Pergamon

Neuropharmacology 39 (2000) 1443–1450

NEURO PHARMACOLOGY

www.elsevier.com/locate/neuropharm

Selective somatostatin sst_2 receptor blockade with the novel cyclic octapeptide, CYN-154806

W. Feniuk *, E. Jarvie, J. Luo, P.P.A. Humphrey

Glaxo Institute of Applied Pharmacology, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, UK

Accepted 8 February 2000

Abstract

The cyclic octapeptide, CYN-154806, inhibited specific [^{125}I]-[Tyr^{11}]-SRIF binding to CHO-K1 cell membranes expressing human recombinant somatostatin (SRIF) sst_2 receptors (pIC_{50} 8.58) or rat $sst_{2(a)}$ and rat $sst_{2(b)}$ receptors (pIC_{50} 8.35 and 8.10, respectively). The affinity of CYN-154806 at other human somatostatin receptor types was at least 100 times lower (pIC_{50} 5.41–6.48). In functional studies, CYN-154806 inhibited SRIF-induced increases in extracellular acidification (EAR) in CHO-K1 cells expressing h sst_2 receptors (pK_B 7.92) but had no effect on UTP-induced increases in EAR. CYN-154806 also blocked SRIF-induced increases [^{35}S]-GTPγS binding in CHO-K1 cell membranes expressing h sst_2 receptors as well as rat $sst_{2(a)}$ and rat $sst_{2(b)}$ receptors (pK_B 7.81, 7.68 and 7.96, respectively). In marked contrast, no blockade was observed at h sst_5 receptors in concentrations as high 10 μM. The antagonistic activity of CYN-154806 was also studied in isolated tissue preparations that are known to express endogenous SRIF receptors. Thus CYN-154806 blocked SRIF, but not DAMGO-induced inhibition of neurogenic contractions in rat isolated vas deferens and guinea-pig ileum (pK_B 7.79 and 7.49, respectively). CYN-154806 had no effect on SRIF-28 induced inhibition of neurogenic contractions in guinea-pig vas deferens. The results demonstrate that CYN-154806 is a highly potent specific and selective SRIF sst_2 receptor blocking drug. Furthermore, sst_2 receptors mediate SRIF-induced inhibition of neurogenic contractions in rat vas deferens and guinea-pig ileum but not guinea-pig vas deferens which is thought to be mediated by sst_5 receptors.

Keywords: Somatostatin; sst_2 receptors; CYN-154806; Somatostatin receptor blockade

1. Introduction

The tetradecapeptide, somatostatin (somatotropin release inhibitory factor, SRIF) was originally discovered in hypothalamic extracts and is known to be an endogenous inhibitor of the release of growth hormone from the pituitary gland (Krulich et al., 1968; Brazeau et al., 1973). However, the identification of SRIF in extrahypothalamic sites within the brain and peripheral tissues (Johansson et al., 1984; Reichlin, 1983) suggests that SRIF may subserve a wider role, in addition to the regulation of growth hormone secretion. It is present in synaptosomes and is released from nerve terminals in a Ca2+ dependent manner (Epelbaum, 1986). Exogenously administered SRIF produces many effects within the central nervous system (for a review see Schindler et al., 1996) and differentially modulates the release of other neurotransmitters within the brain. For example, the release of noradrenaline is inhibited by SRIF in rat hypothalamus (Gothert, 1980) whilst dopamine, glutamate and GABA release is enhanced in rat striatum (Hathway et al., 1998).

The effects of SRIF are mediated via its interaction with specific membrane bound receptors belonging to the seven transmembrane spanning superfamily of G-protein coupled receptors. With such a diversity of action, it is not surprising that several receptor types exist and the human genes for at least five receptor types (termed sst_1–sst_5) have now been cloned. In both mice and rats, the sst_2 receptor can exist in two splice variant forms, $sst_{2(a)}$ and $sst_{2(b)}$, differing in length and composition of their respective carboxy termini (Vanetti et al., 1992; Schindler et al., 1998). Using recombinant

* Corresponding author. Tel.: +44-1223-334-069; fax: +44-1223-334-178.

E-mail address: wf2776@glaxowellcome.co.uk (W. Feniuk).

0028-3908/00/$ - see front matter
PII: S0028-3908(00)00035-6

expression systems, selective peptide agonists have been identified for many of these receptors and more recently non-peptide agonists for each of the receptors have been identified in combinatorial libraries constructed on the basis of molecular modelling of known peptide agonists (Rohrer et al., 1998). Whilst the availability of such peptide and non-peptide agonists has undoubtedly aided our ability to pharmacologically characterise SRIF receptors which are endogenously expressed in tissues and cells (Feniuk et al., 1995; Dimech et al., 1995; McKeen et al., 1995; Lauder et al., 1997), determination of the physiological significance of such receptors requires the identification of potent, specific and selective receptor blocking drugs.

Recently Bass et al. (1996, 1997) identified the first potent selective peptidic sst_2 receptor blocking drug, CYN-154806. This novel disulfide-linked cyclic octapeptide (Ac–4NO_2–Phe-c (D-Cys–Tyr-D-Trp–Lys–Thr–Cys)-D-Tyr–NH_2) displayed sub-nanomolar affinity for rat sst_2 receptors recombinantly expressed in CHO cells, and reversed SRIF-induced inhibition of forskolin stimulated cAMP formation as well as SRIF-induced growth of yeast cells expressing rat sst_2 receptors. In the present study we have further investigated the SRIF receptor selectivity of CYN-154806 in CHO-K1 cell membranes expressing human sst_1–sst_5 receptors as well as rat $sst_{2(a)}$ and $sst_{2(b)}$ receptors. Using cells expressing recombinant human sst receptors, we have also studied the effect of this novel peptide on sst_2-mediated increases in extracellular acidification and increases in ^{35}S-GTPγS binding. Previous studies, using peptide agonists, suggested that the inhibitory effect of SRIF on autonomic neurotransmission in some peripheral tissues was sst_2 mediated (Feniuk et al., 1993, 1995; Feniuk and Humphrey, 1994). We have therefore additionally studied the effect of CYN-154806 on SRIF-induced inhibition of neurotransmission in the rat isolated vas deferens and guinea-pig ileum preparations.

A preliminary account of some of these findings has been presented to the British Pharmacological Society (Feniuk et al., 1998).

2. Methods

2.1. Radioligand binding

Radioligand binding studies were carried out using the method essentially described by Castro et al. (1996). CHO-K1 cell membranes (approximately 1–3 μg protein) expressing h sst_1–h sst_5 and rat $sst_{2(a)}$ or $sst_{2(b)}$ receptors, in 50 mM Tris–HCl (pH 7.4) were incubated with 0.03 nM [^{125}I]-[Tyr^{11}]-SRIF for 90 min in the absence or presence of increasing concentrations of SRIF or CYN-154806. The Tris buffer contained $MgCl_2$ (5 mM), leupeptin (10 μg ml^{-1}), soyabean trypsin inhibitor (1 μg ml^{-1}), and bacitracin (0.2 mg ml^{-1}). The levels of receptor expression (pmol mg^{-1} protein) were h sst_1 (4.0±0.4), h sst_2 (4.1±0.7), h sst_3 (2.8±0.3), h sst_4 (17.9±1.9), h sst_5 (2.8±0.5), r $sst_{2(a)}$ (10.3±0.1) and r $sst_{2(b)}$ (7.3±1.6). Non-specific binding was defined with 1 μM SRIF. The assay was terminated by rapid filtration over Whatman GF/C glass fibre filters pre-soaked in 0.5% polyethylenimine followed by 3×3 ml washes of 50 mM Tris–HCl buffer. Radioactivity on the filters was determined using a Canberra Packard Cobra II auto-γ counter. Data are the mean from three to four individual experiments each carried out in duplicate. Analyses of the competition curves were made using the assumption that the ligand bound to a single site and pIC_{50} (−log of the concentration of competing drug causing 50% inhibition of specific binding) and Hill sloes (nH) are determined using Graphpad Prism software.

2.2. Microphysiometry

SRIF-induced increases in extracellular acidification rates (EAR) were measured by microphysiometry using the Cytosensor® (Molecular Devices) as previously described in detail (Smalley et al., 1998). CHO-K1 cells expressing h sst_2 receptors were seeded into Cytosensor microphysiometer (Molecular Devices) cups at a density of 5.0×10^5 cells/cup, approximately 18 h prior to experimentation. Cups were placed in the Cytosensor chamber and perfused at 37°C with bicarbonate free DMEM containing 1 g l^{-1} bovine serum albumin at a rate of 120 μl min^{-1}. The rate of extracellular acidification was measured during a 10 s period whilst perfusion was stopped, followed by 43 s of normal flow. All drugs were made up in bicarbonate free DMEM. Agonist-induced increases in EAR were measured over a period of 3 min 40 s during which time increases in EAR had reached a plateau. Cells were allowed to equilibrate for 30 min before being challenged with a single concentration of UTP (3 μM) which was used as an internal standard and then continuously perfused with either normal media or media containing CYN-154806. The UTP challenge was repeated 30 min later followed by progressively increasing concentrations of SRIF at 30 min intervals. Concentration effect curves to SRIF were determined from the magnitude of the SRIF-induced increases in EAR and plotted as a percentage of the initial challenge response to UTP.

2.3. Agonist-induced activation of [^{35}S]-Guanosine-5′-O-(3-thio)triphosphate ([^{35}S]-GTPγS) binding

Assays were carried out in Unifilter 96 GFB plates (Canberra Packard). Agonist-induced activation of [^{35}S]-GTPγS binding was determined using the methods described in detail by Williams et al. (1997). CHO-K1 cell membranes (2–6 μg protein) expressing recombinant

sst receptors were incubated for 90 min at room temperature in a 10 mM HEPES buffer, pH 7.4, containing 150 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 0.3 μM GDP. Increasing concentrations of SRIF were pre-incubated with membranes for 90 min prior to the addition of 0.2 nM [^{35}S]-GTPγS. A 30 min incubation with [^{35}S]-GTPγS was used to determine agonist activated [^{35}S]-GTPγS binding. Non-specific binding (routinely less than 5% of total binding) was determined in the presence of 10 μM GTPγS. Reactions were determined by vacuum filtration using a Packard Filtermate harvester and filters were allowed to dry for 20 min. The amount of radioactivity bound was determined after the addition of 50 μl Microscint-O (Packard) scintillation fluid using a Canberra Packard Topcount Scintillation Counter. SRIF-induced increases in [^{35}S]-GTPγS binding were determined in the absence or continuous presence of CYN-154806. SRIF-induced increases in [^{35}S]-GTPγS binding were calculated as an increase above basal binding and concentration effect curves plotted as a percentage of the maximum increase obtained with the highest concentration of SRIF tested.

2.4. Isolated tissue preparations

The methods used were essentially those previously described in detail (Feniuk et al., 1995). In brief, male Dunkin Hartley guinea-pigs (200–450 g) or Wistar rats (200–350 g) were humanely killed and lengths of terminal ileum or pairs of vasa deferentia removed and placed in a modified Krebs solution of the following composition (mM): NaCl (118), $NaHCO_3$ (25), KCl (4.7), $MgSO_4.7H_2O$ (0.6), KH_2PO_4 (1.2), $CaCl_2.6H_2O$ (1.3), D-glucose (11.1). Preparations of guinea-pig ileum, guinea-pig vas deferens and rat vas deferens were mounted between a pair of platinum electrodes in 4 ml organ baths for recording of isometric tension changes using a Dynamometer UF1 force transducer from a resting tension of approximately 0.5 g. Bath temperature was maintained at 37–38°C and the Krebs solution continuously gassed with a 95% O_2/5% CO_2 gas mixture. All preparations were allowed to equilibrate for periods of at least 30 min before electrical field stimulation was employed. The parameters for electrical stimulation were guinea-pig ileum (0.1 Hz, 0.1 ms continuous), guinea-pig vas deferens (5 Hz, 0.5 ms, 1.5 s every 30 s) and rat vas deferens (0.1 Hz, 0.5 ms continuous) using supramaximal currents of 700–800 mA delivered from Digitimer D330 multistimulator.

Control concentration-effect curves to SRIF or SRIF-28 (guinea-pig vas deferens) on neurogenically mediated contractile responses were obtained by non-cumulative addition of sequentially increasing concentrations of drug at 15 min intervals. Drugs were washed from the bath at the point where the inhibitory effect had just reached its maximum repsonse. Preparations were then left for 30 min before concentration-effect curves were repeated in either the absence (control) or presence of antagonist which was present for the 30 min period between curves and remained in contact with the tissue for the remainder of the experiment. Agonist effects were measured as an inhibition of the neurogenically mediated contraction and concentration-effect curves plotted as a percentage of the maximum agonist response obtained in the same preparation.

2.5. Data analysis

Agonist pEC_{50} values ($-\log_{10}$ of agonist concentration producing 50% of the maximum response) were determined by non-linear regression from individual SRIF concentration-effect curves using GraphPad Prism software. Mean values are presented as the arithmetic mean±sem from at least four experiments. Agonist concentration ratios (geometric mean) were calculated by comparing individual pIC_{50} values in the absence and presence of antagonists and pK_B estimates derived from individual agonist concentration ratios determined at each antagonist concentration using the Gaddum–Schild equation [(pK_B=$\log_{10}$ (conc ratio-1)$-\log_{10}$ (antagonist conc.)]. Mean values are expressed as arithmetic mean±sem.

2.6. Drugs

SRIF and SRIF-28 (Peninsula) were prepared as 1 mM stock solutions in distilled water, aliquoted and stored at −80°C until use. [I^{125}]-Tyr^{11}-SRIF (2000 Ci m mol^{-1}) was purchased from Amersham International. ^{35}S-GTPγS (100–1500 Ci mmol^{-1}) was purchased from Du Pont; Ac–4NO_2–Phe-c (D-Cys–Tyr-D-Trp–Lys–Thr–Cys]-D-Tyr–NH2 (CYN-154806) was custom synthesised by Neosystem Laboratories, Strasbourg, France. UTP and DAMGO were purchased from RBI.

3. Results

3.1. Radioligand binding

Both SRIF and CYN-154806 caused a concentration-dependent inhibition of specific [I^{125}]-[Tyr^{11}]-SRIF binding in CHO-K1 cell membranes expressing recombinant sst receptors. SRIF displayed the highest apparent affinity at human and rat sst_2 receptors whilst the affinities at the other recombinant sst receptor types were approximately 3–10-fold lower (see Table 1). CYN-154806 also showed selectivity for the recombinant sst_2 receptor types but unlike SRIF, the selectivity with respect to binding at the other receptor types was much greater (Table 1). There was little difference in the affinity of CYN-154806 at human (pIC_{50} 8.58) sst_2 receptors and

Table 1
Inhibition of specific [I^{125}]-[Tyr^{11}]-SRIF binding in CHO-K1 cell membranes expressing recombinant SRIF receptors[a]

	SRIF pIC_{50}	nH	CYN-15486 pIC_{50}	nH
h sst_1	9.25±0.02	1.2±0.1	5.41±0.04	0.8±0.1
h sst_2	10.17±0.02	1.1±0.1	8.58±0.07	0.9±0.1
h sst_3	9.21±0.02	1.0±0.1	6.07±0.04	0.8±0.1
h sst_4	8.92±0.05	1.1±0.1	5.76±0.09	0.7±0.1
h sst_5	9.50±0.07	1.0±0.1	6.48±0.09	1.1±0.1
r $sst_{2(a)}$	10.39±0.11	1.4±0.1	8.35±0.19	0.7±0.1
r $sst_{2(b)}$	10.17±0.04	1.3±0.1	8.10±0.08	0.7±0.1

[a] Values are mean±sem. Data on human and rat recombinant SRIF receptors are from four and three individual experiments, respectively, each carried out in duplicate.

the two rat sst_2 splice variants ($sst_{2(a)}$ pIC_{50} 8.4; $sst_{2(b)}$ pIC_{50} 8.1).

3.2. Microphysiometry

The basal rate of extracellular acidification (EAR) in CHO-K1 cells expressing h sst_2 receptors was 169±15 μV s^{-1} (approximately 0.17 pH unit min^{-1}). SRIF caused a concentration-dependent increase in EAR (pEC_{50} 9.45±0.18) with a maximum change of 108±6 μV s^{-1}. In the presence of CYN-154806 (0.1 and 1.0 μM) which itself had little effect on basal EAR, concentration-effect curves to SRIF were displaced to the right with no apparent suppression of the maximum response (Fig. 1). The SRIF concentration ratios [geometric mean (range)] were 13.1 (6.7–24.2) and 79.3 (38.2–149.7) in the presence of CYN-154806 (0.1 and 1.0 μM), respectively, giving an overall mean pK_B estimate of 7.99±0.12.

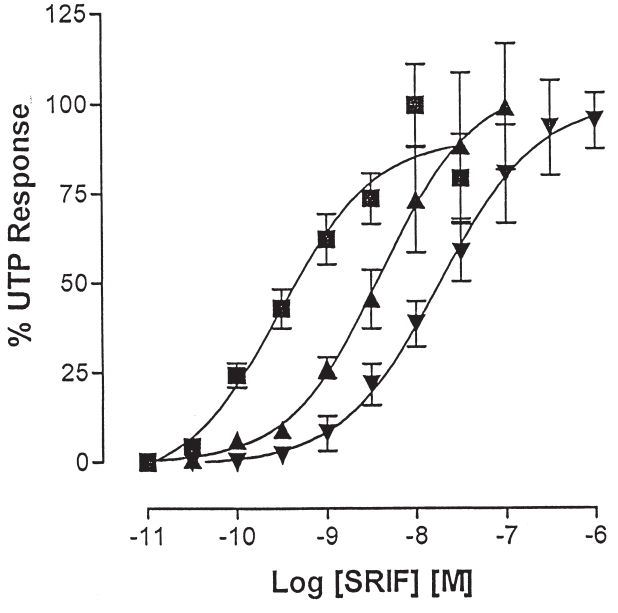

Fig. 1. SRIF-induced increases in extracellular acidification rates in CHO-K1 cells expressing h sst_2 receptors in the absence (■) and presence of CYN-154806 0.1 μM (▲) or 1.0 μM (▼). SRIF-induced increases in extracellular measured as a percentage of the increase in acidification produced by a standard UTP (3 μM) challenge. All values are mean±sem from three separate experiments performed in duplicate.

CYN-154806 (0.1 and 1.0 μM) had no effect on response to UTP (3 μM). UTP-induced increases in EAR were 111±18 and 114±11 μV s^{-1} (before exposure to CYN-154806) and 100±16 and 107±9 μV s^{-1} after a 30 min exposure to CYN-154806 (0.1 and 1.0 μM), respectively.

3.3. Agonist-induced increases in [^{35}S]-GTPγS binding

SRIF caused a concentration-dependent increase in basal [^{35}S]-GTPγS binding in CHO-K1 cell membranes expressing h sst_2, r $sst_{2(a)}$, r $sst_{2(b)}$ and h sst_5 receptors with respective pEC_{50} values of 7.75±0.16, 7.89±0.13, 7.79±0.19 and 7.01±0.29. The percentage increases above basal [^{35}S]-GTPγS binding were, respectively, 156±12, 293±59, 344±23 and 209±12%.

In the presence of CYN-154806 (0.1 and 1.0 μM), control concentration-effect curves to SRIF were displaced to the right in a concentration-dependent manner with no apparent suppression of the maximum response in CHO-K1 cell membranes expressing h sst_2, r $sst_{2(a)}$ and r $sst_{2(b)}$ receptors (Fig. 2). The respective estimated pK_B values were 7.81±0.05, 7.68±0.07 and 7.96±0.08. In marked contrast, CYN-154806 had no effect on SRIF-induced increases in ^{35}S-GTPγS binding in membranes expressing h sst_5 receptors, even in concentrations as high as 10 μM (Fig. 2).

3.4. Isolated tissue studies

SRIF caused a concentration-dependent inhibition of neurogenic contractions in rat isolated vas deferens (EC_{50} 34 (27–56) nM). CYN-154806 (0.1, 0.3 and 1 μM) had no effect on neurogenic contractions but displaced the SRIF concentration-effect curves to the right with no depression of the maximum response (Fig. 3). The respective SRIF concentration-ratios in the presence of CYN-154806 (0.1, 0.3 and 1 μM) were, 6.7 (5.5–9.3),

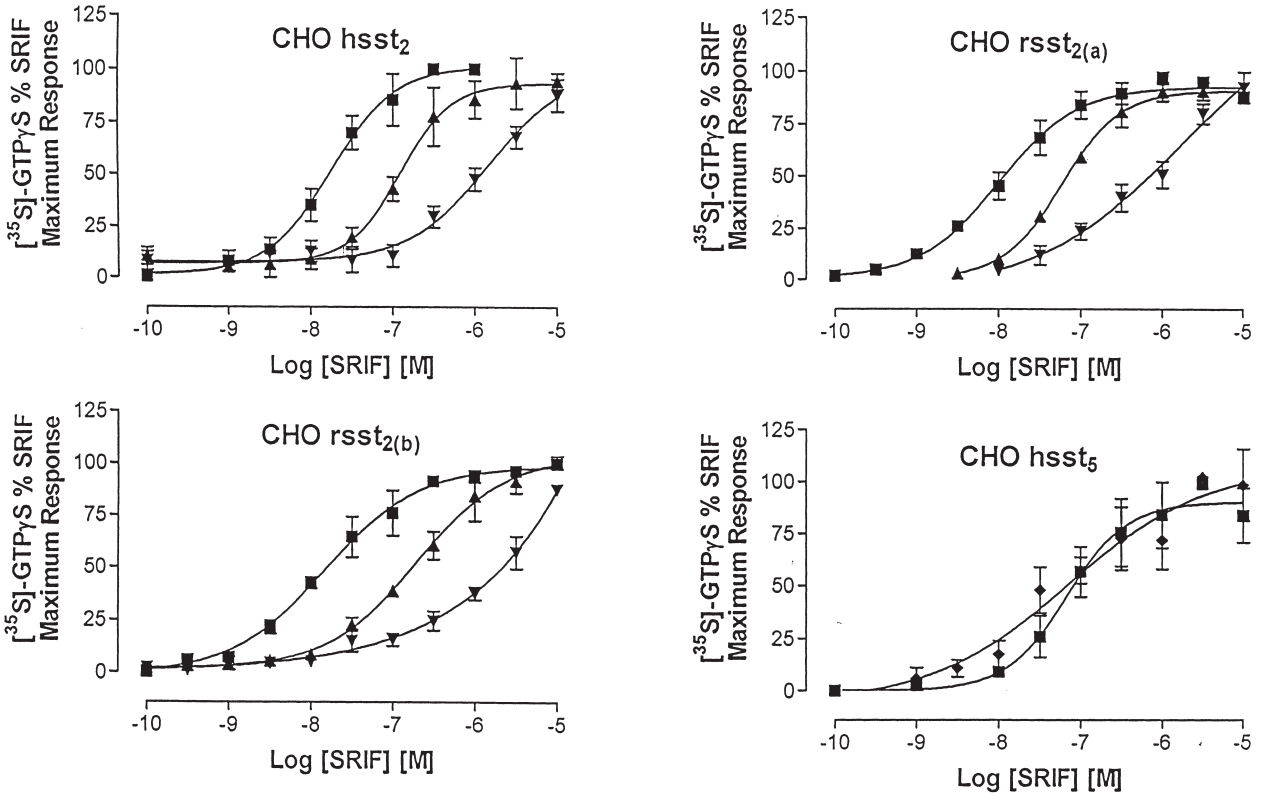

Fig. 2. SRIF-induced increase in [^{35}S]-GTPγS binding in CHO-K1 cells expressing h sst_2, r $sst_{2(a)}$, r $sst_{2(b)}$ or h sst_5 receptors. Concentration-effect curves to SRIF in the absence (■) and presence of CYN-154806 0.1 μM (▲), 1.0 μM (▼) or 10.0 μM (◆). All values are mean±sem from at least four experiments.

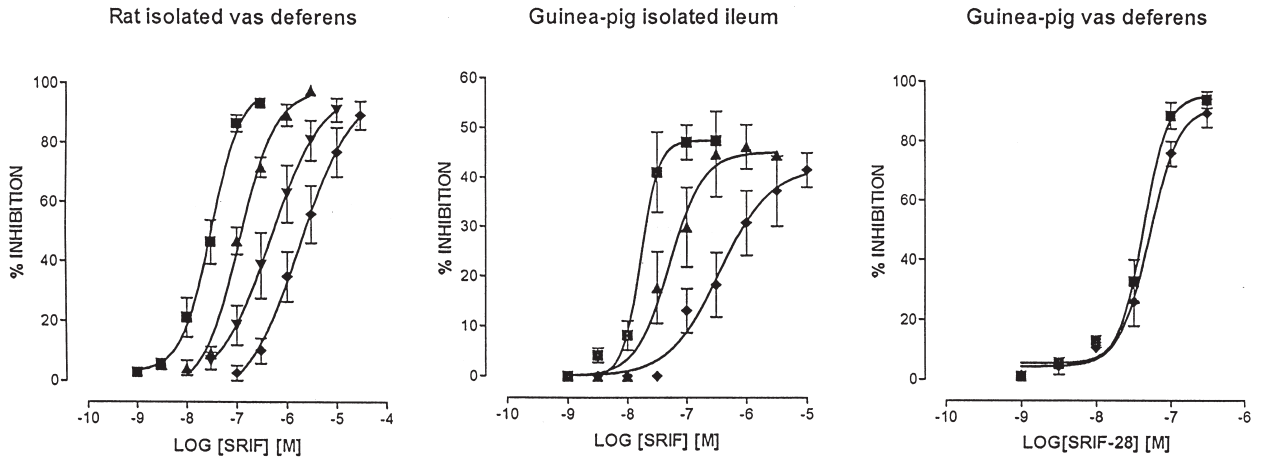

Fig. 3. SRIF-induced inhibition of neurogenically mediated contractions in rat isolated vas deferens, guinea-pig isolated ileum and guinea-pig vas deferens in the absence (■) and presence of CYN-154806 0.1 μM (▲), 0.30 μM (▼) or 1.0 μM (◆). All values are mean±sem from at least four experiments.

19.5 (14.4–32.8) and 67.9 (45.6–129.6) giving an overall mean pK_B estimate of 7.79±0.05.

SRIF [EC_{50} 43 (35–50) nM] also inhibited neurogenic contraction in guinea-pig isolated ileum (Fig. 3) and these responses were also antagonised by CYN-154806 (0.1 and 1 μM). The SRIF concentration-ratios were 5.0 (3.6–8.8) and 25.8 (11.4–96.6), respectively, giving an overall mean pK_B estimate of 7.49±0.11.

CYN-154806 (1 μM) had no effect on the inhibitory action of the μ opiate agonist, DAMGO, in either the rat isolated vas deferens or guinea-pig isolated ileum (concentration-ratios 0.81 (0.50–1.15) and 0.96 (0.91–1.10), respectively).

SRIF-28 [EC_{50} 44 (15–80)] caused a concentration-dependent inhibition of neurogenic contractions in guinea-pig vas deferens (Fig. 3), however CYN-154806

(1 μM) had no effect on this response [concentration-ratio 1.9 (1.2–3.0)].

4. Discussion

Whilst numerous studies have explored the distribution of SRIF receptor types and transcripts in both the brain and periphery (see Section 1), our understanding of the physiological role of the individual receptors is still poorly understood. Mice in which the sst_2 gene has been inactivated show no distinctive behavioural phenotype (Zheng et al., 1997; Martinez et al., 1998), although the physiological importance of the sst_2 receptor in regulating growth hormone and gastric acid secretion was highlighted. Indeed, these studies have corroborated the conclusions which have been drawn by comparing the agonistic potencies of a number of synthetic SRIF analogues displaying differing affinities for the individual receptor types on pituitary and gastric mucosal function (Wyatt et al., 1996; Hoyer et al., 1994). Whilst such studies have proved useful, definitive receptor characterisation in tissues and cells expressing multiple receptor types awaits the identification of specific and selective receptor blocking drugs.

The initial identification by Bass and colleagues (1996, 1997) of a novel disulphide-linked cyclic octapeptide (CYN-154806) [Ac–4NO_2–Phe-c (D-Cys–Tyr-D-Trp–Lys–Thr–Cys]-D-Tyr–NH_2, which displayed sub-nanomolar binding affinity for rat sst_2 receptors recombinantly expressed in CHO cells, and which reversed SRIF-induced inhibition of forskolin stimulated cAMP formation as well as SRIF-induced growth of yeast cells expressing rat sst_2 receptors, prompted us to investigate further the pharmacology of CYN-154806 on individual human as well as rat SRIF receptors.

The objectives of this study were, first to use radioligand binding with [I^{125}]-[Tyr^{11}]-SRIF to determine the overall selectivity of CYN-154806 for different recombinant SRIF receptors expressed in CHO-K1 cell membranes. Secondly, we wanted to compare the functional effects of this peptide at recombinant sst_2 receptors with those endogenous receptors in isolated tissue preparations where previous studies had suggested sst_2-mediated inhibition of neurotransmission (Feniuk et al., 1993, 1995).

In radioligand binding studies, CYN-154806 was found to be a highly potent and selective ligand for recombinantly expressed sst_2 receptors, displaying at least 100-fold selectivity for sst_2 over other SRIF receptor types. Unlike antagonists for other peptide receptors such as NK1 receptors (Walpole et al., 1998), CYN-154806 did not differentiate between human and rat sst_2 receptors and inhibited specific [I^{125}]-[Tyr^{11}]-SRIF binding with equal affinity. We have recently shown (Schindler et al., 1998) that like the mouse (Vanetti et al., 1992), the rat sst_2 receptor exists as two splice variants termed $sst_{2(a)}$ and $sst_{2(b)}$ differing in length and composition of their carboxy termini. Reisine and colleagues (Reisine et al., 1993) have shown that the affinities of a number of synthetic SRIF peptide analogues are similar for both mouse sst_2 receptor isoforms and indeed, in the present study, CYN-154806 did not differentiate between the rat splice variants.

Further studies were performed in order to determine the effect of CYN-154806 on SRIF-induced activation of human and rat receptors recombinantly expressed in CHO-K1 cells. These studies included SRIF-induced increases in extracellular acidification, using the technique of microphysiometry, and SRIF-induced increases in [^{35}S]-GTPγS binding. The former technique allows continuous monitoring of cellular metabolic activity by measuring extracellular acidification rates (McConnell et al., 1992) and has been used to characterise a number of G-protein coupled receptors recombinantly expressed in different cell lines, including h sst_2 and h sst_4 receptors (e.g. Castro et al., 1996; Smalley et al., 1998). The latter technique relies on the receptor-stimulated exchange of GDP for [^{35}S]-GTPγS (instead of GTP) and therefore provides a quantitative measure of signal transduction close to the level of receptor activation. We have previously used this technique to characterise in detail, h sst_5-receptors expressed in CHO-K1 cells (Williams et al., 1997).

SRIF (pEC_{50} 9.45) was a highly potent agonist at causing increases in EAR in CHO-K1 cells expressing h sst_2 receptors. In the presence of increasing concentrations of CYN-154806, concentration-effect curves to SRIF were displaced to the right in a concentration-dependent manner with no suppression of the SRIF maximum response, demonstrating the surmountable nature of the blockade. The blockade observed following treatment with CYN-154806 was specific since UTP-induced increases in EAR, mediated via the activation of endogenously expressed $P2Y_2$ receptors, were unchanged.

SRIF also caused concentration-dependent increases in agonist-induced increases in [^{35}S]-GTPγS binding in CHO-K1 cell membranes expressing h sst_2 as well as r $sst_{2(a)}$ and r $sst_{2(b)}$ receptors. The potency of SRIF (pEC_{50} approximately 7.8) was about 50-fold weaker than observed in the microphysiometer studies. This finding suggests that SRIF-induced increases in EAR involve highly efficient coupling between receptor and effector and that only a small degree of GDP exchange for GTP is required to induce significant increases in EAR (see Kenakin, 1993). As was observed in the microphysiometry studies, CYN-154806 caused a concentration-dependent rightward displacement of SRIF-induced increases in [^{35}S]-GTPγS binding in CHO-K1 cell membranes expressing h sst_2 as well as r $sst_{2(a)}$ and r $sst_{2(b)}$ receptors. The antagonistic potency of CYN-154806 was similar in

all of these studies (pK_B 7.68–7.96) and similar to the potency observed in the microphysiometer experiments (pK_B 7.92). Importantly, CYN-154806 had no effect on SRIF-induced increases [^{35}S]-GTPγS binding in CHO-K1 cell membranes expressing h sst_5 receptors further demonstrating its selectivity of action shown in the initial radioligand binding studies with [I^{125}]-[Tyr^{11}]-SRIF.

Although these studies have clearly demonstrated the antagonistic activity of CYN-154806 at sst_2 receptors recombinantly expressed in CHO-K1 cells, further studies were carried out in order to determine its effects in isolated tissues expressing endogenous SRIF receptors (rat isolated vas deferens and guinea-pig isolated ileum). In both the rat vas deferens and guinea-pig ileum, SRIF causes concentration-dependent inhibition of neurogenically-mediated contractile responses via receptors, which on the basis of agonist potency comparisons, have the characteristics of sst_2 receptors (Feniuk et al., 1993, 1995; Feniuk and Humphrey, 1994). The maximum SRIF-induced inhibition of neurogenic contractions in the guinea-pig ileum (approximately 50%) was much smaller than that observed in the rat vas deferens (approximately 95%) and resulted in steep agonist concentration-effect curves in the former preparation. Since responses to high concentrations of SRIF in the guinea-pig ileum are susceptible to desensitisation (Feniuk et al., 1995), it is possible that the steepness of the SRIF concentration effect in this preparation results from a suppressed maximum response brought about by such desensitisation. Nevertheless, as was seen in CHO-K1 cells expressing either human or rat sst_2 receptors, CYN-154806 caused a concentration-dependent rightward displacement of the concentration-effect curves to SRIF in both the rat isolated vas deferens and guinea-pig ileum. The antagonistic potency of CYN-154806 (pK_B values of 7.79 and 7.49, respectively) was not different to that calculated from studies with recombinantly expressed human and rat sst_2 receptors, providing further evidence that SRIF-induced inhibition of neurotransmission in both the rat vas deferens and guinea-pig ileum is sst_2 mediated. Both somatostatin and the sst_2-receptor selective peptide have been shown to interact with μ-opioid receptors (Terenius, 1976; Maurer et al., 1982) and indeed sst_2 receptors share an approximate 40% homology to opioid receptors (Yamada et al., 1992). We have previously shown that the inhibitory effect of SRIF on neurogenically mediated contractions in the guinea-pig ileum is not susceptible to blockade by naloxone (Feniuk et al., 1993) and therefore not mediated via an action at μ-opioid receptors. However, it was important to further define the specificity of action of CYN-154806 and to investigate its effect on the inhibitory actions of the μ-opioid receptor agonist, DAMGO in both the rat vas deferens and guinea-pig isolated ileum. CYN-154806 (1 μM) had no effect on the inhibitory effect of DAMGO in either isolated tissue preparation.

We have previously shown that SRIF and SRIF-28 also cause an inhibition of neurogenic contractions in guinea-pig isolated vas deferens (Feniuk et al., 1995) and on the basis of agonist potency comparisons suggested that this effect is not mediated by action at sst_2 receptors. Since CYN-154806 (1 μM) had no effect on the inhibitory effect of SRIF-28 in the guinea-pig vas deferens, an action at sst_2 receptors can now be definitively excluded. The receptor type mediating inhibition in the guinea-pig vas deferens is still unknown but we have postulated on the basis of agonist studies that it is likely to be an sst_5 receptor (Feniuk et al., 1995).

In summary, the results from this study have shown that CYN-154806 is a potent, specific and selective antagonist at human, rat as well as guinea-pig SRIF sst_2 receptors. In all studies the antagonism was surmountable. Whilst the in vivo activity and pharmacokinetics of this cyclic peptide is still unknown, CYN-154806 is a useful tool with which to further explore the significance of sst_2 receptors expressed in both the central and peripheral nervous system. Indeed our recent study (Hicks et al., 1998) describing the ability of CYN-154806 to inhibit SRIF-induced activation of an outward K^+ current in anterior cingulate cortical neurones, suggests that sst_2 receptors may play, amongst other things, an important role in pain perception and that centrally penetrant sst_2 agonists may lead to a new class of analgesic drug.

References

Bass, R.T., Buckwalter, B.L., Patel, B.P., Pausch, M.H., Price, L.A., Strnad, J., Hadcock, J.R., 1996. Identification and characterization of novel somatostatin antagonists. Mol. Pharmacol. 50, 709–715.

Bass, R.T., Buckwalter, B.L., Patel, B.P., Pausch, M.H., Price, L.A., Strnad, J., Hadcock, J.R., 1997. Erratum. Mol. Pharmacol. 51, 170.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., Guillemin, R., 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179, 77–79.

Castro, S.W., Buell, G., Feniuk, W., Humphrey, P.P.A., 1996. Differences in the operational characteristics of the human recombinant somatostatin receptor types, sst_1 and sst_2, in mouse fibroblast (Ltk−) cells. Br. J. Pharmacol. 117, 639–646.

Dimech, J., Feniuk, W., Latimer, R.D., Humphrey, P.P.A., 1995. Somatostatin-induced contraction of human isolated saphenous vein involves sst_2 receptor-mediated activation of L-type calcium channels. J. Cardiovasc. Pharmacol. 26, 721–728.

Epelbaum, J., 1986. Somatostatin in the central nervous system: physiology and pathological modifications. Prog. Neurobiol. 27, 63–100.

Feniuk, W., Dimech, J., Humphrey, P.P.A., 1993. Characterization of somatostatin receptors in guinea-pig isolated ileum, vas deferens and right atrium. Br. J. Pharmacol. 110, 1156–1164.

Feniuk, W., Dimech, J., Jarvie, E.M., Humphrey, P.P.A., 1995. Further evidence from functional studies for somatostatin receptor heterogeneity in guinea-pig isolated ileum, vas deferens and right atrium. Br. J. Pharmacol. 115, 975–980.

Feniuk, W., Humphrey, P.P.A., 1994. Somatostatin-induced inhibition of neurotransmission in the mouse isolated vas deferens is resistant to pertussis toxin. Eur. J. Pharmacol. 261, 333–337.

Feniuk, W., Jarvie, E.M., Luo, J.A., Humphrey, J.A., Humphrey, P.P.A., 1998. Functional studies with the novel somatostatin (SRIF) sst_2 receptor blocking drug Ac–NH–4NO_2–Phe-c[D-Cys–Tyr-D-Trp–Lys–Thr–Cys]–Tyr–NH_2 (CYANAMID 154806). Br. J. Pharmacol. 123, 111.

Gothert, M., 1980. Somatostatin selectively inhibits noradrenaline release from hypothalamic neurones. Nature 288 (5786), 86–88.

Hathway, G.J., Emson, P.C., Humphrey, P.P.A., Kendrick, K.M., 1998. Somatostatin potently stimulates in vivo striatal dopamine and gamma-aminobutyric acid release by a glutamate-dependent action. J. Neurochem. 70, 1740–1749.

Hicks, G.A., Feniuk, W., Humphrey, P.P.A., 1998. Outward current produced by somatostatin (SRIF) in rat anterior cingulate pyramidal cells in vitro. Br. J. Pharmacol. 124, 252–258.

Hoyer, D., Lubbert, H., Bruns, C., 1994. Molecular pharmacology of somatostatin receptors. Naunyn Schmiedebergs Arch Pharmacol. 350, 441–453.

Johansson, O., Hokfelt, T., Elde, R.P., 1984. Immunohistochemical distribution of somatostatin-like immunoreactivity in the central nervous system of the adult rat. Neuroscience 13, 265–339.

Kenakin, T.P., 1993. Pharmacological Analysis of Drug–Receptor Interactions, 2nd edition. Raven Press, New York.

Krulich, L., Dhariwal, A.P., McCann, S.M., 1968. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology 83, 783–790.

Lauder, H., Sellers, L.A., Fan, T.P., Feniuk, W., Humphrey, P.P.A., 1997. Somatostatin sst_5 inhibition of receptor mediated regeneration of rat aortic vascular smooth muscle cells. Br. J. Pharmacol. 122, 663–670.

Martinez, V., Curi, A.P., Torkian, B., Schaeffer, J.M., Wilkinson, H.A., Walsh, J.H., Tache, Y., 1998. High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. Gastroenterology 114, 1125–1132.

Maurer, R., Gaehwiler, B.H., Buescher, H.H., Hill, R.C., Roemer, D., 1982. Opiate antagonistic properties of an octapeptide somatostatin analog. Proc. Natl. Acad. Sci. 79, 4815–48177.

McConnell, H.M., Owicki, J.C., Parce, J.W., Miller, D.L., Baxter, G.T., Wada, H.G., Pitchford, S., 1992. The cytosensor microphysiometer: biological applications of silicon technology. Science 257, 1906–1912.

McKeen, E.S., Feniuk, W., Humphrey, P.P.A., 1995. Somatostatin receptors mediating inhibition of basal and stimulated electrogenic ion transport in rat isolated distal colonic mucosa. Naunyn Schmiedebergs Arch. Pharmacol. 352, 402–411.

Reichlin, S., 1983. Somatostatin. N. Engl. J. Med. 309, 1495–1501.

Reisine, T., Kong, H., Raynor, K., Yano, H., Takeda, J., Yasuda, K., Bell, G.I., 1993. Splice variant of the somatostatin receptor 2 subtype, somatostatin receptor 2B, couples to adenylyl cyclase. Mol. Pharmacol. 44, 1016–1020.

Rohrer, S.P., Birzin, E.T., Mosley, R.T., Berk, S.C., Hutchins, S.M., Shen, D.M., Xiong, Y., Hayes, E.C., Parmar, R.M., Foor, F., Mitra, S.W., Degrado, S.J., Shu, M., Klopp, J.M., Cai, S.J., Blake, A., Chan, W.W., Pasternak, A., Yang, L., Patchett, A.A., Smith, R.G., Chapman, K.T., Schaeffer, J.M., 1998. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. Science 282, 737–740.

Schindler, M., Humphrey, P.P.A., Emson, P.C., 1996. Somatostatin receptors in the central nervous system. Prog. Neurobiol. 50, 9–47.

Schindler, M., Kidd, E.J., Carruthers, A.M., Wyatt, M.A., Jarvie, E.M., Sellers, L.A., Feniuk, W., Humphrey, P.P.A., 1998. Molecular cloning and functional characterization of a rat somatostatin sst2(b) receptor splice variant. Br. J. Pharmacol. 125, 209–217.

Smalley, K.S., Feniuk, W., Humphrey, P.P.A., 1998. Differential agonist activity of somatostatin and L-362855 at human recombinant sst_4 receptors. Br. J. Pharmacol. 125, 833–841.

Terenius, L., 1976. Somatostatin and ACTH are peptides with partial antagonist-like selectivity for opiate receptors. Eur. J. Pharmacol. 1976, 211–213.

Vanetti, M., Kouba, M., Wang, X., Vogt, G., Hollt, V., 1992. Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). FEBS Lett. 311, 290–294.

Walpole, C., Ko, S.Y., Brown, M., Beattie, D., Campbell, E., Dickenson, F., Ewan, S., Hughes, G.A., Lemaire, M., Lerpiniere, J., Patel, S., Urban, L., 1998. 2-Nitrophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-N-benzyl-N-methylamide (SDZ NKT 343), a potent human NK1 tachykinin receptor antagonist with good oral analgesic activity in chronic pain models. J. Med. Chem. 41, 3159–3173.

Williams, A.J., Feniuk, W., Humphrey, P.P.A., 1997. Somatostatin5 receptor-mediated [^{35}S]guanosine-5′-O-(3-thio)triphosphate binding: agonist potencies and the influence of sodium chloride on intrinsic activity. Mol. Pharmacol. 51, 1060–1069.

Wyatt, M.A., Jarvie, E., Feniuk, W., Humphrey, P.P.A., 1996. Somatostatin sst_2 receptor-mediated inhibition of parietal cell function in rat isolated gastric mucosa. Br. J. Pharmacol. 119, 905–910.

Yamada, Y., Post, S.R., Wang, K., Tager, H.S., Bell, G.I., Seino, S., 1992. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. Proc. Natl. Acad. Sci. 89, 251–295.

Zheng, H., Bailey, A., Jiang, M.H., Honda, K., Chen, H.Y., Trumbauer, M.E., Van der Ploeg, L.H., Schaeffer, J.M., Leng, G., Smith, R.G., 1997. Somatostatin receptor subtype 2 knockout mice are refractory to growth hormone-negative feedback on arcuate neurons. Mol. Endocrinol. 11, 1709–1717.