



## The putative somatostatin antagonist cyclo-somatostatin has opioid agonist effects in gastrointestinal preparations

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### ABSTRACT

**Aims:** Specificity of receptor antagonists used is crucial for clarifying physiological/pathophysiological roles of the respective endogenous agonist. We studied the effects (somatostatin antagonist and possibly other actions) of cyclo-somatostatin (CSST), a putative somatostatin receptor antagonist on the guinea-pig small intestine, a preparation where somatostatin causes inhibition of nerve-mediated contractions.

**Main methods:** In isolated organ experiments, half-maximal cholinergic “twitch” contractions of the guinea-pig small intestine were evoked or tonic contractions of the rat stomach fundus strip (in the presence of physostigmine) were elicited by electrical field stimulation. The effects of somatostatin (somatostatin-14), CSST, naloxone, as well as of direct smooth muscle stimulants were examined.

**Key findings:** Somatostatin (10 nM–1 μM) caused transient inhibition of the twitch contraction, in a naloxone-insensitive manner. Surprisingly, CSST (0.3–1 μM) also inhibited twitch contractions (more than 50% reduction at 1 μM). This effect was prevented by the opioid receptor antagonist naloxone. Responses to acetylcholine or histamine were not or only minimally inhibited by CSST (up to 3 μM). CSST (0.3 μM in the absence or 1–10 μM in the presence of naloxone) failed to inhibit the effect of somatostatin. The SST<sub>2</sub> receptor antagonist CYN-154806 (3 μM) attenuated the effect of somatostatin and failed to evoke naloxone-sensitive inhibition of the twitch response. The naloxone-sensitive inhibitory effect of CSST on cholinergic contractions was also confirmed in the rat stomach fundus preparation.

**Significance:** Cyclo-somatostatin exerts opioid agonist activity in the two preparations tested, while it does not behave as a somatostatin-receptor antagonist in the guinea-pig intestine.

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### Introduction

Cyclo-somatostatin (CSST) (Fries et al., 1982) is a non-selective somatostatin receptor antagonist used both in vitro and in vivo. Given the multiple effects of somatostatin as neural or hormonal modulator, we set out to test CSST against somatostatin, in the hope of finding a universal but specific somatostatin receptor antagonist for in vitro experiments. This substance binds at all somatostatin receptors (see Cycloantagonist SA in Siehler et al., 1999), although some effects of somatostatin may be resistant to CSST inhibition (Shirahase et al., 1993). Of the isolated organs, the guinea-pig ileum is one of the few that show motor responses to somatostatin, namely an inhibition of the cholinergic “twitch” contractions in response to single electrical shocks that are brief enough not to excite smooth muscle cells directly (Guillemin, 1976; Furness and Costa, 1979; Cohen et al., 1979; Jhamandas and

Elliott, 1980; Vizi et al., 1984; Feniuk et al., 1995) or partly cholinergic contractions to the neuropeptides neurotensin or caerulein (Monier and Kitabgi, 1981). Although it has been argued that the inhibitory action of somatostatin is indirect (Vizi et al., 1984), it does not seem to involve opioid receptors, as unequivocally shown by all of the above studies that utilized naloxone, an opioid antagonist (Guillemin, 1976; Jhamandas and Elliott, 1980; Monier and Kitabgi, 1981).

The guinea-pig ileum is a standard isolated organ preparation of experimental pharmacology, capable of detecting a high number of drug effects, including neuromodulators and smooth muscle-active drugs. Before using CSST for antagonizing endogenous somatostatin, we tested its effects in this preparation. To our surprise, we found that CSST was not effective against somatostatin, while it inhibited cholinergic contractions in a naloxone-reversible manner. This finding prompted us to test the effects of CYN-154806, a selective SST-2 receptor antagonist (Bass et al., 1996; Feniuk et al., 2000), a substance that inhibits the effect of somatostatin on the cholinergic “twitch” (Feniuk et al., 2000). The rat stomach fundus strip was chosen thereafter for investigating if the opioid agonist-like effect of CSST can be demonstrated in the gastrointestinal tract of another species of laboratory animals.

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## Materials and methods

### Animals and preparation

Guinea-pigs (short-haired, colored) of either sex weighing 300–500 g, or male Wistar rats weighing 300–360 g were stunned by a blow to the occiput and bled out from the carotid arteries. Whole segments of the guinea-pig ileum (approximately 2 cm in length) were set up as preparations. Rat stomachs were opened along the lesser and greater curvatures and the two halves were cut into longitudinally-oriented strips of approximately 2 cm in length. All preparations were suspended in organ baths containing 5 ml of oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Krebs–Henseleit solution at 37 °C. Composition of the bathing solution was as follows (mM), NaCl 119, NaHCO<sub>3</sub> 25, KCl 2.5, MgSO<sub>4</sub> 1.5, CaCl<sub>2</sub> 2.5, KH<sub>2</sub>PO<sub>4</sub> 1.2, and glucose 11. Movements of the tissues were recorded isotonicly, by means of lever transducers and bridge amplifiers (Hugo Sachs – Harvard Apparatus, March – Hugstetten, Germany). The load on the tissues was 7 mN (guinea-pig small intestine) or 5 mN (rat fundus strip).

### Experimental protocols

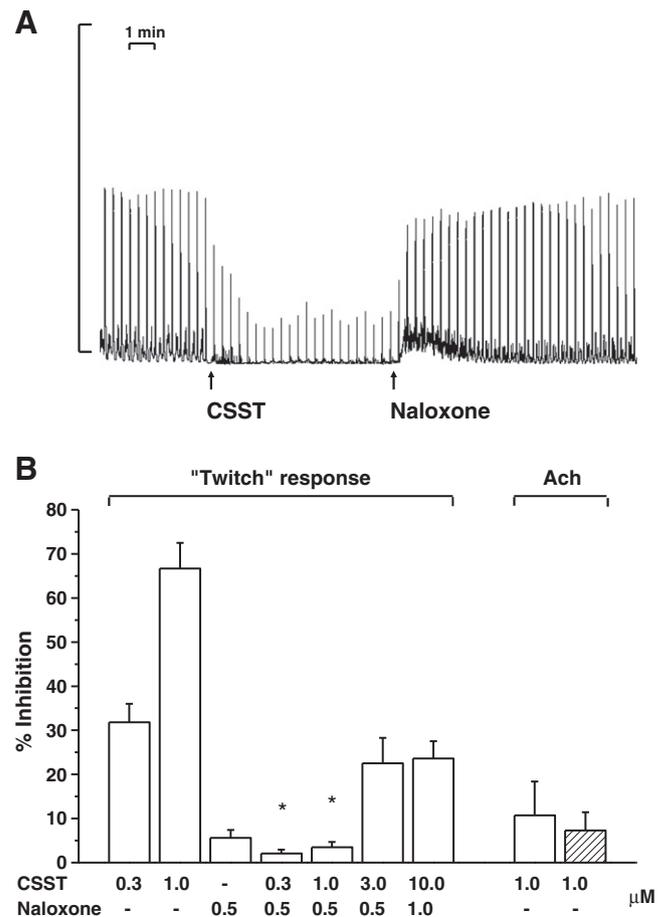
The experiments commenced after an equilibration period of 40 min (guinea-pig ileum) or 75 min (rat stomach). In the guinea-pig preparation, cholinergic “twitch” responses of approximately half-maximal size were evoked by electrical field stimulation (near-maximal voltage of 15 V/cm, 0.1 ms pulse width, single electrical shocks at 0.05 Hz), applied by means of a high-performance stimulator (Experimetria, Budapest, Hungary), through a pair of platinum wire electrodes, placed at the top and the bottom of the organ bath. In some experiments, approximately half-maximal longitudinal contractions were evoked by acetylcholine or histamine. After uniform responses had been obtained with these drugs or electrical field stimulation, the effects of somatostatin, CSST or CYN-154806 were tested following no pretreatment or various pretreatments (the drug of pretreatment was also present in the “baseline” period). Since the response to somatostatin was not fully reproducible upon repeated administration, this substance was also administered only once to each preparation (except in those experiments where reproducibility of the effect itself was investigated). Comparisons were made for independent groups of preparations. Unless indicated otherwise, CSST or CYN-154806 was also administered only once to each preparation. Contact times for the drugs are given below.

A similar protocol has been chosen for the rat fundus as well, with the following differences. First, a frequency of 1 Hz of electrical stimulation (voltage and pulse width as above) was needed to evoke half-maximal cholinergic (atropine – 0.5 μM – sensitive) contractions. Second, even 1-Hz stimulation could only evoke cholinergic contraction if the preparations were incubated with the cholinesterase inhibitor physostigmine (0.1 μM) throughout the experiment. We have chosen this tool to obtain cholinergic responses because elevating the stimulation frequency could lead to a diminishment or loss of opioid sensitivity of the responses evoked. Trains of stimuli of 25 s were used, so that contractile responses reached their peak during stimulation. If such trains were applied once in 45 min, responses proved reproducible. The effect of CSST was studied either in the absence or in the presence of naloxone. Guanethidine (3 μM) was present throughout these experiments for excluding responses mediated by sympathetic neurons, although preliminary experiments indicated no obvious effect of this drug to the response evoked by electrical stimulation.

### Drugs

Acetylcholine chloride, CYN-154806 (Ac-4NO<sub>2</sub>-Phe-c (D-Cys-Tyr-D-Trp-Lys-Thr-Cys)-D-Tyr-NH<sub>2</sub>), guanethidine sulfate, histamine dihydrochloride, naloxone hydrochloride, phentolamine hydrochloride,

propranolol hydrochloride, tetrodotoxin, and theophylline were purchased from Sigma, Budapest, Hungary; somatostatin, cyclo-(7-aminoheptanoyl-Phe-D-Try-Lys-Thr[BZL]) (cyclo-somatostatin; CSST), and ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) were from Tocris, Bristol, UK. Morphine hydrochloride and physostigmine hydrochloride were of pharmaceutical grade. Stock solutions of 100 mM (acetylcholine, histamine), 10 mM (CYN-154806, naloxone, propranolol, phentolamine, theophylline) or 1 mM (somatostatin, CSST, tetrodotoxin) were prepared in physiological saline. ODQ (10 mM) was dissolved in DMSO. Dilutions of drugs were made in physiological saline if necessary. Most substances were administered into the organ bath in volumes of 0.2–1 μl/ml bath solution (theophylline in 10 μl/ml). The solvents were devoid of any pharmacological effect. Contact times of the drugs were as follows: 3 min for somatostatin, histamine, and acetylcholine, 5 min for CSST, 10 min for CYN-154806, and 15–20 min for the rest of the drugs.



**Fig. 1.** A, inhibition of cholinergic “twitch” contractions of the guinea-pig ileum in response to electrical field stimulation by the putative somatostatin receptor antagonist cyclo-somatostatin (CSST; 1 μM) and reversal of this effect by the opioid receptor antagonist naloxone (0.5 μM). Parameters of stimulation were, square-wave pulses at 0.05 Hz, 0.1 ms pulse width, and near-maximal voltage of 15 V/cm. Vertical calibration, maximal longitudinal spasm due to histamine (10 μM). B, influence of CSST (concentrations as indicated) on cholinergic “twitch” responses of the guinea-pig ileum in response to electrical field stimulation or on contractions evoked by acetylcholine (ACh; as indicated above the columns). Note that inhibition of the “twitch” response was largely prevented by naloxone (administered as pretreatment for 15 min). The last two columns indicate the slight decrease of acetylcholine-evoked contractions after the administration of CSST (open column) or of CSST in tetrodotoxin-pretreated preparations (hatched column). Concentrations other than those indicated in the figure were 30–50 nM for acetylcholine and 0.5 μM for tetrodotoxin. Number of experiments for each column was n = 5–8. \* – significant differences from the appropriate controls (first two columns; Mann–Whitney test). (In spite of the reproducibility of the CSST-induced inhibition, the effect of naloxone pretreatment was studied on independent groups of preparations).

## Expression of data and statistical methods

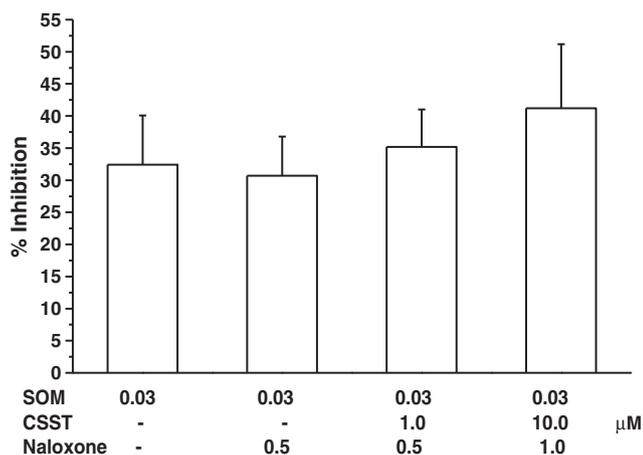
The effects of drugs were expressed as % reduction of the pre-drug contraction amplitude (cholinergic contractions in response to electrical field stimulation, longitudinal spasms evoked by histamine, 50 nM–0.3  $\mu$ M or acetylcholine, 30 or 50 nM; kept constant within an experiment). Data are presented as mean  $\pm$  SEM. Statistical comparisons were made with the Mann–Whitney test (2 independent samples), using % inhibitions. A probability of  $P < 0.05$  or less was taken as statistically significant.

## Results

### Guinea-pig ileum

CSST (0.3–1  $\mu$ M) inhibited cholinergic “twitch” responses (Fig. 1A, B), in a concentration-dependent manner. The action of CSST was quickly reversed by rinsing (more than 50% fading in 5 min). The effect of CSST (0.3–1  $\mu$ M) was prevented by a pretreatment of naloxone (0.5  $\mu$ M; Fig. 1B) or reversed by an administration of this opioid antagonist (Fig. 1A). CSST (3  $\mu$ M) exerted a moderate inhibitory action in the presence of naloxone (0.5  $\mu$ M). Naloxone by itself did not influence the “twitch” response (Fig. 1B). Naloxone at 0.5  $\mu$ M was able to reverse the maximal inhibitory effect of 1  $\mu$ M morphine on the “twitch” response ( $n = 5$ , data not shown).

Two combinations of inhibitors were tested on the CSST-induced reduction of the “twitch” response. Theophylline (a  $P_1$  purinoceptor antagonist; 140  $\mu$ M) plus ODQ (an inhibitor of the soluble guanylate cyclase; 3  $\mu$ M), administered between the first and second administrations of CSST (1  $\mu$ M, 30 min apart) failed to reduce the effect of CSST. Similarly, a combination of the adrenergic  $\alpha$ -receptor antagonist phenolamine and the  $\beta$ -receptor antagonist propranolol (0.3  $\mu$ M each) did not influence the inhibitory action of CSST, whereas naloxone (0.5  $\mu$ M) fully inhibited the action of CSST (1  $\mu$ M) also in this experimental protocol ( $n = 3$  for all three pretreatments, data not shown). A full reproducibility of the effect of CSST (0.3 and 1  $\mu$ M) has been verified in 6 experiments (two administrations with a washout interval of 40 min;  $32.5 \pm 4.2$  vs.  $32.4 \pm 3.5\%$  inhibition at 0.3  $\mu$ M CSST for the first and second administrations, respectively; the corresponding data for 1  $\mu$ M of CSST were  $53.7 \pm 2.7$  vs.  $59.3 \pm 3.6\%$  inhibition;  $n = 6$  for both pairs of data). It should be noted, however, that the effect of naloxone on the CSST-induced inhibition was studied on independent groups of preparations.



**Fig. 2.** Inhibition of cholinergic “twitch” contractions (vertical axis) of the guinea-pig ileum in response to electrical field stimulation by somatostatin (30 nM) in the presence of naloxone (0.5 or 1  $\mu$ M) or naloxone plus CSST (1 or 10  $\mu$ M). Note that there is no sign of somatostatin receptor antagonist action of CSST. Number of experiments for each column was  $n = 6$ –7.

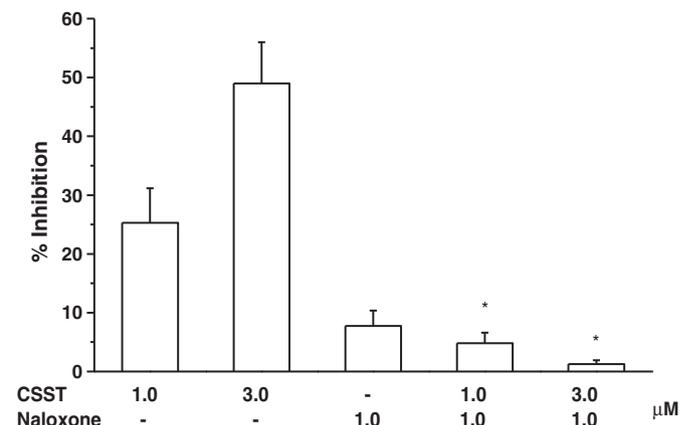
The inhibitory effect of CSST was not exerted at a smooth muscle level, as shown by experiments where histamine (50 nM–0.3  $\mu$ M) or acetylcholine (30 or 50 nM) was administered in the absence and following the administration of CSST (0.3–3  $\mu$ M). Histamine was tested in the presence of atropine (0.5  $\mu$ M). CSST at 1  $\mu$ M ( $n = 4$ ) or 3  $\mu$ M ( $n = 3$ ) proved fully ineffective against histamine-induced, half-maximal contractions. With acetylcholine, a slight, statistically not significant decrease was found at 1  $\mu$ M CSST (Fig. 1B), both in the absence and in the presence of the  $Na^+$ -channel inhibitor tetrodotoxin (0.5  $\mu$ M). Similar results were found with 3  $\mu$ M of CSST ( $n = 4$ , data not shown), i.e. the reduction (if any) has not become larger.

Pilot experiments have shown that a partial inhibition of the “twitch” response by morphine (30 or 300 nM) was not reversed by CSST (0.3  $\mu$ M) ( $n = 4$  for either morphine concentration). Instead, an additional inhibitory effect was seen upon the administration of CSST. These data provide no indication for an opioid receptor antagonist effect of CSST, at least not against morphine.

In accordance with earlier data (see Introduction) somatostatin caused concentration-related, partial and transient inhibition of the “twitch” response (no reduction at 10 or 100 pM,  $n = 3$  each; 8% reduction at 1 nM,  $n = 9$ ; 27% reduction at 10 nM,  $n = 8$ ; 34.5% reduction at 0.1  $\mu$ M,  $n = 7$ ; 53.5% reduction at 1  $\mu$ M,  $n = 7$ ). Half-time of fading of the effect was around 100 s with all effective concentrations. The effect of somatostatin was not reproducible; at 1  $\mu$ M its amplitude was approximately halved at each repetition of administration (at 30 min intervals;  $n = 6$ ; the contact time of the drug was extended from 3 to 5 min in these 6 experiments). In the presence of naloxone (0.5  $\mu$ M) the action of somatostatin (10–100 nM) was not smaller than that with no pretreatment ( $n = 5$ –7, data not shown).

Experiments concerning the possible somatostatin antagonist action of CSST in the guinea-pig ileum yielded completely negative results. In preparations with partially inhibited “twitch” response in the presence of 0.3  $\mu$ M of CSST somatostatin (30 nM) still caused the usual inhibition of the “twitch” response ( $n = 4$ , data not shown). In the presence of naloxone (for preventing the opioid-like effect of CSST) CSST (1 or 10  $\mu$ M) also failed to reduce the inhibitory effect of somatostatin (30 nM; Fig. 2). The concentration of naloxone was raised from 0.5 to 1  $\mu$ M in those experiments where the highest concentration of CSST (10  $\mu$ M) was tested.

The  $SST_2$  receptor antagonist CYN-154806 was tested in a concentration of 3  $\mu$ M, which is several-fold higher than the reported  $IC_{50}$  of this antagonist on the guinea-pig ileum (Feniuk et al., 2000). CYN-154806 did not influence cholinergic “twitch” contractions in some



**Fig. 3.** Inhibitory action of CSST (concentrations as indicated) on cholinergic contractions of the isolated, physostigmine-treated rat fundus strip, in the absence and in the presence of naloxone. Responses were evoked by electrical field stimulation (1 Hz for 25 s). Number of experiments for each column was  $n = 6$ –7. \* – significant differences from the appropriate controls (first two columns; Mann–Whitney test). Note that naloxone alone failed to have an effect on the responses but prevented the inhibitory action of CSST.

preparations, while in others a modest inhibition (less than 10% reduction) was clearly observed. For the entire group of 9 preparations, reduction of the “twitch” response only amounted to  $3.7 \pm 1.1\%$ . A similar degree of inhibition was, however, observed in naloxone ( $0.5 \mu\text{M}$ ) pretreated preparations as well ( $5.6 \pm 2.8\%$  reduction,  $n = 5$ ). CYN-154806 ( $3 \mu\text{M}$ ) inhibited the effect of somatostatin (tested in the single concentration of  $30 \text{ nM}$ ). In the absence of CYN-154806 somatostatin caused a  $25.3 \pm 3.8\%$  reduction of the “twitch” ( $n = 7$ ) and in the presence of the antagonist the reduction amounted to  $7.8 \pm 3.9\%$ ,  $n = 9$ ;  $P < 0.01$ , Mann–Whitney test. Naloxone pretreatment ( $0.5 \mu\text{M}$ ) did not modify the antagonist effect of CYN-154806 ( $n = 4$ ; data not shown).

#### Rat stomach fundus strip

Electrical field stimulation evoked contraction that was fully inhibited (in fact, reversed into non-adrenergic relaxation) by atropine ( $0.5 \mu\text{M}$ ). CSST ( $3$  or  $10 \mu\text{M}$ ) inhibited these responses (Fig. 3). This inhibitory effect was prevented by naloxone ( $1 \mu\text{M}$ ). Naloxone alone failed to influence nerve-mediated cholinergic contractions of the fundus (Fig. 3). As could be expected, morphine ( $0.1$  or  $1 \mu\text{M}$ ) also inhibited cholinergic contractions of the rat fundus ( $56$  and  $70\%$  reduction, respectively,  $n = 5$ ); the effect of  $1 \mu\text{M}$  morphine was fully prevented by naloxone ( $1 \mu\text{M}$ ,  $n = 4$ , data not shown).

#### Discussion

It is well known that morphine and other opioid agonists reduce the release of acetylcholine in the guinea-pig ileum (Paton, 1957 and numerous subsequent studies). Cholinergic contractions of the rat stomach fundus have also been found to be inhibited by morphine (Dehpour et al., 1994). The present data clearly indicate for the first time that the putative somatostatin receptor antagonist CSST exerts an opioid agonist-like effect in the guinea-pig ileum, as well as in the rat fundus strip, against cholinergic contractions evoked by electrical field stimulation. This latter finding indicates that the opioid-like effect of CSST is not confined to the guinea-pig. The somewhat lower potency of CSST on the rat stomach preparation, as compared with the guinea-pig intestine might be simply the consequence of stimulation parameters ( $1 \text{ Hz}$  vs. single shocks at  $0.05 \text{ Hz}$ , respectively), but this problem has not been studied further.

CSST fails to inhibit the effect of somatostatin in the ileum, at least in the presence of naloxone (administered to prevent the reduction of ileum contraction by CSST). Somatostatin seems to exert its inhibitory action in the guinea-pig via  $\text{SST}_2$ -like receptors (Feniuk et al., 1995, 2000); autoradiographic data lend some support to this conclusion (Fehlmann et al., 2000). However, Foong et al. (2010) have demonstrated the presence of both  $\text{SST}_1$  and  $\text{SST}_2$  receptors in the guinea-pig ileum submucous plexus. The inhibitory action of somatostatin is insensitive to naloxone, as shown by the present experiments and indicated by data of the literature (see Introduction). In accordance with the results of Feniuk et al. (2000), the specific  $\text{SST}_2$  receptor antagonist CYN-154806 inhibited the effect of somatostatin in the guinea-pig ileum. This compound (also a cyclic somatostatin analog) failed to exert a naloxone-sensitive reduction of the cholinergic “twitch” response. Also the inhibitory action of CSST ( $1 \mu\text{M}$ ) was not influenced by an addition of CYN-154806, as shown by our pilot experiments (Bartho et al., unpublished observations).

Lack of a considerable inhibitory action of CSST on the cholinergic contractions in the presence of naloxone, as well as on histamine- and acetylcholine-induced contractions (in the absence of naloxone) excludes a direct smooth muscle-depressant effect of this compound. Failure of ODQ, theophylline, phentolamine and propranolol to inhibit the effect of CSST indicates that guanylate-cyclase-mediated mechanisms

(such as an effect of nitric oxide or carbon monoxide),  $\text{P}_1$  purinoceptors, adrenergic  $\alpha$  or  $\beta$  receptors, respectively, do not participate in this process. It should be noted that propranolol, at the concentration used in this study, was able to inhibit the relaxation of precontracted, atropine-treated ileum in response to mesenteric nerve stimulation, as shown in previous experiments in our laboratory, although it may show limited effect against exogenous  $\beta$ -receptor agonists (Horinouchi and Koike, 2000).

Some somatostatin analogs show opioid receptor antagonist effect (Maurer et al., 1982; Peterson et al., 1985); opioid antagonist somatostatin analogs include cyclic peptides (see among others Shook et al., 1986, 1987; Walker et al., 1987), but neither opioid receptor antagonist, nor agonist activity has been reported for CSST. Our data provide pharmacological evidence for an opioid receptor agonist effect of CSST; no sign of antagonist action was found, at least not against morphine. Opioid agonist activity of CSST might explain some in vivo findings, such as an antinociceptive effect of central administration of this peptide (Bartsch et al., 2005).

#### Conclusion

The current data indicate that CSST should be carefully tested for specific somatostatin receptor antagonist properties in any system it is used in; special care should be taken to check opioid agonist-like effects. Some previous data of the literature obtained with CSST would need to be revisited.

#### Conflict of interest

The authors declare no conflict of interest.

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#### References

- Bartsch T, Levy MJ, Knight YE, Goadsby PJ. Inhibition of nociceptive dural input in the trigeminal nucleus caudalis by somatostatin receptor blockade in the posterior hypothalamus. *Pain* 2005;117:30–9.
- Bass RT, Buckwalter BL, Patel BP, Pausch MH, Price LA, Strnad J, et al. Identification and characterization of novel somatostatin antagonists. *Mol Pharmacol* 1996;50:709–15.
- Cohen ML, Wiley KS, Yaden E, Slater IH. In vitro actions of somatostatin, D-Val<sup>1</sup>, D-Trp<sub>8</sub>-somatostatin and glucagon in rabbit jejunum and guinea-pig ileum. *J Pharmacol Exp Ther* 1979;211:423–9.
- Dehpour AR, Delfan A, Mousavizadeh K, Mortazavi SR. Effects of atropine, pirenzepine, clonidine, and morphine on biphasic response of rat gastric fundus to field stimulation. *Gen Pharmacol* 1994;25:951–5.
- Fehlmann D, Langenegger D, Schuepbach E, Siehler S, Feuerbach D, Hoyer D. Distribution and characterisation of somatostatin receptor mRNA and binding sites in the brain and periphery. *J Physiol Paris* 2000;94:265–81.
- Feniuk W, Dimech J, Jarvie EM, Humphrey PP. Further evidence from functional studies for somatostatin receptor heterogeneity in guinea-pig isolated ileum, vas deferens and right atrium. *Br J Pharmacol* 1995;115:975–80.
- Feniuk W, Jarvie E, Luo J, Humphrey PP. Selective somatostatin  $\text{sst}_2$  receptor blockade with the novel cyclic octapeptide, CYN-154806. *Neuropharmacology* 2000;39:1443–50.
- Foong JP, Parry LJ, Gwynne RM, Bornstein JC. 5-HT<sub>1A</sub>,  $\text{SST}_1$ , and  $\text{SST}_2$  receptors mediate inhibitory postsynaptic potentials in the submucous plexus of the guinea pig ileum. *Am J Physiol Gastrointest Liver Physiol* 2010;298:G384–94.
- Fries JL, Murphy WA, Sueiras-Diaz J, Coy DH. Somatostatin antagonist analog increases GH, insulin, and glucagon release in the rat. *Peptides* 1982;3:811–4.
- Furness JB, Costa M. Actions of somatostatin on excitatory and inhibitory nerves in the intestine. *Eur J Pharmacol* 1979;56:69–74.
- Guillemin R. Somatostatin inhibits the release of acetylcholine induced electrically in the myenteric plexus. *Endocrinology* 1976;99:1653–4.
- Horinouchi T, Koike K. Functional identification of beta3-adrenoceptors in the guinea-pig ileum using the non-selective beta-adrenoceptor antagonist (+/–)-bupranolol. *J Auton Pharmacol* 2000;20:253–8.

- Jhamandas K, Elliott J. Comparative effects of somatostatin and enkephalins on the guinea pig ileum and the rat vas deferens. *Can J Physiol Pharmacol* 1980;58:1389–92.
- Maurer R, Gaehwiler BH, Buescher HH, Hill RC, Roemer D. Opiate antagonistic properties of an octapeptide somatostatin analog. *Proc Natl Acad Sci U S A* 1982;79:4815–7.
- Monier S, Kitabgi P. Effects of  $\beta$ -endorphin, met-enkephalin and somatostatin on the neurotensin-induced neurogenic contraction in the guinea-pig ileum. *Regul Pept* 1981;2:31–42.
- Paton WDM. The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br J Pharmacol Chemother* 1957;12:119–27.
- Peterson LJ, Hirling LD, Pelton JT, Hruby VJ, Burks TF. A comparison of the opioid antagonist properties of novel somatostatin analogs in vitro. *Proc West Pharmacol Soc* 1985;28:165–7.
- Shirahase H, Kanda M, Shimaji H, Usui H, Rorstad OP, Kurahashi K. Somatostatin-induced contraction mediated by endothelial TXA<sub>2</sub> production in canine cerebral arteries. *Life Sci* 1993;53:1539–44.
- Shook JE, Pelton JT, Kazmierski W, Lemcke PK, Wire WS, Hruby VJ, et al. Comparison of the opioid antagonist properties of a cyclic somatostatin analog in vitro and in vivo. *NIDA Res Monogr* 1986;75:205–8.
- Shook JE, Pelton JT, Lemcke PK, Porreca F, Hruby VJ, Burks TF. Mu opioid antagonist properties of a cyclic somatostatin octapeptide in vivo: identification of mu receptor-related functions. *J Pharmacol Exp Ther* 1987;242:1–7.
- Siehl S, Seuwen K, Hoyer D. Characterisation of human recombinant somatostatin receptors. 1. Radioligand binding studies. *Naunyn-Schmiedeberg's Arch Pharmacol* 1999;360:488–99.
- Vizi ES, Horváth T, Somogyi GT. Evidence that somatostatin releases endogenous substance(s) responsible for its presynaptic inhibitory effect on rat vas deferens and guinea pig ileum. *Neuroendocrinol* 1984;39:142–8.
- Walker JM, Bowen WD, Atkins ST, Hemstreet MK, Coy DH. Mu-opiate binding and morphine antagonism by octapeptide analogs of somatostatin. *Peptides* 1987;8:869–75.