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Somatostatin Receptor Subtypes in Human Thymoma and Inhibition of Cell Proliferation by Octreotide *in Vitro*

DIEGO FERONE, MARTIN P. VAN HAGEN, DIK J. KWEKKEBOOM, PETER M. VAN KOETSVELD, DIANA M. MOOY, ELGIN LICHTENAUER-KALIGIS, AGNES SCHÖNBRUNN, ANNAMARIA COLAO, STEVEN W. J. LAMBERTS, AND LEO J. HOFLAND

Departments of Internal Medicine III (D.F., M.P.v.H., P.M.v.K., D.M.M., E.G.R.L.-K., S.W.J.L., L.J.H.), Immunology (M.P.v.H.), and Nuclear Medicine (D.J.K.), Erasmus University, 3015 AD Rotterdam, The Netherlands; Department of Integrative Biology and Pharmacology, University of Texas (A.S.), Houston, Texas 77225; and Department of Molecular and Clinical Endocrinology and Oncology, Federico II University (D.F., A.C.), 80131 Naples, Italy

ABSTRACT

Somatostatin (SS) and SS receptor (SSR) subtypes, code-named sst_{1-5}, are heterogeneously expressed in the normal human thymus. This suggests their involvement in controlling the immune and/or neuroendocrine functions in this organ. Moreover, recently a high *in vivo* uptake of [^{111}In-DTPA-D-Phe^1]octreotide has been reported in patients bearing thymoma. The present study characterizes *in vivo* and *in vitro*, functional SS-binding sites in a human thymoma.

A high uptake of [^{111}In-DTPA-D-Phe^1]octreotide was observed in the chest of a patient with myasthenia gravis due to a cortical thymoma. Specific binding of [^{125}I-Tyr^{11}]SS-14 was found on a membrane preparation of the surgically removed thymoma. Scatchard analysis showed high affinity binding sites (K_d, 47.5 ± 2.5 pmol/L) with low maximum binding capacity (23.5 ± 2.5 fmol/mg membrane protein). RT-PCR analysis showed the presence of sst_1, sst_{2A}, and a predominant sst_3 messenger RNA (mRNA) expression in the tumor tissue. Primary cultured tumor cells expressed sst_3 mRNA only. In contrast to the normal thymus, SS mRNA was not expressed. By immunohistochemistry, the tumor cells highly expressed sst_3 receptors, weakly expressed sst_1 receptors, and showed no immunostaining for sst_{2A} receptors. sst_{2A} immunoreactivity was found in the stromal compartment of the tumor, particularly on the endothelium of small intratumoral blood vessels. In primary cultured tumor cells, both SS and octreotide (10 nmol/L) significantly inhibited [^{3}H]thymidine incorporation by 40.6% and 43.2%, respectively.

The following conclusions were reached. 1) As this tumor displayed a high immunoreactivity for sst_3 and the cultured tumor cells expressed the sst_3 mRNA only, this SSR may be the subtype involved in the inhibition of epithelial tumor cell proliferation by octreotide *in vitro*. 2) A loss of endogenous SS production in this thymoma might be implicated in the uncontrolled cell growth. 3) In this case, the sst_3 may play a role in determining the uptake of [^{111}In-DTPA-D-Phe^1]octreotide by *in vivo* SS receptor scintigraphy. (*J Clin Endocrinol Metab* **85:** 1719–1726, 2000)

THE THYMUS is the primary lymphoid organ where cytokines and thymic hormones participate in regulating the proliferation and differentiation of T cells (1). These factors are produced by the thymic stroma. The preponderant cell type in the stroma is of epithelial origin, consisting of at least two distinct subsets, cortical and medullary thymic epithelial cells (TEC) (2). Recently, attention has been drawn to the presence of neuroendocrine and peripheral hormones, which seem to participate in an autocrine and paracrine manner to the functioning of TEC (3). Some of these hormones have well characterized actions on immune cell differentiation and proliferation, whereas others, such as somatostatin (SS), have been identified, but not investigated for their function within the thymus (4).

The biological effects of SS, including the inhibition of hormone and exocrine secretion as well as the modulation of neurotransmission and cell proliferation, are mediated through five different G protein-coupled, high affinity, membrane receptor subtypes (sst_{1-5}) (5, 6). SS receptors (SSR) have been demonstrated in normal tissues and in many tumors at the protein and messenger ribonucleic acid (mRNA) levels by receptor binding studies on tissue homogenates, by *in vivo* and *in vitro* SSR autoradiography, by *in situ* hybridization, as well as by RT-PCR (7, 8). Furthermore, SSR subtypes have been localized at the cellular level by immunohistochemistry using a specific antipeptide antibody in a series of human tumors and in normal human pancreas as well (9–13). Localization studies of SSR on specific cell types within SSR-positive tissues demonstrated heterogeneity of receptor distribution (14).

Octreotide, an octapeptide SS analog, binds with high affinity to sst_2 and with a relatively lower affinity to sst_3 and sst_5 (15). Imaging techniques with [^{111}In-DTPA-D-Phe^1]octreotide have visualized SSR in various human neoplasms *in vivo*. In many cases, a positive scan predicts a good response to treatment with octreotide (16, 17).

We have recently characterized the SS and SSR subtype distribution pattern within the normal human thymus (18).

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Address all correspondence and requests for reprints to: Dr. Leo J. Hofland, Department of Internal Medicine III, Room Bd 277, University Hospital Dijkzigt, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. E-mail: hofland@inw3.azr.nl.

SS and sst_1, sst_{2A}, and sst_3 mRNAs were found in thymic tissue, whereas enriched cultured TEC selectively express sst_1 and sst_{2A} as well as SS mRNA (18). Moreover, successful treatments with octreotide have been reported in two patients with thymoma (19, 20). To unravel the functional significance of SSR in human thymomas, we investigated in the present study the expression and functional role of SS and SSR subtypes in a cortical thymoma. *In vivo* SSR scintigraphy was performed in a patient admitted for myasthenia gravis. SSR expression on the tumor tissue was investigated using *in vitro* SSR binding studies, RT-PCR, and immunohistochemistry. Moreover, the effects of SS and octreotide on cell proliferation were evaluated in a primary cell culture of the thymoma.

Materials and Methods

In vivo study

Scintigraphy with [^{111}In-DTPA-D-Phe^1]octreotide was performed as previously reported (21). Briefly, planar and single photon emission tomography (SPECT) images of the chest were obtained 24 h after the injection of 228 megabecquerels [^{111}In-DTPA-D-Phe^1]octreotide (Mallinkrodt, Inc., Petten, The Netherlands). Imaging studies were performed using a two-headed γ-camera (Picker 2000, Picker Instruments, Cleveland, OH) for planar imaging and a three-headed γ-camera (Picker 3000) for SPECT studies, both equipped with a medium energy collimator. Acquisition time for planar spot images was 15 min.

Samples

The thymoma was surgically removed from a female patient (age, 55 yr). Samples from this tumor and from three normal thymuses (from children undergoing cardiac surgery) were used in the present study. The protocol was in accordance with the Helsinki Doctrine on Human Experimentation. Informed consent was obtained from the patients or their parents. Samples were taken directly at the operation, quickly frozen on dry ice, and stored at −80 C for autoradiographic and RT-PCR studies or fixed in 10% paraformaldehyde overnight for immunohistochemistry. An additional specimen of the tumor was used for the establishment of a primary culture.

SS receptor autoradiography and RT-PCR

Receptor autoradiography was carried out on 10-micrometer thick cryostat sections using [^{125}I-Tyr^{11}]SS-14 and [^{125}I-Tyr^3]octreotide as radioligands (SA, ~2000 Ci/mmol) as previously described (18). RT-PCR was performed as previously reported (18). The sizes of PCR products were 318, 332, 651, 221, 323, 223, 349, and 762 bp, for sst_1, sst_{2A}, sst_{2B}, sst_3, sst_4, sst_5, SS, and β-actin, respectively.

SS receptor binding studies

The method of membrane isolation and the reaction conditions were described previously (18). [^{125}I-Tyr^{11}]SS-14 and [^{125}I-Tyr^3]octreotide were used as radioligands.

Immunohistochemical localization of sst_1, sst_{2A}, and sst_3 receptors

Paraffin-embedded sections (5 μm) were deparaffinized, rehydrated, exposed to microwave heating (in citric acid buffer) at 100 C for 15 min, rinsed in tap water followed by phosphate buffer solution, and subsequently incubated for 15 min in normal goat serum (1:10 dilution in phosphate buffer solution and 5% BSA). The sections were then incubated overnight at 4 C with the sst_1 (R1–201) and sst_{2A} (R2–88) antibodies (gifts from Dr. A. Schönbrunn) (22, 23) in a dilution of 1:500 (12) and with the sst_3 antibodies (Biotrend, Cologne, Germany) in a dilution of 1:3000. Finally, a standard streptavidin-biotinylated alkaline phosphatase or peroxidase complex (ABC kit, Biogenix, San Ramon, CA) was used according to the manufacturer's recommendations to visualize the bound antibodies. Negative controls for immunohistochemistry included 1) omission of the primary antibody, and 2) preabsorption of the antibodies with the respective immunizing receptor peptides (at a concentration of 100 nmol/L).

Cell dispersion and cell culture

Normal and neoplastic TEC were isolated as previously described (18). Briefly, the thymoma tissue was enzymatically dissociated with collagenase (Sigma, St. Louis, MO; 2 mg/mL) for 1 h at 37 C. The dispersed cells were precultured for 5–7 days in 15 mg/cm^2 collagen type I (Collagen S, type I, Roche Molecular Biochemicals, Mannheim, Germany)-precoated 75-cm^2 flasks (Costar, Cambridge, MA) at a density of 5×10^6 cells/flask in 10 mL culture medium. The culture medium, the same as that used during the experiments, was MEM D-valine (Life Technologies, Inc., Paisley, Scotland) supplemented with 10% FCS, penicillin (10^5 U/L), fungizone (0.5 mg/mL), and L-glutamine (2 mmol/L). The pH of the medium was adjusted to 7.4. The cells were cultured at 37 C in a humid CO_2 incubator. After this period, cell viability detected by trypan blue exclusion was 90%, and the cells that had not attached to the flasks, mainly thymocytes, were harvested.

The isolation of epithelial cells was indirectly performed using sheep antimouse IgG-coated magnetic beads and an ASO_2 antihuman fibroblast primary antibody kit (Dianova GmbH, Hamburg, Germany). This mouse monoclonal antibody reacts specifically with membrane-bound protein of human fibroblasts of different origins. The magnetic beads were coated with the specific primary antibody and mixed with the target cell suspension to form bead-rosetted cells (fibroblasts), which were collected at the tube wall and isolated using a magnetic separator rack. For a complete negative selection, the supernatant, containing the epithelial cells that were not bound to the beads was treated once again with the coated beads. This remaining suspension containing isolated epithelial cells was used for the experiments. The cells (20,000/well) were seeded in 1 mL culture medium in 24-well collagen type I-precoated plates (Costar) and allowed to attach for 24 h. Thereafter, test substances were added, and the cells were incubated for 72 h. Cell proliferation was measured by adding 1 μCi [*methyl*-^{3}H]thymidine (91 Ci/mmol; Amersham Pharmacia Biotech, Houten, The Netherlands)/well during the last 24 h, as described in detail previously (18). For RT-PCR studies, neoplastic TEC were seeded in collagen-coated flasks (see above) and grown to confluence. Thereafter, the cells were harvested, and mRNA was isolated as described previously (18). For keratin staining, cells were cultured on collagen-coated glass coverslips. The cells were fixed for 10 min with methanol at the end of the incubation period. Cytokeratin staining was performed with a PAP Kit System (code K518, DAKO Corp., Glostrup, Denmark). Cultured thymocytes were collected and processed for mRNA analysis by RT-PCR as previously described (18).

Test substances

SS-14 (Bachem, Hannover, Germany) and octreotide (Novartis, Basel, Switzerland) were used at a concentration of 10 nmol/L.

Statistical analysis

Data are expressed as the mean ± SEM. Binding experiments were performed at least twice, and the data shown for the displacement study are derived from triplicate values. In functional studies, there were four wells per treatment group. The data were analyzed by ANOVA to determine overall differences between treatment groups. When significant differences were found, a comparison between treatment groups was made using the Newman-Keuls test. SSR binding data were analyzed by the method of Scatchard.

Results

Scintigraphy with [^{111}In-DTPA-D-Phe^1]octreotide

A high uptake of [^{111}In-DTPA-D-Phe^1]octreotide was detected after 24 h in the mediastinum (Fig. 1, A and B). Computed tomographic scanning of the chest confirmed the diagnosis of thymic enlargement (Fig. 1C).

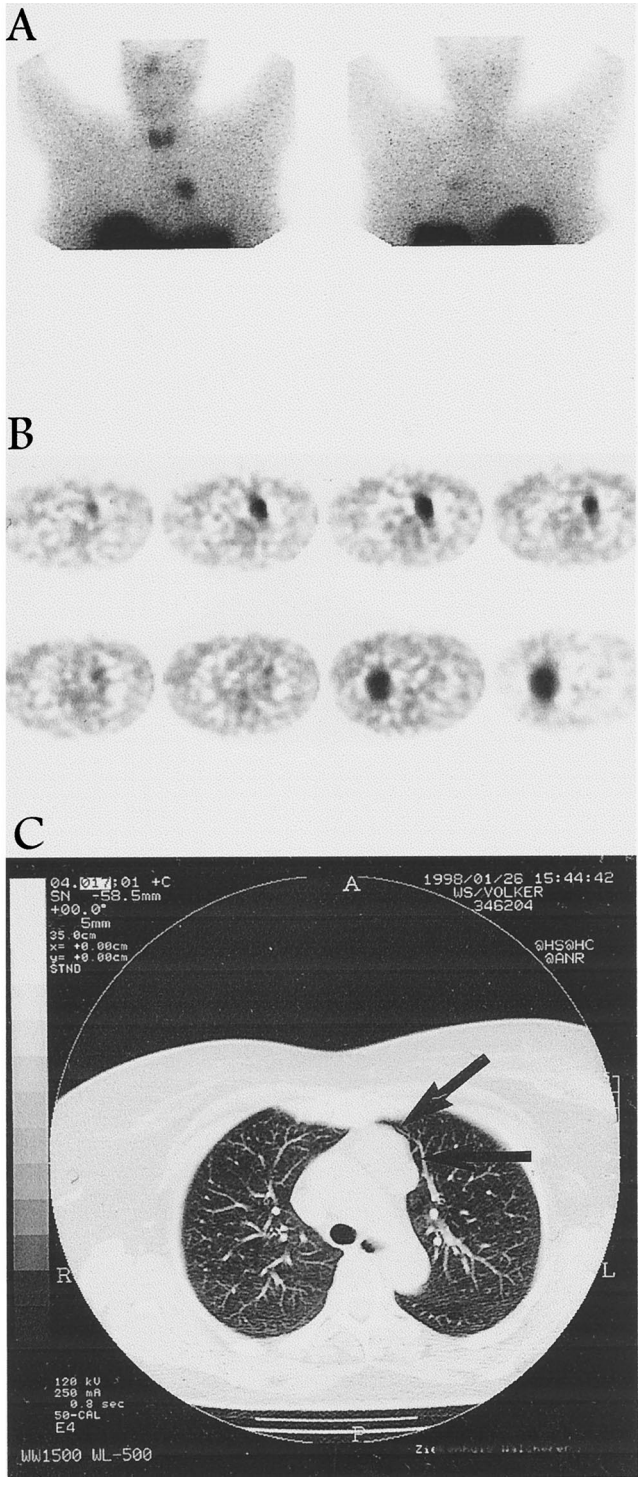

FIG. 1. A, Planar images of the chest 24 h after injection of [^{111}In-DTPA-D-Phe^1]octreotide. Anterior (*left*) and posterior (*right*) views. There is normal uptake in the thyroid and some accumulation in the nose region due to common cold. An abnormal uptake in the region of the left hilum is seen. B, Transversal SPECT images of the chest 24 h post-injection. Images are from cranial to caudal. The *left side* of the images represents the right side of the patient. In the *top row*, the accumulation at the site of the thymoma is seen, located left-sided in the anterior mediastinum. The last two images in the *bottom row* show uptake in the liver. C, Computed tomographic scan of the chest. The *arrows* indicate the extent of the thymic mass.

SS and SSR (subtype) expression

At autoradiography, no specific binding of [^{125}I-Tyr^{11}]SS-14 or [^{125}I-Tyr^3]octreotide was found in the thymoma (data not shown). However, Scatchard analysis of [^{125}I-Tyr^{11}]SS-14 binding on an enriched membrane preparation of the thymoma tissue revealed high affinity binding sites with an apparent K_d of 47.5 ± 2.5 pmol/L and a low maximum binding capacity (B_{max}) of 23.5 ± 2.5 fmol/mg membrane protein. Figure 2A shows a representative experiment. No specific binding was detectable on the tumor-derived cultured thymocytes. In addition, octreotide (100 nm) displaced [^{125}I-Tyr^{11}]SS-14 binding on membrane preparations of the thymoma tissue significantly less compared with SS-14 (49.5% *vs*. 90.8%, respectively; Fig. 2B). On normal human thymus membranes high affinity binding sites for [^{125}I-Tyr^3]octreotide were detected [K_d, 200 ± 70 pmol/L; B_{max}, 4.7 ± 0.3 fmol/mg membrane protein (mean ± SEM)].

By RT-PCR, sst_1, sst_{2A}, and a predominant sst_3 mRNA expression was detected in the thymoma tissue (Table 1). Conversely, mRNAs encoding for SS, sst_{2B}, sst_4, and sst_5 were absent (Table 1). In the cultured neoplastic TEC, RT-PCR analysis showed the presence of sst_3 mRNA only (Table 1). In cultured thymocytes only mRNA for β-actin was detected (Table 1). As previously described (18), the normal thymus expressed SS, sst_1, sst_{2A}, and sst_3 mRNAs, whereas cultured TEC expressed SS, sst_1, and sst_{2A} mRNAs (Table 1). By immunohistochemistry, a high immunoreactivity for sst_3 receptors and a very weak immunostaining for sst_1, but not for sst_{2A}, receptors was observed on the tumor cells (Fig. 3, A and C). sst_{2A} was weakly expressed in the stroma, mainly on endothelium of few small intratumoral vessels (Fig. 3B). In all cases the immunostaining could be completely abolished by preabsorption with 100 nmol/L of the respective antigen peptides (Fig. 3, D, E, and F).

In vitro effect of SS and octreotide on [^{3}H]thymidine incorporation of cultured neoplastic TEC

SS-14 (10 nm) and the SS analog octreotide (10 nm) significantly inhibited [^{3}H]thymidine incorporation by 40.6% and 43.2%, respectively, in the cultured tumor cells (Fig. 4). The epithelial origin of the cultured tumor cells was confirmed by immunocytochemical staining for cytokeratin that revealed a 100% pure epithelial cell culture.

Discussion

Human thymoma is the most common tumor of the anterio-superior mediastinum and is composed of cytologically bland, neoplastic TEC accompanied by a variable admixture of benign lymphocytes (24). This tumor is frequently associated with paraneoplastic phenomena, such as myasthenia gravis, and/or other autoimmune-related disorders and hematological abnormalities (24). The microenvironment is abnormal in thymoma, and it differs from the normal thymus in the prevalence of cortical areas and the deficiency of medullary ones (25).

A high *in vivo* uptake of [^{111}In-DTPA-D-Phe^1]octreotide has been detected in patients bearing thymoma or thymic carcinoid, whereas the normal or hyperplastic thymus is

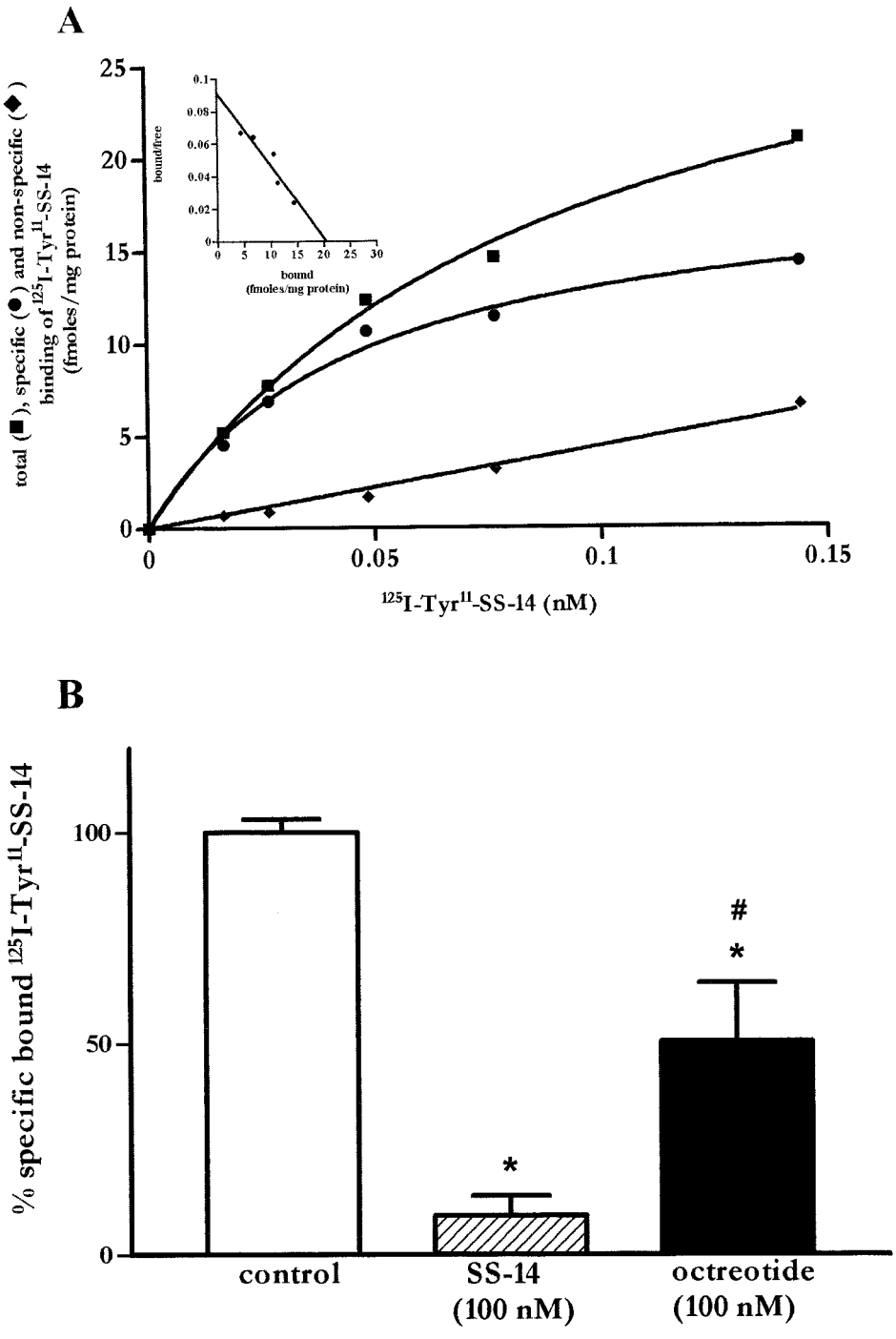

FIG. 2. Expression of SSR in a membrane preparation of the human thymoma. A, Saturation curves indicate total (■), nonspecific (◆), and specific (●) binding of [^{125}I-Tyr^{11}]SS-14. *Inset*, Scatchard analysis of [^{125}I-Tyr^{11}]SS-14 binding to thymoma tissue membranes (K_d, 50 pmol/L; B_{max}, 21 fmol/mg membrane protein). B, Displacement of [^{125}I-Tyr^{11}]SS-14 binding by SS-14 (100 nm; ▨) and octreotide (100 nm; ■). *, $P < 0.01$ *vs.* control; #, $P < 0.01$ *vs.* SS-14. Control, 922.3 ± 3.2 cpm; SS-14, 84.7 ± 4.7 cpm; octreotide, 465.7 ± 14 cpm. Data are derived from triplicate values and are the mean ± SEM.

not visualized (20, 26). Moreover, successful treatments with octreotide and prednisone and with octreotide alone have been reported in two patients with thymoma (19, 20). In one case the treatment resulted in both tumor size reduction and an improvement in the autoimmune-related disease (19). The precise mechanisms involved in this effect are unclear, however. In a previous study, using *in vitro* SSR autoradiography, Reubi *et al.* did not find SS-binding sites in four thymomas of undefined histological subtype, whereas SSR receptors were detectable in the normal thymus (27). The lack of *in vitro* detection of SSR in human thymoma seems in contrast with the compelling evidence that tumors originating from SSR-positive tissues generally express SSR (8, 28).

We have recently characterized SSR subtype expression in the normal human thymus (18). sst_1 and sst_{2A} mRNAs were expressed in the thymic tissue and isolated TEC, whereas sst_3 mRNA was detectable in thymic tissue only and not on enriched cultured TEC or cultured thymocytes (18). As several different subsets of epithelial cells and thymocytes have been described within the human thymus, the sst_3 receptor might be expressed on another cell subset or might undergo

TABLE 1. Somatostatin and somatostatin receptor subtype mRNA expression in normal human thymus and in a cortical thymoma as determined by RT-PCR

Samples	RT-PCR							
	sst_1	sst_{2A}	sst_{2B}	sst_3	sst_4	sst_5[a]	SS[b]	β-Actin
Normal human thymus	+	+	−	+	−	−	+	+
Normal TEC[c]	+	+	−	−	−	−	+	+
Thymocytes[d] (normal thymus)	−	−	−	−	−	−	−	+
Thymoma	±	±	−	+	−	−	−	+
Neoplastic TEC	−	−	−	+	−	−	−	+
Thymocytes[d] (thymoma)	−	−	−	−	−	−	−	+

[a] Somatostatin receptor subtypes 1–5.
[b] Somatostatin.
[c] Thymic epithelial cells.
[d] Cultured thymocytes.

rapid down-regulation during culturing. In fact, it is well known that the expression of neuropeptide receptors on immune cells is dynamically regulated and depends on the traffic of these cells through and within lymphoid compartments and homing in the tissues (29, 30). Moreover, preliminary data show sst_{2A} immunoreactivity on stromal cells in the medullary compartment of normal human thymus (31). All of these findings are in agreement with the evidence of a strong compartmentalization of neuroendocrine peptide receptors in lymphoid tissue (32).

In the present report we found a high uptake of [^{111}In-DTPA-D-Phe^1]octreotide in a patient with cortical thymoma and, in agreement with the study by Reubi *et al*. (27), a lack of SSR-binding sites by autoradiography performed on cryostat sections from the tumor tissue. However, we were able to detect a very low density of specific high affinity binding sites of [^{125}I-Tyr^{11}]SS-14 on an enriched membrane preparation from the thymoma tissue. By RT-PCR, we found mRNAs encoding for sst_1, sst_{2A}, and sst_3 in the thymoma tissue, whereas mRNA encoding for sst_3 was detectable in primary cultured neoplastic cells. Conversely, no binding was detectable, and no mRNAs encoding for SSR subtypes were found on the tumor-derived cultured thymocytes. Consonant with the presence of sst_1 mRNA and the low affinity of octreotide for sst_3 receptors, we found that octreotide only partially displaced [^{125}I-Tyr^{11}]SS-14 binding on cell membrane from this tumor. To further characterize the heterogeneous expression of SSR subtypes in this tumor, we used three recently developed polyclonal SSR antibodies, highly specific for sst_1, sst_{2A}, and sst_3 receptors (11, 22, 23). By immunohistochemistry, we observed a high immunoreactivity for sst_3 receptors and a very weak expression of sst_1 receptors on the tumor tissue. Conversely, the tumor cells did not express sst_{2A} receptors, whereas sst_{2A} immunoreactivity was localized in the stroma, mainly on the endothelium of scattered small intratumoral vessels. These findings confirm the predominant presence of sst_3 in this tumor. In fact, the mRNA encoding for this protein has been detected by RT-PCR on both tumor tissue and cultured tumor cells, and our hypothesis is that this cortical thymoma might have arisen from a subset of thymic sst_3-expressing cells. However, the diffuse immunoreactivity for sst_3 in the tumor tissue suggests the presence of this SSR subtype on reactive thymocytes as well. Although mRNA encoding for sst_3 was undetectable in cultured thymocytes from both thymoma and normal thymus, our recent observation showed the presence of SS-binding sites on freshly isolated thymocytes (31). This finding might explain the diffuse immunoreactivity for sst_3 in the thymoma and confirm the evidence that neuropeptide receptor expression in lymphoid tissues might rapidly change in conditions different from those in the natural microenvironment (33).

sst_3 receptors have been shown to display the highest amount of agonist-dependent receptor internalization compared to the other SSR subtypes (34). Although the affinity of octapeptide SS analogs for sst_3 is lower compared with that for sst_2, the high internalization of sst_3 receptors might be the possible mechanism regulating the *in vivo* uptake of [^{111}In-DTPA-D-Phe^1]octreotide within thymomas. Indeed, the present study demonstrates that sst_3 is the subtype predominantly expressed in this tumor, displaying a significant *in vivo* uptake of [^{111}In-DTPA-D-Phe^1]octreotide. Another suggestive hypothesis, which can not be completely excluded, is the presence of a novel unidentified SSR involved in determining the *in vivo* uptake of the radiotracer.

Cell subsets containing neuropeptide hormones are present in the cortical areas of normal thymus as well as in thymoma (35). The identification of SS and its receptors in the normal thymus strongly supports the paracrine actions of the peptide in modulating the immuno- and/or neuroendocrine functions in this organ (18, 27). The pattern of distribution of SS-binding sites observed in normal thymus seems to be still preserved in thymic hyperplasia associated with myasthenia gravis, but seems to be lost in thymoma (27). Furthermore, in the present case, SS mRNA was not detectable in either thymoma tissue or the primary cultured tumor cells, whereas it was detected in normal human thymic tissue and in normal cultured TEC (18). This finding suggests that the loss of local SS production might be implicated in the uncontrolled cell growth and/or the tumor autoimmune-related diseases and possible tumor pathogenesis. In support of this hypothesis, we found that the *in vitro* administration of SS-14 and the SS analog octreotide at high concentration (10 nmol/L) significantly inhibited [^{3}H]thymidine incorporation in the cultured tumor cells. Both compounds displayed a comparable ability in inhibiting [^{3}H]thymidine incorporation by cultured cells. As SS-14 binds sst_3 with higher affinity than octreotide, a difference in the percentage of inhibition should have been observed. However, taking into consideration that we measured the uptake of [^{3}H]thymidine after 72 h, we might have

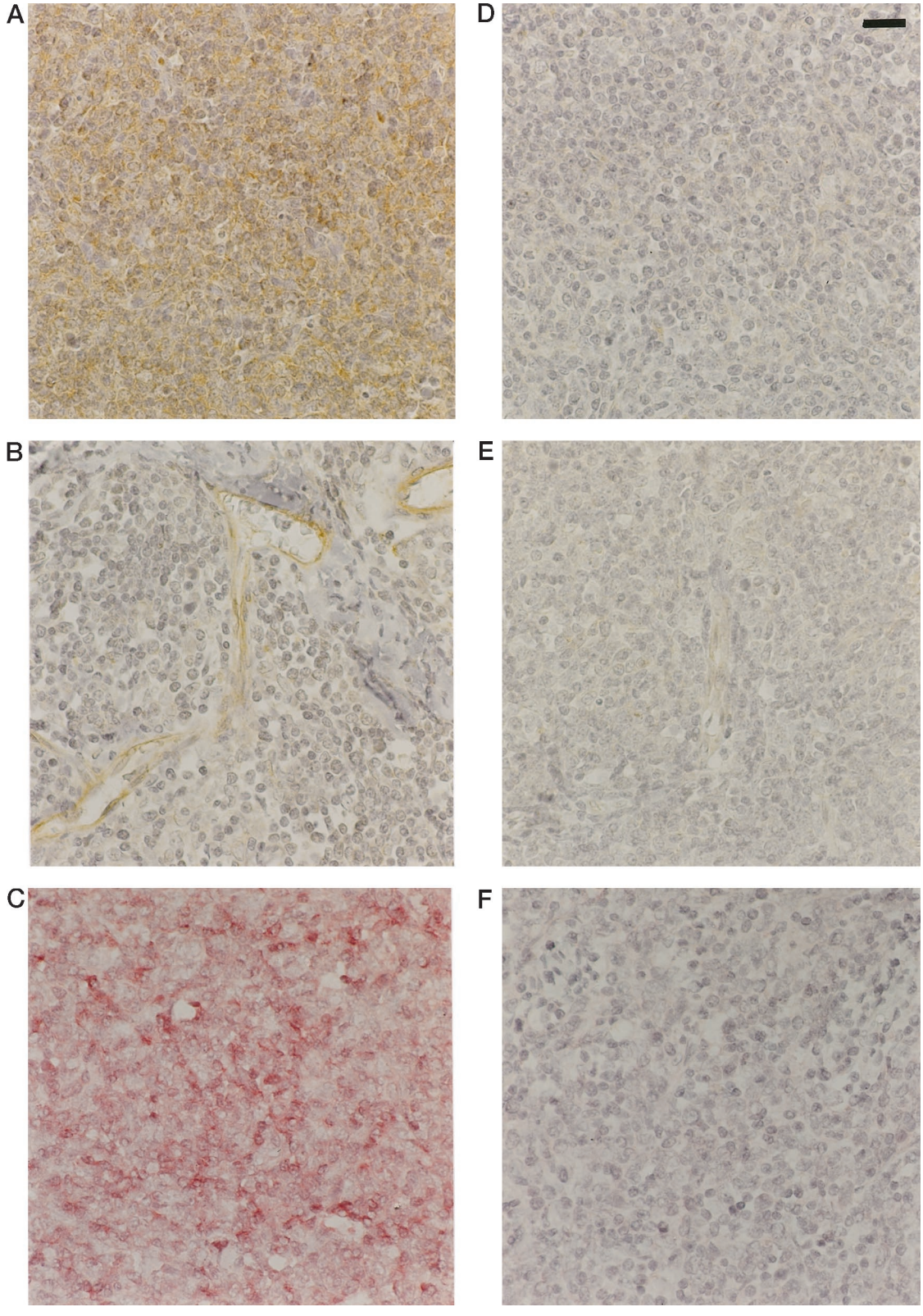

FIG. 3. Immunohistochemical detection of sst_1, sst_{2A}, and sst_3 receptors in the thymoma. *Left*: A, Weak diffuse sst_1 immunoreactivity within the tumor. Section developed with 3,3′-diaminobenzidine; B, sst_{2A} immunoreactivity located on the endothelium of an intratumoral vessel. Section developed with 3,3′-diaminobenzidine; C, high diffuse sst_3 immunoreactivity within the tumor. Section developed with New Fucsine/Naphtol AS-MX. *Right*: D–F, Adjacent sections showing displacement of immunostaining after the preabsorption of the antibodies with 100 nM of the respective peptide antigens. The sections are slightly counterstained with hematoxylin. Magnification, ×400. *Bar,* 25 μm.

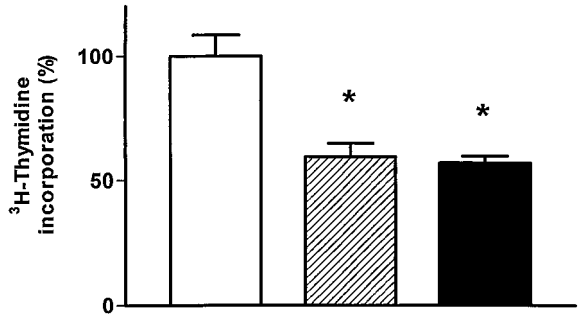

FIG. 4. Effects of SS and octreotide on [^{3}H]thymidine incorporation in a primary culture of the thymoma. Neoplastic thymic epithelial cells were incubated in MEM and 10% FCS for 72 h in quadruplicate without or with 10 nmol/L of the drugs indicated. Values are expressed as a percentage of [^{3}H]thymidine incorporation by control cells and are the mean ± SEM (n = 4 wells). *, $P < 0.01$ *vs.* control. ▨, SS-14, ■, octreotide. The control value of [^{3}H]thymidine incorporation was 182.3 ± 15.9 cpm.

underestimated the true inhibition degree by SS-14 due to a lower stability of the latter compound. The inhibition of cell proliferation by SS-14 and octreotide, and the predominant expression of sst_3 in the thymoma are in line with emerging data regarding signal transduction pathways linked to SSR that show a cell cycle-dependent induction of apoptosis by octreotide (36). Octreotide-induced apoptosis seems signaled through the sst_3 and associated with dephosphorylation-dependent conformational change in wild-type p53 (37). Although controversial data about alterations of p53 expression have been reported in thymic epithelial tumors (38, 39), SS-induced apoptosis might represent an additional mechanism involved in the regulation of cell proliferation in human thymus. Furthermore, this finding may form the basis for the inhibition of tumor growth in patients with thymoma treated with octreotide. As octreotide binds to sst_{2A}, sst_3, and sst_5, but not to sst_1, and considering that in the present case sst_{2A} and sst_5 are not expressed in the tumor cells, the *in vitro* effect of SS and octreotide seems to be mediated directly by sst_3.

In conclusion, although further investigations are required, the loss of SS production in combination with a predominant expression of sst_3 receptor in this thymoma might be implicated in uncontrolled cell growth and tumor pathogenesis. These data support the physiological paracrine/autocrine role of SS in the human thymic microenvironment and indicate that a disturbance in this system might be involved in the pathogenesis of thymic neoplasms. Furthermore, SSRs are localized in different complementary cell compartments in the normal and neoplastic human thymus. At least three SSR subtypes seem to control neuroendocrine function as well as the cell growth in this organ. Moreover, the sst_3 subtype may play an important role in determining the uptake of [^{111}In-DTPA-D-Phe^1]octreotide during *in vivo* SSR scintigraphy. The inhibition of cell proliferation by octreotide through sst_3 may explain the reported successful treatment of thymoma with this SS analog. Finally, SSR scintigraphy and SS analog treatment of thymomas may represent a new diagnostic and therapeutic approach to these tumors.

Acknowledgments

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