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Pharmacological profile of somatostatin and cortistatin receptors

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Abstract

Somatostatin (SRIF) and cortistatin (CST) are two endogenous peptides with high sequence similarities that act as hormones/neurotransmitters both in the CNS and the periphery; their genes although distinct result from gene duplication. Their receptors appear to be common, since the five known SRIF receptors (sst1–sst5) have similar subnanomolar affinity for SRIF and CST, whether the short (SRIF-14, CST-14, CST-17) or the long versions (SRIF-28, CST-29) of the peptides. Whether CST targets specific receptors not shared by SRIF, is still debated: MrgX2 has been described as a selective CST receptor, with submicromolar affinity for CST but devoid of affinity for SRIF; however the distribution of CST and MrgX2 is largely different, and there is no MrgX2 in rodents. A similar situation arises with the GHS receptor GHS-R1a, which displays some preferential affinity for CST over SRIF, but for which there is no evidence that it is activated by CST *in vivo*. In both cases, one may argue that submicromolar affinity is not the norm of a GPCR for its endogenous neuropeptide. On the other hand, all receptors known to bind SRIF have similar high affinity for CST and both peptides act as potent agonists at the sst1–sst5 receptors, whichever transduction pathway is considered. In addition, $[^{125}I][Tyr^{10}]CST_{14}$ labels sst1–sst5 receptors with subnanomolar affinity, and $[^{125}I][Tyr^{10}]CST_{14}$ binding in the brain is overlapping with that of $[^{125}I][Tyr^{0}]SRIF_{14}$. The functional differences reported that distinguish CST from SRIF, have not been explained convincingly and may relate to ligand-driven transductional selectivity, and other complicating factors such as receptor dimerisation, (homo or heterodimerisation), and/or the influence of accessory proteins (GIPs, RAMPS), which remain to be studied in more detail.

Keywords: Somatostatin; Cortistatin; MrgX2; GHS-R1a; GPCRs; Radioligand binding; Second messengers

1. Somatostatin and cortistatin

Somatostatin (somatotropin release-inhibiting factor, SRIF) is a cyclic peptide widely expressed throughout the CNS, in endocrine tissues and in the gastrointestinal tract (GIT). The 14 amino acid peptide, somatostatin (SRIF-14), was first identified and isolated from ovine hypothalamic extracts as a potent inhibitor of growth hormone release from the pituitary. Subsequently, a longer N-terminally extended form (SRIF-28) was identified (see Guilllemin, this issue). A rat neuropeptide with strong homology to SRIF was cloned more recently, and named cortistatin (CST) due to its exquisite brain selective expression (de Lecea et al., 1996): depending on the species, it is a tetradecapeptide, sharing up to 11 amino acids with SRIF. The human homologue of CST seems to be a heptadecapeptide (CST-17). By analogy with SRIF, there may also be a larger isoform of CST, i.e., CST-29. Both peptides are produced as prepropeptides which are then processed to the final forms (see Vaudry, this issue), their genes are the products of gene duplication (see Tostivint et al., 2006). In fish, there are even 3 different genes coding for SRIF like peptides.

SRIF exerts a wide range of biological actions, including inhibition of secretion of growth hormone, insulin, glucagon and gastrin as well as other hormones secreted by the pituitary and GIT. Somatostatin also acts as a neuromodulator in the CNS and shows anti-proliferative effect on a wide range of cancer cells (Hoyer et al., 1995; Weckbecker et al., 2003). Although, various physiological parameters, including transitions between sleep phases, consolidation of short- and long-term memory, and locomotor activity, respond in an apparently peptide-specific manner to SRIF and CST, the latter has nanomolar affinity for each known SRIF receptor subtype. The existence of specific CST receptors has not been demonstrated so far, although, it was suggested that the MAS related gene receptor MgrX2 and/or the GH secretagogue receptor GHS-R1a may play such a role (see below).

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2. SRIF and CST receptors

SRIF and CST act via a family of G protein-coupled receptors, of which five subtypes (sst_1–sst_5) were cloned and characterized from various species (Hoyer et al., 1995; Weckbecker et al., 2003). Sequence homology is 39–57% among the five subtypes, each being highly conserved across species. Based on structural and operational features, they are divided into two groups: SRIF-1 includes sst_2, sst_3 and sst_5 receptors while SRIF-2 consists of sst_1 and sst_4 receptors, distinguished by high affinity of cyclic peptides such as octreotide (SMS-201-995), seglitide (MK-678) and lanreotide (BIM-23014) for the SRIF-1 group. In humans, SRIF receptors are encoded by five non-allelic genes, which have been identified on chromosomes 14, 17, 22, 20 and 16. Genes coding for sst_1, sst_3, sst_4 and sst_5 are intronless, whereas in rodents the gene for sst_2 contains three introns which result in the generation of two receptor protein variants: the unspliced sst_{2A}, and sst_{2B} which is spliced in the carboxy terminal part of the gene, and differs from sst_{2A} by the length of its carboxy tail. In addition, SRIF receptors have also been cloned from chicken and fish tissues (e.g., Zupanc et al., 1999; Lin et al., 2002; Nunn et al., 2002).

Studies utilizing subtype selective SRIF analogs in both *in vivo* and *in vitro* experiments demonstrate that sst_2 receptors are the major player in the SRIF receptor family with broad inhibitory effects on the endocrine secretion, e.g., growth hormone, insulin, glucagon, gastrin, cholecystokinin, vasoactive intestinal peptide, and secretin, as well as the exocrine secretion, e.g., gastric acid, intestinal fluid, and pancreatic enzymes. sst2 receptors also seem to play a major role in various forms of gastro enteropancreatic (GEP) cancers, in epilepsy and pain (Vezzani and Hoyer, 1999; Weckbecker et al., 2003; Rubio et al., 2007). The sst1 receptor may function as an autoreceptor at least in basal ganglia and the eye. sst3 receptors are enigmatically localized to neuronal cilia, and sst3 antagonists have marked behavioral effects. sst4 receptors are highly expressed in the lung, but their role remains to be defined, although in the mouse they modulate epileptic activity. sst_5 receptors mediate inhibition of insulin release from the pancreatic β-cells in addition to regulating growth hormone release. Currently, octreotide and lanreotide are in clinical use for the treatment of acromegaly, diarrhea and various gastroenteropancreatic tumors, whereas [^{111}In]penteotride is used for whole body tumor imaging.

There is accumulating evidence that CST and SRIF may produce different effects both in the brain as described very early on (de Lecea et al., 1996, 1997; Braun et al., 1998; Vassilaki et al., 1999; Schweitzer et al., 2003) and in the periphery, especially in the immune system (Dalm et al., 2003, 2004; Gonzalez-Rey et al., 2006a,b; Ferone et al., 2006; Rubio et al., 2007). It is thus legitimate to ask whether such differences can be explained by the existence of CST-specific receptors as has been suggested for MgrX2 and/or GHS-R1a. On the other hand, there is also quite some evidence that shows CST and SRIF to have very similar actions, mediated by the known sst1–sst5 receptors (Siehler et al., 1998a; Criado et al., 1999; Spier et al., 2005; Gottero et al., 2004; Grottoli et al., 2006).

3. Pharmacological profiles of mammalian and fish SRIF/CST receptors

The five so-called SRIF receptors (sst1–sst5) were cloned in mammals before CST was discovered (Hoyer et al., 1995). These GPCRs were thus characterized/named accordingly as SRIF receptors, each with a distinct profile and distribution (Fehlmann et al., 2000). Similarly, several SRIF receptors were cloned from fish and assigned by homology to the sequence of mammalian receptors (e.g., sst2, sst3, sst5) (Zupanc et al., 1999; Lin et al., 2002). One of the prime questions that arose when CST was discovered and reported to have different functional effects in the brain when compared with SRIF (de Lecea et al., 1996, 1997; Fukusumi et al., 1997), related to the nature of the receptors being activated by either SRIF or CST. In other words, are there selective receptors for one (CST) or the other of the peptides (SRIF), are they partly overlapping or are they entirely shared? We therefore undertook to synthesise CST and analogues (CST-14, CST-17, [Tyr^{10}]CST_{14}), and radiolabel an iodinated CST analogue ([^{125}I][Tyr^{10}]CST_{14}). A number of studies were then carried out to compare SRIF and CST binding at recombinant human sst1–sst5 receptors (and some fish receptors), determine and compare their affinities for CST and SRIF analogues (Siehler et al., 1998a,b), establish the pharmacological profiles of the sites labeled by [^{125}I][Tyr^{10}]CST_{14} and [^{125}I]LTT-$SRIF_{28}$ (as well as other known SRIF receptor radioligands), and last but not least, characterize the effects of the CST analogues at the known SRIF receptors using various second messenger paradigms (Siehler et al., 1999a,b; Siehler and Hoyer, 1999a,b,c). The first point to be made is that [^{125}I][Tyr^{10}]CST_{14} and [^{125}I]LTT-$SRIF_{28}$ bind with high affinity to very similar densities of receptor sites in CCL39 cells expressing human ss1–sst5 receptors. [^{125}I][Tyr^{10}]CST_{14} in the five cells lines was characterized by the following pK_d (−log mol/l) and B_{max} values (fmol/mg) for $hsst_{1-5}$ receptors: 10.02 ± 0.04, 220 ± 30; 9.45 ± 0.09, 340 ± 70; 10.06 ± 0.11, 340 ± 50; 9.67 ± 0.14, 340 ± 110 and 10.33 ± 0.03, 5630 ± 330, respectively. The B_{max} values were very similar to those obtained in saturation experiments using [^{125}I]LTT-$SRIF_{28}$. Both radioligand were similarly affected by the presence of GppNHp, suggesting strongly an agonist type of binding to a GPCR. Table 1 summarises and compares the affinity values of SRIF and CST analogues labeled with [^{125}I][Tyr^{10}]CST_{14} or [^{125}I]LTT-$SRIF_{28}$. It is clear that CST and SRIF analogues have very similar high affinities for all five sst1–sst5 receptors, CST-17 being the most potent ligand at sst1, sst4 and sst5 receptors, whereas it is almost equipotent with SRIF-14 at sst3 receptors, and somewhat less potent than SRFIF-14 at sst2 receptors, but in every case the affinity is around 1 nM (sst2) and up to 0.3 nM at sst1, compared to the best affinity of SRIF-14 at sst2 (0.1 nM). These values are customary of endogenous neuropeptides for their cognate receptors. Although, not shown here, we have demonstrated previously that the pharmacological profile defined at every single receptor labeled with [^{125}I][Tyr^{10}]CST_{14} or [^{125}I]LTT-$SRIF_{28}$ is nearly superimposable, both in terms of absolute affinity values for a large number of analogues and thus rank order of affinity.