RADIOIMMUNOASSAY FOR SOMATOSTATIN RECEPTOR TYPE 2

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Objective. To develop radioimmunoassay for somatostatin receptor type 2 (SSTR2) and search for its presence in certain rat tissues.

Methods. Anti-SSTR2 antiserum has been raised in New Zealand white rabbits immunized with a conjugate of synthetic SSTR2 with bovine serum albumin. Radioiodination of SSTR2 was performed by chloramin T method followed by purification of radioiodinated material on Sephadex G-25 column.

Results. The obtained antibody did not crossreact with SSTR1, SSTR3, SSTR4, SSTR5, hypothalamic hormones, pituitary hormones, neuropeptides or gut hormones. The assay was performed with a double antibody system. SSTR2 was extracted from the tissues with acid acetone. The dilution curve of acid acetone-extracts of rat hypothalamus in the radioimmunoassay system was parallel to the standard curve. The recovery of tissue SSTR2 was about 89 % , and the intra-assay and inter-assay variations were 4.9 % and 7.8 %, respectively. SSTR2 was found in the hypothalamus, cerebrum, cerebellum, pituitary, stomach and testis.

Conclusions. These data suggest that this assay system is suitable for the estimation of SSTR2 in the tissues.

Key words: Somatostatin receptor type 2 – New radioimmunoassay – Peptide synthesis – Tissue contents in rat

Somatostatin(SSTR) is a tetradecapeptide, which was originally isolated from hypothalamus and shown to be a powerful inhibitor of many endocrine and exocrine secretions (Reichlin 1983). SST induces its biological activity by interacting with specific high-affinity cell-surface receptors. The properties of SST receptors (SSTR) have been analyzed by using a variety of biochemical approaches. These studies have revealed that heterogeneities exist in the structure of SSTR. Recently, five specific subtypes of SSTR have been cloned (Patel 1999).

We previously reported that SSTR2 was widely distributed in the rat body using an immunohistochemical method (Mitsuma et al. 1996). In the present study, we report the development of a radioimmunoassay for SSTR2 and its application in rats.

Materials and Methods

Animals. Male Wistar rats weighing 250 g were housed in a temperature (22 °C) and humidity (60 %) controlled room with 12 h illumination cycle. They were fed with laboratory chow and water ad libitum. After twelve hours fasting, the rats were sacrificed under sodium pentobarbital anesthesia (60 mg/kg) and the tissues (brain, stomach and testis) were obtained. The brain was removed and the hypothalamus, cerebrum and cerebellum were separated ac-

Table 1
A schematic diagram of the assay procedure for SSTR2

Standard or samples	0.1 ml
Antibody (1:1000)	0.1 ml
SSTR2-I-125	0.1 ml
Buffer	0.5 ml
incubated for 24 hours at 4 °C	
Added second antibody solution at 4 °C	
Incubated 24 hours at 4 °C	
Centrifuged at 3000 rev/min at 4 °C	
Decanted supernatants	
Counted (precipitates)	
Calculated bound/total count (B/T)	

cording to the method previously described (Mitsuma et al. 1991).

Drugs. Synthetic hypothalamic hormones, pituitary hormones, neuropeptides and gut hormones were purchased from the Protein Research Foundation (Osaka, Japan). Bovine serum albumin (BSA) and glutaraldehyde were obtained from the Wako Chem. Co. Ltd. (Tokyo, Japan). Sephadex G-25 was purchased from Pharmacia (Sweden). Other substances were purchased from Sigma (MO, USA).

Generation and characterization of anti-SSTR2 antibody, Peptides corresponding to the following sequences of the rat SSTR2 were synthesized using a solid phase method by an automated peptide synthesizer, followed by purification with HPLC: SNQTEPYYDMT, according to the method previously described (Hirooka et al. 1993). The peptides were conjugated on an equal weight basis to bovine serum albumin by the method previously described for anti-GHRH antibody (Mitsuma et al. 1983), using glutaraldehyde. New Zealand white rabbits were immunized with the emulsion of 1 mg of this conjugate in 1 ml water in complete Freund's adjuvant (1:2, v/v) which was injected into the foot pad at intervals of three weeks. Blood was drawn one week after each injection. The presence of anti-SSTR2 antiserum was checked by immunoprecipitation method as reported elsewhere (Hirooka et al. 1992).

Radioiodination of SSTR2 and purification. Radioiodination of SSTR2 was performed with the chloramin T method according to the report (Greenwood et al. 1963). The radioiodinated materials were chromatographed on a Sephadex G-25 (1x20 cm), eluted with 0.01 M phosphate buffer (pH 7.4) and collected in one ml fractions. The first peak was ^{125}I labeled SSTR2 and the second peak was free ^{125}I. Specific activity was calculated to be approximately 200 micro Ci/μg.

Assay buffer. The assay buffer consisted of 0.01 M phosphate buffer (pH 7.4) with 0.1 % BSA, 0.1 % mercaptoethanol and 0.1 % triton X-100.

Assay procedure. Double antibody radioimmunoassay system was used and the schematic diagram of the procedure was shown in Table 1.

Extraction of SSTR2 from the tissues. The extraction of SSTR2 was performed by the method previously described (Mitsuma et al. 1999). The freshly obtained tissues were weighed and placed in 0.5 ml acid acetone, homogenized and centrifuged. The supernatants were dried under the air stream in a water bath (56 °C). The recovery of this extraction method was evaluated by adding a known amount of synthetic SSTR2 to the tissues. The recovery was found to be approximately 89 %.

Results

Preparation of anti-SSTR2 antiserum. All three rabbits used responded to the immunization and developed antibodies at a final dilution 1:1000 or higher. The antiserum used in this study was obtained one week after the fourth injection and showed a specific binding at a final dilution of 1:8,000.

Specificity of antiserum. The specificity of anti-SSTR2 is shown in Table 2. No crossreactivity was observed with SSTR1, SSTR3, SSTR4, SSTR5, hypothalamic hormones, pituitary hormones, neuropeptides and gut hormones.

Standard curve and dilution of acid acetone extracted hypothalamus. The detection limit in this system was calculated as 10 pg/ml. Parallel curve was obtained for the dilution of acid acetone-extracted hypothalamus.

Recovery experiment Recovery of SSTR2 was evaluated with known amount of SSTR2 to the hypothalamus and measured with the radioimmunoassay. The recovery was approximately 100 %.

Intra-assay and inter-assay variation. The intra-assay variation was 4.9%,and the inter-assay variation was 7.8 %.

Table 2
Relative reactivity of hypothalamic hormones, pituitary hormones, neuropeptides or gut hormones in SSTR2 radioimmunoassay system

SSTR2	100	Adrenomedullin	0.001
SSTR1	0.001	Endothelin-1	0.001
SSTR3	0.001	Endothelin-2	0.001
SSTR4	0.001	Endothelin-3	0.001
SSTR5	0.001	ACTH	0.001
Somatostatin	0.001	TSH	0.001
TRH	0.001	LH	0.001
GHRH	0.001	FSH	0.001
CRH	0.001	Prolactin	0.001
LHRH	0.001	GH	0.001
Neurotensin	0.001	Alpha-MSH	0.001
Beta-endorphin	0.001	Vasopressin	0.001
Leucine-enkephalin	0.001	Oxytocin	0.001
Dynorphin	0.001	Secretin	0.001
Alpha-neoendorphin	0.001	Glucagon	0.001
Beta-neoendorphin	0.001	Gastrin	0.001
Substance P	0.001	Insulin	0.001
VIP	0.001	GLP-1	0.001
CGRP	0.001		
ANP	0.001		
BNP	0.001		
CNP	0.001		

Arbitary values of SSTR2 = 100

Table 3
Immunoreactive SSTR2 in rat organs (ng/g wet weight)

Hypothalamus	5.3 ± 0.3 ng/g
Cereberum	8.0 ± 0.5 ng/g
Cerebellum	8.1 ± 0.6 ng/g
Pituitary	1.6 ± 0.2 ng/g
Stomach	8.4 ± 0.7 ng/g
Testis	1.4 ± 0.4 ng/g

The values are shown by mean+SE in each group of seven rats

SSTR2 in the tissues. SSTR2 was found in the central nervous system, stomach and testis shown in Table 3.

Discussion

A specific method for measurement of SSTR2 is essential for the investigations of physiological and pathophysiological significance of this peptide. The present study tried to develop a SSTR2 radioimmunoassay for measuring SSTR2. The antibody used in this study did not crossreact with SSTR1, SSTR3, SSTR4, SSTR5, hypothalamic hormones, pituitary hormones, neuropeptides and gut hormones. The data indicate that this antibody is specific to SSTR2 and suitable for the estimation of SSTR2 in a radioimmunoassay system. Because iodinated SSTR2 is not commercially available to date, we performed radioiodination with chloramin T method. The elution profile showed two peaks: the first peak was ^{125}I labeled SSTR2 and the second peak was free ^{125}I. The first peak was used as the radioactive ligand. The dilution curve of acid acetone-extracted hypothalamus was found parallel to the standard curve, and the results of recovery experiment, intra-assay and inter-assay variation were satisfactory. These data suggest that this assay system is suitable to measure SSTR2 in the tissues.

The present study demonstrated that SSTR2 was found not only in the central nervous system ,but also in stomach and testis. These data support the previous report which showed that SSTR2 is widely distributed in rat body by immunohistochemical study (Mitsuma et al. 1996). Furthermore, present finding is comparable with other previous reports in which the binding of labeled SST was used (Srikant et al. 1981; Epelbaum et al. 1985; Martin et al. 1991). Recently, it has been reported that SSTR2 mRNA is expressed in the central nervous system, anterior pituitary and gastrointestinal tract (Bruno et al. 1992; Yamada et al. 1992). We confirmed this in protein levels. SST presents in these organs and has many actions upon these organs (Patel et al. 1978; Reichlin 1983). Thus, these data suggest that the action of SST in these organs may be mediated via SSTR2 and that this peptide act in a paracrine or autocrine way through the SSTR2. We also found SSTR2 in the testis, although its physiological significance in this organ remained unclear.

The present data suggest that this assay system is suitable to measure SSTR2 in the tissues.

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