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Expression of somatostatin receptor subtypes in human pituitary adenomas — immunohistochemical studies

Ekspresja różnych podtypów receptora somatostatynowego w gruczolakach przysadki — badania immunohistochemiczne

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Abstract

Background: The highly variable expression of SSTR subtypes in pituitary adenomas (PA) may partially explain why the subgroup of somatotropinomas or other adenomas do not respond to the therapeutic action of currently used long-acting somatostatin analogues like octreotide or lanreotide.
Material and methods: Our study summarizes the data on expression of all somatostatin receptor subtypes (SSTR 1–5), extended for 2A and 2B SSTR isoforms, revealed by means of immunohistochemistry in dependence to different hormonal phenotype of the tumour.
Results: The pattern of SSTR immunostaining (estimated according to the percentage frequency of appearance) was in acromegaly: **SSTR 5 > SSTR 1 > SSTR 2A = SSTR 3 > SSTR 2B**, in prolactinomas: **SSTR 2B = SSTR 3 = SSTR 5 > SSTR 1 = SSTR 2A**, in gonadotropinomas: **SSTR 3 > SSTR 2B > SSTR 1 = SSTR 2A > SSTR 5**, in corticotropinomas: **SSTR 2A > SSTR 1 = SSTR 3 > SSTR 5 > SSTR 2B**. In PA immunonegative for pituitary hormones, we noticed only a weak staining of all receptor subtypes including SSTR 4. In plurihormonal adenomas with positive GH phenotype the staining pattern was: **SSTR 5 > SSTR 1 = SSTR 2B** and in plurihormonal PA with negative GH phenotype: **SSTR 1 = SSTR 5 > SSTR 2A = SSTR 2B = SSTR 3**. In plurihormonal adenoma with ACTH immunopositivity, the staining pattern was: **SSTR = SSTR 2A = SSTR 3 = SSTR 5.** SSTR 1 and SSTR 5 were the most frequent subtypes of somatostatin receptor in plurihormonal adenomas without ACTH expression.
Conclusions: Human PA represents a group of tumours with a much more differentiated appearance of somatostatin receptor subtypes. It is very important to determine the SSTR profile individually for each tumour to make an appropriate decision as to therapeutic strategy choice. Apart from applying SSTR 2 and SSTR 5-preferring octreotide and lanreotide — newly synthesized multiligand analogues, such as SOM 230, KE 108, or other SST selective analogues, may represent a further useful approach for the treatment, especially in cases other than somatotropinoma or thyrotropinoma. **(Pol J Endocrinol 2009; 60 (4): 240–251)**

Key words: *somatostatin receptor subtypes, pituitary tumours, immunohistochemistry*

Streszczenie

Wstęp: Duża różnorodność ekspresji podtypów receptora somatostatynowego SSTR 1–5 w gruczolakach przysadki (PA) może częściowo wyjaśniać, dlaczego w guzach somatotropowych lub innych obserwuje się brak odpowiedzi na działanie analogów somatostatyny o przedłużonym działaniu, takich jak octreotyd i lanreotyd, wiążących się głównie z podtypem receptora 2 i 5.
Materiał i metody: W pracy podsumowano wyniki badania metodą immunohistochemiczną ekspresji wszystkich podtypów receptora somatostatynowego SSTR 1–5, łącznie z izoformami 2A i 2B, w zależności od fenotypu hormonalnego guza.
Wyniki: Wzory immunoekspresji SSTR 1–5, określone na podstawie procentowej częstości występowania, przedstawiały się następująco: w akromegalii — **SSTR 5 > SSTR 1 > SSTR 2A = SSTR 3 > SSTR 2B**, w guzach prolaktynowych: **SSTR 2B = SSTR 3 = SSTR 5 > SSTR 1 = = SSTR 2A**, w guzach gonadotropowych — **SSTR 3 > SSTR 2B > SSTR 1 = SSTR 2A > SSTR 5**, w guzach kortykotropowych — **SSTR 2A > SSTR 1 = SSTR 3 > SSTR 5 > SSTR 2B**. W guzach immunonegatywnych dla hormonów przysadkowych zanotowano jedynie słaby odczyn dla wszystkich podtypów receptora, łącznie z SSTR 4. W guzach wielohormonalnych GH-dodatnich, ekspresja receptora była następująca — **SSTR 5 > SSTR 1 = SSTR 2B**, a w guzach wielohormonalnych GH-ujemnych — **SSTR 1 = SSTR 5 > SSTR 2A = = SSTR 2B = SSTR 3**. W guzach wielohormonalnych o fenotypie ACTH-dodatnim — **SSTR 1 = SSTR 2A = SSTR 3 = SSTR 5**. W guzach wielohormonalnych ACTH-ujemnych najczęściej występowały SSTR 1 i SSTR 5.
Wnioski: Podtypy receptora somatatostatynowego SSTR 1 i SSTR 5 występują w ludzkich gruczolakach przysadki w sposób bardzo zróżnicowany. Dlatego też, aby podjąć właściwą decyzję dotyczącą sposobu ich leczenia, należy określić indywidualny dla każdego guza, profil SSTR. Nowo zsyntetyzowane multiligandy, takie jak SOM 230, KE 180 lub selektywne analogi, mogą być użyteczne w leczeniu zwłaszcza innych guzów, niż te wydzielające GH lub TSH. **(Endokrynol Pol 2009; 60 (4): 240–251)**

Słowa kluczowe: *podtypy receptora somatostatynowego, gruczolaki przysadki, immunohistochemia*

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Introduction

Long-acting somatostatin analogues, octreotide, and lanreotide are largely used in the medical treatment of growth hormone-secreting pituitary adenomas (PA) (somatotropinomas) in patients suffering from acromegaly because they inhibit the enhanced growth hormone secretion and in some cases cause tumour shrinkage. The same effectiveness of somatostatin analogues was observed in a rare subtype of pituitary adenoma, thyrotropinoma, secreting thyrotropin (TSH), and led to a rare form of hyperthyroidism. Somatostatin (SST) and its analogues act via a specific receptor (SSTR) present on the target cells. Five subtypes of the SST receptor have been identified so far, i.e. SSTR 1-5 with two splicing variants (2A and 2B) of the type 2 receptor [1, 2]. These receptors belong to a group of 7 transmembrane domains linked with the G protein and are encoded by 5 genes which are present on separate chromosomes [3, 4]. The particular SSTR subtype can occur alone or coexist together with other subtypes in the same cell. There could be a considerable variation in SSTR expression between the different adenoma types and among tumours of the same type. The diversity and coexistence of several somatostatin receptor subtypes in one tumour unables unequivocal prediction of which receptor subtype would react with a given somatostatin analogue. This highly variable expression of SSTR subtypes in pituitary adenomas may partially explain why a subgroup of somatotropinomas or other pituitary adenomas do not respond to the therapeutic action of the currently used long-acting somatostatin analogues: octreotide and lanreotide. Therefore, apart from applying SSTR 2 and SSTR 5-preferring octreotide and lanreotide — newly synthesized multiligand analogues, such as SOM 230 (pasireotide), KE 108, or other SST selective analogues, may represent a further useful approach for the treatment of pituitary adenomas. Trials with somatostatin/dopamine (SST/DA) chimeras in types of pituitary tumours also have been undertaken [5]. Therefore, it is very important to determine the individual SSTR profile for each tumour to make an appropriate decision as to the therapeutic strategy choice. The immunohistochemical (IHC) technique performed on paraffin-embedded tissue specimens obtained from surgically removed tumours which are routinely used for histopathological examinations seems the best *ex vivo* in vitro method in detecting cellular distribution of SST receptors [6]. It searches the expression of receptors at the level of receptor protein and gives us insight into the receptor's cellular localization. This method should be considered supplementary to, or even as effective as, methods of molecular biology or radiodiagnostic imaging. The usefulness of this method can even be considered superior, due to lower costs of reagents (molecular biology), shorter time of providing results, and lack of using radioactive reagents (receptor scintigraphy). We have already proven this in our previous studies concerning immunohistochemical detection of SSTR expression in "clinically non-functioning " human pituitary adenomas (CNFPA), adrenal gland tumours, thyroid tumours, and neuroendocrine tumours using antibodies specific for a given receptor subtype [7–10]. The aim of this study was to summarize the data on expression of all somatostatin receptor subtypes (SSTR 1–5), extended for a 2A and 2B SSTR isoforms, by means of immunohistochemistry in order to differentiate hormonal phenotype of the pituitary adenoma.

Material and methods

This project received the approval of the Ethics Committee of the Medical University of Łódź no.: RNN/97/ /06/KE dated 16.05.2006. We investigated 66 pituitary adenomas removed by transsphenoidal adenomectomy. Many of them manifested themselves as clinically non-functioning pituitary tumours before surgery. Based on detailed clinical recognition, hormone levels in patients' blood, and detailed immunohistochemical hormonal estimation after surgery, definitive qualification into particular groups of pituitary adenomas was possible. All samples were immunostained with specific mono- and polyclonal antibodies directed to pituitary hormones or α subunits to determine the hormonal phenotype of the adenoma. After final qualification, our material contained: 9 acromegalic patients (8 female — F,1 male — M, aged 23–60, mean 43 years), 3 prolactinomas (2 F, 1 M, aged 36–57, mean 46.3 years), 22 specimens recognized as gonadotropinoma (8 women, 14 men, aged 39–73, mean 52 years), 5 patients with Cushing disease (4 women, 1 man, aged 29–47, mean 38.6 years), and 4 patients with hormonally immunonegative adenoma (2 women and 2 men, aged 40–72, mean 57 years). Twenty-three tumours were recognized as plurihormonal adenomas (10 F, 13 M, aged 41–71, mean 55.6 years). This last group was divided into 4 subgroups, depending on GH and ACTH immunoexpression. For somatostatin receptor subtype determination, Bouin-Hollande fixed, dehydrated, and paraffin embedded 8-μm sections were immunostained using commercially available rabbit polyclonal antisera raised against carboxyl-terminal fragments of specific human somatostatin receptor subtypes (GRAMSCH Laboratories, Schwabhausen, Germany): SSTR 1 (named SS-840 antibody, corresponding to amino acid sequence 377–391 of the receptor's peptide chain), SSTR 2A (SS-800, specific for 355–369 sequence), SSTR 2B (SS-860, specific for 342–356 sequence), SSTR 3 (SS-850, specific for 381–395

Table I. *Expression of somatostatin receptor subtypes in somatotropic pituitary adenomas, determined by immunohistochemistry method (IHC)*

Tabela I. *Ekspresja podtypów receptora somatostatynowego SSTR 1–5 w guzach somatotropowych, oceniana metodą immunohistochemiczną (IHC)*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2355	Acromegaly F — 43 years old	GH+ PRL+	++ cytopl/ mem	++/+++ cytopl/ mem	++ cytopl/ mem	+ cytopl	negative	++/+++ cytopl/ mem
2.	2391/3	Acromegaly F — 23 years old	GH+ LH+	++ cytopl	+ cytopl	+ cytopl	++ cytopl	negative	+/++ mem/ cytopl
3.	2507	Acromegaly F — 46 years old	GH+ PRL+ αSU+ TSH+single cells	++/+++ cytopl	++ cytopl	++/+++ cytopl	++ cytopl	negative	+++ cytopl
4.	2356	Acromegaly F — 56 years old	GH+ PRL+	++/+++ cytopl	++/+++ mem	++/+++ cytopl	+/–	negative	++/+++ cytopl
5.	1543/2	Acromegaly F — 66 years old	GH+	+/++ cytopl	+/++ cytopl	+/++ cytopl	+/++ cytopl	negative	+++ cytopl
6.	2441/3	Acromegaly F — 60 years old	GH+ PRL+ LH+	+ cytopl	++ mem/ cytopl	+/–	++ mem cytopl	negative	++ mem/ cytopl
7.	1839/4	Acromegaly M — 59 years old	GH+ PRL+αSU+ single cells, TSH+single cells	++/+++ mem/ cytopl	+/++ mem/ cytopl	++/+++ mem/ cytopl	++ mem/ cytopl	negative	++/+++ mem/ cytopl
8.	2430/2	Acromegaly F — 28 years old	GH+ PRL+ LH+ αSU+, TSH+ single cells	++ cytopl	++/+++ cytopl	+/++ cytopl	++ cytopl	negative	++ cytopl
9.	1539/5	Acromegaly F — 30 years old	GH+ PRL+	++ mem/ cytopl	++ mem/ cytopl	++ mem/ cytopl	+++ cytopl	negative	++/+++ mem/ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

sequence), SSTR 4 (SS-880, specific for 374–388 sequence) and SSTR 5 (SS-890, specific for 350–364 sequence). The immunohistochemical procedures were performed as previously described [11]. The working dilution of antibodies was 1: 1000 (diluted in 0.05 M TRIS buffer, pH 7.6 containing 2% goat serum). Following overnight incubation at 4°C in a humidified chamber with primary antibodies, the cells were treated with anti-rabbit IgG biotinylated goat antibody (1:800, DAKO, Denmark) and streptavidin complex (Strept ABC/HRP, DAKO, Denmark). The immunoreaction was visualized with 3.3′-diaminobenzidine (DAB, DAKO, Denmark) solution. For negative controls, the primary antibody was omitted and normal goat serum was used.

The immunoreactive intensity for specific receptor proteins was scored semiquantitatively using a descriptive scale as follows: strong staining (+++), moderate staining (++), weak staining (+), and trace staining (+/–). Only strong (+++) or moderate (++) staining was considered positive of the given subtype of SSTR in the further descriptions. Subcellular distribution pattern of SSTR subtypes — membranous or cytoplasmic — was also determined.

Results

Somatotropinoma

We observed that SSTR 1, 2A, 2B, 3, and 5 subtypes coexist in each of the investigated samples with different intensities, without SSTR 4 (see Table I). This receptor subtype did not occur in any of the investigated samples. In our experiment, we found in the majority of specimens the cytoplasmic localization of the receptor, although membrane-localized immunopositivity also occurred. In 7/9 (77.8%) patients we noticed strong and moderate immunostaining of **SSTR1**; in the remaining

Table II. *Expression of somatostatin receptor subtypes in prolactinomas, determined by IHC*

Tabela II. *Immunohistochemiczna ocena występowania podtypów receptora somatostatynowego SSTR 1–5 w gruczolakach prolaktynowych*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2353/2	Prolactinoma F — 46 years old	PRL+	+/++ cytopl/mem	+ cytopl/ mem	++ cytopl/ mem	++ cytopl/ mem	negative	+++ cytopl/ mem
2.	1058	Prolactinoma F — 57 years old	PRL+	+++ cytopl/mem	+++ cytopl/ mem	++ cytopl/ mem	++ cytopl/ mem	negative	+++ cytopl/ mem
3.	1237	Prolactinoma M — 36 years old	PRL+	++ cytopl	+++ cytopl	++ cytopl	+++ cytopl	negative	+++ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

two the reaction was weak to moderate. **SSTR 2A** and **SSTR3** staining with strong and moderate intensity was observed in 6/9 (66.6%) of samples. In 3 others the immunoreaction was trace to moderate. Strong and moderate intensity of **SSTR 2B** appeared in 5/9 (55.5%) of cases. The 4 remaining cases exhibited moderate to pale reaction. In 8/9 (88.8%) of patients, **SSTR 5** immunostaining was strong to moderate, and only one specimen showed moderate to weak reaction. The pattern of SSTR expression in acromegaly (as estimated according to the percentage frequency of appearance) was **SSTR 5 > SSTR 1 > SSTR 2A = SSTR 3 > SSTR 2B**.

Prolactinoma

Strong and moderate staining of **SSTR 1** and **SSTR 2A**, which was distributed both in the cytoplasm and the membranes or only in the cytoplasm, was shown in 2/3 specimens (66.7%) (Table II). **SSTR 2B, 3 and 5** immunoreactivity with this grade of intensity was observed in all three cases (100%) (Fig. 1–6). The SSTR 4 in all of them was negative. The pattern of immunostaining in prolactinomas was: **SSTR 2B = SSTR 3 = = SSTR 5 > SSTR 1 = SSTR 2A**.

Gonadotropinoma

Only 4 out of 22 (18.2%) gonadotropinomas showed strong and moderate **SSTR 1** immunoreaction, and one expressed this receptor subtype with weak to moderate intensity (Table III). The remaining 17 cases were shown as negative to weak grades of staining. The same score (18.2%) was observed in a case of **SSTR 2A**, with negative to weak immunostaining in the remaining 18 samples. Expressions of **SSTR 2B, SSTR 3, and SSTR 5** were roughly similar and represented 5, 6, and 3 out of 22 samples (22.7%, 27.3%, 13.6%) respectively. Four out of 22 tumours presented SSTR 4 immunopositivity with trace to weak grades of intensity. In the investigated adenoma cells with different intensity of immunoreaction, cytoplasmic and membrane-localized immunopositivity was observed. We also found a local strong to moderate staining in the endothelium (endoth) in one specimen. The pattern of immunostaining in this subtype of pituitary adenomas was: **SSTR 3 > SSTR 2B > SSTR 1 = SSTR 2A > SSTR 5**.

Corticotropinoma

Our experiment demonstrated highly variable expression of SSTR subtypes in five ACTH–secreting pituitary adenomas (Table IV). We observed a mixed distribution pattern of receptors — cytoplasmic and membranous. Strong and moderate staining of **SSTR 1**, which was distributed both in the cytoplasm and the membranes, or in the cytoplasm only, was shown in 4/5 specimens (80%), **SSTR 2A** in 5/5 (100%), **SSTR 2B** in 2/5 (40%), **SSTR 3** in 4/5 (80%), and **SSTR 5** in 3/5 (60%). In contrast to other subtypes of pituitary adenomas, **SSTR 4** immunoreactivity of this subtype was not negative, as in the majority of cases, but weak positive in 4/5 (80%). In corticotropinoma the SSTR 1, 2A, and 3 seem to be the dominant forms of somatostatin receptor, with expression pattern: **SSTR 2A > SSTR 1 = SSTR 3 > SSTR 5 > SSTR 2B**.

Inactive adenomas

In four pituitary adenomas without immunohistochemical reactivity of any hormone, we noticed trace, weak, or negative staining in all of the investigated receptor subtypes, including SSTR 4 (Table V).

Plurihormonal adenomas

Plurihormonal adenoma — GH(+)

This group of adenomas, in spite of expression of GH and some other pituitary hormones, did not reveal acro-

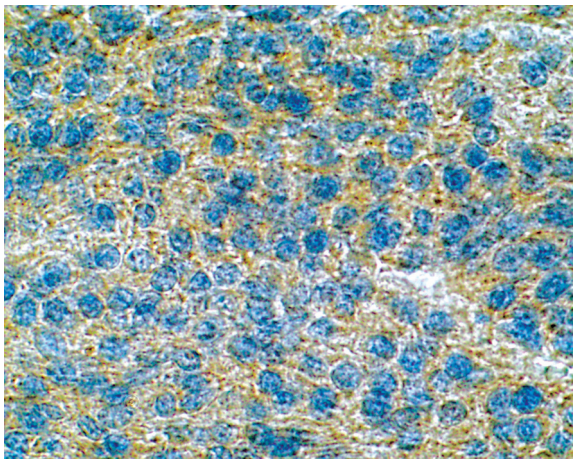

Figure 1. *SSTR 1 expression in a prolactinoma patient with weak to moderate intensity (no. 2353) (+/++ cytopl-mem) (× 600)*

Rycina 1. *Ekspresja SSTR 1 w gruczolaku prolaktynowym o intensywności odczynu od słabej do średniej (nr 2353) (+/++ cytopl-mem) (× 600)*

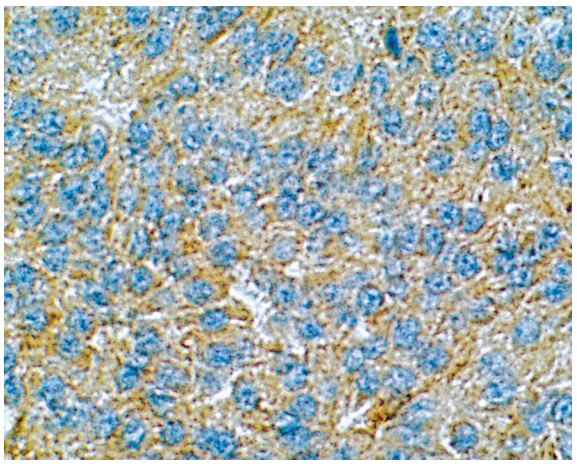

Figure 2. *Weak SSTR 2A expression in a prolactinoma patient (no. 2353) (+ cytopl-mem) (× 600)*

Rycina 2. *Odczyn SSTR 2A o słabym natężeniu w gruczolaku prolaktynowym (nr 2353) (+ cytopl-mem) (× 600)*

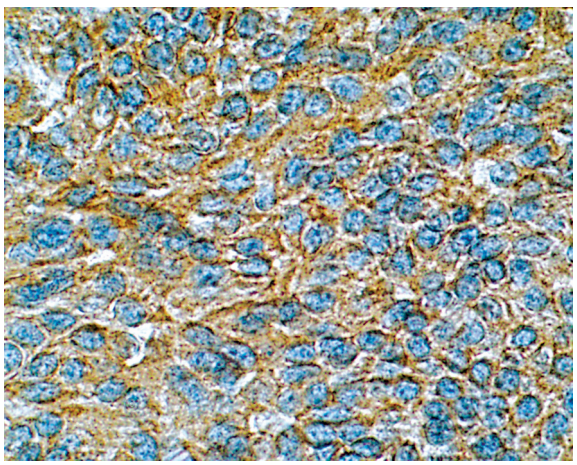

Figure 3. *Moderate immunostaining of SSTR 2B in a prolactinoma patient (no. 2353) (++ cytopl-mem) (× 600)*

Rycina 3. *Umiarkowany odczyn SSTR 2B w gruczolaku prolaktynowym (nr 2353) (++ cytopl-mem) (× 600)*

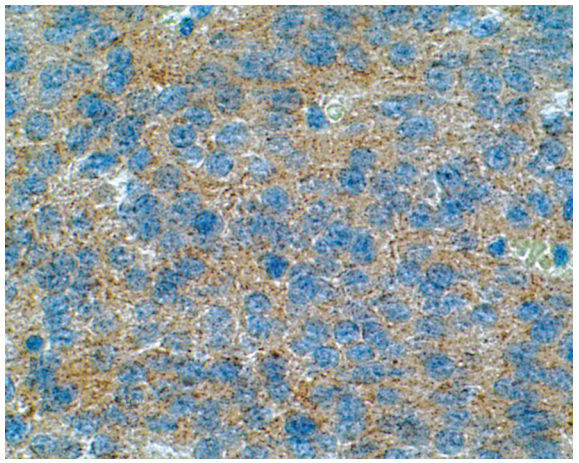

Figure 4. *Moderate SSTR 3 expression in a prolactinoma patient (no. 2353) (++ cytopl-mem) (× 600)*

Rycina 4. *Umiarkowana ekspresja SSTR 3 w gruczolaku prolaktynowym (nr 2353) (++ cytopl-mem) (× 600)*

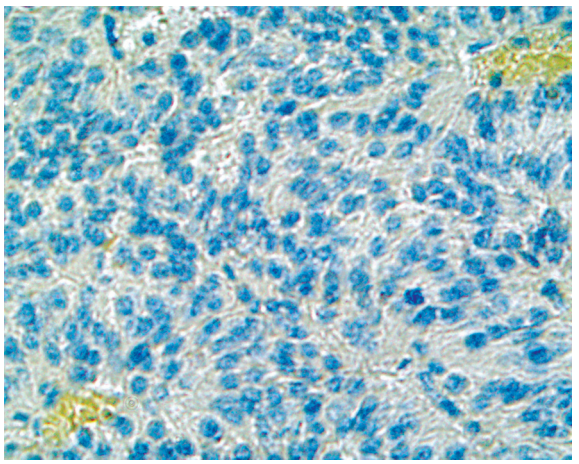

Figure 5. *Negative SSTR 4 expression in a prolactinoma patient (no. 2353) (× 400)*

Rycina 5. *Ujemny odczyn SSTR 4 w gruczolaku prolaktynowym (nr 2353) (× 400)*

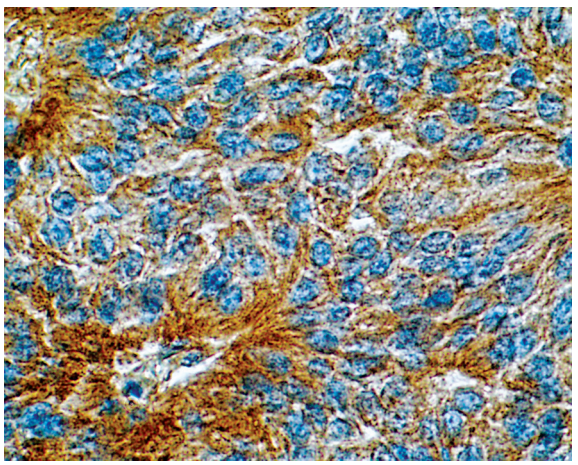

Figure 6. *Strong SSTR 5 staining in a prolactinoma patient (no. 2353) (+++ cytopl-mem) (× 600)*

Rycina 6. *Silna ekspresja SSTR 5 w gruczolaku prolaktynowym (nr 2353) (+++ cytopl-mem) (× 600)*

Table III. *Expression of somatostatin receptor subtypes in gonadotroph pituitary adenomas, determined by IHC*

Tabela III. *Występowanie podtypów receptora somatostatynowego SSTR 1–5 w guzach gonadotropowych, oceniane metodą immunohistochemiczną*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2387/3	Gonadotropinoma M — 64 years old	free β-LH+	++ cytopl	+ cytopl	++ cytopl	+ mem/ cytopl	+/– cytopl	+/++ mem/ cytopl
2.	2289	Gonadotropinoma M — 41 years old	free β-LH+	+/– cytopl	+ cytopl	negative	++ mem/ cytopl	negative	+/++ cytopl
3.	2395	Gonadotropinoma F — 44 years old	free β-LH+	+/++ cytopl	+ cytopl	+ cytopl	+ cytopl	negative	+ cytopl
4.	1500	Gonadotropinoma F — 56 years old	αSU+FSH+ LH+	negative	+/–	+/–	negative	negative	+
5.	1602	Gonadotropinoma F — 72 years old	FSH+LH+ αSU+	+	+	+	+	negative	+
6.	1476	Gonadotropinoma M — 47 years old	FSH+	+/–	+	+	+/–	negative	+
7.	1609	Gonadotropinoma M — 47 years old	FSH+	+/– cytopl	+ cytopl	+/– cytopl	+/– cytopl	+ cytopl	++ cytopl
8.	1646	Gonadotropinoma M — 47 years old	αSU+ LH+FSH+ single cells	+	+/–	+	+	negative	+
9.	1802/5	Gonadotropinoma M — 47 years old	αSU+ FSH+	+ cytopl	+ cytopl	+ cytopl	+++ cytopl	negative	+ cytopl
10.	1662/5	Gonadotropinoma M — 48 years old	FSH+	negative	negative	+/–	negative	negative	+
11.	2299	Gonadotropinoma M — 48 years old	αSU+ LH+ single cells	+++ mem/ cytopl ++/+++ endoth	++ mem ++/+++ endoth	+++ mem/ cytopl ++/+++ endoth	+++ cytopl ++/+++ endoth	negative	+++ cytopl ++/+++ endoth
12.	1667/4	Gonadotropinoma F — 53 years old	αSU+ FSH+ LH+	+	+/–	negative	negative	negative	+
13.	1925/2	Gonadotropinoma F — 53 years old	αSU+ FSH+ LH+	+++ cytopl/ mem	+++ cytopl/ mem	++ cytopl/ mem	++ cytopl/ mem	negative	+ cytopl/ mem
14.	1283	Gonadotropinoma F — 49 years old	FSH+ LH+ αSU+	+	+	+	+/–	negative	+
15.	1825	Gonadotropinoma F — 49 years old	FSH+ LH+ αSU+ single cells	+++ cytopl	+++ cytopl	+++ cytopl	+++ cytopl	+/– cytopl	+++ cytopl
16.	1538/3	Gonadotropinoma M — 39 years old	free-βLH+	+	+	+	+	+	+
17.	1054	Gonadotropinoma M — 39 years old	αSU+	+/–	+/–	+	+/–	negative	+
18.	1057	Gonadotropinoma M — 51 years old	αSU+ FSH+ LH+	+	negative	+/–	+/–	negative	+
19.	1083	Gonadotropinoma F — 73 years old	αSU+	+	+	+	?	negative	+
20.	1603/3	Gonadotropinoma M — 43 years old	αSU+ FSH+	+	+/–	+	+	negative	+
21.	1610/1	Gonadotropinoma M — 70 years old	αSU+	+	+	+	+	negative	+
22.	2785/2	Gonadotropinoma M — 59 years old	βSU+ LH+	+ cytopl	++ mem/ cytopl	++ cytopl /cytopl	++ mem	negative	+ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; endoth: endothelium; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

Table IV. *Expression of somatostatin receptor subtypes in corticotroph adenomas of pituitary, determined by IHC*

Tabela IV. *Ekspresja podtypów receptora somatostatynowego SSTR 1–5 w guzach kortykotropowych, oceniana metodą immunohistochemiczną (IHC)*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2340/2	Corticotropinoma M — 37 years old	ACTH+	+++ cytopl	+++ cytopl	++/+++ cytopl	+++ cytopl	+ cytopl	+++ cytopl
2.	1680	Corticotropinoma F — 43 years old	ACTH+	+ cytopl	++ cytopl	++ cytopl	++ cytopl	+/– cytopl	+ cytopl
3.	1660/1	Corticotropinoma F — 47 years old	ACTH+	+++ mem/ cytopl	+++ mem/ cytopl	+ mem/ cytopl	+++ mem/ cytopl	+/– mem/ cytopl	+++ mem/ cytopl
4.	2385/1	Corticotropinoma F — 29 years old	ACTH+	+++ mem/ cytopl	+++ mem/ cytopl	+ mem/ cytopl	+++ mem/ cytopl	+ mem/ cytopl	++ mem/ cytopl
5.	2284/4	Corticotropinoma F — 37 years old	ACTH+ PRL+(20% of cells)	+++ mem/cytopl	++ cytopl	+ cytopl	+/– cytopl	negative	+ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

Table V. *Expression of somatostatin receptor subtypes in inactive adenomas of the pituitary gland, determined by IHC*

Tabela V. *Ekspresja SSTR 1–5 w guzach immunonegatywnych dla hormonów przysadkowych*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	1128	Adenoma inactivum F — 68 years old	negative IHC	+	+/–	+	negative	+	+
2.	1201	Adenoma inactivum F — 40 years old	negative IHC	+	+	+	+	+	+
3.	1537	Adenoma inactivum M — 72 years old	negative IHC	+	+	+	+	+	+
4.	1800	Adenoma inactivum M — 48 years old	negative IHC	+	negative	+	+/–	negative	+

strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

megaly or another pituitary hyperfunction. Thus, all of them also belong to "clinically non-functioning" pituitary adenomas (CNFPA).

In plurihormonal adenomas with positive GH phenotype of the tumour we found strong and moderate intensity of staining both of **SSTR 1** and **SSTR 2B** at the same level in 3/7 (42.8%) with mixed membrane and cytoplasmic localization (Table VI). **SSTR 2A** and **SSTR3** immunopositivity was also at the same low level in 1/7 (14.3%). In another 4 specimens, weak to moderate staining of SSTR 2A was observed. Negative or pale SSTR 3 reaction in 4 adenomas was noticed. Only one of the tumours representing **SSTR 4** immunopositivity was found. **SSTR 5** was detected in 4/7 (57.1%) cases with moderate to strong intensity and in the next two cases (28.5%), with weak to moderate intensity. Local strong or moderate staining in the endothelium was observed in some specimens. The receptors were distributed mainly in the area of cytoplasm with a few specimens showing mixed membranous – cytoplasmic localization.

The staining pattern was: **SSTR 5 > SSTR 1 = SSTR 2B (57.1% and 42.8%, respectively)**.

Plurihormonal adenoma — GH(–)

Cytoplasmic and membranous distribution of somatostatin receptor subtypes with different intensity was detected in pituitary plurihormonal adenomas with negative GH phenotype of the tumour (Table VII). Strong and moderate immunoreactivity of **SSTR 1** and **SSTR 5** was detected in 3/4 (75%) samples. The SSTR **2A, 2B, and 3** staining was defined as strong or moderate in 2 out of 4 examined specimens (50%). No presence of **SSTR 4** was found in 3/4 (75%) patients. In

Table VI. *Expression of somatostatin receptor subtypes in plurihormonal adenomas of the pituitary gland with positive GH phenotype, determined by IHC*

Tabela VI. *Ekspresja podtypów receptora somatostatynowego SSTR 1–5 w guzach wielohormonalnych przysadki, GH-dodatnich, oceniana metodą immunohistochemiczną (IHC)*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2293/1	Plurihormonal adenoma F — 54 years old	GH+ αSU+ FSH+ LH+ PRL+	+ cytopl	+/–	++ cytopl	+/–	negative	++/+++ cytopl ++ endoth
2.	2294/6B	Plurihormonal adenoma M — 41 years old	GH+ αSU+ LH+	++ mem/ cytopl	+/–/+ mem/ cytopl + endoth	+/–	negative	negative	+/++ cytopl ++ endoth
3.	2295/2	Plurihormonal adenoma M — 71 years old	GH+ αSU+ PRL+ FSH+ single cells LH+single cells	++ cytopl +++ endoth	+ cytopl +/+++ endoth	++ cytopl + endoth	negative	negative	++ mem/ cytopl ++ endoth
4.	2361/4	Plurihormonal adenoma M — 44 years old	GH+ FSH+ LH+αSU+	+ cytopl	+/++ cytopl	negative	negative	negative	+/–
5.	2452/4	Plurihormonal adenoma F — 69 years old	GH+ LH+ PRL+	+ cytopl/ mem	+/++ cytopl/ mem	+/–	+ cytopl	negative	++/+++ cytopl
6.	2782/2	Plurihormonal adenoma M — 46 years old	GH +LH+ FSH+ αSU+	+/++ mem/ cytopl	+/++ mem/ cytopl	+/–/+ cytopl	++ mem/ cytopl	negative	+/++ mem/ cytopl
7.	2345	Plurihormonal adenoma F — 64 years old	GH+ PRL+ LH+ ACTH+	+++ cytopl/ mem	++ cytopl	+++ cytopl/ mem	+ cytopl	+/–	+++ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; endoth: endothelium; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

Table VII. *Expression of somatostatin receptor subtypes in plurihormonal adenomas of the pituitary gland with GH-immunonegative reaction, determined by IHC*

Tabela VII. *Ekspresja podtypów receptora somatostatynowego SSTR 1–5 w guzach wielohormonalnych przysadki, GH-ujemnych, oceniana metodą immunohistochemiczną (IHC)*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	1930/1	Plurihormonal adenoma M — 62 years old	αSU+ single cells PRL+ ACTH+	+/–	+	+/–	negative	+/–	+
2.	2279/2	Plurihormonal adenoma M — 53 years old	PRL+ ACTH+	+/+++ cytopl +++ endoth	+ cytopl/ mem (+++ single cells cytopl) +++ endoth	+++ endoth +/+++ cytopl	++ cytopl/ mem ++ endoth	negative	+/+++ cytopl ++ endoth
3.	2292/1	Plurihormonal adenoma F — 57 years old	PRL+ ACTH+	++ cytopl	++ cytopl	+/–/+ cytopl	+/–/+ cytopl	negative	++/+++ mem/ cytopl
4.	2286/4	Plurihormonal adenoma F — 57 years old	PRL+ ACTH+	+++ cytopl	+++ cytopl	+++ cytopl	+++ cytopl	negative	+++ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; endoth: endothelium; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

Table VIII. *Expression of somatostatin receptor subtypes in plurihormonal adenomas with ACTH immunopositivity, determined by IHC*

Tabela VIII. *Ekspresja podtypów receptora somatostatynowego SSTR 1–5 w guzach wielohormonalnych przysadki, ACTH-dodatnich, oceniana metodą immunohistochemiczną (IHC)*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2122/1	Plurihormonal adenoma F — 60 years old	ACTH+ PRL+ αSU+ LH+ 5% of cells	+ cytopl	++ cytopl	+ cytopl	+ cytopl	negative	+/– cytopl
2.	2283/5	Plurihormonal adenoma F — 60 years old	ACTH+ PRL+	+/++ mem/ cytopl	+/–/+ cytopl	+/–/ + cytopl	++/+++ mem/ cytopl	negative	+/++ cytopl
3.	1930/1	Plurihormonal adenoma M — 62 years old	ACTH+ PRL+ αSU+ single cells	+/–	+	+/–	negative	+/–	+
4.	2279/2	Plurihormonal adenoma M — 53 years old	ACTH+ PRL+	+/+++ cytopl +++ endoth	+ cytopl/ mem +++ single cells (cytopl) +++ endoth	+/+++ cytopl +++ endoth	++ cytopl/ mem ++ endoth	negative	+/+++ cytopl ++ endoth
5.	2292/1	Plurihormonal adenoma F — 57 years old	ACTH+ PRL+	++ cytopl	++ cytopl	+/–/+ cytopl	+/–/+ cytopl	negative	++/+++ mem/ cytopl
6.	2286/4	Plurihormonal adenoma F — 57 years old	ACTH+ PRL+	+++ cytopl	+++ cytopl	+++ cytopl	+++ cytopl	negative	+++ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; endoth: endothelium; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

one of them, this subtype of receptor was demonstrated in pale staining (25%). Local strong or moderate staining in the endothelium was observed in one specimen (no. 2279). Summary SSTR 1 and SSTR 5 were the dominant forms of somatostatin receptor subtypes in this phenotype of pituitary adenoma. The staining pattern was: **SSTR1 = SSTR 5 > SSTR 2A = SSTR 2B = = SSTR 3 (75% and 50%, respectively)**.

Plurihormonal adenoma — ACTH(+)

In 6 examined specimens of plurihormonal adenoma with ACTH immunopositivity, SSTR subtypes 1, 2A, 2B, 3, and 5 appeared and the staining was defined as strong to moderate in 3/6 specimens (50%) (**SSTR 1, 2A, 3, 5**) and in 2/6 (33.3%) patients in case of **SSTR 2B** (Table VIII). There was no observed **SSTR 4** immunostaining in 5/6 (83.3%) cases, and in 1 sample the reaction was pale. In this group of pituitary tumours, as was seen earlier, we noticed a local strong or moderate staining in the endothelium of the same specimen (no. 2279). The receptors were distributed mainly in the area of the cytoplasm with a few having mixed membranous–cytoplasmic localization. The staining pattern was: **SSTR 1 = SSTR 2A = SSTR 3 = SSTR 5 (50%).**

Plurihormonal adenoma — ACTH(–)

The plurihormonal adenomas without ACTH expression were characterized by the coexistence of all the investigated somatostatin receptor subtypes at variable levels in each tumour (Table IX). They demonstrated cytoplasmic and/or membrane-localized immunopositivity. **SSTR 1** and **SSTR 5** dominated with strong and moderate staining in 3/6 specimens (50%); the remaining specimens had less marked (moderate to weak) immunopositivity. **SSTR 2A, 2B, and 3** subtypes where observed only in 2/6 tumours (33.3%) with strong to moderate immunoreactivity, while the remaining tumours represented moderate to weak or even pale to negative staining. It is interesting that in this group of pituitary tumours, we observed negative SSTR 3 immunostaining more frequently than in others (50%). Only one of the tumours represented **SSTR 4** immunopositivity. SSTR 1 and SSTR 5 were the most frequent subtypes of somatostatin receptor in this type of pituitary tumour. **SSTR 1 = SSTR 5 (50%).**

Table IX. *Expression of somatostatin receptor subtypes in plurihormonal adenomas without ACTH expression, determined by IHC*

Tabela IX. *Ekspresja podtypów receptora somatostatynowego SSTR 1–5 w guzach wielohormonalnych przysadki, ACTH-ujemnych, oceniana metodą immunohistochemiczną (IHC)*

No	No of patient		Hormonal phenotype	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	2345	Plurihormonal adenoma F — 64 years old	GH+ PRL+ LH+	+++ cytopl/ mem.	++ cytopl	+++ cytopl/ mem	+ cytopl	+/–	+++ cytopl
2.	2294/6	Plurihormonal adenoma M — 41 years old	αSU+ LH+ GH+	++ mem/ cytopl	+/–/+ mem/ cytopl + endoth	+/–	negative	negative	+/++ cytopl ++ endoth
3.	2295/2	Plurihormonal adenoma M — 71 years old	αSU+ GH+ PRL+	++ cytopl +++ endoth	+ cytopl +/+++ endoth	++ cytopl + endoth	negative	negative	++ mem/ cytopl ++ endoth
4.	2361/4	Plurihormonal adenoma M — 44 years old	FSH+ LH+ GH+ αSU+	+ cytopl	+/++ cytopl	negative	negative	negative	+/–
5.	2424/4	Plurihormonal adenoma M — 44 years old	LH+ αSU+ PRL+ FSH+ single cells	+ cytopl	++ cytopl	+/–	+++ mem/ cytopl	negative	+++ mem/ cytopl
6.	2782/2	Plurihormonal adenoma M — 46 years old	LH+ FSH+ αSU+ GH+5% of cells	+/++ mem/ cytopl	+/++ mem/ cytopl	+/–/+ cytopl	++ mem/ cytopl	negative	+/++ mem/ cytopl

mem: membranous localization; cytopl: cytoplasmatic localization; endoth: endothelium; strong staining (+++), moderate staining (++), weak staining (+), and pale staining (+/–)

Discussion

In the present study we have shown the wide distribution of all of the SSTR subtypes in pituitary adenomas with variable levels both in different groups of pituitary adenomas as well as in individual tumours. Our results confirm, in part, previous studies concerning GH-secreted [6, 12] and clinically non-functioning pituitary adenomas [6, 9]. Therefore, these two types of adenoma were the most frequently investigated in earlier studies. In the present study in acromegalic patients, we observed the following pattern of expression: SSTR 5 > SSTR 1 > SSTR 2A = SSTR 3 > SSTR 2B. Previously, it was shown that SSTR 2A and SSTR 5 appeared with 100% frequency but SSTR 2B and SSTR 3 were less expressed. None of the tumours presented SSTR 4 immunopositivity [12]. These results are generally in agreement with earlier results of other authors [13–16] although they were led mainly by molecular biology methods (RT-PCR — reverse transcriptase or real-time polymerase chain reaction). In 23 somatotropinomas examined by Taboada et al. [13], SSTR 5 mRNA (messenger ribonucleic acid) was the dominant isoform of SSTR in 52% of the tumours, while SSTR 2 mRNA was dominant in 39%. The SSTR pattern observed by the quoted authors was: SSTR 5 > SSTR 2 > SSTR 3 > > SSTR 1 > > > SSTR 4. According to Hofland and Lamberts [14], in human GH-secreting pituitary adenomas, both SSTR 2 and SSTR 5 are involved in the regulation of GH secretion. Using in situ hybridization techniques with selective oligoprobes, Schaer *et al.* [15] revealed a very high incidence of SSTR 5 subtype in growth hormone-producing pituitary adenomas. Nielsen et al. [16] investigated SSTR expression by combining molecular biology (RT-PCR) and in vivo receptor scintigraphy in 7 acromegalic patients. SSTR 2 mRNA was present in all cases, whereas SSTR 4 was absent in all samples. It is of interest that in the group of acromegalic patients partially responsive or resistant to long-term treatment with somatostatin analogues, Matrone et al. [17] demonstrated the expression of SSTR in 1 in 9/16 (56.25%) cases. They suggested the potential usefulness, in the treatment of this group of patients, of somatostatin analogues selective for SSTR 1, for example BIM-23745. We found the expression of SSTR 1 in 77.8% samples of somatotropinomas but we did not have full information about the earlier treatment of the patients.

The group of prolactinomas was scarce because currently this type of tumour is usually treated pharmacologically with D2 receptor ligands. Only patients who are resistant to the pharmacological therapy undergo the pituitary surgery. In our study SSTR 2B, 3, and 5

were equally expressed (100%) while SSTR 1 and 2A were rarely observed. In Hofland and Lambert's study [14], in prolactinomas, SSTR 5 is the key receptor in regulating responsiveness to SS. SSTR 2 is presented with low abundance. Pawlikowski [6] summarized the data from different studies on SSTR expression in prolactinomas. The majority of prolactinomas expressed SSTR 1, 2, and 5, whereas SSTR 3 was detectable only in 1 in 3 cases. SSTR3 was expressed in all of the 3 prolactinomas studied by Greenman and Melmed [18] by reverse transcription coupled to polymerase chain reaction. SSTR 5 was highly expressed in one prolactinoma, but the remaining two prolactinomas had low levels of expression of SSTR 5. RT-PCR quantitative analysis performed by Jaquet et al. [19] demonstrated the presence of SSTR 5 mRNA in all prolactinomas studied. This receptor subtype was dominant in 7 of 10 tumours. The SSTR1 transcript was also highly expressed in these tumours. The SSTR 2 transcripts, although detected in all tumours, were at a much lower level of expression. To summarize, high SSTR 5 expression is common for all the above-mentioned prolactinomas, independently of the method used.

Gonadotropinoma is usually diagnosed before surgery as a clinically non-functioning pituitary adenoma (CNFPA), but immunohistochemical examination after adenomectomy revealed positive staining for LH and FSH or their αSU and/or β-subunit. Therefore, in this way we qualified this group of patients as diagnosed with gonadotroph pituitary adenoma. Our results revealed somatostatin receptor subtypes only in 6 (SSTR 3), 5 (SSTR 2B), 4 (SSTR 1, SSTR 2A) and 3 (SSTR 5) out of 22 investigated tumours with expression pattern: SSTR 3 > SSTR 2B > SSTR 1 = SSTR 2A > SSTR 5 (27.3%, 22.7%, 18.2 %, and 13.6%, respectively). This is in agreement with the results of Taboada et al.[13] where SSTR 3 mRNA exhibited the highest quantity in NFPA, followed by SSTR 2. Our earlier study [9] showed 100% immunopositivity of SSTR 2B and SSTR 5; other subtypes were less abundant. Hofland [14] confirmed the existance of SSTR 2 in a significant proportion of gonadotrophs. Although the dates reported by the above-mentioned authors are not fully concordant, in all of them the expression of SSTR 4 was scarce. In the heterogenous group of clinically non-functioning pituitary adenomas, truly inactive adenomas are next to gonadotropinomas and plurihormonal adenomas. Somatostatin receptor subtype immunoreactivity in inactive tumours was observed in almost all specimens but was faintly detectable. It is interesting that SSTR 4 staining was more frequent than in other tumours with 100% weak immunostaining. This observation corroborates with our earlier study [9]. We noticed a similar, weak SSTR 4 reaction in 4/5 of patients only in corticotropinomas. In addition, this type of pituitary adenoma also expressed multiple receptor subtypes with a frequency of 100% of SSTR 2A, 80% of SSTR 1 and SSTR 3, 60% of SSTR 5, and 40% of SSTR 2B. As in the former types of pituitary tumours, there is no complete agreement in results obtained by different authors [14, 20] although their reports suggest that somatostatin receptor subtype 5 is the most important in regulating ACTH secretion in Cushing's disease. Our findings do not confirm the prevalence of SSTR 5 in ACTH–secreting adenomas. However, our group of tumours manifesting themselves as Cushing's disease was rather scarce and consequently our observations are not conclusive. The lower mean age of patients with Cushing disease (38.6 years) as compared to that with other patients with pituitary tumours is worth consideration.

This study is the first to describe the distribution of somatostatin receptor subtypes in pituitary plurihormonal adenomas. Our aim was to answer the question of whether the immunopositivity of GH or ACTH is linked to a more abundant expression of particular SSTR subtypes. Twenty-three patients with plurihormonal adenomas were divided into 4 subgroups depending on GH and ACTH immunoexpression. The common feature for all of them was the frequently marked immunopositivity of SSTR 1 and SSTR 5. Especially in the group of plurihormonal adenomas without GH expression, we observed an increased appearance of SSTR 1 and SSTR 5. So we can say that in the plurihormonal adenomas, expression of somatostatin receptor subtypes does not depend on GH secretion by tumour cells, and even contrary, the fact of GH absence in the tumour promotes to increase SSTR production in the cells. It is also worth mentioning that SSTR 3 immunonegativity is more frequent than in other types of tumours (42.8% and 50% frequency in GH-positive and ACTH-negative subgroups, respectively).

We conclude that human pituitary adenomas represent a group of tumours with highly differentiated appearance of somatostatin receptor subtypes. Depending on the investigated method used, one can observe the existence of the same SSTR subtype in the same group of tumours at variable levels. The molecular biology method (mainly PCR) is now more often used to investigate receptor expression but it is burdened with some doubts. IHC technique gives us information about receptor expression at the protein level. PCR method gives results at the level of mRNA. However, despite the gene expression it could be posttranslational modification of SSTR without protein synthesis. Thus, mRNA detection alone is not sufficient to confirm the presence of receptor protein, which is the molecular target for somatostatin and its analogues [6, 21]. Moreover, using this method we could not obtain the data concerning

the localization of receptor immunopositivity at the cellular level. Membranous or cytoplasmic receptor protein distribution within the cell can tell us about receptor activity. Because SSTR belongs to a family of 7 transmembrane domains linked with the G protein receptors, one could conclude that only membrane-localized immunostaining is compatible with the functional role of somatostatin receptors; earlier experience confirms this thesis [6, 21, 22].

Conclusions

We conclude that human pituitary adenomas represent a group of tumours with highly differentiated appearance of somatostatin receptor subtypes. It is very important to determine the individual SSTR profile for each tumour by means of immunohistochemistry to make an appropriate decision as to the therapeutic strategy choice. Apart from applying SSTR 2 and SSTR 5-preferring octreotide and lanreotide, newly synthesized multiligand analogues such as SOM 230 (pasireotide), KE 108, or other SST selective analogues may represent a further useful approach for treatment, especially in cases other than GH- or TSH-secreting pituitary adenomas.

Competing interests

The authors declare that they have no competing interests.

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