

Sequence analysis of the promoter region of the rat somatostatin receptor subtype 1 gene

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Abstract

Somatostatin receptor (SSTR) subtype genes are differentially expressed in brain and various peripheral tissues. RNA blotting and semiquantitative PCR analyses have revealed low levels of SSTR1 mRNA in the gastrointestinal tract and relatively high levels in GH₃ anterior pituitary cells. As a first step in the investigation of the regulation of SSTR1 gene expression, we isolated a genomic fragment that contains the promoter region and determined the transcriptional initiation site. The SSTR1 gene lacks introns and TATA and CAAT motifs, but possesses several consensus recognition sequences for the transcription factors GCF and AP-2. The presence, also, of two Pit-1 binding sites could explain the high SSTR1 mRNA levels in GH₃ cells.

Key words: Pit-1; Pituitary; GCF; AP-2

1. Introduction

The diverse biological activities of the peptide hormone somatostatin are mediated by at least five different receptors, termed SSTR1-5 [1–7], that belong to the superfamily of G-protein coupled receptors each of which contains seven transmembrane domains. When expressed individually in mammalian cells the five receptor subtypes exhibit distinct pharmacological properties, yet, when activated, all inhibit adenylate cyclase [8]. RNA blot analysis and nuclease protection and in situ hybridization experiments show a spatially and temporally different localization of the individual SSTR mRNAs in brain and peripheral tissues [9]. For instance, in rat brain areas such as the cortex and the magnocellular preoptic nucleus SSTR1 transcripts are first observed at prenatal day 14 and increase in number until prenatal day 20 [5]. However, SSTR1 mRNA levels then decrease in the magnocellular preoptic nucleus during further development being barely detectable by in situ hybridization in adult animals; in contrast, they remain unchanged in cortex. The heterogeneous mRNA distributions point to different physiological roles for the various receptor subtypes and reflect the complex regulation of expression of the five genes.

By RNA blot analysis and polymerase chain reaction (PCR) amplification we have further examined the distribution of SSTR mRNAs in peripheral organs. To gain

a first insight into the mechanism of gene regulation, we have also isolated and characterized the rat SSTR1 gene.

2. Materials and methods

2.1. Isolation of genomic clones

A rat genomic library constructed in lambda DASH II (Stratagene) was screened by standard protocols [10] with a 1,276 bp fragment that contained the entire coding region of the rat SSTR1 cDNA [3]; this was obtained by PCR amplification using the primers 5'-TGAGCTGTGAGCTTGGAG-3' and 5'-TCAAAGCGTGCTGATCCT-3'. Positive plaques were purified and one clone, λ rg1.1, was chosen for further analysis.

2.2. Mapping, subcloning and sequencing

The 19 kb insert of λ rg1.1 was analyzed by restriction mapping and Southern blot hybridizations. Appropriate restriction fragments were subcloned into pBluescript II SK+ (Stratagene) and sequenced either manually [11] or with the help of an Applied Biosystems model 373A DNA Sequencer.

2.3. Primer extension

Primer extension was performed essentially as described [10] using poly(A)⁺ RNA from GH₃ cells and a ³²P end-labeled antisense 30-base oligonucleotide 5'-GACGCTCACCTTGCGCCCTTGCTGCCTGC-3' corresponding to nucleotides 94–123 of the cloned rat SSTR1 cDNA [3]. 20 μ g RNA was mixed with 5×10^4 cpm of primer (specific activity 10^7 cpm/pmol) in 30 μ l hybridization buffer which contained 80% (v/v) formamide, 40 mM PIPES (pH 6.4), 1 mM EDTA (pH 8.0) and 0.4 M NaCl. Following incubation at 80°C for 5 min the mixture was hybridized overnight at 32°C. After extension for 1 h at 37°C with 200 units M-MLV reverse transcriptase (Stratagene), products were analyzed in 6% denaturing polyacrylamide gels.

2.4. RNA blot analysis and semiquantitative PCR analysis

Preparation of total RNA, isolation of poly(A)⁺ RNA, RNA blot and semiquantitative PCR analysis were carried out as described previously [12]. As hybridization probe for RNA blot analysis, the complete SSTR1 cDNA [3] was radiolabeled by the random priming method [10].

3. Results and discussion

Previous studies have shown that SSTR1 [3] and

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Abbreviations: SSTR, somatostatin receptor; GCF, GC-factor; AP-2, activator protein 2.

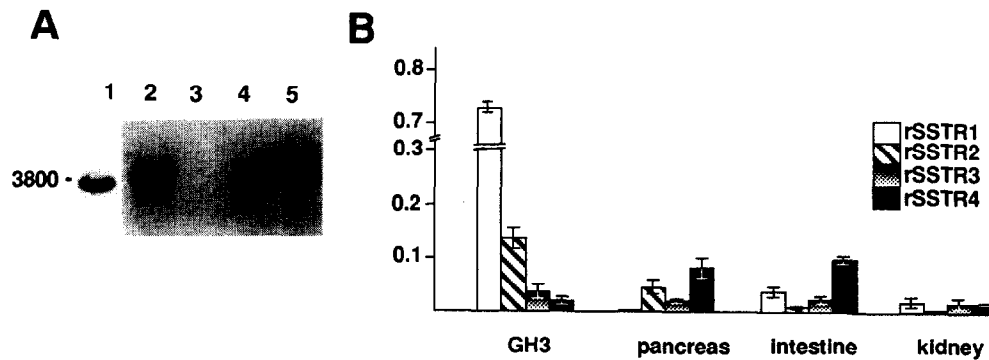


Fig. 1. (A) Northern blot analysis of rat SSTR1 mRNA in peripheral tissues. Poly(A)⁺ RNA (10 µg) isolated from GH₃ cells (1), stomach (2), pancreas (3), duodenum (4) and jejunum (5) was electrophoresed, blotted and hybridized with a radiolabelled SSTR1 cDNA probe. (B) Relative abundance of SSTR1–4 mRNAs analyzed by semiquantitative PCR. Amplified cDNAs generated using specific primers were dot-blotted, hybridized with subtype-specific oligonucleotide probes and quantified using a PhosphorImager as described previously [12].

SSTR3 [5] mRNAs are present in various rat brain regions [5,12]. To investigate the possible physiological roles of the different SSTRs, the distributions of SSTR mRNAs in peripheral tissues were analyzed. RNA blot analysis revealed high levels of the 3.8 kb SSTR1 transcript in rat anterior pituitary GH₃ cells and lower levels in stomach, duodenum and jejunum (Fig. 1A). No SSTR1 transcripts were detected in the pancreas, and no SSTR3 mRNA has been observed in any of the above tissues when using this technique (data not shown). To analyze the SSTR mRNA distributions in greater detail and at higher sensitivity, semiquantitative PCRs have been employed. Fig. 1B reveals that the different tissues contain distinct sets of SSTR mRNAs. Thus, SSTR1 probably has an important role in anterior pituitary GH₃ cell function. SSTR2 also seems to be involved in pituitary function, while SSTR4 may be important for gastrointestinal and pancreatic physiology.

These results, which are in line with previous reports [9] strongly suggest that SSTR gene expression is subject to finely tuned regulatory mechanisms. In order to begin to examine the molecular details of this regulation, the SSTR1 gene was isolated. Sequence analysis of a 7.4 kb *EcoRI*–*HindIII* fragment revealed that it contains the entire previously reported cDNA sequence [3], which lacks introns, about 2 kb of 5'-flanking DNA and 1.7 kb of 3'-flanking DNA (Fig. 2).

The transcriptional start site of the SSTR1 gene was determined using poly(A)⁺ RNA from GH₃ cells that contain a relatively large amount of the corresponding mRNA. Fig. 3 shows a major primer-extended product, corresponding to a guanosine residue, 178 bp upstream from the translation initiation site. In addition, three minor extension products are observed corresponding to positions –2, –1 and +2 of the SSTR1 gene (Fig. 2).

The nucleotide sequence upstream of the SSTR1 mRNA start site contains neither a canonical TATA box nor a CAAT box but possess sequences having a high GC content. These features are reminiscent of promoters

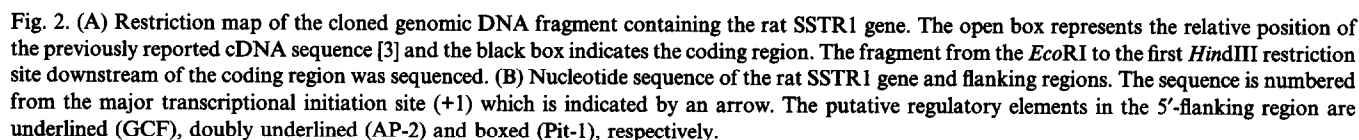
of ubiquitously-expressed housekeeping genes [13,14] such as the human dehydrofolate reductase, hypoxanthine phosphoribosyl transferase and phosphoglycerate kinase genes [15–17]. However, promoters lacking a TATA box but containing GC rich sequences can also be found in genes involved in growth control such as the gene for the human epidermal growth factor receptor [18] and the cellular proto-oncogene Ha-ras [19]. In addition, it is worth noting that the promoter for another G-protein coupled receptor, namely the rat serotonin 5-HT₂ receptor, also lacks a TATA motif [20].

Table 1

Putative regulatory elements in the 5'-flanking region of the rat SSTR1 gene

Pit-1	5'	3'
consensus	A/T T A T C/T C A T	
	-1952 T T A T T C A T	-1959
	-110 A T A T C C A T	-117
GCF	5'	3'
consensus	G/C C G G/C G/C G/C C	
	-1084 G C G G C G C	-1078
	-1054 G C G C G G C	-1060
	-1010 C C G C G C C	-1004
	-954 G C G G G G C	-960
	-656 G C G C C G C	-650
	-233 C C G C C C C	-239
	-200 G C G C C C C	-194
	-192 C C G C C G C	-186
AP-2	5'	3'
consensus	C C C A/C N G/C G/C G/C	
	-1374 C C C A T C C C	-1381
	-1343 C C C C A G G C	-1350
	-1127 C C C C A C C C	-1120
	-906 C C C C C C C	-913
	-197 C C C C C C G	-190
	-99 C C C A G C G C	-106

Consensus sequences recognized by the transcription factors are from [26]. The numbering of the putative regulatory elements corresponds to that of Fig. 1.



recognition sequences for another transcription factor interacting with GC boxes, AP-2 [22], are observed. Two of these are located within the first 200 bp upstream of the mRNA initiation site. Interestingly, two elements that have the consensus sequence of the binding site for the transcription factor Pit-1 (also called growth hormone factor 1) [23,24], are found at positions -107 and -1,951. Pit-1 is a POU-domain protein, which is



Fig. 3. Primer-extension analysis of the 5'-terminus of the rat SSTR1 mRNA. Poly(A)⁺ RNA from the GH₃ cell-line (1) and an equal amount of yeast tRNA (2) were hybridized with a ³²P-labelled oligonucleotide complementary to part of the rat SSTR1 cDNA sequence and then subjected to reverse transcription. The same oligonucleotide was used in a sequencing reaction with the genomic clone. Synthesized DNA products of the C reaction (C; exposure time 16 h) were run as a marker in parallel with the primer-extended products (exposure time 48 h).

restricted to the adult anterior pituitary and has been implicated in the regulation of the tissue specific expression of both the prolactin and growth hormone genes [25]. The presence of Pit-1 recognition sequences in the rat SSTR1 gene might explain the relatively high levels of SSTR1 mRNA in the pituitary [9] and the pituitary-derived, prolactin- and growth hormone-secreting GH₃ cell-line (Fig. 1). This hypothesis is attractive because it suggests that peptide hormone genes and the gene for a receptor involved in the control of peptide hormone secretion are subject to common transcriptional regulatory mechanisms. However, evidence in support of a functional role for the identified sites must await further experiments.

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