Expression and Localization of Somatostatin Receptor SSTR1, SSTR2, and SSTR3 Messenger RNAs in Primary Human Tumors using in Situ Hybridization

J. C. Reubi, J. C. Schaer, B. Waser, and G. Mengod

Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Berne, Switzerland [J. C. R., J. C. S., B. W.], and Departamento de Neuroquimica, Centre d'Investigació i Desenvolupament, CSIC, Barcelona, Spain [G. M.]

ABSTRACT

Somatostatin receptor gene expression of SSTR1, SSTR2, and SSTR3 subtypes was evaluated by in situ hybridization in 55 human primary tumors shown to contain a high density of somatostatin receptors in binding assays. All 55 tumors expressed at least one SSTR subtype. Of 55 somatostatin receptor-positive tumors, 46 had SSTR2 mRNA; all 46 were characterized as having receptors with a high affinity for the synthetic analogue octreotide. Of 55 tumors, 12 expressed SSTR1, and 14 expressed SSTR3 mRNA. The subtype SSTR1 was expressed alone in 4 cases, SSTR2 was expressed alone in 33 cases, and SSTR3 was expressed alone in one case. In 4 cases, all 3 SSTR were expressed simultaneously. The cases having SSTR1 mRNA were identified in binding experiments with [125I]-labeled somatostatin-14 and -28 analogues rather than with [125I]-([Tyr5]-octreotide. Whereas meningiomas, neuroblastomas, pituitary adenomas, small cell lung carcinomas, lymphomas, and breast tumors expressed primarily a high abundance of SSTR2, carcinoids, islet cell carcinomas, medullary thyroid carcinomas, and ovarian tumors had a mixed distribution of the somatostatin receptor subtypes. This is the first demonstration of the presence of SSTR1, SSTR2, and SSTR3 in primary human tumors using in situ hybridization. Since these somatostatin receptor subtypes probably mediate distinct somatostatin actions, it may be worthwhile to search for subtype-specific analogues to use for the treatment and diagnosis of these tumors.

INTRODUCTION

In the last few years, the presence of SS-Rs has been reported in a large number of human primary tumors and tumor cell lines (1—3), using SS-R-binding techniques, either with in vitro homogenate binding assays or SS-R autoradiography. In the rat and human brain, the use of different iodinated SS analogues made it possible to differentiate pharmacologically between various SS-R subtypes, in particular those with high (SS1 subtype) or low (SS2 subtype) affinity for octreotide (4—6). Whereas most tumors expressed the SS1 subtype, characterized by a high affinity for SS-14, SS-28, and octreotide, a number of tumors clearly had the SS2 subtype, with a high affinity for SS-14 and SS-28 but a low affinity for octreotide (7). The tumors having SS2 receptors included some GEP tumors: in these tumors, this receptor subtype was shown to be functionally relevant since the excess hormone released by the tumor was inhibited by SS-14 and SS-28, but not by octreotide, in vitro as well as in vivo (2). In pituitary adenomas and meningiomas, only a minority of the SS-R-positive cases appeared to express the SS2 subtype preferentially (1). However, approximately 50% of the receptor-positive medullary thyroid carcinomas expressed the SS2 subtype (8). Finally, we found that all SS-R-positive ovarian tumors were of the SS2 subtype (9).

The recent cloning of several SS-R genes has increased our understanding of SS-R structure and function. To date the human SS receptor subtypes SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 have been cloned and partially characterized. These subtypes belong to a superfamily of receptors that have seven membrane-spanning domains (10—13). They have distinct, often overlapping, patterns of expression in human organs such as the brain, the pituitary, the gastrointestinal tract, the pancreas, and the kidneys. All five receptor subtypes can functionally couple to the inhibition of adenyl cyclase. Pharmacological studies showed that all five human subtypes bind SS-14 and SS-28 with a high affinity, whereas the SSTR2 subtype preferentially binds the octapeptide analogue octreotide with very high affinity. Although the SS-R subtypes share many biochemical and pharmacological characteristics, their sequences are relatively divergent and may promote the diverse biological actions of SS because of potentially disparate receptor-coupled signal transduction mechanisms.

The present study was designed to evaluate which SS-R subtypes are expressed in selected SS-R-positive primary human tumors, representative of all types of SS-R-positive tumors, including pituitary adenomas, meningiomas, neuroblastomas, GEP and breast tumors, lymphomas, MTC, SCLC, and ovarian carcinomas. In situ hybridization of SSTR1, SSTR2, and SSTR3 mRNAs was used and compared with in vitro receptor binding on adjacent sections.

MATERIALS AND METHODS

Specimens. All tested tumors had been surgically removed, immediately frozen on dry ice, and stored at —70°C before use. The tumors were cut on a cryostat (Leitz 1720) into 10- or 20-μm sections and mounted on precleaned microscope slides. The sections used for receptor autoradiography were stored at —20°C until processed further. Adjacent sections used for in situ hybridization were air dried and fixed with 4% formaldehyde, washed with phosphate-buffered saline, dried, and stored at 4°C under dry conditions until processed further.

The tumors were selected on the basis of their high content of SS receptors in binding assays. The various tumor types tested are listed in Table 1. Most of these tumors had been used in previous studies (9, 14—20) for other purposes.

In Situ Hybridization Histochemistry. Crystalline sections were used for SSTR1, SSTR2, and SSTR3 mRNA detection by in situ hybridization. The protocol followed was essentially that described in detail previously for SS mRNA in situ hybridization (21-23). Forty-eight-base long oligonucleotides complementary to the bases coding for amino acids 2—17 and 252—267 of the human SSTR1 mRNA sequence (10), 31—46 and 237—252 of the human SSTR2 mRNA sequence (10), and 228—243 and 366—381 of the human SSTR3 mRNA sequence (11) were synthesized and purified on a 20% polyacrylamide-8 M urea sequencing gel (Microsynth, Windisch, Switzerland).

They were labeled at the 3′-end by using [α-32P]dATP (>3000 Ci/mmole; Amersham, Aylesbury, Buckinghamshire, United Kingdom) and terminal deoxynucleotidyltransferase (Boehringer, Mannheim, Germany) to specific activities of 0.9—2.0 x 10⁶ Ci/mmol (22, 23).

Several control experiments were carried out with the probes used in the present study in order to determine the specificity of the hybridization signal obtained (22, 23). The hybridization pattern obtained with two oligonucleotides complementary to different regions of SSTR1, SSTR2, or SSTR3 mRNA, when used independently as hybridization probes in consecutive sections of tumor tissue, was the same for both probes, with similar exposure times. The...
RESULTS

A comparison of the expression of mRNA for SSTR1, SSTR2, and SSTR3 with the binding data using two different radioligands, \(^{125}\text{I}-[\text{Ty}]\text{-octreotide}\) and \(^{125}\text{I}-[\text{LTT}]\text{-SS-28}\) is given in Table 1. In the pituitary tumors (7 GH and one TSH adenomas) the presence of high amounts of mRNA for SSTR2 was identified in all cases, whereas mRNA for SSTR1 was not found. SSTR3 mRNA was detected in 3 of 8 cases. In the corresponding binding experiments, the two radioligands used were similarly able to identify a high density of SS-R; SS-28 and octreotide both showed high-affinity binding. Fig. 1 shows SSTR2 mRNA and octreotide-binding sites in one GH- and one TSH-secreting pituitary adenoma. In all 10 meningiomas tested only SSTR2 mRNA was identified (Table 1). Fig. 2 shows an example of a meningioma containing SSTR2 mRNA and high-affinity binding sites for \(^{125}\text{I}-[\text{Ty}]\text{-octreotide}\) and for \(^{125}\text{I}-[\text{LTT}]\text{-SS-28}\) in the tumor tissue exclusively. In GEP tumors, more diversity and variability in the SS-R subtype pattern were found with all possible combinations expressed (Table 1). As shown in Fig. 3, one islet cell carcinoma had the mRNAs for all 3 subtypes; all 3 were expressed homogeneously in the whole specimen, but SSTR2 mRNA was much more abundant than the other two. The expression of all 3 subtypes was also found in a second islet cell carcinoma and in one carcinoid (Table 1). As shown in Fig. 4, another carcinoid had one tumor part expressing only SSTR1 mRNA and another part expressing SSTR1 and SSTR2 mRNAs. Receptor autoradiographic experiments demonstrated 2 different receptor subtypes discriminated by the 2 different SS-R radioligands, confirming the results previously published for this case (17). Breast tumors, lymphomas, SCLCs, and neuroblastomas had an abundance of SSTR2 in the majority of the cases compared to the two other subtypes. On the contrary, MTC and ovarian tumors had SSTR2 receptors less frequently. As shown in Fig. 5, the MTC 2/59 has only
SOMATOSTATIN RECEPTOR EXPRESSION IN PRIMARY HUMAN TUMORS

had a high density of $^{125}$I-[Tyr$^3$]-octreotide as well as $^{125}$I-[LTT]-SS-28 binding sites but no detectable SSTR1, SSTR2, or SSTR3 mRNAs.

**DISCUSSION**

This study shows for the first time the presence of mRNA for three SS-R subtypes, SSTR1, SSTR2, and SSTR3, in primary human tumors, revealed by *in situ* hybridization histochemistry. Human tumors have, therefore, the potential to express all three SS-R subtypes. However, each individual tumor only rarely expresses the three types simultaneously. The mRNA most frequently and most abundantly expressed in these tumor specimens is that for the SSTR2 subtype since it was found in all pituitary adenomas, meningiomas, neuroblastomas, breast tumors, and SCLCs as well as in a majority of GEP tumors and lymphomas. The mRNAs for SSTR1 and for SSTR3 in general were found less frequently: they were most regularly found in GEP tumors, MTC, and ovarian cancers. In the majority of cases, the mRNA for the SS-R subtypes was homogeneously distributed in the whole tumor tissue, suggesting that most tumor cells are expressing the receptor, an observation also confirmed with the present autoradiography experiments. Except in one carcinoid case, in which SSTR1 and SSTR2 mRNA were clearly localized in different tumor regions, it was not possible to evaluate whether the different subtypes, when simultaneously expressed in a single tumor, are localized on the same tumor cells.

An important aspect of this study is the comparison of the expression of the different mRNAs with the receptor detection using *in vitro* binding data with $^{125}$I-[Tyr$^3$]-octreotide and $^{125}$I-[LTT]-SS-28 in autoradiographic studies on adjacent sections. The study clearly demonstrates that all tumors expressing the mRNA for the SSTR2 receptor subtype have a high affinity for octreotide and can be labeled with the octapeptide $^{125}$I-[Tyr$^3$]-octreotide. Conversely, the four tumors tested in the present study, which were shown to express only receptors of the SSTR1 subtype and not of the SSTR2 subtype were found to bind $^{125}$I-[LTT]-SS-28 or $^{125}$I-[Tyr$^3$]-SS-14 but not labeled octreotide.

SSTR1 receptors which can be detected only with $^{125}$I-[LTT]-SS-28 but not with $^{125}$I-[Tyr$^3$]-octreotide.

All tumors expressing SSTR2 mRNA had SS-R identified by binding experiments with $^{125}$I-[Tyr$^3$]-octreotide and $^{125}$I-[LTT]-SS-28 and were shown to have high affinity for octreotide and SS-28. Those four tumors expressing only SSTR1 mRNA, however, had SS-R identified with $^{125}$I-[LTT]-SS-28, but not with $^{125}$I-[Tyr$^3$]-octreotide; they showed high affinity for SS-28 but low affinity for octreotide. Those 3 cases with SSTR1 and SSTR3 mRNAs but no SSTR2 mRNA were all found to have $^{125}$I-[LTT]-SS-28 and $^{125}$I-[Tyr$^3$]-octreotide binding, as shown in Fig. 6 in the example of an insulin-secreting ICC. This suggests that octreotide binding may also identify to some extent the SSTR3 receptor subtype. However, the ovarian carcinomas 6/B, the only tumor having only SSTR3 receptor expression, only bound $^{125}$I-[LTT]-SS-28.

Additional exceptions were the carcinoids 8/WT and 10/LE, which

---

**Fig. 1.** SSTR2 somatostatin receptor subtype in a GH-producing pituitary adenoma (A and B) and in a TSH-producing pituitary adenoma (C and D). A and C, autoradiograms showing high amounts of SSTR2 mRNA in the tumors. B and D, autoradiograms showing total binding of $^{125}$I-[LTT]-SS-28 in adjacent sections of the tumor tissue. Nonspecific binding (in presence of $10^{-8}$ M SS-28) was negligible. Bar, 1 mm.

**Fig. 2.** SSTR2 somatostatin receptor subtype in a meningioma. A, hematoxylin-eosin-stained section. Bar, 1 mm. B, autoradiogram showing high amounts of SSTR2 mRNA. C, autoradiogram showing total binding of $^{125}$I-[Tyr$^3$]-octreotide in adjacent sections of the tumor tissue. D, autoradiogram showing total binding of $^{125}$I-[LTT]-SS-28 in adjacent sections of the tumor tissue. Receptors are located in the tumor but not in the connective tissue.

**Fig. 3.** SSTR1, SSTR2, and SSTR3 receptor subtypes in an insulinoma. A, hematoxylin-eosin-stained section. Bar, 1 mm. B, autoradiogram showing high amounts of SSTR2 mRNA. C, autoradiogram showing total binding of $^{125}$I-[Tyr$^3$]-octreotide in adjacent sections of the tumor tissue. D, autoradiogram showing total binding of $^{125}$I-[L TT]-SS-28 in adjacent sections of the tumor tissue. Receptors are located in the tumor but not in the connective tissue.
good correlation between SS-R binding using receptor autoradiography and mRNA detection using in situ hybridization histochemistry (17). Interestingly, the three tumors expressing SSTR1 and SSTR3 only, one ICC, one MTC, and one lymphoma, were shown to have a strong 125I-[Tyr\(^3\)]-octreotide binding and also a significant 125I-[Tyr\(^3\)]-octreotide binding. Since tumors with SSTR1 alone have no affinity for octreotide, it is likely that the SSTR3 component is responsible for the 125I-[Tyr\(^3\)]-octreotide binding. This may be explained by the fact that octreotide still has some affinity to SSTR3 stably expressed in various cell types, with the concentration producing 50% inhibition varying depending on the study considered (11, 25–27), between 3

Therefore, 125I-[Tyr\(^3\)]-octreotide in binding experiments may select, among the SSTR1 and SSTR2 subtypes, the tumors expressing SSTR2 receptors. These SSTR2 receptors are likely to be identical (24) with an SS-R subtype, named SS-1 receptor in earlier pharmacological reports and found to have a high affinity not only for SS-14 and SS-28 but also for small synthetic SS analogues such as octreotide or MK 678 (4–6). The carcinoid case having two tumor regions, one region expressing the two SS-R subtypes SSTR1 and SSTR2 and the other region expressing SSTR1 only, is an outstanding example of the
and 35 nm. The lack of octreotide binding in the only tumor having only SSTR3 mRNA, an ovarian tumor, may be due to the relatively low density of these receptors.

The carcinoids 8/WT and 10/LE, which have a high density of $^{125I}$-[Tyr$^3$]-octreotide receptors but no SSTR1, SSTR2, or SSTR3 mRNAs, may be tumor specimens expressing a further SS-R subtype, either not yet discovered or possibly related to the rat SS receptor subtype identified by O’Carroll et al. (28), having also a high affinity for octreotide (rSSTR5).

This study is not meant to give information about the exact incidence of the mRNAs for SS-R in the various types of primary human tumors; indeed, a selection of tumors identified as strongly SS-R positive in binding experiments was chosen for the present purpose. The study, however, demonstrates for the first time that human tumors shown previously to have binding sites for SS also have the corresponding mRNAs: this is the case for pituitary adenomas (14), meningiomas (22), GEP tumors (17), neuroblastomas (20), breast tumors (19), lymphomas (29), and SCLCs (18), most often characterized by having the SSTR2 subtype. Furthermore, the study confirms other binding data suggesting that selected tumors, such as MTC (8), ovarian cancers (9), or several GEP tumors (15, 17), are characterized by receptor subtypes other than SSTR2, also previously known as SS-1. We do not know to what extent the in situ hybridization histochemistry technique presently used would be sensitive enough to detect mRNAs in tumors with a low density of SS-R measured with binding methods. Neither can we exclude that a very low abundant mRNA for one of the subtypes would have remained undetected with this technique.

The exact function mediated by the 3 SS-R subtypes is not yet clearly established. Interestingly, a preliminary study has suggested that the SSTR2 subtype may be responsible for the antiproliferative effects of SS analogues (30). The fact that a majority of human primary tumors as well as tumor cell lines (31) express this particular receptor type is promising for the therapeutic potential of SS analogues in such tumors. Also of importance is the fact that these SSTR2 receptors have a high affinity for small, stable SS analogues, such as octreotide or lansopride, used worldwide for the treatment of acromegaly and GEP tumors by inhibition of hormone release.

ACKNOWLEDGMENTS

We thank U. Horisberger and U. Läderach for excellent technical assistance.

Some of the tumors used in this study and investigated previously for other purposes were kindly provided by Dr. A. Landolt, Dr. R. Seiler, Dr. J. Fockens, Dr. L. Kvolis, Dr. C. Moertel, Dr. W. Häcki, Dr. V. Macaulay, Dr. S. W. J. Lamberts, and Dr. J. Laisonne.

REFERENCES

10. Yamada, Y., Post, S., Wang, K., Tager, H., Bell, G., and Seino, S. Cloning and functional characterization of a human somatostatin receptor subtype identified by O’Carroll et al. (28), having also a high affinity for octreotide (rSSTR5).
Expression and Localization of Somatostatin Receptor SSTR1, SSTR2, and SSTR3 Messenger RNAs in Primary Human Tumors using \textit{in Situ} Hybridization


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/13/3455