



# Somatostatin receptors in GH-secreting pituitary adenomas — their relationship to the response to octreotide

## Receptory somatostatynowe w gruczolakach przysadki wydzielających GH — związek z odpowiedzią na oktreotydy

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### Abstract

Twenty pituitary adenomas, surgically removed from patients suffering from acromegaly, were studied. The tumours were immunostained with anti-GH and anti-PRL antibodies and with antibodies raised against particular subtypes of somatostatin receptors (rsst1–5). Expression of rsst immunoreactivity was scored using the following scale: 0 — negative reaction, 1 — weak reaction, 2 — moderate reaction, 3 — strong reaction. In 15 patients in whom the GH response to the acute administration of 200 µg octreotide was tested the correlation between the expression of rsst and the percentage of GH drop was estimated. All the tumours were GH-immunopositive and in the majority (14/20) co-expression of PRL was also found. All the adenomas examined expressed rsst2A (20/20) and rsst5 (12/12) receptor proteins. Receptors sst2B and rsst3 were found in all but one of the tumours examined (19/20 and 11/12, respectively). None of tumours investigated presented rsst4 immunopositivity. The mixed (GH/PRL) adenomas showed a tendency to a higher expression of rsst2A + rsst2B and a greater response to octreotide administration. A significant positive correlation was found between rsst2A + rsst2B expressions and a drop in GH after octreotide.

To conclude, the GH-inhibiting effect of octreotide depends on the intensity of expression of both rsst2A and rsst2B. Both isoforms of rsst2 mediate the same biological response (inhibition of GH secretion) in GH-secreting and GH/PRL-secreting adenomas.

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**Key words:** acromegaly, somatostatin receptors, octreotide

### Streszczenie

Zbadano 20 gruczolaków przysadki, usuniętych operacyjnie u chorych z akromegalią. Na skrawkach parafinowych guzów wykonano odczyny immunohistochemiczne z użyciem przeciwciał anti-GH i anti-PRL oraz przeciwciał przeciwko poszczególnym podtypom receptorów somatostatynowych (rsst1–5). Nasilenie odczynu na rsst oceniano z użyciem następującej skali: 0 — odczyn negatywny, 1 — odczyn słaby, 2 — odczyn umiarkowany, 3 — odczyn silny. U 15 chorych zbadano korelację między nasileniem odczynów na rsst a odpowiedzią GH na podanie 200 µg oktreotydu. Wszystkie badane guzy były immunopoztywne dla GH, a większość wykazywała także ko-ekspresję PRL (14/20). Wszystkie badane gruczolaki wykazywały ekspresję rsst2A (20/20) i rsst5 (12/12). Receptory sst2B i rsst3 występowały także we wszystkich badanych guzach poza jednym (odpowiednio: 19/20 i 11/12). W żadnym z badanych w tym kierunku guzów nie stwierdzono obecności rsst4. Mieszane gruczolaki (GH/PRL) wykazywały tendencje do wyższej ekspresji rsst2A + rsst2B i zarazem silniejszej odpowiedzi na oktreotydy, niż guzy monohormonalne. Stwierdzono znamiennej korelację dodatnią między sumą ekspresji rsst2A + rsst2B a spadkiem GH po podaniu oktreotydu. Podsumowując, hamujący wydzielanie GH efekt oktreotydu zależy od nasilenia ekspresji receptorów sst2A i sst2B. Obie izoformy receptora sst2 w guzach wydzielających hormon wzrostu pośredniczą w tej samej odpowiedzi biologicznej (hamowaniu wydzielania GH). (Endokrynol Pol 2008; 59 (3): 196–199)

**Słowa kluczowe:** akromegalia, receptory somatostatynowe, oktreotydy

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### Introduction

Inhibition of growth hormone (GH) secretion was the first known physiological effect of somatostatin. Because acromegaly is a disease caused by excess of GH, long-

acting somatostatin analogues were soon introduced to treat it. In the 1990s the five subtypes of somatostatin receptor were cloned (for review see: 1). Moreover, one subtype (sst2) exists in two splicing variants, called 2A and 2B, and it was suggested that these two variants



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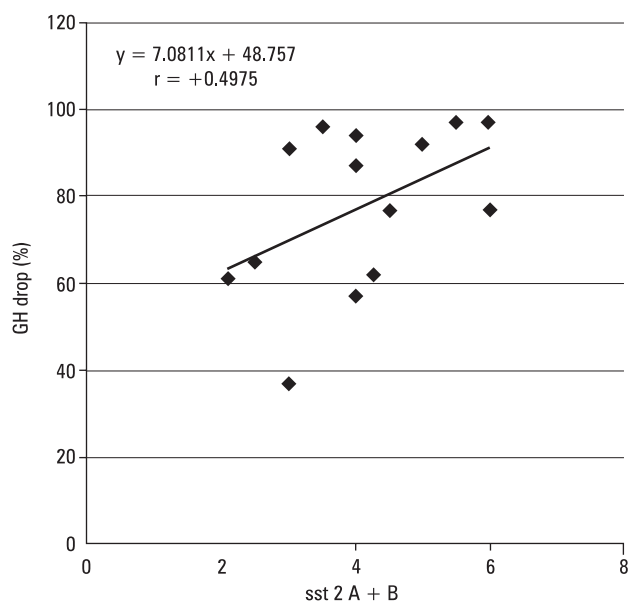
may mediate different biological effects [2, 3]. It was shown that inhibition of GH secretion by somatostatin is mediated by subtypes 2 and 5 [4], although the role of subtype 1 cannot be excluded [5]. Long-acting somatostatin analogues applied in the medical treatment of acromegaly, octreotide and lanreotide bind preferentially to the receptor subtype 2. Earlier studies on somatostatin receptors in GH-secreting pituitary adenomas showed the expression of mostly *sst2* and *sst5* [6–10]. The expression of *sst1* and *sst3* was variable, and *sst4* was only detected in one study [6]. However, the majority of published studies concerned the expression of receptors at the level of mRNA and not at the level of receptor proteins. The aim of the present study was to re-evaluate the data on somatostatin receptors in GH-secreting adenomas by means of immunohistochemistry and to answer the question concerning the relationship between receptor protein expression and responsiveness to the long-acting somatostatin analogue octreotide.

## Material and methods

Twenty pituitary adenomas, surgically removed from patients suffering from acromegaly, were studied. The tumours were immunostained with antibodies against the pituitary hormones (GH, PRL, LH, FSH, TSH, free-alpha SU, ACTH). To detect particular subtypes of somatostatin receptor (*rsst1*–*5*) we used primary antibodies raised against the specific regions of *sst* receptor proteins and obtained from Gramsch Laboratories (Schwabhausen, Germany). The expression of *rsst* immunoreactivity was scored using the following scale: 0 — negative reaction, 1 — weak reaction, 2 — moderate reaction, 3 — strong reaction. In 15 patients the effect of the GH response to the acute administration of octreotide was tested. Octreotide (Sandostatin Novartis) was administered subcutaneously in a dose of 200 µg and GH concentration in the blood serum were measured just before the injection (0') and after 60', 120', and 240'. The maximal drop was expressed as a percentage of the basal value. The correlation between the expression score of *rsst* and the percentage GH drop was assessed using Pearson's coefficient of correlation and analysed by a t-test.

## Results

All the tumours investigated were GH-immunopositive. In the majority of tumours (14/20) co-expression of PRL was also found. Moreover, LH was co-expressed in 4 adenomas, TSH in 2, FSH in one and free-alpha SU also in one tumour. All the adenomas examined expressed *rsst2A* (20/20) and *rsst5* (12/12) receptor proteins.



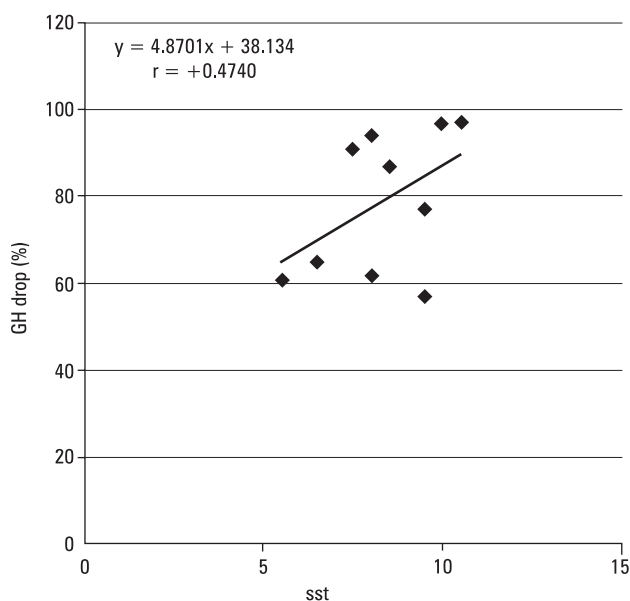
**Figure 1.** Correlation between the summarised score of *rsst2A*+*rsst2B* expression and the drop in GH after octreotide administration

**Rycina 1.** Korelacja między sumą ekspresji *rsst2A*+*rsst2B* a zmniejszeniem stężenia GH po podaniu okretotydu

Receptors *sst2B* and *rsst3* were found in all but one of the tumours (19/20 and 11/12, respectively). None of the tumours presented *rsst4* immunopositivity. The mixed (GH/PRL) adenomas showed a tendency towards higher expression of *rsst2A* + *rsst2B* ( $4.32 \pm 0.28$  vs.  $3.65 \pm 0.61$ , means  $\pm$  SEM) and a greater response to octreotide administration (respective values of GH level drop =  $85.8 \pm 4.1\%$  vs.  $53.6 \pm 8.1\%$ ). However, these differences were not statistically significant, probably because of the low number of cases secreting GH alone in our material. A significant positive correlation was found between the summarised score of *rsst2A* + *rsst2B* expressions and the drop in GH after octreotide administration (Fig. 1). The tendencies towards a positive correlation between the summarised score of *rsst2A* + *rsst2B* + *rsst3* + *rsst5* (Fig. 2) and towards a negative correlation between *rsst2A*/*rsst2B* expression ratio (Fig. 3) and the octreotide effect were also observed.

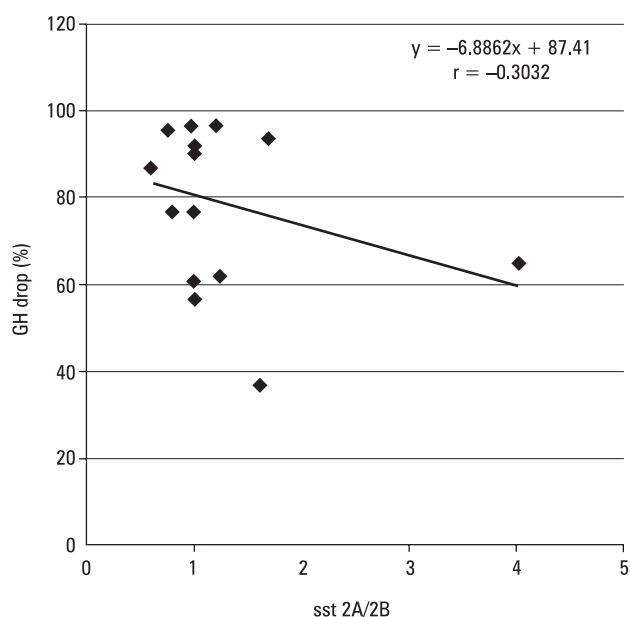
## Discussion

As in the earlier studies [11–13] we found that the majority of pituitary adenomas express not only GH but also PRL and, less frequently, glycoprotein hormones. Results of our immunohistochemical study on the expression of somatostatin receptors in pituitary adenomas excised in patients with acromegaly show a prevalence of *rsst2* and *rsst5* and are concordant with earlier observations cited in the Introduction. They also



**Figure 2.** Correlation between the summarised score of *rsst2A* + *rsst2B* + *rsst3* + *rsst5* (*sst*) and the drop in GH after octreotide administration

**Rycina 2.** Korelacja między sumą ekspresji *rsst2A* + *rsst2B* + *rsst3* + *rsst5* (*sst*) a zmniejszeniem stężenia GH po podaniu okreotydu



**Figure 3.** Correlation between *rsst2A/rsst2B* expression ratio and the octreotide effect on GH secretion

**Rycina 3.** Korelacja między stosunkiem ekspresji *rsst2A* i *rsst2B* a wpływem okreotydu na wydzielanie GH

show that the GH-inhibiting effect of octreotide depends on the intensity of expression of both *rsst2A* and *rsst2B*. Recently it has been shown that the response to octreotide (both *in vivo* and *in vitro*) is positively corre-

lated with the immunohistochemical expression of *rsst2A* [14]. However, expression of *rsst2B* was not estimated in this study. Alderton et al. [2] showed that CHO cells transfected with different isoforms of *rsst2* differentially responded to somatostatin *in vitro*. The *rsst2A* variant mediated growth inhibition, whereas *rsst2B* exerted the opposite effect. In our earlier study on human non-functioning pituitary adenoma cells *in vitro* we observed similar effects. We showed that the expression of *rsst2A* (but not *rsst2B*) correlated positively with the inhibitory effect of somatostatin on tumoural cell viability [15] or chromogranin A and alpha-subunit secretions [3]. The results of the present study do not support the hypothesis concerning the opposite effects of the A and B variants of *rsst2* with respect to the control of GH secretion. It is evident that both isoforms of *rsst2* mediate the same biological response (inhibition of GH secretion) in GH-secreting and GH/PRL-secreting adenomas. However, we cannot exclude the possibility that these opposite effects of *rsst2* isoforms may concern the antiproliferative action in these adenomas. Further study is needed to answer this question.

The question of whether the hormonal phenotype of pituitary adenomas in acromegalic patients detected by means of the immunohistochemical investigation is related to *rsst* expression and somatostatin analogue effectiveness remains unclear. However, the observations made in this study suggest that co-expression of PRL may be accompanied by a higher expression of both isoforms of *rsst2* and a stronger inhibitory response to octreotide.

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## References

1. Melań-Mucha G, Mucha S. Somatostatin receptors: distribution in normal tissues and transduction mechanisms. In: Pawlikowski M (ed.). Somatostatin analogs in diagnosis and therapy. Landes Bioscience, Austin 2007; 7–20.
2. Alderton F, Fan TP, Schindler M. Rat somatostatin *sst2(a)* and *sst2(b)* receptor isoforms mediate opposite effects on cell proliferation. *Br J Pharmacol* 1998; 125: 1630–1633.
3. Pawlikowski M, Ławnicka H, Pisarek H et al. Effects of somatostatin-14 and the receptor-specific somatostatin analogs on chromogranin A and alpha-subunit (alpha-SU) release from “clinically nonfunctioning” pituitary adenoma cells incubated *in vitro*. *J Physiol Pharmacol* 2007; 58: 179–188.
4. Shimon I, Yan X, Taylor JE et al. Somatostatin receptor (SSTR) subtype — selective analogues differentially suppress *in vitro* growth hormone and prolactin in human pituitary adenomas. Novel potential therapy for functional pituitary tumors. *J Clin Invest* 1997; 100: 2386–2392.
5. Matrone C, Pivonello R, Colao A et al. Expression and function of somatostatin receptor subtype 1 in human growth hormone secreting pituitary tumors deriving from patients partially responsive or resistant to long-term treatment with somatostatin analogs. *Neuroendocrinology* 2004; 79: 142–148.

6. Panetta R, Patel YC. Expression of mRNA for all five human somatostatin receptors (hSSTR1-5) in pituitary tumors. *Life Sci* 1995; 56: 333–342.
7. Greenman Y, Melmed S. Expression of three somatostatin subtypes in pituitary adenomas: evidence of preferential SSTR5 expression in the mammosomatotroph lineage. *J Clin Endocrinol Metab* 1994; 79: 724–729.
8. Miller GM, Alexander JM, Bikkal HA et al. Somatostatin receptor subtype gene expression in pituitary adenomas. *J Clin Endocrinol Metab* 1995; 80: 1386–1392.
9. Nielsen S, Mellekjær S, Rasmussen LM. Expression of somatostatin receptors on human pituitary adenomas in vivo and ex vivo. *J Endocrinol Invest* 2001; 24: 430–437.
10. Makowska AM, Matyja E, Dudziak M et al. Expression of three somatostatin receptor subtypes (SSTR2A, SSTR3 and SSTR5) in secreting (HGH, PRL, ACTH) and non-functioning pituitary adenomas. In: Program & Abstracts, Endo2003, Philadelphia, June 19–22, 2003; P1-637 (abstract).
11. Scheithauer BW, Kovacs K, Horvath E. The Adenohypophysis. In: *Bloodworth Endocrine Pathology*, Lechago J, Gould VE (ed.). Williams and Wilkins, Baltimore 1997: 85–152.
12. Salehi F, Cohen S, Syro LV et al. Plurihormonality in pituitary adenomas associated with acromegaly. *Endocr Pathol* 2006; 17: 291–296.
13. Coculescu M, Badiu C, Galoiu S et al. Evolution under complex therapy of acromegaly due to a pituitary plurihormonal adenoma with colocalisation of GH and FSH. *Acta Endocrinol* 2006; 2: 337–348.
14. Ferone D, de Herder WW, Pivonello R et al. Correlation of in vitro and in vivo somatotrophic adenoma responsiveness to somatostatin analogs and dopamine agonists with immunohistochemical evaluation of somatostatin and dopamine receptors and electron microscopy. *J Clin Endocrinol Metab* 2008 (in press).
15. Gruszka A, Kunert-Radek J, Radek A et al. The effect of selective sst1, sst2, sst5 somatostatin receptor agonists, a somatostatin/dopamine (SST/DA) chimera and bromocriptine on “clinically non-functioning” pituitary adenomas in vitro. *Life Sci* 2006; 78: 689–693.