NEURON-SPECIFIC ENOLASE IS PRODUCED BY NEUROENDOCRINE TUMOURS

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Summary Neuron-specific enolase (NSE) is a neuronal form of the glycolytic enzyme enolase, which was first found in extracts of brain tissue, and was later shown to be present in APUD (amine precursor uptake and decarboxylation) cells and neurons of the diffuse neuroendocrine system but not in other peripheral cells. 90 neuroendocrine neoplasias (APUDomas) (including islet-cell tumours, phaeochromocytomas, medullary thyroid carcinomas, oat-cell tumours, and APUDomas of the gut, pancreas, and lung) reacted strongly with antisera to NSE. In addition, large amounts of the enzyme were found by radioimmunoassay in the tumours (mean 1626±479 SEM ng of NSE/mg protein), whereas control non-endocrine neoplasias contained less than 15 ng NSE/mg protein. Thus NSE, a specific enzyme produced in the neural and endocrine systems, was found to be produced in considerable quantities by all types of APUDomas but not in any non-endocrine tumours. NSE seems to be a useful and easily detected marker which may be used to distinguish endocrine from non-endocrine neoplasias. Clinical detection of endocrine tumours is difficult and such tumours are often missed. Use of NSE as a marker may avoid this.

Introduction

NEURON-SPECIFIC enolase (NSE) was originally extracted from bovine brain[1] and was later characterised as a specific neuronal isomer of the widely distributed glycolytic enzyme, 2-phospho-D-glycerate hydrolase (EC 4.2.1.11).[2] It was at first considered that the gene coding for this isomer was restricted to neurons, and that it was only present in the central nervous system. NSE was later found in endocrine (APUD, amine precursor uptake and decarboxylation) cells of the central and peripheral divisions of the diffuse neuroendocrine system.[3] Subsequently it has been found in all components of this system, including those of the gut,[4] pancreas,[3,4] lung,[5] pituitary,[3] thyroid,[3] and adrenal glands.[3]

We detected this enzyme in various endocrine tumours, by both immunostaining of tumour cells and radioimmunological measurement of extractable enzyme.

Material and Methods

Tissue Preparation

We investigated 90 endocrine tumours (APUDomas) (table I). Classification of these tumours as endocrine was based on clinical observations; conventional histology; peptide immunocytochemistry; silver impregnation; and ultrastructural morphology.[6,7] Specimens from both primary and secondary tumours were included in this study and were fixed in routine histological fixatives (Bouin's fluid or formalin) or promptly frozen, freeze-dried, and fixed in benzoquinone or formaldehyde vapour,[5] to preserve the antigenicity of peptides and the enzyme.

Oat-cell carcinoma and hela cells were obtained from human ascitic fluid and grown in RPMI-1640 medium, supplemented with 10% fetal calf serum, 10 mmol non-essential aminoacids, 100 units/ml penicillin, 100 μg/ml streptomycin, and 7·5 μg/ml insulin. Oat-cell carcinomas were passage 34 and hela cells passage 24. Both were released from culture with 0·02% EDTA and assayed by radioimmunoassay as previously described.[9]

TABLE I—NSE IMMUNOSTAINING IN TUMOURS AND PEPTIDE PRESENT

Tumours	No. tested	No. with NSE	Single product	+ 1 other peptide	> other peptide
Endocrine tumours:					
VIPomas	15	15	4	9	2
Gastrinomas	10	10	6	3	1
Glucagonomas	12	12	1	4	7
Insulinomas	10	10	5	2	3
Phaeochromocytomas	14	14	–	5	–
Medullary thyroid carcinoma	10	10	6	–	–
Gut carcinoids	8	8	4(5HT)	–	–
Oat-cell carcinoma	5	5	–	–	–
Ganglioneuroblastomas	3	3	2(VIP)	–	–
Lung carcinoid	1	1	–	–	–
Chemodectoma	1	1	–	–	–
Vagal paraganglioma	1	1	–	–	–
Non-endocrine tumours:	11	0	–	–	–
Carcinomas (4 stomach, 3 rectum, 3 colon, 1 caecum)					

VIP = vasoactive intestinal polypeptide.
5HT = 5-hydroxytryptamine.

Antisera

Purified NSE was prepared from rat brain as previously described[10] and antisera were raised in New Zealand white rabbits.[11] All the peptide primary antibodies (table II) were raised in rabbits except for anti-insulin which was raised in guineapigs. Antisera to corticotrophin, calcitonin, growth hormone, and insulin were obtained from Wellcome Laboratories, and anti-bovine and anti-human pancreatic polypeptides (BPP and HPP) were kindly donated by Dr Ronald Chance from Lilly Laboratories.

Immunocytochemistry

Sections (5 μm) thick were cut and immunostained by the unlabelled antibody peroxidase anti-peroxidase (PAP) method.[12] Primary antibodies (PAP method) were applied after dilution in

TABLE II—PEPTIDE ANTIBODIES

Antibodies to:	Region directed	Working dilution
Corticotropin	Not known	1/2000
Bombesin	C-terminal	1/4000
Calcitonin	Mid/N-terminal	1/2000
Cholecystokinin	Mid 9–20	1/5000
Glucagon	N-terminal	1/5000
Endorphin	Mid/N-terminal	1/6000
Gastrin	C-terminal	1/2000
Gastric inhibitory polypeptide	Mid	1/12000
Growth hormone	Not known	1/7500
Met-enkephalin	C-terminal	1/2000
Motilin	C-terminal	1/600
	N-terminal	1/1000
Neurotensin	Whole molecule	1/8000
Rat NSE	Not known	1/4000
Pancreatic polypeptide (BPP and HPP)	Not known	1/12000
Serotonin (5HT)	Amino side chain	1/1000
Secretin	Not known	1/6000
Somatostatin	Not known	1/4000
Substance P	C-terminal	1/16000
Vasoactive intestinal polypeptide	C-terminal	1/20000
Insulin	Not known	1/800

0·01 mol/l phosphate-buffered saline (PBS) (pH 7·0) as shown in table II, for 18–24 h at 4°C. The sections were then incubated for 30 min with goat anti-rabbit IgG (Miles Laboratories, 1/200 dilution), followed by rabbit PAP (UCB Bioproducts 1/300, 30 min) and stained with 0·05% 3,3'-diaminobenzidine-tetrahydrochloride with 0·03% hydrogen peroxide in PBS. Sections were counter-stained with haematoxylin. Controls included the application of non-immune rabbit serum or previously neutralised antibodies instead of specific active primary antisera. Neutralisation was achieved by the addition of either pure NSE (0·1 nmol/ml) or synthetic peptides (1–10 nmol/ml) to the corresponding diluted antisera. The PAP complex was also used alone, or with goat anti-rabbit IgG as the first layer, then developed as usual with diaminobenzidine and hydrogen peroxide.

Serial Sections

To determine the possible synchronous production of both peptide and enzyme, serial (4 μm) sections were stained with the enzyme antibody and the antibody corresponding to the peptide hormone(s) known to be present in a particular tumour.

Radioimmunoassay

We used a double-antibody solid-phase radioimmunoassay with ^{125}I-labelled NSE purified from human brain to determine NSE concentration. The assay was performed as previously described.[9] The detection limit ranged from 100 pg to 5 ng per assay tube. Tissue extracts were prepared by homogenising the tumour tissue in 10 volumes of "tris" phosphate buffer (10 mmol/l, pH 7·3) by means of a Brinkman "Polytron D" (setting 6 for 10 s). The resulting homogenate was centrifuged at 100 000 *g* 1 h and the supernatant was saved for analysis. All NSE values were expressed as ng NSE/mg soluble protein. Protein was determined by the method of Lowry.[13]

Results

All 90 endocrine tumours, whether primary or metastatic, secreting a single hormone or more than one, were strongly immunostained by NSE antibodies (figs. 1 and 2). Although better immunostaining was obtained after vapour fixation, satisfactory results were also achieved with routinely fixed material. The intensity of the immunostaining bore no relation to the amount and type of stored peptide (figs. 3 and 4). All APUDomas contained large amounts of extractable NSE (table III).

The very low enzyme concentration detected in non-endocrine neoplasias were seen, by immunostaining, to be due to the presence of NSE in associated non-tumour ganglion cells (fig. 5) or nerve fibres. The intensity of the immunostaining correlated well with the amount of enzyme present in each tumour (table III).

Fig. 1—NSE immunostaining in an ileal carcinoid (PAP method).

bv = blood vessel. Reduced by ⅓ from × 360

Fig. 2—A pancreatic VIPoma immunostained by PAP method with antibodies to NSE.

Variability of degree of immunostaining is not related to amount of peptide stored. This is better illustrated in fig. 3 and 4. Reduced by ⅓ from x 520.

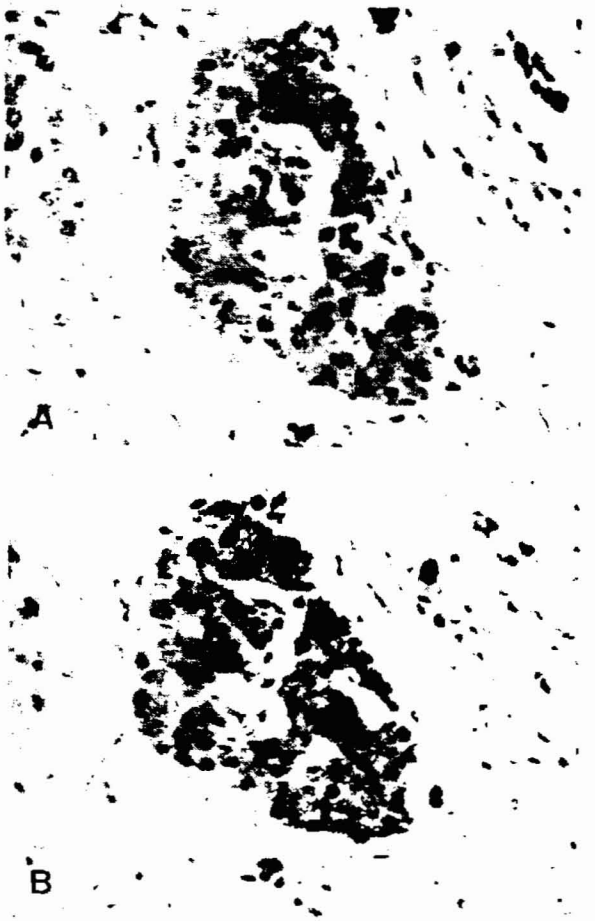

Fig. 3—Immunostaining of NSE and peptide.

(A) A cluster of tumour cells immunostained with specific antibodies to pancreatic glucagon.

(B) 4μm section serial to section A, immunostained with antibodies to NSE (PAP method). Note similar intensity of peptide and enzyme immunoreactivity.

Reduced by ⅓ from x 400.

Fig. 4—Comparative immunostaining of NSE peptide.

(A) A cluster of tumour cells, from the same glucagonoma as in fig. 3, poorly immunoreactive to specific glucagon antibodies.

(B) 4 μm section serial to section A, showing the same cluster of tumour cells strongly immunoreactive to NSE antibodies (PAP method).

Reduced by ⅓ from x 320.

Fig. 5—A gastric carcinoma with non-reactive tumour cells (long arrows) and densely NE immunoreactive non-tumour ganglion cells (short arrows) (PAP method).

Reduced by ⅓ from x 150.

TABLE III—NSE LEVELS IN VARIOUS TUMOURS

Tumour*	NSE (ng/mg protein)	Immunocytochemistry
Glucagonomas	381	+
	766	+
	1271	+ +
	1295	+ +
Insulinoma	362	+
Gut carcinoid	420	+
Medullary thyroid carcinoma	50	+
Phaeochromocytomas	394	+
	1116	+ +
Ganglioneuroblastoma	3871	+ +
Paraganglioma	1724	+ +
Oat-cell carcinomas	3889	ND
	5602	ND
Rectal carcinoma	14·7	–
Hela cells	9·2	ND
	11·8	ND

*Tissues were surgical specimens with the exception of oat cells and hela cells, which were tissue cultures.
ND = not done.

Discussion

We demonstrated that NSE is present in large amounts in tissue extracts from all classes of peripheral neuroendocrine tumours (APUDomas), including islet-cell tumours, phaeochromocytomas, medullary thyroid carcinomas, oat-cell carcinomas and carcinomas of the gut, pancreas, and lung. The concentrations correlated very well with strong NSE immunostaining.

The ability to stain specifically all types of endocrine and neural tumours is particularly valuable, since there are many postulated hormonal systems whose tissue of origin has not so far been demonstrated. Investigation of tumours composed of cells from such systems may help to elucidate these hormonal systems. Previous stains (i.e., argentaffinity or argyrophilia), although popular with pathologists, are clearly unreliable.[14] At present, conventional electron microscopy and peptide immunocytochemistry (at the light and electron microscopical levels) are probably the most accurate methods for diagnosing peptide-secreting endocrine tumours. Antisera to a wide variety of peptides have been produced, but few are presently available to a routine pathology department. The finding of a reliable and sensitive immunostaining method (which uses a single antibody and conventional fixatives) for the identification of APUDomas of all types is therefore of great value.

NSE staining was always intense even in those tumours whose secretory rate was so high that insufficient stored hormone was available for a definitive hormone staining reaction to develop. In such cases, conventional specific staining is of very little value and the detection of NSE antibodies is an excellent method for the demonstration of endocrine tumours. We found that NSE can be detected by radioimmunoassay in plasma samples, and that significantly raised levels are seen in patients with endocrine neoplasia. This raises the possibility of our using plasma NSE measurement as an additional and simple diagnostic method. Further investigations may thus result in a valuable diagnostic test for the routine screening and subsequent therapeutic monitoring of tumours.

F. J. T. is a visiting fellow in the Histochemistry Unit (fellowship from Fundacion Gran Mariscal de Ayuacucho (Venezuela). A. J. A. B. is a visiting colleague in the Histochemistry Unit supported by the Brazilian Research Council (CNPq). This work was generously supported in part by the Cancer Research Campaign. Oat-cell carcinoma cultures were obtained from Dr Adi F. Gazdar, of the National Cancer Institute, Veterans Administration Medical Oncology Branch, U.S.A.

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Hypothesis

COELIAC DISEASE: A GRAFT-VERSUS-HOST-LIKE REACTION LOCALISED TO THE SMALL BOWEL WALL?

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Summary In coeliac disease, gluten, or one of its fractions, combines with a gut-wall macrophage or lymphocyte to form a lymphoid cell which is recognised by the host as foreign. It is proposed that this cell, rather than being eliminated as the target of a cell-mediated attack by the host, becomes autonomous and initiates a graft-versus-host (GvH)-like reaction. The reaction is largely confined to the gut wall and its associated lymphoid tissue. The severe cachexy and the peripheral lymph-node and splenic atrophy may be explained as features of chronic GvH disease or a "runting" syndrome. Untreated coeliac disease may lead to lymphomatous transformation indistinguishable from the lymphoma of experimental chronic GvH disease.

INTRODUCTION

COELIAC disease is a disease of the small bowel caused by the cereal protein, gluten. It has not been established which fraction of gluten causes the disease, but gluten withdrawal from the diet leads to a remission. The mucosal damage is associated with malabsorption and steatorrhoea, and is characterised by villous atrophy, crypt hyperplasia, increased mononuclear-cell infiltration between the mucosal epithelial cells, and an increase in plasma cells in the lamina propria.[1] There may be mesenteric node hyperplasia,[2] and usually spleen and peripheral lymph-node atrophy.[3] Malignant transformation may occur, characteristically to lymphoma:[4] this was usually described as a reticulum-cell sarcoma, or histiocytic lymphoma, but the cells can now be shown to be of B-lymphocyte lineage and the lymphoproliferation is better designated immunoblastic sarcoma.[4] More rarely coeliac disease is associated with autoimmune diseases.[1,5] There is a strong association with the HLA-DW3 antigen and HLA-B8.[6]

CELLULAR INVOLVEMENT IN COELIAC DISEASE

Intra-epithelial lymphocyte counts are very high in untreated coeliac disease, but they fall towards normal after a number of years on a gluten-free diet.[7]

Tests for cell-mediated immunity (CMI) to gluten fractions have not been conclusive, but suggest a role for a cellular mechanism in gluten intolerance.[1] Studies using mucosal lymphocytes have shown more convincing evidence of local CMI to gluten fractions.[8] A high incidence of tuberculin anergy has been demonstrated in coeliac disease,[9] and in the untreated condition there is impairment of lymphocyte transformation to the mitogen phytohaemagglutinin (PHA):[1] both observations indicate non-specific impairment of CMI.

MODELS FOR COELIAC DISEASE

Small bowel involvement in human GvH disease is common; the ileum is usually more involved than the jejunum, but the histological appearances may be similar to untreated coeliac disease.[10]

In a mouse model of GvH (runt) disease, intestinal involvement was shown to be proportional to the amount of spleen cells infused into the host.[11] The early stages of GvH—with attenuation of the villi, crypt hyperplasia, increase in intra-epithelial mononuclear cells with only a very slight increase in lamina propria cells—were similar to the appearances in untreated coeliac disease. Mucosal ulceration, seen in advanced stages of GvH, occurs in 5–10% of coeliac disease.

Allografts of small intestine, transplanted in mice, are rejected within 1–3 weeks.[12] The histological changes of established rejection are very similar to both untreated coeliac disease and GvH disease, with the exception that the mononuclear cellular infiltrate is mainly in the lamina propria, and only a few cells are in an intra-epithelial position. Rejection progresses to total mucosal ulceration and smooth muscle destruction.

GRAFT-VERSUS-HOST DISEASE

GvH disease in man occurs after bone-marrow transplantation. In animals there are several types of model, but for a GvH reaction to occur the host must be able to tolerate the donor cells, the donor cells must be immunocompetent T lymphocytes, and the host must provide an immunogenic stimulus for these cells in the form of an incompatibility between donor and host histocompatibility