brown JC (1979) Pancreatic α- and β-cell responses to GIP infusion in normal man. Am J Physiol 237:E185–E191

11. Flemming P, Grothe W, Maschek H, Petry KU, Wellmann A, Georgii A (1996) The site of inhibin production in ovarian neoplasms. Histopathology 29:465–468
12. Furukawa M, Eto Y, Kojima I (1995) Expression of immunoreactive activin A in fetal rat pancreas. Endocr J 42:63–68
13. Halvorson LM, DeCherney AH (1996) Inhibin, activin, and follistatin in reproductive medicine. Fertil Steril 65:459–469
14. Hildebrandt RH, Rouse RV, Longacre TA (1997) Value of inhibin in the identification of granulosa cell tumors of the ovary. Hum Pathol 28:1387–1395
15. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique. J Histochem Cytochem 25:577–589
16. Kogawa K, Nakamura H, Sugino K, Takio K, Titani K, Sugino H (1991) Activin-binding protein is present in pituitary. Endocrinology 128:1434–1440
17. Krummen LA, Woodruff TK, Deguzman G, Cox ET, Baly DL, Mann E, Garg S, Wong WL, Cossum P, Mather JP (1993) Identification and characterization of binding proteins for inhibin and activin in human serum and follicular fluids. Endocrinology 132:431–443
18. Lan HY, Mu W, Nikolic-Paterson DJ, Atkins RC (1995) A novel, simple, reliable, and sesitive method for multiple immunoenzyme staining: use of microwave oven heating to block antibody crossreactivity and retrieve antigens. J Histochem Cytochem 43:97–102
19. La Rosa S, Sessa F, Capella C, Riva C, Leone BE, Klersy C, Rindi G, Solcia E (1996) Prognostic factors in nonfunctioning pancreatic endocrine tumours. Virchows Arch 429:323–333
20. Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R (1986) Pituitary FSH is released by a heterodimer of the β-subunits from the two forms of inhibin. Nature 321: 779–782
21. Mason DY, Abdulaziz B, Falini H, Stein H (1983) Double immunoenzymatic labelling. In: Polak JM, Van Noorden S (eds) Immunocytochemistry. Practical applications in pathology and biology. Wright, Bristol pp 113–128
22. Mason J, Niall HD, Seeburg DH (1986) Structure of two human ovarian inhibins. Biochem Biophys Res Commun 135: 957–964
23. Massagué J (1987) The TGFβ family of growth and differentiation factors. Cell 49:437–438
24. Mathews LS (1994) Activin receptors and cellular signaling by the receptor serine kinase family. Endocr Rev 15:310–325
25. Meunier H, Rivier C, Evans RM, Vale W (1988) Gonadal and extragonadal expression of inhibin α, βA and βB subunits in various tissues predicts diverse functions. Proc Natl Acad Sci USA 85:247–251
26. Nakamura T, Takio K, Eto Y, Shibai H, Titani K, Sugino H (1990) Activin-binding protein from rat ovary is follistatin. Science 247:836–838
27. Nakamura T, Sugino K, Titani K, Sugino H (1991) Follistatin, an activin-binding protein, associates with heparan sulfate chain of proteoglycans on follicular granulosa cells. J Biol Chem 266:19432–19437
28. Ogawa K, Abe K, Kurosawa N, Kurohmaru M, Sugino H, Takahashi M, Hayashi Y (1993) FEBS Lett 319:217–220
29. Ogawa K, Ono K, Kurohmaru M, Hayashi Y (1995) Effect of streptozotocin injection on expression of immunoreactive follistatin and βA and βB subunits of inhibin/activin in rat pancreatic islets. Eur J Endocrinol 132:363–369
30. Penabad JL, Bashey HM, Asa SL, Haddad G, davis KD, Herbst AB, Gennarelli TA, Kaiser VB, Chin WW, Snyder PJ (1996) Decreased follistatin gene expression in gonadotroph adenomas. J Clin Endocrinol Metab 81:3397–3407
31. Rindi G, Luinetti O, Cornaggia M, Capella C, Solcia E (1993) Three subtypes of gastric argyrophil carcinoids and the gastric neuroendocrine carcinoma: a clinicopathologic study. Gastroenterology 104:994–1006
32. Rishi M, Howard LN, Bratthauer G, Tavassoli FA (1997) Use of monoclonal antibody against human inhibin as marker for sex cord-stromal tumors of the ovary. Am J Surg Pathol 21: 583–589
33. Roberts V, Meunier H, Vaughan J, Rivier C, Vale W, Sawchenko P (1989) Production and regulation of inhibin in pituitary gonadotropes. Endocrinology 124:552–554
34. Schneyer AL, Rzucidlo DA, Sluss PM, Crowley WF Jr (1994) Characterization of unique binding kinetics of follistatin and activin or inhibin in serum. Endocrinology 135:667–674
35. Shimasaki S, Koga M, Esch F, Cooksey K, Mercado M, Koba A, Ueno N, Ying SY, Ling N, Guillemin R (1988) Primary structure of the human follistatin precursor and its genomic organization. Proc Natl Acad Sci USA 85:4218–4222
36. Shimasaki S, Koga M, Buscaglia ML, Simmons DM, Bicsak TA, Ling N (1989) Follistatin gene expression in the ovary and extragonadal tissues. Mol Endocrinol 3:651–659
37. Shimonaka M, Inovye S, Shimasaki S, Ling N (1991) Follistatin binds to both activin and inhibin through the common beta-subunit. Endocrinology 128:3313–3315
38. Stefaneanu L (1997) Pituitary sex steroid receptors: localization and function. Endocr Pathol 8:91–108
39. Stewart AG, Milborrow HM, Ring JM, Crowther CE, Forage RG (1986) Human inhibin genes: genomic characterization and sequencing. FEBS Lett 206:329–334
40. Totsuka Y, Tabuchi M, Kojima I, Shibai H, Ogata E (1988) A novel action of activin A: stimulation of insulin secretion in rat pancreatic islets. Biochem Biophys Res Commun 156: 335–339
41. Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J (1986) Purification and characterization of a FSH-releasing protein from porcine ovarian follicular fluid. Nature 321:776–779
42. Viale G, Doglioni C, Gambacorta M, Zamboni G, Coggi G, Bordi C (1992) Progesterone receptor immunoreactivity in pancreatic endocrine tumors. Cancer 70:2268–2277
43. Wada M, Shitani Y, Kosaka M, Sano T, Hizawa K, Saito S (1996) Immunohistochemical localization of activin A and follistatin in human tissues: Endocr J 43:375–385
44. Weeks DL, Melton DA (1987) A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGFβ. Cells 51:861–867
45. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA (1988) Novel regulators of bone formation: molecular clones and sctivities. Science 242: 1528–1534
46. Yasuda H, Inoue K, Shibata H, Takeuchi T, Eto Y, Hasegawa Y, Sekine N, Totsuka Y, Mine T, Ogata E, Kojima I (1993) Existence of activin-A in A- and D-cells of rat pancreatic islet. Endocrinology 133:624–630
47. Ying SY (1988) Inhibins, activins and follistatin: gonadal proteins modulating the secretion of follicle-stimulating hormone. Endocr Rev 9:267–293