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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 oktreotyd

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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 ug 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, somatostain receptors, octreotide*

Streszczenie

Zbadano 20 gruczolaków przysadki, usuniętych operacyjne u chorych z akromegalią. Na skrawkach parafinowych guzów wykonano odczyny immunohistochemiczne z użyciem przeciwciał anty-GH i anty-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 korelacje między nasileniem odczynów na rsst a odpowiedzią GH na podanie 200 μg oktreotydu. Wszystkie badane guzy były immunopozytywne 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 oktreotyd, niż guzy monohormonalne. Stwierdzono znamienną 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, oktreotyd*

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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 sst 4 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 ug 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.

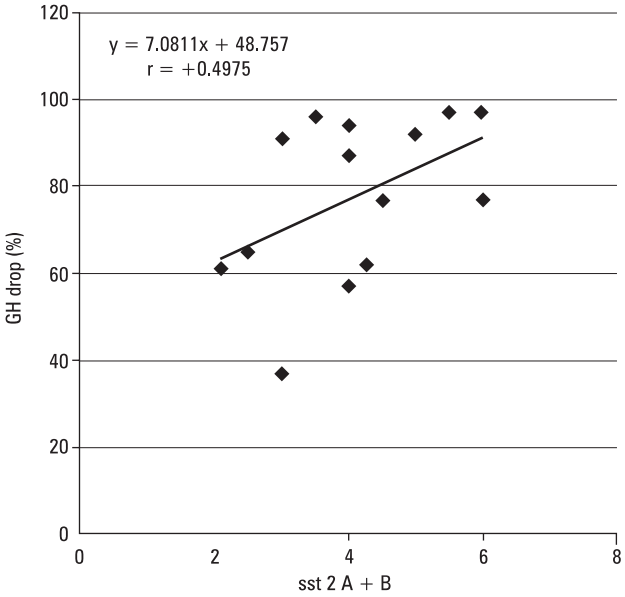

Figure 1. *Correlation between the summarised score of rsst2A+rsst2B expression and the drop in GH after octreotide administration*

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

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 ± 0.28 *vs.* 3.65 ± ± 0.61, means ± SEM) and a greater response to octreotide administration (respective values of GH level drop = 85.8 ± 4.1% *vs.* 53.6 ± 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

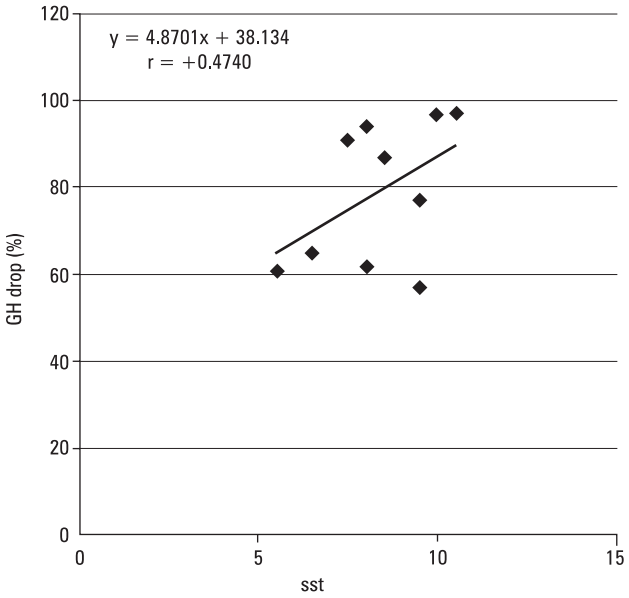

Figure 2. *Correlation between the summarised score of rsst2A + rsst2B + rsst3 + rsst5 (sst) and the drop in GH after octreotide administration*

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

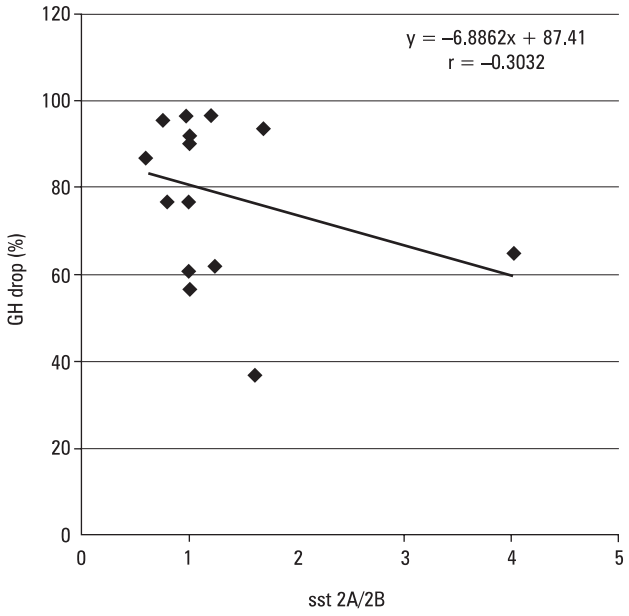

Figure 3. *Correlation between rsst2A/rsst2B expression ratio and the octreotide effect on GH secretion*

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 correlated 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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