NOTE

Immunohistochemical Localization of Somatostatin Receptor Type 2A in Rat and Human Tissues

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Abstract. In this study, we elucidated the cellular localization of somatostatin receptor (SSTR) by immunohistochemistry using an antibody specific for SSTR type 2A (SSTR2A) in various organs of rat and human. SSTR2A expression was basically similar in rat and human, except in the pancreas and adrenal cortex. In the pituitary gland, the posterior lobe and the majority of growth hormone cells and some ACTH and TSH cells expressed SSTR2A. In rat adrenal gland, the zona glomerulosa strongly expressed SSTR2A, whereas zone-specific immunoreactivity was not observed in human. The adrenal medulla moderately expressed SSTR2A in both rat and human. SSTR2A immunoreactivity was observed in islet cells and some ductal cells in human pancreas, and also in acinar cells of rat pancreas. In gastrointestinal (GI) tract, the majority of crypt cells and nerve plexuses strongly expressed SSTR2A. The number of SSTR2A positive cells was much more than that of chromogranin A positive endocrine cells. In the kidney, the glomerular capillaries and collecting tubules, but not proximal tubules, showed immunoreactivity. SSTR2A immunoreactivity was observed not only in endocrine cells but also in non-endocrine cells.

Key Words: Somatostatin receptor type 2A, Immunohistochemistry, Pancreas, Gastrointestinal tract, Adrenal gland

Somatostatin regulates endocrine and exocrine functions and the growth inhibition of various organs, such as the pituitary gland, pancreas and gastrointestinal (GI) tract, by acting on the somatostatin receptor (SSTR) of target organs [1, 2]. Five types of SSTR genes have been cloned and were shown to produce 6 types of proteins [3, 4]; however, SSTR2B has been detected only in rat, but not in human tissues. Messenger RNA of SSTR1-5 was widely expressed in the brain and peripheral organs and displays an overlapping but characteristic pattern that is subtype-selective and tissue- and species-specific [5, 6]. Yamada et al. reported the cloning, functional expression, and tissue distribution of SSTR1 and SSTR2, and reported that mRNA of SSTR1 and SSTR2 were expressed at highest levels in rat jejunum and stomach, and in cerebrum and kidney, respectively [7]. Tissue localization of SSTR has been investigated mainly by ligand binding autoradiography and in situ hybridization histochemistry [8]. To elucidate the exact cellular distribution of SSTR, however, immunohistochemical investigation is needed. Recently, specific antibodies against SSTR...
subtypes have been raised, and immunohistochemical studies of SSTR have been carried out [9-17]. In a preliminary study, we stained rat and human pancreas using the SSTR2A antibody, and found that the tissue localizations of immunoreactivity was different from each other. This encouraged us to carry out a comparative study of the expression of SSTR2A in various organs of rat and human, selecting the representative endocrine and non-endocrine organs which have been recognized to be affected by somatostatin.

Materials and Methods

Adult male rats (LEA/Hkm) were sacrificed by chloroform anesthesia. Whole internal organs were immediately removed and fixed in 10% buffered formalin for 18 hr at 4°C and embedded in paraffin. In conducting this research, the investigators adhered strictly to the Guidelines for the Care and Use of Laboratory Animals of Tohoku University. Normal human tissues including pituitary gland, pancreas, intestine, adrenal gland and kidney obtained by surgery or autopsy within 3 hours after death were fixed in 10% buffered formalin for 18 hrs at 4°C and embedded in paraffin. Human tissues were chosen at random without regard to age, sex, or kind of disease.

Immunohistochemistry: Formalin-fixed, paraffin-embedded tissues were serially cut into 3 µm thick sections which were then dewaxed and rehydrated. Antigen retrieval of sections by incubation in 0.01 M citric acid buffer (pH 6.0) and autoclaving for 5 min at 120°C was performed before the immunohistochemical procedures. Immunohistochemical staining for SSTR2A was performed with the streptavidine-biotin-complex method as previously reported [18]. Sheep polyclonal antibody against SSTR2A was raised against a synthetic peptide corresponding to the predicted amino acids 347-366 of the carboxy-terminus of rat SSTR2A as previously described [9]. Western blotting and adsorption test was done to confirm the specificity of the affinity-purified SSTR2 antibody. The peptide sequence is identical in rat, human and mouse SSTR2A. The working dilution of SSTR2A was 1:100. A rabbit anti-sheep IgG-biotinylated antibody (Vector Laboratories, Burlingame, CA) diluted 1:100 was used as the second antibody. For the control study, normal sheep serum was used instead of the primary antibody. Tissues of pancreas and pheochromocytoma were simultaneously stained as a positive controls.

To determine the colocalization of SSTR2A and peptide hormones in the pituitary gland and pancreas, serial sections were cut and two adjacent sections were paired for immunostaining of SSTR2A and the following peptide hormones. For pituitary gland, growth hormone (GH), prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), ACTH and thyroid-stimulating hormone (TSH) were examined, and for pancreas, insulin, glucagon, somatostatin and pancreatic polypeptide were examined. Furthermore, we carried out double immunostaining using the Envision system (DAKO, Glostrup, Denmark,) for pancreatic peptides and then stained SSTR2A by the ABC system with alkaliphasatase labeled avidin (Histofine, Nichirei, Tokyo). Peptide hormones were colorized by diaminobenzidine as brown and SSTR2A was colorized by a mixture of nitroblue tetrazolium solution and 5-bromo-4-chloro-3-indolyl phosphate solution as blue (Boehringer Mannheim Biochemica, Mannheim, Germany). Sources and working dilutions of these peptide hormones were as follows: GH (DAKO, 1 : 4000), prolactin (DAKO, 1 : 1), LH (DAKO, 1 : 4000), FSH (DAKO, 1 : 3000), ACTH (INCASTAR, Stillwater, MN, 1 : 4000), TSH (DAKO, 1 : 3000), insulin (DAKO, 1 : 3000), glucagon (DAKO, 1 : 3000), somatostatin (DAKO, 1 : 3000), pancreatic polypeptide (DAKO, 1 : 3000). Chromogranin A (DAKO, 1 : 1000) was also stained for in the GI tract.

Results

Immunohistochemical reaction for SSTR2A was localized to the cell membrane in the majority of reactive cells, as well as to the cytoplasm in endocrine cells of pituitary gland and pancreas islets. Immunohistochemical results are summarized in Table 1. Immunoreactive intensity was evaluated in comparison with the pancreatic islets, which were simultaneously stained as positive controls as follows: materials that stained as strongly as pancreas + + +; positively but less than pancreas, + +; weakly positive, +.
In human pituitary gland, there were many SSTR2A-immunoreactive cells (Fig. 1). Serial sections stained by antibodies for SSTR2A and pituitary hormones revealed that the majority of GH-producing cells, some of the ACTH-producing cells, especially those invading the posterior lobe, and TSH-producing cells, contained SSTR2A. The intensity of immunostaining was weaker in the invading corticotrophs than that of GH-producing cells. LH-producing cells, FSH-producing cells and prolactin-producing cells were not identified as SSTR2A positive. Folliculostellate cells were negative for SSTR2A.

In rat adrenal gland, only the zona glomerulosa of the cortex was strongly positive (Fig. 2). In human adrenal gland, cortical cells showed weak immunoreactivity without definite differences in cortical zonation. Rat and human adrenal medulla had moderate immunoreactivity.

In human and rat pancreas, SSTR2A immunoreactivity was strongly positive on the cell membrane and weaker in the cytoplasm of almost all endocrine cells including insulin, glucagon, somatostatin and pancreatic polypeptide cells. Some ductal cells were also very strongly positive. Acinar cells were negative in human but positive in rat pancreas. Double immunostaining of SSTR2A and pancreatic hormones clearly demonstrated that immunoreactive intensity of SSTR2A was stronger in insulin cells and somatostatin cells than in glucagon cells and pancreatic polypeptide cells in both human (Figs. 3, 4) and rat pancreas (Figs. 5, 6).

In human GI tract, SSTR2A immunoreactivity was observed in many crypt cells (Fig. 7) and also

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**Table 1. Immunohistochemical Distribution of SSTR 2A in Human and Rat Tissues**

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Rat</th>
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<tr>
<td>Pituitary gland</td>
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<td>anterior lobe</td>
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<td>GH cells</td>
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<td>n.e.</td>
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<td>ACTH cells</td>
<td>++</td>
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<td>TSH cells</td>
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<td>LH, FSH cells</td>
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<td>PRL cells</td>
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<td>posterior lobe</td>
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<td>Pancreas islet</td>
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<tr>
<td>insulin cells</td>
<td>+++</td>
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<td>glucagon cells</td>
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<td>somatostatin cells</td>
<td>++++</td>
<td>+</td>
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<td>pancreatic polypeptide cells</td>
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<td>+</td>
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<td>duct</td>
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<td>acinar cells</td>
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<td>GI tract</td>
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<td>crypt cells</td>
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<td>nerve plexus</td>
<td>++++</td>
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<td>(Auerbach's &amp; Meissner's)</td>
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<td>Adrenal gland</td>
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<tr>
<td>cortex (zona glomerulosa)</td>
<td>-</td>
<td>++</td>
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<tr>
<td>medulla</td>
<td>+</td>
<td>+</td>
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<td>Kidney</td>
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<tr>
<td>glomerulus (capillary)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>collecting tubules</td>
<td>++++</td>
<td>+</td>
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<tr>
<td>proximal tubules</td>
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_n.e._: not examined

+++ : strong immunoreactivity

++  : moderate immunoreactivity

+   : weak immunoreactivity

-   : no immunoreactivity
The present study shows the cellular localization of SSTR2A protein in various normal human and rat organs as revealed by immunohistochemistry. Immunohistochemical reactivity was localized in the cell membrane of the majority of cells, and in the cytoplasm of endocrine cells of the pituitary gland and pancreas islets. The distribution of SSTR2A in the cytoplasm and on the surface membrane of endocrine cells is consistent with the previous finding that somatostatin-binding sites are present in secretory-granule membranes from pancreatic islets [19].

There have been a few previous studies of SSTR in rat pituitary gland [15, 20]. Using double immunostaining of pituitary hormones and SSTR, Mezey et al. found that SSTR2A and 5 were widely distributed in the pituitary gland and that both are present in a large percentage of GH cells. SSTR2A was also expressed in about a third of gonadotrophs and thyrotrophs [20]. Here we demonstrated for the first time that the invading corticotrophs weakly immunostained SSTR2A. In a study of human pituitary glands and tumors by reverse-transcriptase polymerase chain reaction (RT-PCR) followed by Southern blotting, all 5 SSTR subtype mRNAs were shown to be expressed in the fetal pituitary gland, while the adult pituitary was positive for 4 subtypes, lacking only SSTR4 mRNA [21]. Of 15 human pituitary tumors positive for SSTR mRNA analyzed, 14 expressed more than one subtype. The SSTR2 mRNA was expressed in all human pituitary tumors as the 2A variant, there being no detectable transcript for SSTR2B [21]. Our study revealed that not only GH cells but also some invading corticotrophs and thyrotrophs also showed SSTR2A expression.

Among the 5 SSTRs, mRNA for SSTR2A was the most frequently expressed (87% of the tumors) [21]. This implies that SST analogues such as SMS 201-995, known to interact with SSTR2 but not with SSTR1, act on pituitary tumors mainly via the SSTR2 subtype [21]. Another study of SSTRI and SSTR2 in human pituitary tumors revealed that SSTR subtypes 1 and 2 are heterogeneously expressed in different pituitary adenoma cell types [22]. No correlation was detected among tumor size, level of

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**Fig. 1.** Many SSTR2A immunoreactive cells are observed in the anterior lobe (A) and invading corticotrophs (I) of the human pituitary gland. Immunoreactivity of SSTR2A is weaker in the invading corticotrophs than in the anterior pituitary gland.

**Fig. 2.** SSTR2A immunoreactivity is observed in zona glomerulosa cells of rat. Zona fasciculata and zona reticularis show no immunoreactivity.

**Fig. 3.** Double immunostaining of insulin and SSTR2A in the human pancreas. Insulin cells show a mixture of brown (insulin) and blue (SSTR2A). Some cells show only blue (SSTR2A).

**Fig. 4.** Double immunostaining of glucagon and SSTR2A in the human pancreas. Glucagon cells located in peripheral area of islets show a mixture of brown (glucagon) and some blue (SSTR2A).

**Fig. 5.** Double immunostaining of insulin and SSTR2A in the rat pancreas (Figs. 5 and 6 are the same islet). Almost all black cells show a mixture of brown (insulin) and blue (SSTR2A). Peripheral blue cells around the black cells are positive for SSTR2A but negative for insulin.

**Fig. 6.** Double immunostaining of glucagon and SSTR2A in the rat pancreas. Peripheral brown cells seem positive for only glucagon, but if we compare them to Fig. 5, these cells are also positive for SSTR2A. Thus the peripheral brown cells are a mixture of blue and brown. This means that immunoreactivity of SSTR is weaker in glucagon cells than in insulin cells.

**Fig. 7.** There are numerous cells immunoreactive for SSTR2A in crypt cells of human gastric mucosa (SSTR2A immunostaining).

**Fig. 8.** Serial sections of human stomach show that the number of chromogranin A positive cells are less than that of SSTR2A-positive cells (Chromogranin A immunostaining. Figs. 7 and 8 are serial sections).
hormonal hypersecretion, and SSTR expression status [22].

In rat adrenal gland, only the zona glomerulosa showed strong immunoreactivity for SSTR2A. Somatostatin inhibits angiotensin II-stimulated aldosterone secretion [1]. Our results indicate that somatostatin directly inhibits aldosterone secretion via SSTR2A on the cells of the zona glomerulosa. Neuroendocrine differentiation in normal adrenal cortex, mainly in zona glomerulosa, has been demonstrated by expression of neuron specific enolase, synaptophysin, neuronal cell adhesion molecule and neurofilaments [23, 24]. Our detection of SSTR2A in zona glomerulosa may provide some evidence of neuroendocrine differentiation in this zone. Adrenocortical zonation is not clear in human and the expression of SSTR2A is also not clearly defined in relation to zonation. The adrenal medulla of the rat and human showed moderate immunoreactivity to SSTR2A.

The present study revealed that the pattern of SSTR2A expression in the pancreas of rat and human is similar. SSTR2A strongly expressed in the majority of islet cells including insulin and somatostatin cells and ductal cells, and less strongly in glucagon cells and pancreatic polypeptide cells. The acinar cells were positive in rat but not in human. Hunyady et al. [11] first reported the immunohistochemical localization of SSTR protein in the rat (adult, male, intact Sprague-Dawley) pancreas, in which all acinar cells and the glucagon- and pancreatic polypeptide-immunoreactive cells were intensely labeled for SSTR2A, while no signal was detected in somatostatin cells. A very few insulin-immunoreactive cells were also labeled for SSTR2A, but the signal in these cells was weaker than that in exocrine, glucagon and pancreatic polypeptide cells [11]. Such results are quite different from the present ones, and while the reason for this discrepancy is not clear, it may depend on differences in the antibodies and kinds of rats used. Immunohistochemical results are direct evidence of the presence of the SSTR2 protein in the exocrine as well as endocrine pancreas, as previously revealed biochemically [25]. There are some discrepancies in the distribution of SSTR subtypes in pancreatic islets between the human and rat. Although Moldovan et al. [26] reported that insulin secretion is inhibited by an SSTR2A selective analogue in isolated perfused human pancreas, this analogue (DC32-87) has been found not to be SSTR2 selective [5]. Pharmacological data also suggest that different SSTR subtypes are expressed in glucagon and insulin cells in the rat endocrine pancreas, with glucagon secretion being most sensitive to SSTR2-selective analogues and insulin secretion most sensitive to SSTR5-selective analogues in rats [27, 28, 29]. Recently Kumar et al. [16] analyzed SSTR subtype specific expression in human pancreatic islets and found SSTR2 in insulin and glucagon cells, with insulin, glucagon, and somatostatin cells each expressing multiple SSTR isoforms. The present study using double immunohistochemical staining revealed that SSTR2A was expressed strongly in the majority of cells of human and rat pancreatic islets, especially in insulin and somatostatin cells, but less intensely in glucagon and pancreatic polypeptide cells. Furthermore, one interesting difference between rat and human is that the majority of rat acinar cells expressed SSTR2A, but human acinar cells did not. This is important when evaluating the pharmacological effects of somatostatin analogue using rat.

In rat and human intestine, the Meissner's and Auerbach's nerve plexuses (submucosal and myenteric plexuses, respectively) and GI tract mucosa were always strongly immunoreactive to SSTR2A [30, 31]. Reubi [31] reported that human GI tract expressed SSTRs in the GI tract mucosa, the peripheral nervous system, and gut-associated lymphoid tissue, where the SSTR are preferentially located in germinal centers. In addition, Sternini et al. [10] reported an immunohistochemical study of SSTR2A using antisera against the C-terminus peptide (361-369) and demonstrated that SSTR2A antibody primarily stained interstitial cells of Cajal. Although our study could not confirm the immunoreactivity in gut-associated lymphoid tissues, the majority of crypt cells of the mucosa of the GI tract and many epithelial cells of the surface of the duodenal villi were strongly labeled by SSTR2A antibody in addition to peripheral nervous tissue. When we compared the number of cells immunoreactive to SSTR2A and chromogranin A which is a representative marker for neuroendocrine cells, the former was more than the latter. This means that somatostatin regulates not only endocrine cells but also non-endocrine cells in the GI tract.

In the human and rat kidney, SSTR2A immuno-
reactivity was observed in collecting tubules and in glomerular capillaries. Some somatostatin inhibits antidiuretic hormone (ADH)-mediated water absorption in the kidney [1], our study shed light on the mechanism of somatostatin-ADH interaction.

Immunohistochemistry is the most useful tool with which to define the cellular localization of SSTR. More precise cellular detection would greatly contribute to our understanding of the physiological function of somatostatin in various organs and to the establishment of treatments utilizing somatostatin analogues for various diseases. Certain unexpected physiological changes associated with somatostatin analogue therapy may be explained by clarifying the tissue localization of SSTR2A. Further detailed histological studies combined with biological or pharmacological functions are necessary in each of the organ presented here, as well as in other organs not described in the present study.

References


