



Does the response of GH-secreting pituitary adenomas to octreotide depend on the cellular localization of the somatostatin receptor subtypes SSTR2 and SSTR5?

Czy odpowiedź gruczolaków przysadki wydzielających hormon wzrostu na oktreotyd zależy od komórkowej lokalizacji podtypów SSTR2 i SSTR5 receptora somatostatynowego?

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Abstract

Introduction: The immunohistochemical examination of somatostatin receptor (SSTR) subtypes expression in different endocrine tumours, including pituitary adenomas, revealed membranous or cytoplasmic distribution of SSTR1–5. Currently used long-acting somatostatin analogue octreotide prefers SSTR2 and SSTR5 subtypes. In an earlier study a positive correlation between the summarized score of SSTR2A + SSTR2B expressions and growth hormone (GH) response to octreotide administration was found, independently of receptor distribution within the cell. In this study we searched for the relationship between the GH inhibitory response to acute octreotide administration and SSTR2A, SSTR2B, and SSTR5 cellular localization.

Material and methods: Thirteen acromegalic patients underwent a test of acute administration of octreotide before surgery. The drop in GH was defined as the percentage of the basal value. The pituitary adenomas from these patients were immunostained to determine the hormonal phenotype and expression of SSTR subtypes. The subcellular distribution pattern of SSTR subtypes — membranous or cytoplasmic — was determined.

Results: In the majority of specimens, cytoplasmic localization of receptor subtypes was observed, although membrane or mixed cytoplasmic-membranous localized immunopositivity also occurred. The drop in GH after octreotide administration varied between 57.1–96.7% (mean 82.1%). Among the patients with the cytoplasmic localization of SSTR5, the decrease in GH was significantly higher ($92.0 \pm 7.0\%$). A tendency towards the higher response in patients with cytoplasmic localization of SSTR2A and 2B was also observed (86.8% and 87.0%, respectively).

Conclusions: It seems that cytoplasmic localization of SSTR5, SSTR2A, and SSTR2B is connected with enhanced GH inhibition after octreotide administration. It is possible that this somatostatin analogue alters the localization of subtypes SSTR2A and SSTR2B through the receptor internalization. As a consequence, the SSTR-immunopositivity in cell cytoplasm is increased. The cytoplasmic but not the membranous localization is connected with the higher responsiveness to the octreotide in *somatotropinomas*.

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Key words: octreotide, somatostatin receptor subtypes, somatotropinoma

Streszczenie

Wstęp: Badania immunohistochemiczne ekspresji podtypów receptora somatostatynowego (SSTR1–5, *somatostatin receptor 1–5*) w guzach endokrynnych wykazują ich obecność zarówno w błonie komórkowej, jak i na terenie cytoplazmy komórek guza. Szeroko stosowany obecnie syntetyczny analog somatostatyny — oktreotyd wykazuje powinowactwo głównie do podtypów SSTR2 i SSTR5. W naszych wcześniejszych badaniach zauważono pozytywną korelację między zsumowanym wskaźnikiem (*score*) nasilenia odczynu immunohistochemicznego SSTR2A i 2B, a hamowaniem sekrecji hormonu wzrostu (GH, *growth hormone*) po podaniu oktreotydu u chorych z akromegalią, niezależnie od lokalizacji receptorów w komórkach guza. Obecne badania autorów pracy dotyczą ewentualnej współzależności pomiędzy hamowaniem GH w odpowiedzi na oktreotyd a komórkową lokalizacją SSTR2A, 2B i 5.

Materiał i metody: Zbadano 13 chorych z akromegalią, u których przed operacją wykonano krótki test z oktreotydem. W gruczolakach przysadki określono immunohistochemicznie ich fenotyp hormonalny oraz występowanie i lokalizację podtypów SSTR.

Wyniki: W większości przypadków obserwowano cytoplazmatyczną ekspresję badanych podtypów SSTR, aczkolwiek występowała również lokalizacja błonowa i mieszana błonowo-cytoplazmatyczna. Spadek wydzielania GH w teście hamowania oktreotydem zawierał się w przedziale pomiędzy 57,1–96,7% (średnio 82,1%). Największy znamienny spadek wydzielania GH ($92,0 \pm 7,0\%$) po podaniu oktreotydu wystąpił u pacjentów z cytoplazmatyczną lokalizacją SSTR5 w komórkach guza. Zauważono także tendencję do silniejszej odpowiedzi u pacjentów, u których również SSTR2A i 2B miały położenie cytoplazmatyczne (odpowiednio 86,8% i 87,0%).

Wnioski: Wydaje się, że cytoplazmatyczne, a nie błonowe położenie SSTR2A, 2B i 5, jest powiązane z silniejszym hamowaniem GH w teście z oktreotydem. Możliwe, że oktreotyd w procesie internalizacji receptora zmienia jego położenie z błonowego na cytoplazmatyczne i zwiększa jego immunopozytywność w cytoplazmie badanych komórek guza.

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Słowa kluczowe: oktreotyd, podtypy receptora somatostatynowego, guzy somatotropowe przysadki

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Introduction

The immunohistochemical investigation of somatostatin receptor subtypes (SSTR) expression in different endocrine tumours, including pituitary adenomas, revealed membranous or cytoplasmic distribution of SSTR1–5 within the investigated tumour cells. As the SSTR belongs to a family of seven transmembrane domains linked with the G protein receptors [1, 2], one could conclude that only the membrane-localized immunostaining is compatible with the functional role of somatostatin receptors. Earlier experience supports such a thesis [3–5]. Therefore, some authors suggest that in neuroendocrine tumours, only the membranes located SSTR2 are active although the active SSTR3 and SSTR5 subtypes possess the cytoplasmic localization [6, 7]. On the other hand, in earlier studies [8–11] we described membranous, cytoplasmic, and mixed membranous-cytoplasmic distribution of SSTR1–5 in thyroid, adrenal, and pituitary tumours as well as neuroendocrine tumours, without the statement of their functional state. There is a highly variable expression of SSTR subtypes in these tumours. This fact may partially explain why some of them (for instance a subgroup of *somatotropinomas* or other pituitary adenomas) do not respond to the therapeutic action of the currently used long-acting somatostatin analogues: SSTR2 and SSTR5, preferring octreotide and lanreotide. A significant positive correlation between the summarized score of SSTR2A + SSTR2B expressions and growth hormone (GH) response to octreotide administration was found, independently of receptor distribution within the cell [12]. In this study a tendency towards a positive correlation between the summarized score of SSTR2A + SSTR2B + SSTR3 + SSTR5 to the octreotide treatment was also observed. However, the interdependence of the octreotide effect and somatostatin receptor subtype SSTR2 and SSTR5 distribution was not studied. So in the present study we would like to take into consideration the possible relationship between GH inhibition level to acute octreotide administration and SSTR2A, SSTR2B, and SSTR5 localization in *somatotropinoma* cells.

Material and methods

A group of 13 acromegalic patients (9 females and 4 males, aged 23–67 years, mean age 43.4 years) was investigated. Before surgery, all of the patients underwent a test of acute administration of octreotide (Sandostatin, Novartis) given subcutaneously in a dose of 200 μ g. The growth hormone level was measured in the blood serum at the time intervals 0', 60', 120', and 240' after injection. The drop in GH was defined as the percentage of the basal value. The pituitary adenomas

removed by transsphenoidal adenomectomy from these patients were fixed in Bouin-Hollande fixative and paraffin embedded. All samples were immunostained with specific mono- and polyclonal antibodies directed to pituitary hormones or α subunits to determine the hormonal phenotype of the adenomas. For somatostatin receptor subtype determination, the immunohistochemical procedure was performed as previously described [13]. The 4–5 μ m paraffin sections were immunostained using commercially available rabbit polyclonal antisera raised against carboxyl-terminal fragments of specific human somatostatin receptor subtypes (GRAMSCH Laboratories, Schwabhausen, Germany). 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 (\pm). Subcellular distribution pattern of SSTR subtypes — membranous or cytoplasmic — was also determined. Statistical analysis was performed using Statistica 8 software. The level of statistical significance was set at $p < 0.05$.

Results

In all the investigated tumours the immunohistochemical estimation of hormonal phenotype revealed positive immunostaining of GH. In all but one tumour, PRL was also co-expressed. In the three adenomas, co-expression of LH was found, and another two adenomas co-expressed TSH or free α -SU. All of the adenomas investigated expressed the somatostatin receptor subtypes with different intensity. Only SSTR4 did not occur in any of investigated samples. We found strong and moderate immunostaining of SSTR2A in 9/13 adenomas (69.2%). In the remaining 4 cases the reaction was weak or weak to moderate. Somatostatin receptor 2B was expressed in the same score 9/13 (69.2%). In the remaining 4 patients the reaction was trace, weak, or weak to moderate. The immunopositivity of SSTR5 with strong and moderate grade of intensity appeared in 10/12 (83.3%) of the tumours. In the two adenomas the reaction was weak to moderate. We also determined the immunoreaction for the remaining subtypes: SSTR1 (10/12 = 83.3%) and SSTR3 (9/12 = 75%). In the majority of specimens cytoplasmic localization of receptor subtypes was observed, although membrane or mixed cytoplasmic-membranous localized immunopositivity also occurred. The drop in GH after the octreotide administration varied between 57.1% and 96.7% (mean 82.1%). Thus, all patients were "responders". Table I presents the percentage of GH in dependence to the cellular distribution of the investigated subtypes of SSTR: 2A, 2B, and 5. As can be seen, the decrease in GH in response to the octreotide is significantly higher in the case of

Table I. The correlation between the inhibition of growth hormone (GH) in response to acute octreotide treatment (expressed in percentage rate) and SSTR2A, SSTR2B, and SSTR5 distribution in the somatotropic pituitary adenomas**Tabela I.** Współzależności pomiędzy hamowaniem hormonu wzrostu (GH) w odpowiedzi na krótki test działania oktreotydu a komórkową lokalizacją SSTR2A, 2B i SSTR5 w somatotropowych guzach przysadki

SSTR subtype	Percentage (%) of response GH to acute octreotide treatment with membranous receptor localization	Percentage (%) of response GH to acute octreotide treatment with cytoplasmic receptor localization
SSTR2A	76.3 ± 16.95	87.0 ± 13.95, p > 0.05 (NS)
SSTR2B	78.9 ± 17.2	86.8 ± 13.1, p > 0.05 (NS)
SSTR5	66.4 ± 11.6	92.0 ± 7.0, p < 0.001
SSTR2A+2B+5	73.5 ± 15.4	88.6 ± 11.2, p < 0.01

patients in whom the purely cytoplasmic distribution of SSTR5 was found. There is also a tendency towards a higher response in patients with cytoplasmic localization of SSTR2A and 2B.

Discussion

The pattern of SSTR expression in acromegaly (as estimated according to the percentage frequency of appearance with strong to moderate intensity of staining) was SSTR5 = SSTR1 > SSTR3 > SSTR2A = SSTR2B. It agrees with our earlier observations [10] in which the dominance of SSTR5 and, to a lesser degree, that of SSTR1 was found in *somatotropinomas*. In the group of 12 patients where SSTR5 was estimated, this subtype with cytoplasmic distribution was observed in seven patients and with mixed membranous-cytoplasmic localization in five patients. This membranous-cytoplasmic distribution was accompanied by a lower mean level of GH inhibition after acute octreotide administration, compared with SSTR2A and SSTR2B (see Table I). On the other hand, the cytoplasmic localization of this receptor subtype revealed the strongest mean GH inhibition in this test. A similar tendency with strongest GH inhibition after acute octreotide treatment was found in cases of SSTR2A and SSTR2B localized in the cytoplasm. Only 3 out of 13 patients demonstrated strong or moderate intensity of immunohistochemical staining of SSTR2A with membranous or mixed membranous-cytoplasmic distribution. The other six specimens exhibited evidently only cytoplasmic localization of SSTR2A with this grade of intensity. This fact did not support the view that SSTR2 subtype is the integral membranous form of somatostatin receptor and the rapid targeting and integration of this receptor subtype into the cell membrane is responsible for the failure of its detection in the cytoplasm. Moreover, it seems that, as in the case of SSTR5, only cytoplasmic SSTR2A and SSTR2B were connected with enhanced GH inhibition after

acute octreotide treatment. Consequently, it is possible that this somatostatin analogue alters the localization of both subtypes 2 (2A and 2B) of somatostatin receptor through the receptor internalization and consequently increases the SSTR-immunopositivity in cell cytoplasm. The cytoplasmic localization of immunoreactive receptor proteins may be explained in several ways. Firstly, it may be the result of receptor internalization under the influence of an agonist, as discussed above. Secondly, it may represent the receptor protein *de novo* synthesized within the endoplasmic reticulum. Lastly, we cannot exclude that this localization is an artefact produced during the fixation procedure. Summing up, the data presented above, taken together with our earlier study [12], show that the response of GH-secreting adenoma to octreotide is linked with both the intensity of SSTR immunostaining (which represents the total amount of immunoreactive receptor protein) and the SSTR cellular localization, but, paradoxically, the cytoplasmic and not membranous localization is connected with higher responsiveness.

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