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Review

Cortistatin—Functions in the central nervous system

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Abstract

Cortistatin (CST) is a neuropeptide from the somatostatin (SRIF)/urotensin (UII) family named after its predominantly cortical expression and ability to depress cortical activity, which was discovered a decade ago. *In vitro* assays show CST is able to bind all five cloned somatostatin receptors and shares many pharmacological and functional properties with SRIF. However, distinct from SRIF, CST has been shown to induce slow-wave sleep, reduce locomotor activity, and activate cation selective currents not responsive to somatostatin. Different lines of evidence also indicate that CST, like SRIF, is involved in learning and memory processes. CST-14 may also function as an endogenous anti-convulsant. In addition to its role in cortical synchronization, CST-14 has emerged as an important mediator of immunity and inflammation. This review will cover some of the basic properties of CST in the brain, and will discuss new data on the role of CST in cortical activity.

Keywords: Somatostatin; Sleep; GABA; Gene expression

Contents

1. Cloning of preprocortistatin

As part of our efforts to isolate region-specific transcripts, we isolated a partial cDNA clone from a hippocampal-subtracted library that showed a remarkable expression pattern in scattered cells throughout the cortex and hippocampus (de Lecea et al., 1996). The nucleotide sequence of the full-length cDNA clone suggested that it encoded a novel putative 112 amino acid protein, whose C-terminal revealed a strong similarity with preprosomatostatin. The protein encoded by the cDNA was named preprocortistatin, in recognition of its strong similarity with somatostatin and its predominantly cortical expression. The name cortistatin (CST) also reflects its inhibition of cortical activity (see below).

PreproCST begins with a 27-residue apparent secretion signal sequence. Cleavage of the prepro species to proCST would produce a protein that could be processed at either of two tandem basic amino acid pairs to produce CST-29 and CST-14, analogous to the cleavage of preprosomatostatin at residues 28 and 14 (Gluschankof et al., 1984), or at both basic pairs to additionally produce CST-13 (CST-13). Whereas CST-13 is unrelated to known species, CST-14 shares 11 of 14 residues with somatostatin-14 (SRIF-14), including two cysteine residues that are likely to render the peptide cyclic and the FWKT motif that is critical for SRIF-14 binding to its receptors (Fig. 1) (Veber et al., 1979). This core of hydrophobic amino acids is

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Fig. 1. Primary structure of mammalian SRIF and the rodent and human forms of CST. Note the conserved hydrophobic core of amino acid residues that are critical for somatostatin receptor binding (from Spier and de Lecea, 2000).

also present in urotensin II (UII) and urotensin-related peptide (URP).

CST-14 and SRIF-14 are permuted by one amino acid; the alignment of CST-14 begins at residue 2 of SRIF-14, and CST-14 terminates with a lysine residue beyond the C-terminal cysteine of SRIF-14 (Fig. 1). Although the C-terminal lysine would be susceptible to cleavage by carboxypeptidases, release experiments have demonstrated that this residue is present in the endogenous peptide (Puebla et al., 1999).

A full-length cDNA encoding preproCST has also been obtained from mouse and human (de Lecea et al., 1997b; Fukusumi et al., 1997). The presence of only two cleavage sites in the mouse deduced amino acid sequence shows that processes of the prepropeptide would give rise to two peptides, mCST-44 and mCST-14, analogous to rCST-14. The human nucleotide sequence shows a much lower degree of identity to the rat sequence (71%). Analysis of the putative processing sites in human preproCST revealed that it may be cleaved at two RR sites, giving rise to hCST-29 and a C-terminal 17 residue peptide (hCST-17) that shares 13 of the last 14 residues with rat and mouse CST-14. The Lys-Lys pair that lies just N-terminal to CST-14 in rat and mouse is not conserved in the human sequence. Other possible products that follow the signal sequence (hCST-21 and hCST-31) are not conserved across species, although rCST-31 and hCST-31 share 13 residues clustered in their N-terminal regions that are conserved among the rat, mouse and human prohormone sequences. Rat preproCST is cleaved at the two C-terminal dibasic cleavage sites, KK and KR, to produce rCST-14 and rCST-29 analogously to SRIF-14 and SRIF-28 (Puebla et al., 1999). The lack of evidence for a cleavage at both C-terminal dibasic sites to produce rCST-14 and rCST-13 indicates that rCST-14 and rCST-29 are the major products of the preproCST processing. Thus, although the two peptides are produced approximately equally, the rCST-14 form is preferentially released compared to rCST-29. The presence of CST-14 has been demonstrated *in vivo* in preprosomatostatin knock-out mice. HPLC purification of peptide extracts from SRIF knock-out mice revealed a peak of SRIF-like immunoreactivity, which coincided with synthetic CST-14 (Ramirez et al., 2002). Thus far, there is no evidence of differential biological activities of the different forms of CST (rCST-14, rCST29 and hCST-17).

The nucleotide sequences and chromosomal localizations of preprocortistatin (*cort*, 1p36), SRIF (*SST*, 3q28), Urotensin II (*UTS2*, 1p36) and urotensin-related peptide (*UTS2D*; 3q28) indicate they are products of separate genes, that arouse from a duplication of a common ancestor (Tostivint et al., 2006). A comparative analysis has revealed conserved synteny between the zebrafish *SST3* locus and human chromosome 1p36 region, where the *cort* gene is located, strongly suggesting that the *SST3* gene in nonmammalian species and the *cort* gene in mammals are orthologous (Tostivint et al., 2004).

2. Expression of preprocortistatin in the brain

PreproCST cDNA sequences have been cloned from human peripheral tissues including fetal heart, fetal lung, prostate, colon, kidney and many tumors (Dalm et al., 2004, 2003a; Notas et al., 2004; Rubinfeld et al., 2006). Importantly, preproCST mRNA, but not somatostatin mRNA, has been detected in cells of immune origin (see below).

The distribution pattern of preproCST mRNA, determined by in situ hybridization on rat brain sections, indicates that preproCST mRNA is expressed in scattered neurons throughout the cerebral cortex and hippocampus, and is distinct from preprosomatostatin expression (Fig. 2). However, other areas of the brain also show preproCST mRNA expression, although at much lower levels. For example, in the olfactory bulb, granule GABAergic neurons are positive for preproCST mRNA. In the striatum, a small number of positive cells can be detected, resembling cholinergic or GABAergic interneurons. A few cells in the hypothalamus, corresponding to the periventricular nucleus, are positive for preproCST mRNA. No signals could be detected in the thalamus, mesencephalon, brainstem, cerebellum or spinal cord (de Lecea et al., 1997a,b; Fukusumi et al., 1997).

In the cortex, CST-14 positive cells are especially abundant in layers II–III and VI (Fig. 2B). Interestingly, the distribution of preproCST mRNA positive cells is not uniform in all cortical areas, the visual cortex displays about twice as many preproCST mRNA-containing neurons as the somatosensory cortex. In the hippocampal formation, CST mRNA expression is found in a small subset of non-pyramidal cells in the subiculum and in the stratum oriens of the CA1 and CA3 fields. No preproCST mRNA was detected in the dentate gyrus. Double in situ hybridization experiments have shown that preproCST mRNA co-localized in every instance with either GAD65 or GAD67, demonstrating the GABAergic nature of CST-expressing cells (de Lecea et al., 1997a).

CST-14 and SRIF are expressed in distinct, though partially overlapping sets of GABAergic interneurons neurons in rat (de Lecea et al., 1997a). PreproCST mRNA and SRIF immunoreactivity do not co-localize in layer II–III of the cerebral cortex (Fig. 2D). No CST-positive neurons are present in the hilar region of hippocampus, where SRIF-14 is expressed.

We raised antisera to a peptide fragment corresponding to the rat CST-14 precursor, in a region that does not have any similarity with preprosomatostatin. Immunocytochemical staining with these antisera demonstrated that the distribution of the peptide precursor coincides with that observed by in situ hybridization (Fig. 3). It is noteworthy that cortistatin-immunoreactive neurons and fibers were also detected in the periventricular hypothalamus, a population of cells reminiscent of SRIF-immunopositive neurons (Fig. 3B).

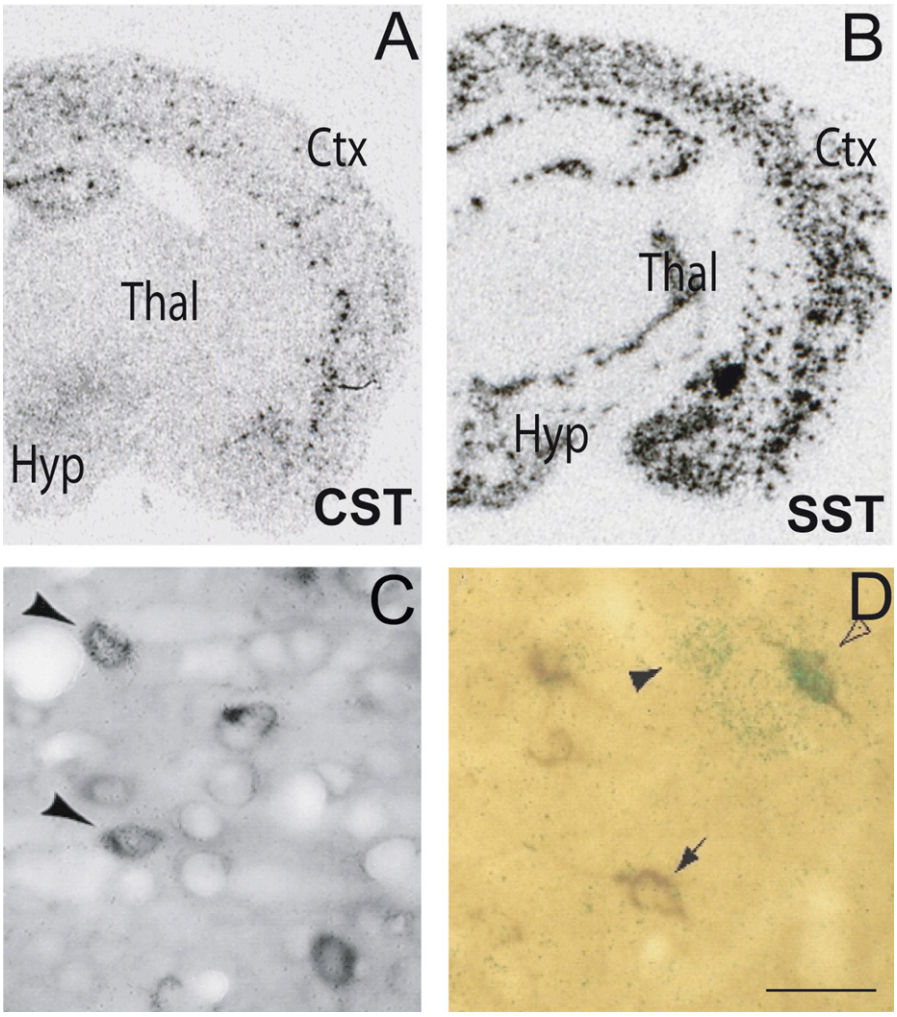

Fig. 2. Expression of preproCST and preproSST mRNAs in the rodent brain. (A) In situ hybridization to preproCST mRNA in the adult wild-type C57BL6 mouse brain. (B) Autoradiograph of an in situ hybridization to mouse preproSST. We have estimated that SST is 20 times more abundant in the brain than CST. Also, note absence of signal for CST in thalamus and hypothalamus. (C) PreproCST mRNA-positive cells are GABAergic, as demonstrated by double in situ hybridization to CST (autoradiographic emulsion) and GAD 67 (DIG-positive cell somata). (D) Partial colocalization of preproCST mRNA and SRIF-14 immunoreactive cells in upper cortical layers (de Lecea et al., 1997a). Bar = 50 μm.

The developmental expression of preprocortistatin mRNA has been characterized by northern blot and by in situ hybridization on brain sections at different developmental stages (de Lecea et al., 1997a). PreproCST mRNA appears at day post-natal 15 and reaches a maximum level during the second post-natal week (between P15 and P20). This pattern of expression correlates with the maturation of cortical interneurons.

The regulation of preproCST mRNA accumulation has been investigated in various paradigms underlying again the differences between the two peptides. PreproSRIF mRNA increases its steady-state concentration fourfold upon kainate injection in the dentate gyrus and CA1, whereas preproCST mRNA does not respond to kainate, indicating that CST and SRIF respond to different signals (Calbet et al., 1999). Also, preproCST mRNA is regulated by the light/dark cycle, with maximum levels before sleep onset. Interestingly, this pattern of regulation is also found in the somatostatin III (PSSIII) gene in goldfish, suggesting that CST-14 and PSSIII may share some functions (Canosa and Peter, 2005).

3. Electrophysiological properties of CST-14

Considering the high level of CST expression in the hippocampus and the hyperpolarizing effect of SRIF on hippocampal neurons, the possible involvement of CST in the physiology of the hippocampus was first investigated by means of current- and voltage-clamp recordings in hippocampal slice preparations (de Lecea et al., 1996). Superfusion of CST-14 hyperpolarizes pyramidal neurons. Unlike SRIF-14, the CST-14 effect develops slowly, reaching a maximum steady effect 6–8 min after the onset of the response. This effect contrasts with a much shorter (2–3 min) time-to-peak of the effect of SRIF-14 on hippocampal neurons under the same experimental conditions (Schweitzer et al., 2003).

To determine the mechanism of the CST-induced inhibition, the effect of CST-14 on the M current (I_M), a non-inactivating voltage-dependent potassium current seen in hippocampal neurons, has been studied (Moore et al., 1988). As previously described for SRIF-14 (Moore et al., 1988; Schweitzer et al.,

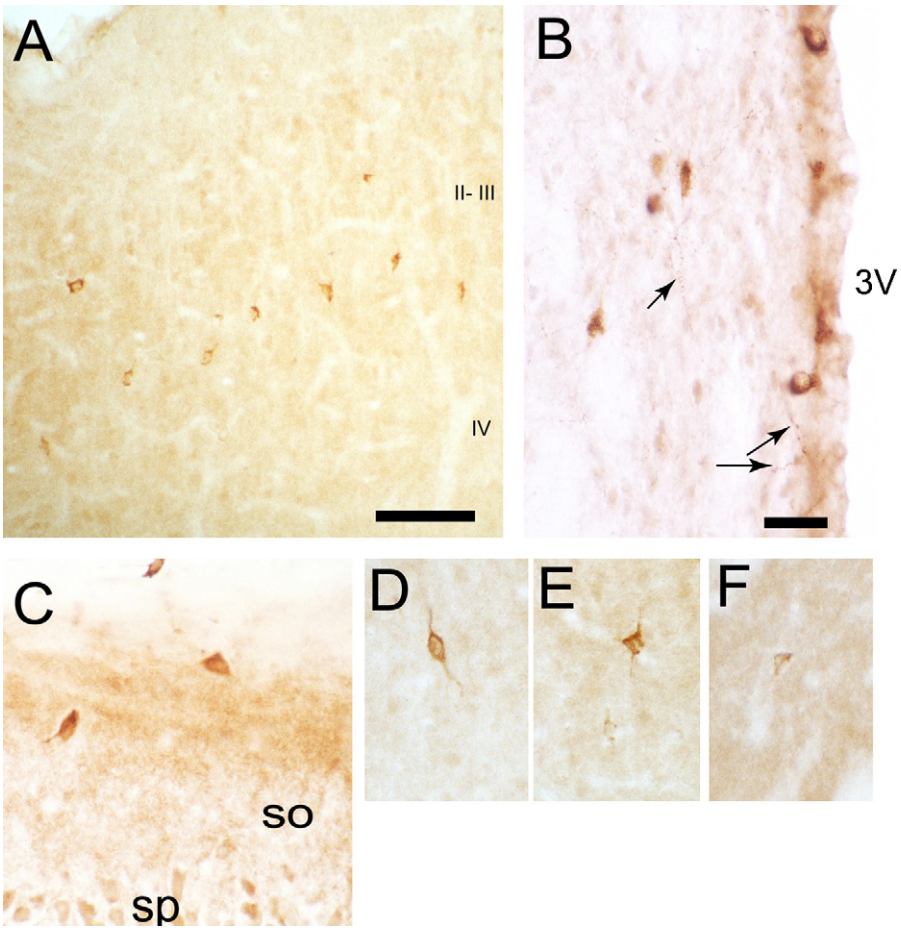

Fig. 3. CST-like immunoreactivity in the rat brain. Staining with antisera to the rat CST precursor shows a distribution of CST-immunoreactive (IR) neurons that is consistent with the in situ hybridization results. In the neocortex (A), scattered, nonpyramidal cells in layers II–III and VI show CST-IR. (bar = 250 μm). (B) A few CST-IR neurons and processes (arrows) were also detected in the periventricular hypothalamus. In the hippocampal formation, CST-immunoreactive neurons were nonpyramidal, and concentrated in the stratum oriens of the CA1. (D–F) Heterogeneous morphologies of CST-immunopositive neurons in the cortex (D), amygdala (E) and striatum (F). Bar for B–F = 50 μm.

1993), CST-14 superfusion increases the amplitude of the I_M relaxation concomitantly with an outward steady-state current, with recovery to control levels upon washout. The same effects were observed following treatment with CST-29 (Schweitzer et al., 2003). Together, these results indicate that CST-14 and SRIF similarly increase K+ conductances in hippocampal neurons, most likely by activating SST receptors. However, CST additionally augments I_h, a voltage-dependent current that plays a key role in the modulation of synaptic integration and regulates oscillatory activity (Schweitzer et al., 2003), supporting the notion that CST-14 interacts with a specific receptor.

In the hippocampus, the nature of cells sensitive to CST has been identified *in vitro* and *in vivo* as glutamatergic neurons. Thus, application of CST-14, as SRIF-14, reduces evoked EPSPs mediated by NMDA and non-NMDA glutamate receptors in CA1 neurons *in vitro*, whereas neither peptide alters IPSPs (Tallent and Siggins, 1997, 1999). *In vivo*, iontophoretic application of CST-14 in anesthetized rats decreases the firing rate of CA1 pyramidal cells induced by NMDA pulses. The modulation of glutamatergic neurons by CST-14 has been observed in extracortical areas. Application of CST-14 in hypothalamic neurons inhibits glutamate-induced responses, probably through the activation of type 2 SRIF receptors (Vasilaki et al., 1999). Together these results suggest that CST generates physiological responses by reducing the activity of excitatory neurons.

4. Pharmacological profile of CST-14

The strong structural similarity between CST-14 and SRIF suggested that CST-14 might bind to somatostatin receptors. Somatostatin binds to five different known receptors, named SSTR1-5, which are members of the 7-transmembrane G-protein coupled receptor superfamily and display distinct, but overlapping expression patterns (Csaba and Dournaud, 2001). rCST-14 and hCST-17 have been shown to displace ^{125}I-SRIF-14 binding to each of the five cloned SSTRs expressed in transfected cell lines, with affinities in the low nanomolar range similar to those of SRIF-14 (Criado et al., 1999; Fukusumi et al., 1997; Siehler et al., 1998). CST-14 has also been shown to be an effective agonist to SSTR(s) expressed by GH4 cells, as measured by inhibition of vasoactive intestinal peptide (VIP) or thyroid-releasing hormone (TRH) induced cAMP accumulation, with indistinguishable efficacy from SRIF-14 (de Lecea et al., 1996). GH4 cells are thought to primarily express SSTR1,

and CST-14 has further been shown to agonize SSTRs 2–5 in transfected CHO cells (Fukusumi et al., 1997).

The anatomical distribution of CST-14 binding sites has been analyzed using autoradiography with ^{125}I Tyr^{10}CST on mouse brain sections. Similarly to SRIF-14 (Leroux et al., 1993), CST-14 shows binding sites throughout the cerebral cortex, especially in the deep layers, hippocampal formation and medial habenula and basolateral amygdala (Spier et al., 2005). Most of the SRIF-14 binding sites in the brain are competed with cold SRIF-14 (100 nM). But, interestingly, 100 nM CST-14 does not compete ^{125}I SRIF-14 labeling in the cortex or amygdala, whereas 100 nM SRIF-14 fully displaces the signal, thus indicating the presence of receptors with different affinities for SRIF-14 and CST-14 (Spier et al., 2005).

Several studies have examined the peptide structures necessary for CST-14 binding to SSTRs. As with SRIF-14, CST-14 does not show any preferred conformation in solution, as determined by circular dichroism and nuclear magnetic resonance (Criado et al., 1999), suggesting that the conformation of the peptides is not an important factor for binding to SSTRs. hCST-17, hCST-15 and hCST-13 all displace ^{125}I-SST-14 binding to SSTR1-5 with similar efficacy (Fukusumi et al., 1997). Surprisingly this indicates that the four N-terminal extracyclic residues of hCST-17 are not important for mediating a detectable pharmacological difference between hCST-17 and SST-14. Using the rationale that the residues contained within the Cys-Cys loop of CST-14 and somatostatin only differ by one amino acid whereas the extracyclic residues are distinct, Criado et al. (1999) examined the role of the extracyclic residues in CST-14 pharmacology. With the cyclic moiety of the peptide utilizing a sequence obtained from octreotide, a potent SRIF analogue, the extracyclic residues were systematically modified. It was shown that both the N-terminal Pro and C-terminal Lys are necessary to elicit CST-14's unique physiological effects on sleep and locomotor activity. With only the N-terminal Pro present, a CST-like effect on locomotor activity but not on sleep was observed, and if only the C-terminal Lys is present, the compound behaves like somatostatin. Analog compounds with the N-terminal proline bound with nanomolar affinities to SSTR3 and 5, but not to SSTRs 1, 2 and 4 (Table 1). Interestingly, SSTR5 mRNA is expressed only at low levels in the brain, while SSTR3 mRNA is expressed abundantly in the cortex and hippocampus. This leads to the possibility that CST-like effects *in vivo* may be mediated through SSTR3. An eight residue peptide based on the structure of CST-14, referred to as CST-8, has been used by several groups in pharmacological studies, and shown weak binding to SSTRs (Sibilia et al., 2006) although it is still unclear whether this compound has the full spectrum of CST-14 activities.

Even though somatostatin signaling is remarkably plastic and up-regulation of SSTRs has been reported in *sst*-deficient mice (Ramirez et al., 2002), ongoing studies on somatostatin receptor-deficient mice (see Tallent et al., in this issue) may yield significant information about the contribution of each receptor subtype on CST signaling.

5. Cortistatin receptors

Neuropeptide receptors are also structurally and functionally related with some cross-talk between neuropeptides and receptors for other neuropeptide families (Civelli et al., 1999). For example, SRIF-14 is able to bind opioid receptors (Pelton et al., 1986). As discussed above, *in vitro* assays show CST binds to and activates the known SSTRs. However, CST-14 does not bind the urotensin II receptor (Nothacker et al., 1999) or μ- and δ-opioid receptors (Connor et al., 1997).

Many of the physiological effects of CST-14 suggest CST's activity is mediated by non-SSTRs. Indeed, it has been shown recently that CST-14 and CST-17, but not SRIF-14, bind to the Growth Hormone (GH)-secretagogue receptors with similar affinity than ghrelin (Deghenghi et al., 2001). The human orphan G-protein coupled receptor MrgX2 has been described as the first human CST selective receptor (Robas et al., 2003). However, the functional significance of this binding activity remains to be ascertained, since MrgX2 is a very promiscuous receptor, binds to other peptides such as proadenomedulin peptide, and only requires a triad of hydrophobic amino acid residues for high affinity binding (Nothacker et al., 2005).

Somatostatin receptor heterodimers (Pfeiffer et al., 2001; Rocheville et al., 2000), receptor accessory molecules, analogous to RAMPs for the calcitonin receptor-like family (Hay

Table 1
Affinities of SRIF-14, CST-14 and analogues to the five human SSTRs

Peptide	hsstr1	hsstr2	hsstr3	hsst4	hsstr5	Sequence
SRIF-14	2.3 ± 0.47	0.23 ± 0.04	1.17 ± 0.23	1.7 ± 0.3	1.4 ± 0.3	Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH
Octreotide	875 ± 180	0.57 ± 0.08	26.8 ± 7.7	>1000	6.8 ± 1.0	D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol
96145	>1000	115 ± 18	934	>1000	26.6 ± 8.8	D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Lys-NH_2
96166	>1000	930 ± 69	27 ± 1.0	>1000	93 ± 39	Pro-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-NH_2
96165	>1000	>1000	105 ± 13	>1000	60.4 ± 19	Pro-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Lys-NH_2
96149	>1000	>1000	>1000	>1000	>1000	Pro-c[Cys-Tyr-D-Trp-Lys-Cys]-Lys-NH_2
CST-14	2.1 ± 0.8	0.5 ± 0.1	3.8 ± 0.9	18.2 ± 2.5	0.9 ± 0.2	Pro- c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys]-Lys

Values are Kd in nM (adapted from Criado et al., 1999).

et al., 2006), or new forms of sst receptors may also explain functional differences between CST-14 and SRIF signaling.

6. CST-14 modulates sleep/wakefulness rhythms

To investigate the physiological function of CST, a first set of experiments investigated the ability of CST to affect the general behavior of rat. For this purpose, the effects of CST-14 on locomotor activity were examined in cannulated, freely moving rats. Intracerebroventricular injection of different amounts (0.1, 0.5 and 1 μg) of synthetic cyclic CST-14 markedly decreased locomotor activity (Criado et al., 1999), an effect opposite to that reported for SRIF-14 at the same doses (Rezek et al., 1976). However, at a higher dose (10 μg) CST-14 infusion induces seizures and barrel rotation, a characteristic behavior of SRIF-14 injection, suggesting that, under saturating conditions CST-14 may act through SSTRs *in vivo*.

To determine the function of CST-14 *in vivo*, de Lecea et al. (1996) infused rats with CST-14 into the brain ventricles and analyzed the electroencephalogram (EEG) as a measure of cortical excitability. Polygraphic monitoring of arousal states subsequent to the administration of CST-14 indicated that rats spent up to 75% of the 4-h recording time in slow-wave sleep compared to 40% in saline-treated control animals. A significant reduction of paradoxical (REM) sleep with the highest dose of CST-14 was also detected. Again, the physiological responses induced by CST-14 are in clear contrast to the reported enhancement of REM sleep with a similar dose of SRIF (Danguir, 1986).

The slow-wave sleep-inducing activity of CST-14 has been replicated by others, using synthetic human CST-17 (Fukusumi et al., 1997). Moreover, CST-14 actively induced sleep when infused during the dark period in rats that have already accomplished their physiological demand of sleep. Consistent with the hypothesis that CST-14 is a sleep factor, sleep deprivation increases the steady-state concentration of preproCST mRNA (Cirelli et al., 2006), and its levels oscillate along the light/dark cycle (LdL, unpublished results).

How does CST-14 induce sleep? CST-14 may enhance the intrinsic activity of cortical neurons by its hyperpolarizing activity on principal cells. Also, regulated release of CST-14 at the appropriate circadian time may antagonize the excitatory effects of ACh and promote sleep. CST-14 may also enhance SW sleep by influencing the activity of neuronal networks by its activity on a hyperpolarization-activated cationic conductance known as the h-current. The h-current induces a small afterdepolarization in thalamocortical cells that causes a refractory period between spindle waves (Luthi and McCormick, 1998). Activation and subsequent deactivation of I_h in thalamocortical cells thus appears to be a key mechanism through which synchronized oscillations are terminated and prevented within defined intervals. Slices from HCN2 mutant mice show complete absence of h-current in thalamocortical neurons, and the mice are highly susceptible to absence seizures (Ludwig et al., 2003). The h-current has also been shown to be important for the establishment of rhythmicity in other neuronal systems (Gauss and Seifert, 2000). CST-14, but not SRIF-14, enhances the amplitude of I_h on hippocampal slices. Interestingly, VIP and PACAP have been shown to increase I_h, thus modifying the firing properties of thalamocortical neurons (Sun et al., 2003). Activation of I_h by CST-14 in cortical networks may thus have a prominent role in regulating the synchronous activity that characterizes slow-wave sleep.

7. Additional functions for cortistatin in the CNS

In addition to its possible role in cortical synchronization, CST-14 appears to have other functions in the central nervous system. For instance, CST-14 may protect against neuronal injury caused by ischemia (Rauca et al., 1999). In this regard, it may be significant that preproCST mRNA showed prominent expression in immature hippocampus at post-natal day 15 that was further increased after kainic-acid-induced seizures (Wilson et al., 2005), which suggests endogenous anti-convulsant activity.

Several groups have shown that icv injection of CST-14 impairs long-term memory in passive avoidance tests (Flood et al., 1997; Sanchez-Alavez et al., 2000). Transgenic mice overexpressing CST-14 in hippocampal neurons do not produce long-term potentiation in the dentate gyrus (Tallent et al., 2005). This effect appears to be mediated by reduction of postsynaptic NMDA receptor function. Thus, in rats, inhibition of glutamatergic neurotransmission by CST-14 may be the cellular basis for long-term memory impairment. Interestingly, CST expression is significantly affected in the cortex of PDAPP transgenic mice, a mouse model of Alzheimer's disease (Winsky-Sommerer et al., 2004). Future experiments with CST knock-out animals may shed light into the possible role of this peptide in cognition.

Detailed gene expression studies have revealed that preproCST is much more widely expressed in peripheral tissues than preprosomatostatin. Indeed, CST-17 has been shown to bind to Growth Hormone (GH) secretagogue receptor in human tissue (Deghenghi et al., 2001). Furthermore, CST-14 inhibits both basal and stimulated GH secretion in the same manner as SRIF (Broglio et al., 2002) and mimics the dual (stimulatory/inhibitory) activity seen in porcine somatotropes (Luque et al., 2006). Increasing evidence also suggests that CST may play a role in the regulatory mechanisms between the neuroendocrine and immune systems. PreproCST mRNA expression has been reported in human lymphoid tissue, immune cells and bone marrow (Dalm et al., 2003b), as well as in the pancreas (Papotti et al., 2003). Dalm and colleagues also observed high expression levels of peproCST mRNA in monocyte-derived macrophages and dendritic cells. In contrast, no SRIF mRNA was detectable in immune cell types. In addition, both preproCST and sst2 mRNAs have been shown to be up-regulated during differentiation of monocytes into both macrophages and dendritic cells. Therefore, it has been proposed that CST-17 would be an endogenous ligand of hsst2 rather than SRIF in the immune system (Dalm et al., 2003b; Ferone et al., 2006).

Also, recently González-Rey et al. have described an intriguing role of CST-14 in different experimental models of inflammation, including ulcerative colitis and arthritis (González-Rey et al., 2007, 2006a,b). CST-14 appears as a perfect anti-inflammatory peptide, since it inhibits proliferation of

Th1 cells and release of pro-inflammatory cytokines (IL-2 and IFN-gamma), while increasing anti-inflammatory signals (IL-10) (reviewed in Rubio et al., 2007). Thus, CST-14 may also exert influences in brain activity and sleep by modulating the release of inflammatory cytokines (Marshall and Born, 2002).

The neuroendocrine activities of CST-14 and -17, including inhibition of GH release in rodents (Baranowska et al., 2006), pigs (Luque et al., 2006) and humans (Gottero et al., 2004; Grottoli et al., 2006) appear indistinguishable from those of SRIF. However, CST and double CST-SRIF knock-out mice do not show major growth abnormalities (de Lecea and Castaño, 2006), raising questions about the existence of alternative pathways that inhibit GH release.

8. Concluding remarks

CST-14 shares homology with SRIF-14 in bioactive peptide amino acid sequence, gene structure, partial coexpression, activation of common receptors and signaling pathways, and neuronal inhibition via the M-current. These observations may suggest a duplication of function between these two related peptides. Indeed, the lack of a significant phenotype in mice lacking the gene for SRIF (Juarez et al., 1997) suggests CST-14 at least partly duplicates SRIF function. However, recent generation of double CST/SRIF mutant mice (de Lecea, unpublished results) and several experiments *in vivo* reveal substantial differences between the two peptides and cumulatively lead to the conclusion that CST-14 is not an alternative, or "back-up" SRIF.

Its relatively restricted distribution in the CNS, compared with other neuropeptides expressed in the neocortex, makes CST-14 an interesting target for pharmacological intervention on diseases in which cortical neurotransmission is impaired, including sleep disorders and epilepsy. The interactions of CST with other transmitter systems may have implications in synaptic plasticity and cognitive function. New roles for CST in immune function and inflammation may also have applications in neurological disease.

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