Endocrine tumours, somatostatin and somatostatin receptors

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Abstract

Somatostatin blocks the release of numerous growth factors and is therefore a potent inhibitor of cell division and/or secretion. It exerts its effects through binding to somatostatin receptors. Five different subtypes of such receptors are identified (SSTR1 to SSTR5), having various tissue expression. The detection of their presence in tumours can be performed on histological sections and has potential therapeutic implications. (Acta gastroenterol. belg., **2004**, 67, **282-284**).

Key words : somatostatin, somatostatin receptors, *in situ* hybridisation, endocrine tumours.

Background

Somatostatin is a regulatory peptide that was originally identified in the hypothalamus as a tetradecapeptide inhibiting growth hormone secretion (1). However, its distribution is not restricted to the hypothalamus since it is found in other regions of the central nervous system as well as in neuroendocrine cells of several organs such as the digestive tract and pancreas (2,3). In the digestive tract, it is synthesised in specialised D cells, which are particularly numerous in the antrum and fundus, located near gastrin and enterochromaffine-like cells. In the pancreas, somatostatin is also located in D cells (4), where they represent, in adults, nearly 10 percent of the total pancreatic endocrine cells (5). It is also observed in the nerves of the myenteric plexus.

Back in the early 70ies, long before the development of immunocytochemistry, it was already possible to specifically detect somatostatin in D cells, thanks to the lead hematoxylin staining method of Solcia. D cells often show long cytoplasmic processes with dilated extremities where the peptide is stored (Fig. 1). This suggests that they have a direct effect on neighbouring cells via a paracrine mode of secretion (6). Somatostatin is synthesised as a precursor molecule called “preprosomatostatin”, which is cleaved by carboxypeptidase and prohormone convertase (PC1 and PC2) during its maturation process (7). Two different forms of somatostatin are biologically active : somatostatin 28 and somatostatin 14 ; they are produced in different cells. Somatostatin 14 predominates in the D cells of the islets of Langerhans and of the stomach as in the central nervous system whereas somatostatin 28 is more abundant in the D cells of the intestinal tract. The inhibitory action of the peptide is not limited to the central nervous system, in the digestive tract for example, somatostatin is known to inhibit almost all digestive and pancreatic hormones. The same holds true for the exocrine secretion from both stomach and pancreas. Somatostatin also interferes with the motricity of the digestive tract. By blocking the release of numerous growth factors, somatostatin is also a potent inhibitor of cell division. The different inhibitory functions of somatostatin are performed through its binding to membranous receptors called “somatostatin receptors” (SSTR). This explains the interest of this peptide and justifies the study of its receptors for therapeutic purposes (8,9).

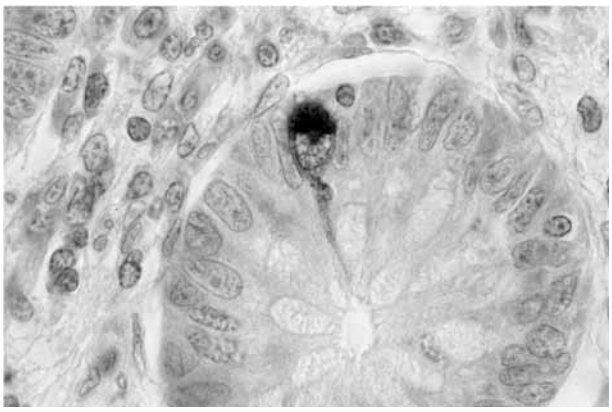

Fig. 1. — D cell in an intestinal crypt showing long cytoplasmic processes and a dilated extremity where the peptide is stored (Objective X 63).

Somatostatin receptors

Subtypes

Five different subtypes of somatostatin receptors have been identified by genetic clonage techniques (SSTR1-5) (10). Distinct genes located on different chromosomes code for these subtypes that differ in both their tissular distribution and concentration (11-13). One of them (SSTR2) is expressed as two distinct forms depending on an alternative splicing of its messenger RNA (14). Both the coupling to secondary messenger of the different SSTRs and their intracellular modes of

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action are also distinct. The affinity of these various subtypes is specific for the different somatostatin analogues (15-17). This has thus a major impact in the detection of the receptors in tumours, and in the choice of a particular somatostatin analogue for a specific therapeutic use.

Tissue expression

SSTRs are widely expressed in normal tissues. In human pancreas for example, insulin-secreting B cells essentially express SSTR1 while glucagon-secreting A cells express SSTR2. SSTR5 is found in both B and D cells (18). This preferential distribution explains that B cell activity is essentially lowered by somatostatin analogues binding SSTR1 while that of glucagon cells depends on ligands specific for SSTR2 (19). In human, 5 subtypes of SSTRs have been detected in the stomach but in other organs, the expression can be restricted to one subtype only as, for example, SSTR3 in the liver.

Numerous tumours also express SSTRs : tumour of the hypophysis, carcinoid tumours, insulinoma, glucagonoma, pheochromocytoma, cancer of the breast, kidney, prostate, meningioma and glioma … The most frequent subtype expressed in these tumours is SSTR2. However, SSTR5 is the most specific subtype for lesions such as breast and insular tumours. As SSTRs are not expressed at the same level in all these lesions, as both content and subtype can vary, and as there are specific ligands for each SSTR type, it is important to determine which precise subtype is actually present in the lesion (8,20-24).

Detection

More than 15 years ago, there were no specific antibodies to the different SSTR subtypes (25). At the time, we tried to identify somatostatin receptor by binding methods, incubating histological sections with somatostatin labelled peroxidase. The sensitivity of the method was poor and the labelling was often difficult to distinguish from the background. Only incubation of fresh sections with radiolabeled somatostatin and further incubation with Rx emulsion as performed by JC Reubi *et al.* gave convincing results (22,26). These methods were effectively very sensitive but background was also important, making a precise recognition and correlation with histological structures sometimes difficult.

Clonage of the different receptor types and the development of *in situ* hybridisation posed the way for important progress in this area. Based on each different SSTR mRNA sequence, we synthesised oligonucleotide probes that are specific for SSTR1, SSTR2 and SSTR5. These probes can be used on histological sections from various tissues and tumours, either obtained at surgery or taken by fine needle biopsies. The mRNA molecular analysis is applied on recent and archived materials fixed in formalin and routinely embedded in paraffin. Histological sections, prepared in RNA-ase free conditions, are incubated with specific oligonucleotide probes, for the different types of SSTRs, labelled with a reporter molecule, such as fluorescein or digoxigenin. The areas where these probes are specifically annealed with the complementary messenger RNA of a particular type of SSTR are then detected, after the immunodetection of the reporter molecule, by the streptavidine peroxidase method. The advantages of these *in situ* hybridisation methods are numerous : the signal may be clearly visualised, its specificity can be strictly controlled, it can be detected even when the number of tumoral cells is very low and thus does not depend on the presence of a high number of tumoral labelled cells in the biopsy. Furthermore, the signal may be quantified as a percentage of labelled cells but also in terms of amount of specific messenger RNA present per cell. This latter analysis relies on densitometric *in situ* quantification, a method as reliable and sensitive as quantitative northern blot (27,28). This can be associated to histological analyses to determine the exact topography of the positive tumour cells. As a result, the intensity of labelling and the precise subtypes of SSTRs located in the lesion are determined and the most appropriate ligand for therapeutic approach may be chosen.

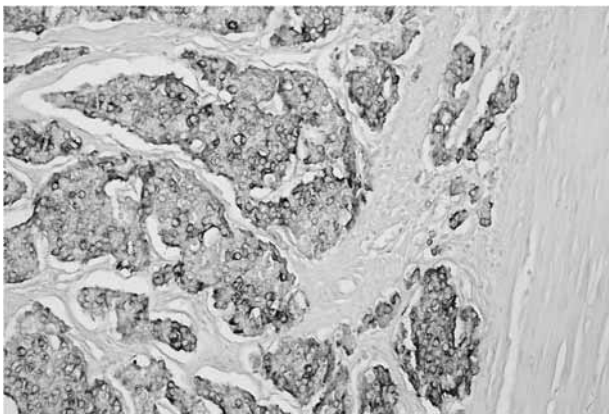

Fig. 2. — SSTR1 mRNA detected by in situ hybridisation in a neuroendocrine tumour of the small intestine (Objective X 10).

From July 2000 to May 2002, we analysed 21 neuroendocrine tumours of the gut and the pancreas with this method. Seventy-two percent of these tumours expressed one or more subtypes of SSTR. SSTR1 mRNA was present in 60% of cases (Fig. 2), SSTR2 mRNA in 55% and SSTR5 mRNA in 40%. Nineteen percent of cases expressed the three subtypes, 50% SSTR1 and 2, 40% SSTR1 and 5 and 36% SSTR2 and 5. In cases without precise diagnosis prior to surgery or in cases with a negative result of octreoscan related to a heavy fibrosis of the endocrine tumour, a positive result by *in situ* hybridisation was particularly useful for further therapeutic decision.

In conclusion, modern pathology offers the opportunity not only to perform accurate diagnosis but also to predict the effects of some therapeutics and to determine whether or not the choice of a specific treatment is appropriate.

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