

# Structural and Compositional Determinants of Cortistatin Activity

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**Cortistatin-14 (CST-14) is a putative novel neuropeptide that shares 11 of its 14 residues with somatostatin-14 (SRIF-14), yet its effects on sleep physiology, locomotor behavior and hippocampal function are different from those of somatostatin. We studied the structural basis for cortistatin's distinct biological activities. As with SRIF-14, CST-14 does not show any preferred conformation in solution, as determined by circular dichroism and nuclear magnetic resonance. Synthetic cortistatin analogs were designed and synthesized based on the cyclic structure of octreotide. Biological assays were carried out to determine their binding affinities to five somatostatin receptors (sst1-5) and their ability to produce changes in locomotor activity and to modulate hippocampal physiology and sleep. The results show that the compound with N-terminal proline and C-terminal lysine amide exhibits cortistatin-like biological activities, including reduction of population spike amplitudes in the hippocampal CA1 region, decrease in locomotor activity and enhancement of slow-wave sleep 2. These findings suggest that both proline and lysine are necessary for cortistatin binding to its specific receptor. *J. Neurosci. Res.* 56:611–619, 1999. © 1999 Wiley-Liss, Inc.**

**Key words:** octapeptide analog; octreotide; somatostatin receptor; cortistatin analogs; electrophysiological techniques

## INTRODUCTION

We recently identified the putative 112 amino acid precursor of a novel neuropeptide rat preprocortistatin (de Lecea et al., 1996). Preprocortistatin begins with a 27-residue apparent secretion signal sequence. Cleavage of the rat preprospecies to procortistatin produces a protein with three tandem basic amino acid pairs, only the most C-terminal of which is conserved in mouse procortistatin. Proteolytic maturation of procortistatin at the

conserved pair produces a 14-residue peptide (CST-14) whose sequence is totally conserved between rat and mouse (de Lecea et al., 1997a).

Cortistatin-14 shares 11 of 14 residues with somatostatin (SRIF-14), including those that have been shown to be responsible for SRIF-14 binding to its receptors (Patel and Srikant, 1997) and the two cysteines that form the cyclic peptide. The cDNA sequences and chromosomal localizations of cortistatin and somatostatin indicate clearly that they are the products of separate genes (de Lecea et al., 1996, 1997a). Synthetic CST-14 has been shown to share several biological properties with SRIF-14 (de Lecea et al., 1996). It binds to somatostatin receptors on GH<sub>4</sub> pituitary cells, inhibits the accumulation of cAMP in those cells, and depresses neuronal activity in hippocampal neurons, probably by enhancing the potassium M-current. However, the effects of CST-14 on cortical electrical activity, locomotor behavior and sleep are distinct from those found for SRIF-14. Moreover, CST-14 was shown to antagonize the effects of acetylcholine on cortical measures of excitability, whereas SRIF-14 enhances acetylcholine release and potentiates acetylcholine responses (Mancillas et al., 1986; Araujo et al., 1990). These observations demonstrate that CST-14 is functionally distinct from SRIF-14 and raise the possibility that CST-14 exerts its activities through an uncharacterized CST-14 selective receptor, although other explanations of different functionalities are possible.

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**TABLE I. Structures and Binding Affinities of Somatostatin, Cortistatin, and Their Synthetic Analogs to hsst1–5**

Peptide	Ki (nM) $\pm$ SEM					Sequence
	hsst1	hsst2	hsst3	hsst4	hsst5	
Somatostatin-14	2.3 $\pm$ 0.47	0.23 $\pm$ 0.04	1.17 $\pm$ 0.23	1.7 $\pm$ 0.3	1.4 $\pm$ 0.3	Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH
Octreotide	875 $\pm$ 180	0.57 $\pm$ 0.08	26.8 $\pm$ 7.7	>1,000	6.8 $\pm$ 1.0	D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol
Compound I	>1,000	115 $\pm$ 18	934	>1,000	26.6 $\pm$ 8.8	D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Lys-NH <sub>2</sub>
Compound II	>1,000	930 $\pm$ 69	27 $\pm$ 1.0	>1,000	93 $\pm$ 39	Pro-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-NH <sub>2</sub>
Compound III	>1,000	>1,000	105 $\pm$ 13	>1,000	60.4 $\pm$ 19	Pro-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Lys-NH <sub>2</sub>
Compound IV	>1,000	>1,000	>1,000	>1,000	>1,000	Pro-c[Cys-Tyr-D-Trp-Lys-Cys]-Lys-NH <sub>2</sub>
Cortistatin-14	2.1 $\pm$ 0.8	0.5 $\pm$ 0.1	3.8 $\pm$ 0.9	18.2 $\pm$ 2.5	0.9 $\pm$ 0.2	Pro-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys]-Lys

SRIF-14 exhibits its biological activities by binding to its G-protein coupled receptors. Five different receptor subtypes have been cloned. SRIF-14 and SRIF-28 bind to all five receptors with nanomolar affinities (Reisine and Bell, 1995). These receptors have distinct, though partially overlapping distributions (Thoss et al., 1995). The SRIF receptor subfamilies can be defined based on their affinities for a series of synthetic analogs (Tran et al., 1985; Hoyer et al., 1994; Schindler et al., 1996).

The SRIF-14 analog octreotide, also known as Sandostatin or SMS201-995 (Bauer et al., 1982), is a cyclic octapeptide with one exocyclic residue at each end of the structure (Table I). Octreotide binds strongly to somatostatin receptor subtypes 2, 3 and 5 (Siehler et al., 1998a). The structural elements involved in interactions between octreotide and somatostatin receptors have been identified by mutagenesis studies (Kaupmann et al., 1995; Nehring et al., 1995; Ozenberger and Hadcock, 1995; Strnad and Hadcock, 1995; Liapakis et al., 1996). In contrast to SRIF-14, which does not have a preferred conformation in solution, octreotide and its cyclic octapeptide analogs have well defined secondary structures in solution (Wynants et al., 1985). The crystal structures of octreotide were determined by Sheldrick and colleagues (Pohl et al., 1995). Recently, Goodman and colleagues synthesized lanthionine analogs of octreotide (Osapay et al., 1997) and proposed a refined model for the somatostatin pharmacophore (Melacini et al., 1997).

Since CST-14 also binds to somatostatin receptors, some of its biological activities may be mediated through somatostatin receptors. Therefore, we designed and synthesized peptides based on the structures of octreotide and its cyclic octapeptide analogs to determine structure–activity relationships of cortistatin. In the current study

we altered the exocyclic residues in cyclic octapeptides to probe for cortistatin activities. Experiments with synthetic CST-14 analogs show that the terminal residues are critical for cortistatin's specific actions, including its inhibitory properties in hippocampal CA1 neurons and its effects on spontaneous locomotor activity and sleep.

## EXPERIMENTAL PROCEDURES

### Peptide Synthesis

The syntheses of cortistatin analogs were accomplished by solid phase peptide methods. The protected peptides were assembled on MBHA Rink amide resin using Fmoc chemistry. After forming the disulfide bridge by oxidation with iodine, the peptides were cleaved from the resin, and the protecting groups were simultaneously removed by trifluoroacetic acid. The desired peptides were purified by RP-HPLC and characterized by HR-MS and NMR.

### Circular Dichroism and NMR Spectroscopy

Ultraviolet spectra were recorded at 5°C on an Aviv 61 DS spectropolarimeter using cell pathlengths of 0.2 cm and 1 cm. Peptide concentrations were between 1 and 10 mM. Peptide concentrations for calculations of molar ellipticities were determined by amino acid analysis. Spectra were recorded for all peptides in 10 mM HEPES. Single scans were obtained by taking points every 0.5 nm with a time constant of 4 sec and using a band of 1.5 mm. Two-dimensional NMR spectra (TOCSY, NOESY and ROESY) of cortistatin were recorded at 278 K on a Bruker AMX500 spectrometer. Spectra were acquired in quadrature mode using time proportional phase incremen-

tation (TPPI). Experiments were recorded with 512 t1 points, each with 2,048 data points over the spectral width of 6,000 Hz. The water peak was suppressed by continuous irradiation during the relaxation delay. The synthetic analogs of cortistatin were studied by NMR under the same conditions as above mentioned, except the samples were dissolved in DMSO- $d_6$  at the concentration of 3–5 mM.

### Radioligand Binding Assay

Receptor binding assays on the cloned human somatostatin receptors were performed using CCL39 cells stably transfected with the different human sst receptors (Siehler et al., 1998a,b), essentially as described (Liapakis et al., 1996). Binding was also measured on membranes from CHO cells transfected with sst receptors from mouse. Data from radioligand binding studies were used to generate inhibition curves. The  $IC_{50}$  values were obtained from curve-fitting performed by the mathematical modeling program FITCOMP. The computer analysis allows for determination of an apparent affinity and an affinity based on the binding to a single site (one-site analysis).

### Physiological Studies and Surgical Procedures

Male Sprague Dawley and Wistar rats (300–440 g) were maintained on a 12-hour light/dark cycle. Rats used in acute electrophysiological studies were anesthetized with halothane (3.0–4.0%; 0.9–1% maintenance), and placed into a stereotaxic apparatus. Microelectrode assemblies were stereotaxically implanted into the hippocampal CA1 as described (Steffensen and Henriksen, 1991). Animals used in chronic electrophysiological and behavioral experiments were anesthetized with halothane (2–3%). In both experimental groups, a guide stainless steel cannula was implanted into the left lateral ventricle as described (Prospero-Garcia et al., 1994; Spina et al., 1996). A group of animals was also implanted with standard electrodes for sleep recordings. The electroencephalogram (EEG) was recorded from two screw electrodes placed on the parietal bone over the hippocampus (4 mm anterior; 3 mm lateral, coordinates relative to bregma) (Paxinos and Watson, 1986). Two wire electrodes inserted in the neck musculature were used to record postural tone through electromyographic activity (EMG). The animals were allowed a minimum of 7 days to recover from the surgical procedures.

### Electrophysiological Recordings in Anesthetized Preparations

Square-wave constant pulses (0.3–1.5 mA; 0.15 ms; 0.1 Hz) were generated and population spike (PS) ampli-

tudes were elicited in the CA1 region as described (Steffensen and Henriksen, 1991). Stimulus/response curves were generated before and after iontophoretic application of the peptide at selected afferent stimulus levels. Paired-pulse curves were generated by testing various intervals (10–260 ms) of orthodromic paired-stimuli (50% maximum PS amplitude). In experiments where peptide treatment produced changes in ongoing PS amplitudes, the stimulation intensity was adjusted to achieve PS amplitudes corresponding to control levels, before performing paired-pulse studies.

### Electrophysiological Recordings in Awake Unrestrained Animals

Rats were continuously recorded for 4 hr after intracerebroventricular (icv) administration of peptides (10:00 AM–2:00 PM). Rats were observed for spontaneous changes in behavior through a one-way window. Sleep recordings were visually scored and four stages were determined: wakefulness, slow-wave-sleep 1 (SWS1), slow-wave-sleep 2 (SWS2) and rapid eye movement (REM) sleep. Total time of each stage was tabulated as a percentage of the total time of recording. The latency to sleep and to the first REM sleep period onset, as well as frequency duration of the individual periods of REM sleep, were also evaluated.

### Locomotor Response

Spontaneous locomotor activity was measured in 16 wire mesh cages (20 × 25 × 36 cm) with two horizontal infrared photocell beams located across the long axis cage, 2 cm above the floor and 16 cm from one another. Beam interruptions and crossovers were recorded every 10 min. On the testing day, after 1 h in the locomotor activity room and a 90-min adaptation period to the wire testing cages, rats' locomotor activity was measured for 3 hours. After icv infusion of peptides, animals were then returned to their locomotor activity cages. All animals were monitored for the following 3 h and experiments were carried out between 5 PM and 12 AM.

### Statistical Analyses

Results for control and drug treatment groups were derived from calculations performed on locomotor activity, evoked field potential, paired-pulse and EEG data and are expressed as means ± S.E.M. Results were compared by a repeated measures analysis of variance (ANOVA), with the Duncan's multiple-range test used for specific comparisons when indicated by ANOVA.

## RESULTS

### Design of Cortistatin Analogs

Since the 12 amino acid loop formed by the covalent linkage of two cysteines has only a single residue difference (Ser/Thr) between SRIF-14 and CST-14, we hypothesized that the distinct biological properties of CST-14 could be explained, at least in part, by differences in the residues at their termini. We took advantage of the well known structural properties of RC160, an octreotide-derived cyclic octapeptide analog of somatostatin, to design and synthesize novel peptides with different substitutions at the exocyclic positions (Table I).

The N-terminal Pro and C-terminal Lys are the major differences between CST-14 and SRIF-14. Thus, in our design we incorporated either or both of them as the exocyclic residues on the cyclic segment, c[Cys<sup>2</sup>-Tyr<sup>3</sup>-D-Trp<sup>4</sup>-Lys<sup>5</sup>-Val<sup>6</sup>-Cys<sup>7</sup>]. These include compound **I** which has a C-terminal Lys-NH<sub>2</sub> and the original N-terminal D-Phe. Compound **II** contains an N-terminal Pro and a truncated C terminus. In compound **III**, N-terminal Pro and C-terminal Lys-NH<sub>2</sub> are placed at the termini of the cyclic peptide. Furthermore, in order to examine whether the type II'  $\beta$  turn in octreotide is still necessary for cortistatin activity, we synthesized compound **IV**, a cyclic heptapeptide counterpart of compound **III**, in which Val<sup>6</sup> is removed to break the  $\beta$  turn.

### Conformational Analyses of Cortistatin and Its Synthetic Analogs in Solution

To assess whether the amino acid substitutions present in CST-14 stabilize the conformation of the peptide compared to SRIF-14, we analyze the conformation of CST-14 in solution by circular dichroism and two-dimensional NMR spectroscopy. As with SRIF-14, CST-14 does not show any stable alpha-helical secondary structure in water at 4°C (10 mM HEPES pH 7.6), as measured by the optical ellipticity of the CD spectrum at 208 and 222 nm. This structural flexibility was found at two different concentrations (not shown). In the NMR study, only sequential NOEs were observed for CST-14. No long-range NOEs or low temperature coefficients of amide protons were observed. Both CD and NMR studies suggest that CST-14 does not adopt a stable secondary conformation in solution.

For the designed octapeptide analogs (compounds **I–III**) of cortistatin, the NOE patterns,  $J_{\text{NH}}$  coupling constants and the low temperature coefficient of the Val<sup>6</sup> amide proton obtained from NMR experiments indicate the existence of a type II'  $\beta$  turn about D-Trp<sup>4</sup> and Lys<sup>5</sup>. In addition, the upfield chemical shifts of the  $\gamma\text{CH}_2$  of Lys<sup>5</sup> suggest the proximity between the side chains of D-Trp<sup>4</sup> and Lys<sup>5</sup>. These findings are consistent with the

conformations of octreotide. Thus, the designed cortistatin analogs adopt a conformation similar to that of octreotide in solution. The heptapeptide analog, compound **IV**, does not exhibit any stable secondary structure under these conditions.

### Binding Affinities of Cortistatin and Its Synthetic Analogs to Somatostatin Receptors

To determine whether the different biological activities of CST-14 could be explained by its preferential affinity to a particular type of somatostatin receptor, we measured the displacement of <sup>125</sup>I-SRIF-14 binding to CCL39 cells transfected with each of the cloned human somatostatin receptors. CST-14 binds to all receptors with similar nanomolar affinities (Table I). The affinity of CST-14 for sst1 is comparable to that of SRIF-14 or SRIF-28 (Schonnbrun and Tashjian, 1980; Patel and Srikant, 1997) and may account for the displacement of <sup>125</sup>I-SRIF-14 described in GH4 cells (de Lecea et al., 1996; Fukusumi et al., 1997). The affinity for sst4 is an order of magnitude less for CST-14 than for SRIF-14. The two peptides have similar affinities for sst2, -3, and -5 receptors.

The binding affinities of synthetic analogs are quite different (Table I). Octreotide has high affinities to sst2 and 5, and moderate affinity to sst3 receptors. With C-terminal Lys-NH<sub>2</sub>, compound **I** has lower affinity to sst2 by 100-fold and is not active on sst1, -3 and -4, while its affinity to sst5 is only modestly lower. Compound **II** which has only the Pro at its N-terminus binds selectively to sst3 at 27 nM. Its affinity to sst5 is about 4 times lower and it does not bind to the other subtypes. When both N-terminal Pro and C-terminal Lys-NH<sub>2</sub> are in place, compound **III** has moderate affinities for sst3 and sst5 receptors (105 nM and 60.4 nM, respectively). It is not active on sst1, -2 and -5 receptors. The heptapeptide analog (compound **IV**) does not bind to any SRIF receptors.

We measured the binding of somatostatin analogues to membranes from CHO cells that had been transfected with the mouse sst receptors. Binding affinities were similar for all receptors and analogues, except sstr5 (data not shown).

### Electrophysiological Effects of Cortistatin-14 Analogs: Hippocampal Function and Sleep

We used in vivo electrophysiological techniques to test the effects of a selected group of compounds on the excitability of hippocampal CA1 neurons and on feed-forward and feedback inhibitory processes mediated in part by hippocampal interneurons using evoked paired-pulse (PP) stimulation (de Lecea et al., 1996). Microiontophoretic application of compounds **I** and **IV** had no



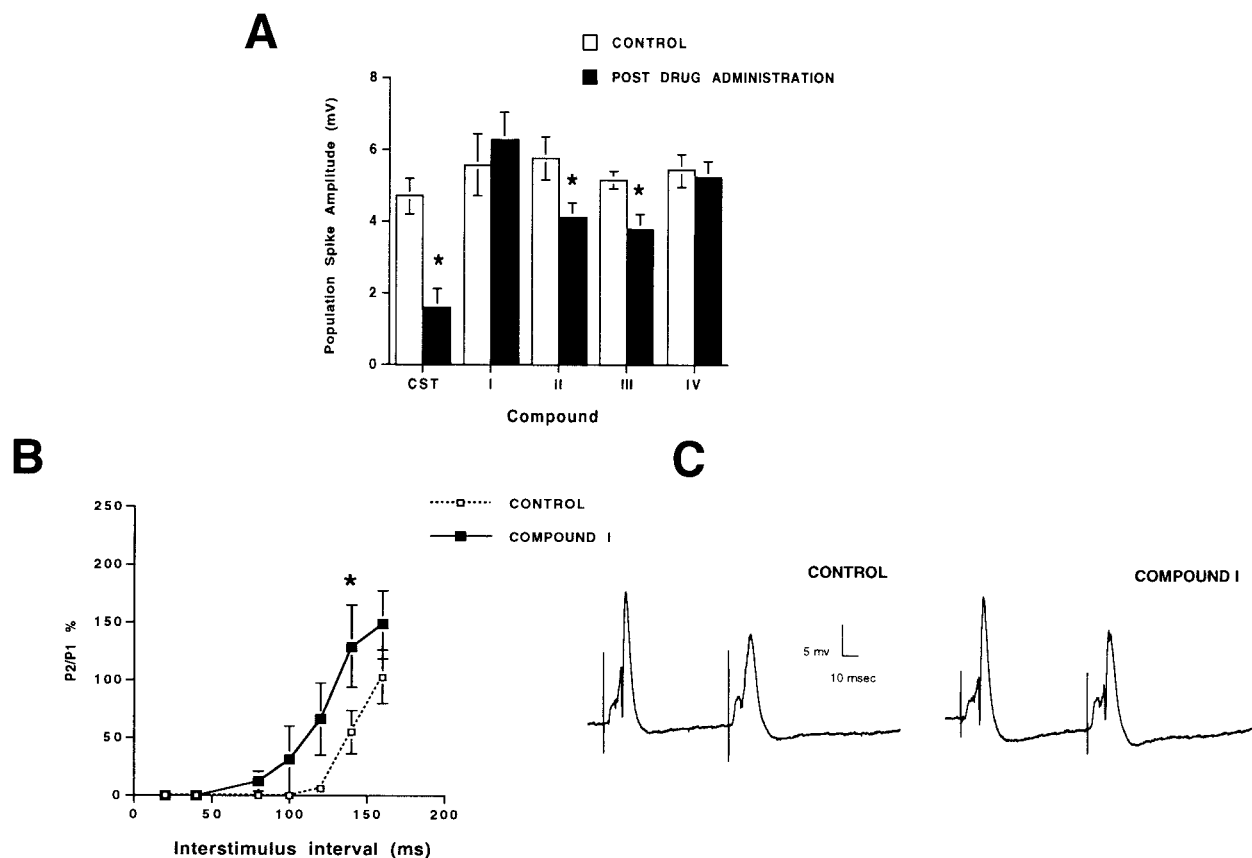


Fig. 1. **A:** Microiontophoretic application of compounds **II** and **III**, like CST-14, significantly reduced PS amplitudes in CA1. Administration of compounds **I** and **IV** had little effect on PS amplitudes. Asterisks represent significance levels at  $P < .05$ . **B:** Iontophoretic application of compound **I** (100–150 nA) reduced PP inhibition ( $*P < .05$ ). **C:** Representative recordings

of field potentials elicited in CA1 by commissural stimulation before and after administration of compound **I**. In the baseline recordings, the second response is completely inhibited at this interstimulus interval (80 ms). Iontophoretic administration of compound **I** reduced PP inhibition.

effect on PS amplitudes ( $P > .05$ ; Fig. 1A) at half-maximal level. In contrast, application of compound **II** or **III**, which contain the N-terminal Pro of CST-14, produces a moderate, but consistent reduction of PS amplitudes ( $P < .05$ ; Fig. 1A) similar to that previously described for CST-14. Administration of compound **II**, **III**, or **IV** has no significant effect on PP responses in CA1 ( $P > .05$ ). However, compound **I** significantly reduced the inhibitory phase of PP responses typical of interstimulus intervals of 80 ms ( $P < .05$ ; Fig. 1B), similar to previous observations with SRIF-14 but not CST-14. These data indicate that compounds **II** and **III** produce CST-14-like effects on hippocampal physiology and suggest that the N-terminal proline is essential for the effects of cortistatin on hippocampal activity.

CST-14 and SRIF-14 display opposite effects on the sleep–wake cycle when administered into the brain ventricles. SRIF-14 and analogs have been shown to increase REM sleep (Rezek et al., 1976; Danguir, 1986;

Beranek et al., 1997), whereas CST-14 selectively enhances slow-wave sleep (de Lecea et al., 1996). We infused up to 10  $\mu$ g of compounds **I–IV** into the brain ventricles of rats and recorded the EEG for 4 hours after injection. Infusion of compounds **I**, **II**, and **IV** (Fig. 2) (1  $\mu$ g) has no statistically significant effect on the sleep–wake cycle ( $n = 5$ ,  $P > .05$ ). By contrast, administration of compound **III**, the N-terminal Pro and C-terminal Lys-NH<sub>2</sub> analog, produces a significant increase in cortical slow waves and a reduction in wakefulness (Fig. 2) ( $n = 5$ ,  $P < .05$ ), similar to the previously reported effects of CST-14.

#### Behavioral Properties of Cortistatin Analogs

Our initial studies indicated that CST-14-treated rats were hypoactive compared with saline-treated rats. These findings contrasted with previous studies demonstrating that icv infusion of SRIF-14 resulted in a

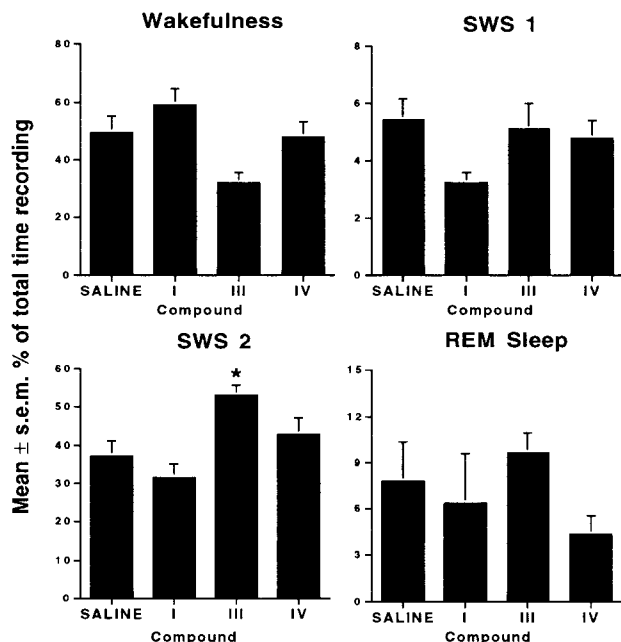


Fig. 2. Effects of CST analogs on the sleep/wake cycle. Like cortistatin, infusion of compound **III** (1  $\mu\text{g}/10 \mu\text{l}$ ) significantly increases the period of SWS2 and slightly decreases wakefulness, whereas compounds **I** or **IV** do not have a statistically significant effect on the states of arousal. \* $P < .05$ .

dramatic increase in locomotor activity, an effect that can develop further into barrel rotation and seizures (Vecsei et al., 1984; Bakhit and Swerdlow, 1986). These same effects have been described for octreotide (Ishikawa et al., 1990). We administered various doses of CST-14 and carried out tests on animals for changes in spontaneous locomotor activity. Intracerebroventricular infusion of CST-14 (0.1 ng to 5  $\mu\text{g}$ ) produces a significant reduction in locomotor activity, as measured by number of beam breaks and crossovers in an activity cage (Fig. 3A). Administration of compound **I** (Fig. 3B), like octreotide or SRIF-14, produces a dose-dependent increase in locomotor activity, which is significantly different from control values at a dose of 5  $\mu\text{g}$ . By contrast, rats infused with compound **III** show a marked reduction in locomotor activity (Fig. 3B). Consistent with these data, rats infused with compound **II** (0.1 and 1  $\mu\text{g}$ ; data not shown) also produce a marked reduction in locomotor activity ( $n = 8$ ;  $P < .05$ ).

## DISCUSSION

We have shown that the recently described neuropeptide CST-14 binds to somatostatin receptors with affinities very similar to those of SRIF-14. Conformational studies using circular dichroism and NMR spectroscopy do not explain the different biological

activities of CST-14 and SRIF-14, since neither peptide has a preferred conformation in solution. However, the octapeptide analogs, compounds **I–III**, have a stable type II'  $\beta$  turn at the Tyr-D-Trp-Lys-Val region. This  $\beta$  turn may also be crucial for cortistatin activities, because heptapeptide analog (compound **IV**), in which the  $\beta$  turn is disturbed, does not bind to any somatostatin receptors and does not have cortistatinlike activities.

The behavioral effects of synthetic peptides show that exocyclic residues are essential in conferring cortistatin's distinct biological response. When both N-terminal Pro and C-terminal Lys-NH<sub>2</sub> are attached to the molecule, compound **III** elicits an electrophysiological and behavioral response very similar to our previous observations with CST-14, including the reduction of population spike amplitudes in the hippocampal CA1 region, decrease in locomotor activity and enhancement of slow-wave sleep (de Lecea et al., 1996). Compound **III**, however, is significantly less potent than CST-14, a difference that could be explained by the size of the loop, rather than to their conformational properties. To the best of our knowledge, compound **III** is the first synthetic analog of cortistatin. With only the N-terminal Pro, compound **II** can reduce CA1 response to commissural stimulation and inhibit locomotor activity, but it has no significant effect on the sleep-wake cycle. If only C-terminal Lys-NH<sub>2</sub> is in place, compound **I** exhibits somatostatinlike bioactivities. Therefore, both N-terminal Pro and C-terminal Lys-NH<sub>2</sub> are necessary in the cyclic octapeptide for cortistatin activities.

Since preprocortistatin mRNA is expressed in cortical and hippocampal nonprojecting interneurons (de Lecea et al., 1997b), it can be assumed that physiological release of CST-14 would have effects on local cortical and hippocampal pyramidal neurons. Based on the anatomical distributions of sst receptors and preprocortistatin mRNAs together with the effects of selective sst ligands on hippocampal physiology and behavior (Raynor et al., 1993), we hypothesized that sst2 and sst3 receptors are the strongest candidates to mediate the effects of CST-14. However, since compounds **II** and **III** do not bind to sst2, their effects must not be mediated through sst2 receptors. It is difficult to decide whether the binding of compounds **II** and **III** to sst5 is physiologically relevant, since sst5 mRNA is expressed only at low levels in adult rat brain (Thoss et al., 1995). Nevertheless, it has recently been described that selective sst5 receptor antibodies are able to identify receptors in basal forebrain and to a lesser extent in limbic structures. Similarly, sst3 receptor mRNA is expressed abundantly in cortical and hippocampal structures, although the corresponding protein has not been formally identified by classical methods (Piwko et al., 1997). Cerebellar expression of sst3, however, re-

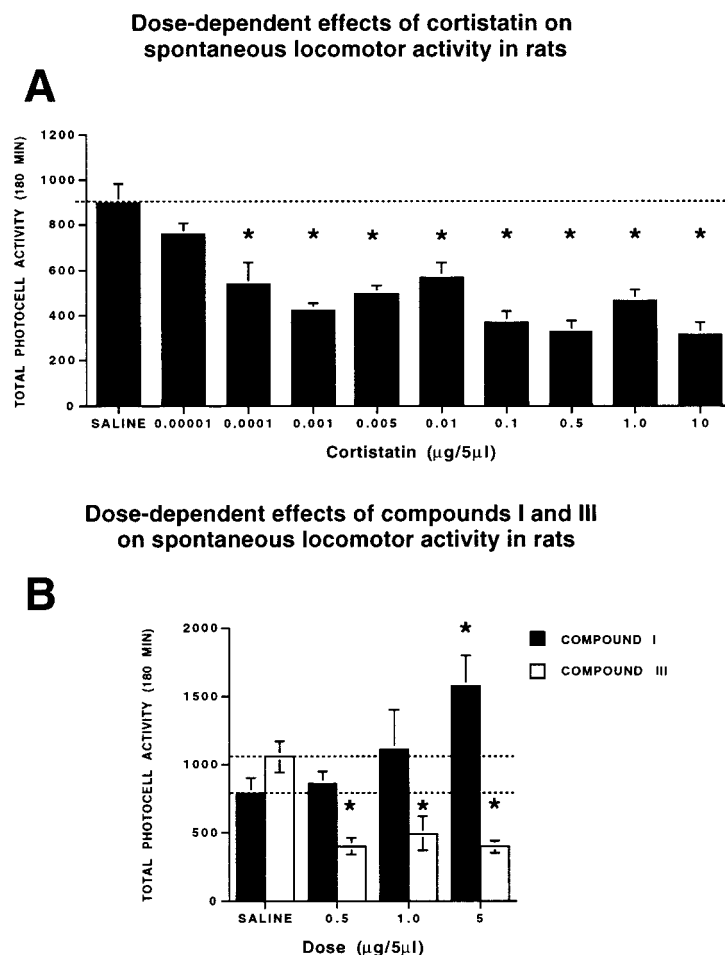


Fig. 3. Effect of intracerebroventricular administration of cortistatin and somatostatin analogs on locomotor activity. **A:** Intracerebroventricular infusion of synthetic cyclic CST-14 produced a marked decrease in locomotor activity ( $P < .05$  vs saline). The highest dose (10 µg) produced barrel rotation in some rats. **B:** Administration of compound I (TT232 (Keri et al., 1996) with an additional lysine at the C terminus) provoked

a dose-dependent increase in spontaneous locomotor activity. By contrast, infusion of compounds II (data not shown) and III, which contain a proline substitution in their N termini, decreased locomotor activity ( $P < .05$  vs saline). Asterisks represent statistical significance at  $P < .05$ ; all doses tested are expressed as micrograms.

mains to be clarified, since cortistatin is absent from this brain region (de Lecea et al., 1997b). Thus, the cortistatin-like effects of these compounds may be explained through their selective binding to sst3 and sst5 receptors. It is also possible that accessory molecules, like the newly discovered RAMP proteins (McLatchie et al., 1998), act in concert with one or more of the sst receptors to form a receptor that binds preferentially CST-14 and compound III. Mu-selective agonists have been shown to enhance EEG synchronization and decrease REM sleep, an effect that can be blocked by naloxone (Garzón et al., 1995; Cronin et al., 1995). It is therefore additionally possible that the octapeptide analogs of cortistatin act through binding to an opioid receptor system, although it has been shown that CST-14 does not bind to mu receptors in rat

locus coeruleus neurons (Connor et al., 1997). Alternatively, the effects of CST-14 and its analogs can be better explained via their binding to a specific, uncharacterized cortistatin receptor.

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