IDENTIFICATION AND PARTIAL SEQUENCE OF SIX SOMATOSTATIN RECEPTORS EXPRESSED IN THE SHARK RECTAL GLAND, SHARK BRAIN AND SKATE KIDNEY

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Somatostatin (SRIF) acts on multiple organ systems and its effects include inhibition of practically every known endocrine and exocrine secretion. In the shark rectal gland, somatostatin is the most potent endogenous inhibitor of chloride secretion. Previous work has suggested at least two sites of action for this inhibitory effect. The first is direct inhibition of adenylate cyclase, resulting in a decrease of cAMP content (Kelley et al., *J. Clin. Invest.*, 85:1629-36, 1990). The other is postulated to occur distal to the generation of cAMP (Silva et al., *Bull. MDIBL*, 23:42-44, 1983). In mammals, somatostatin receptors are G-protein coupled receptors (GPCRs) that are classified into five subtypes: SSR-1, SSR-2, SSR-3, SSR-4, and SSR-5. Overall, there is 39-57% sequence identity within these subtypes with SSR-1 and SSR-4 having the highest identity. However, specific somatostatin-like receptors have not been identified in the shark rectal gland or in any marine species. In these experiments, we sought to use molecular techniques to clone and identify the specific types of SRIF receptors present in the shark rectal gland, shark brain, and skate kidney.

Total RNA was prepared from fresh elasmobranch tissues using TRIZOL® Reagent (Gibco). RNA was subjected to DNAse digest and reverse transcription was carried out with oligo dT primers (Clontech Advantage™). Degenerate primers were designed from two highly conserved regions in all mammalian SRIF receptors in putative intracellular loop 2 (sense primer: GAYMGNTAYYTNGCNGTNGTNCAYCC) and transmembrane region 7 (antisense primer: GGRTTNGCRCANSWRTTNGCRTA). PCR was performed for 35 cycles (30 sec at 95 °C, 30 sec at 58 °C and 1 min at 68 °C) using Expand™ Long DNA polymerase mixture (Boehringer Mannheim). The PCR products (~ 500 bp) were cloned (TA-cloning, Invitrogen), recombinants were screened utilizing blue-white selection, and clones were sequenced at the DNA Sequencing Facility, University of Maine, Orono.

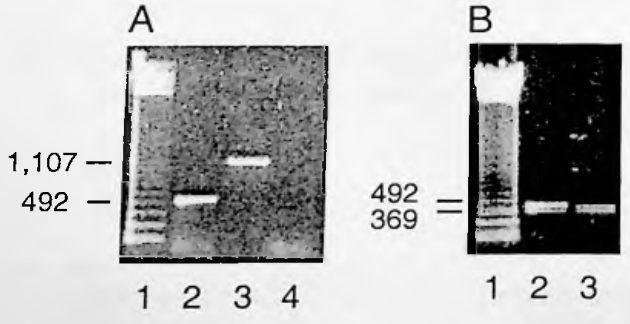

Figure 1. Gel electrophoresis of shark rectal gland PCR products. Panel A: 123 bp ladder (lane 1), band obtained with degenerate somatostatin receptor primers (lane 2), A_0 adenosine receptor (positive control, lane 3) and negative control (no cDNA) using degenerate somatostatin receptor primers (lane 4). Panel B: 123 bp ladder (lane 1), and PCR bands obtained using specific primers for shark SSR-1 and SSR-2 (lane 2 and 3).

Sequencing of six different clones of shark rectal gland PCR products (Fig. 1, panel A, lane 2) yielded two different receptor fragments of 500 bp and 509 bp of nucleotide sequence with an open reading frame of 166 and 169 amino acids, respectively. The putative amino acid sequences had highest homology to mammalian somatostatin receptor type 1 (SSR-1) for the 166 amino acid sequence and to mammalian somatostatin receptor type 2 (SSR-2) for the 169 amino acid fragment. In separate experiments two additional receptor fragments were cloned from shark brain and two others from skate kidney; each had homology to the shark rectal gland

receptors, with notable differences (see below). A partial amino acid alignment of the four shark SRIF receptors cloned from rectal gland and brain is shown in Figure 2.

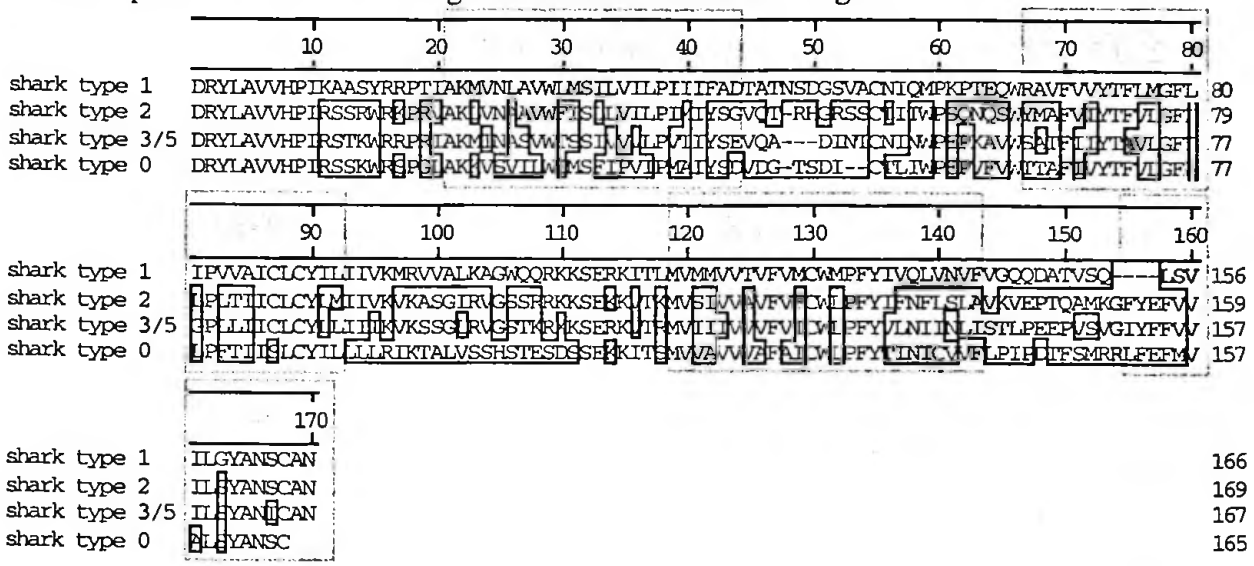

Figure 2. Alignment of four somatostatin receptors from the shark. SSR-1 and SSR-2 were cloned from the rectal gland, SSR-3/5 and SSR-0 originate from the brain. Residues that differ from the shark SSR-1 sequence are boxed. Proposed transmembrane regions are shaded gray.

A phylogenetic tree that includes the six newly identified elasmobranch SRIF receptors is shown in Figure 3.

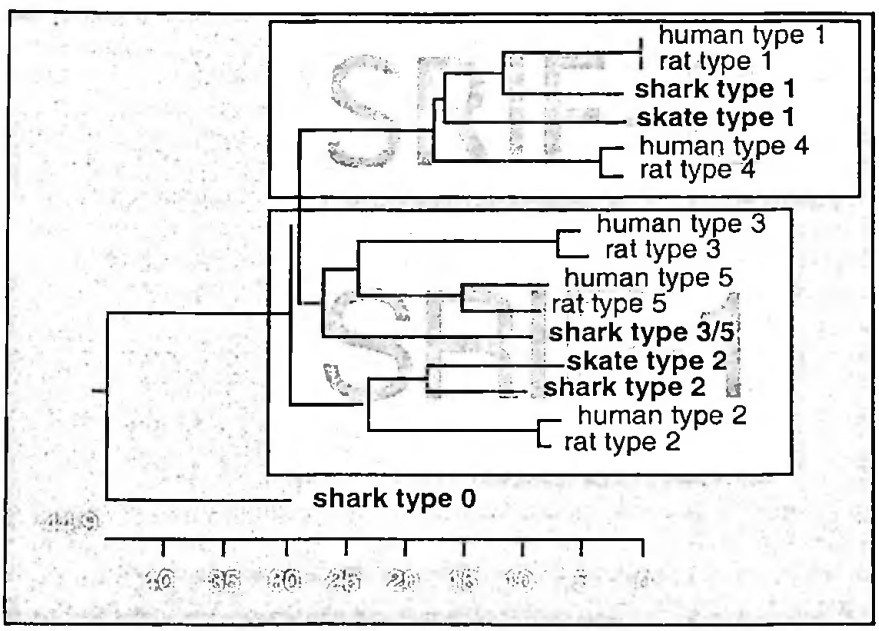

Figure 3. Phylogenetic tree of somatostatin receptors from shark, skate, human, and rat. The alignment was performed on protein sequence from the DRY motif in IC2 to the SCAN motif in TM7 of all receptors. The tree was generated by the MegAlign module of Lasergene software. The length of each pair of branches represents the distance between sequence pairs.

Shark SSR-1 is most similar to the mammalian SSR-1 group with 78.3% similarity to human and rat, but only 63.2% similarity to the skate SSR-1. In contrast, the shark SSR-2 pairs with skate SSR-2 (79.3% similarity), sharing less than 70% similarity with its mammalian counterparts. In shark brain we have identified two novel subtypes of SRIF receptors containing unique sequence. Neither of these new subtypes have been identified in mammals. Shark SSR-

3/5 appears to be the common ancestor of both mammalian SSR-3 and SSR-5 subtypes. The second receptor, which we have called SSR-0, has less than 46.1% similarity to all previously identified somatostatin receptors. SSR-0 does not fall into any of the 5 previously identified subtypes as it is equally different from each. The phylogenetic standing of shark brain SSR-0 in the somatostatin receptor family is comparable to the shark rectal gland A_0 adenosine receptor within its receptor group.

The presence of at least two somatostatin receptors expressed in the rectal gland of the dogfish should help clarify the role of somatostatin in this organ. It is possible that: (a) both receptors are expressed on the rectal gland tubular cell, or (b) one receptor is expressed on the rectal gland cell and the other on the neuro-fibers that infiltrate the rectal gland. The second model is supported by previous experiments on the effect of somatostatin in the shark rectal gland. While somatostatin completely inhibited the stimulation of chloride secretion by forskolin in the perfused rectal gland (Barron et al., *Bull. MDIBL*, 27:136-137, 1988), the same dose (1 μM) of somatostatin applied to cultured cells previously stimulated with forskolin resulted in a 52 ± 10% decrease of the I_{sc} (Epstein et al., *Bull. MDIBL*, 31:117, 1992). This difference could result from an absent pathway in the cultured cell system which lacks the neuro-fibers of the gland.

This work also identifies two previously undescribed SRIF receptor subtypes in the brain of the dogfish shark. One of these, SSR-0, may be related to an ancestral SRIF receptor from which the known subtypes (SSR-1 through SSR-5) evolved. These findings also demonstrate that the shark genome contains both specific GPCR subtypes that are present in mammalian species (for example SSR-1 and SSR-2) in addition to unique GPCR receptor subtypes (SSR-0 and A_0) that apparently are not found in higher vertebrates.

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