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Mediation by $SRIF_1$ receptors of the contractile action of somatostatin in rat isolated distal colon; studies using some novel SRIF analogues

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1 The motor effects of somatostatin-14 (SRIF), and several SRIF peptide analogues were investigated on the rat isolated distal colon. The objective of these studies was to characterize the receptor mediating the contractile action of SRIF by comparing the relative agonist potencies of a range of SRIF analogues.

2 SRIF (1 nM–1 μM) produced concentration-dependent contractions with an EC_{50} value of approximately 10 nM. Contractile responses induced by SRIF were insensitive to atropine (1 μM) or naloxone (1 μM) but abolished by tetrodotoxin (1 μM). Somatostatin-28 ($SRIF_{28}$), also induced concentration-dependent contractions and was equipotent with SRIF. Phosphoramidon (1 μM) and amastatin (10 μM) did not increase the potency of either SRIF or $SRIF_{28}$.

3 The SRIF peptide analogues, octreotide, $SRIF_{25}$, seglitide, angiopeptin and CGP23996 (1 nM–1 μM) produced contractile responses in the rat distal colon, each having similar potency and maximal activity relative to SRIF. The $SSTR_2$ receptor-selective hexapeptide, BIM23027 (0.1 nM–1 μM), and the SRIF stereoisomer, D-Trp^8-SRIF (0.1 nM–1 μM), were the most potent agonists examined being approximately 12 and 7 times more potent than SRIF, respectively. In contrast, the $SSTR_5$ receptor-selective analogue, L362,855, was approximately 120 times weaker than SRIF, whilst the $SSTR_3$ receptor-selective analogue, BIM23056, was inactive at concentrations up to 3 μM.

4 The putative SRIF receptor antagonist, (cyclo(7-aminoheptanoyl Phe-D-Trp-Lys-Thr[Bzl]))(CPP) (1 μM), had no agonist activity and had no effect on contractions induced by SRIF.

5 The contractile actions of BIM23027 and seglitide were subject to pronounced desensitization. Desensitization of preparations by BIM23027 (0.3 μM) abolished the contractile action of SRIF and $SRIF_{28}$ but had no effect on contractions produced by acetylcholine (0.1 nM–1 μM), suggesting that BIM23027, SRIF and $SRIF_{28}$ act via a common receptor mechanism.

6 In conclusion, the rat isolated distal colon contracts in response to SRIF and a number of SRIF analogues. Seglitide and octreotide exhibited similar potency and maximal activity relative to SRIF, suggesting that in the rat colon the receptor mediating contraction belongs to the $SRIF_1$-receptor group, of which the recombinant $SSTR_2$, $SSTR_3$ and $SSTR_5$ receptors appear to be subtypes. The high potency of BIM23027, the weak agonist activity of L362,855 and the lack of activity exhibited by BIM23056 suggests that the SRIF receptor mediating contraction in the rat distal colon is similar to the recombinant $SSTR_2$ receptor.

Keywords: Somatostatin receptors; SRIF; rat colon; contraction; seglitide; BIM23027; BIM23056; L362,855

Introduction

Somatostatin-14 (SRIF), a cyclic tetradecapeptide discovered originally in mammalian hypothalamus (Brazeau *et al.*, 1973), exists in high concentrations within the enteric nervous system of the human and rodent gastrointestinal tract (Costa *et al.*, 1980; Keast *et al.*, 1984; Ekbald *et al.*, 1988). Somatostatin-28 ($SRIF_{28}$), an N-terminally extended form of SRIF, is also found in the gut, but primarily in the endocrine cells of the intestinal mucosa (Pradayrol *et al.*, 1980). SRIF has been reported to be a potent inhibitor of neuroendocrine and exocrine gastrointestinal secretion, intestinal transport and splanchnic blood flow (for review see Gyr & Meier, 1993). The effects of SRIF on gastrointestinal motility are complex. For example, SRIF has been shown to inhibit migrating motor complexes originating in the stomach (Ormsbee *et al.*, 1978), whilst those originating in the intestine appear to be stimulated (Thor *et al.*, 1978; Peeters *et al.*, 1983). In the guinea-pig isolated ileum, SRIF inhibits neurogenically mediated contractions probably through a pre-junctional mechanism (Guillemin, 1976; Feniuk *et al.*, 1993). Under basal conditions in the same preparation, SRIF has been shown to evoke cholinergically mediated contractile responses (Roberts *et al.*, 1993). In conscious dogs, SRIF injected intravenously inhibits both spontaneous and cholecystokinin octapeptide (CCK8)-induced colonic motility (Atanassova *et al.*, 1993).

To date, the receptors mediating these many actions of SRIF in the gut remain poorly characterized. This is largely due to the lack of availability of specific and selective SRIF receptor blocking drugs, and the fact that most agonist potency comparisons have been based solely on differences in the relative potencies of SRIF and $SRIF_{28}$ (Meyers *et al.*, 1980; Brazeau *et al.*, 1981; Hirst *et al.*, 1982). Moreover, with few exceptions (Gu *et al.*, 1992), the potential influence of endogenous peptidases on the reactivity of these peptides appears to have been largely ignored. However, recent molecular cloning studies have revealed a large SRIF receptor family, composed at present of five distinct SRIF receptor types (for review see Bell & Reisine, 1993; Raynor *et al.*, 1993a,b). These have been termed $SSTR_1$ (Yamada *et al.*, 1992a; Li *et al.*, 1992), $SSTR_2$ (Yamada *et al.*, 1992a; Kluxen *et al.*, 1992), and $SSTR_3$ (Yasuda *et al.*, 1992; Meyerhof *et al.*, 1992; Yamada *et al.*, 1992b; Corness *et al.*, 1993) which have been structurally characterized from human, rat and mouse, together with a rat $SSTR_4$ receptor (Bruno *et al.*, 1992). A fifth receptor isolated from the rat by O'Carroll *et al.* (1992), has also been designated $SSTR_4$. It is structurally

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and pharmacologically distinct from the first described rat $SSTR_4$ receptor (Bruno *et al.*, 1992) and for the purpose of the present study (and in the absence of an agreed international nomenclature) will be termed $SSTR_5$. Radioligand binding studies on several mammalian cell lines expressing the different SRIF receptors have identified several peptide analogues that appear to be highly potent, and selective for certain subtypes of SRIF receptor (Raynor *et al.*, 1993a,b). Such compounds include BIM23027 (c[N-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe]), BIM23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2), and L362,855 (c[Aha-Phe-Trp-D-Lys-Thr-Phe]) which selectively bind with subnanomolar affinities to $SSTR_2$, $SSTR_3$ and $SSTR_5$ receptors, respectively (Raynor *et al.*, 1993a,b). However, to date, the functional characteristics of these SRIF analogues in whole tissue preparations, naturally expressing SRIF receptors, remains unexplored.

We have recently demonstrated that SRIF causes a concentration-dependent contraction of the rat isolated colon (McKeen *et al.*, 1994). The aim of this study was to characterize the SRIF receptor mediating this effect by comparing the relative agonist potencies of a range of SRIF analogues, including the recently identified $SSTR_2$, $SSTR_3$ and $SSTR_5$ receptor-selective peptides, BIM23027, BIM23056 and L362,855, respectively. A preliminary account of some of these findings has been presented to the British Pharmacological Society (McKeen *et al.*, 1994).

Methods

Male Sprague-Dawley rats (200–300 g) were humanely killed. The distal colon was removed and placed in 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, D-glucose 11.1, $CaCl_2.6H_2O$ 1.3, (pH 7.4), at room temperature and gassed with 95% O_2/5% CO_2. Preparations were gently cleared of any faecal matter and adhering connective tissue. Segments of colon (approx. 1.5 cm) were mounted in 4 ml organ baths at 37°C in gassed modified Krebs solution of the above composition. Contractile responses were measured isometrically from a resting tension of 1.5 g using a Dynamometer UF1 force transducer, and displayed on a chart recorder (Lectromed Multitrace 8). All preparations were allowed to equilibrate for 30 min during which time tension was maintained at 1.5 g and washed with fresh Krebs every 15 min.

Effect of SRIF analogues

Concentration-effect curves to SRIF were constructed non-cumulatively using increasing sequential concentrations. Each concentration of SRIF was applied for approximately 1 min during which time a peak effect was reached. A washout period of 15 min was used between each SRIF application since in preliminary experiments contractile responses to SRIF were desensitized if shorter periods were employed. The maximum concentration of agonist tested was normally 1 μM. Following a 60 min interval a second concentration-effect curve to a test agonist was established as above. Using a Latin square design, one colonic preparation always acted as a control to monitor spontaneous changes in agonist sensitivity. Thirty minute dosing intervals were used for experiments with BIM23027 and seglitide since preliminary results showed that a 30 min interval with regular washes was required between each drug concentration in order to prevent desensitization.

Effect of peptidase inhibitors and antagonists on responses to SRIF

In some experiments the influence of a combination of the peptidase inhibitors, amastatin (10 μM) and phosphoramidon (1 μM), on the contractile action of SRIF and $SRIF_{28}$ was examined. Agonist concentration-effect curves were obtained before and after a 30 min exposure to the peptidase inhibitors which were in contact with the preparation throughout the second concentration-effect curve. Unless otherwise stated, an identical protocol was used to assess any antagonistic properties of cyclo(7-aminoheptanoyl Phe-D-Trp-Lys-Thr [Bzl])(CPP) (1 μM) or BIM23056 (1 μM).

Effect of desensitization by BIM23027 on responses to SRIF, $SRIF_{28}$ and acetylcholine

Desensitization was achieved by applying BIM23027 (0.3 μM) with a contact time of 4 min, followed by a 1 min washout period before administration of various concentrations of SRIF or $SRIF_{28}$. BIM23027 was applied before each concentration of the test agonist, and a recovery period of 10 min was allowed between successive concentrations. The selectivity of BIM23027 desensitization was determined by investigating the effect of BIM23027 (0.3 μM) pretreatment on acetylcholine-induced (0.1 nM–10 μM) contractions by use of the same protocol as above.

Analysis of results

The contractile responses in the rat distal colon were expressed as a percentage of the maximum contractile response to SRIF obtained in the first (control) concentration-effect curve. When agonist potencies were determined, equi-effective molar ratios (EMRs) were measured from the concentration-effect curves at a point corresponding to 50% of the second agonist maximum (EC_{50}), and corrected for spontaneous changes in agonist sensitivity. The effect of antagonists was studied by measuring the concentration-ratio which was the ratio of the agonist EC_{50} values in the presence and absence of antagonist. All values stated are geometric means (95% confidence limits) of n observations, except for % maxima which are expressed as arithmetic mean ± s.e. mean. Tests for statistically significant differences were carried out using a Student's unpaired t test, and a probability $P<0.05$ was considered significant.

Drugs and solutions

The following drugs were obtained from Sigma Chemical Co. Ltd., SRIF, $SRIF_{28}$, $SRIF_{25}$ cyclo(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr[Bzl]) (CPP), angiopeptin (β-(2-napthyl)-D-Ala-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr amide), phosphoramidon, amastatin, atropine methylnitrate, tetrodotoxin and acetylcholine.

CGP 23996 (cyclo [Ahep-Lys-Asn-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]), BIM23027 (c[N-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe]), BIM23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2), and L362,855 (c[Aha-Phe-Trp-D-Lys-Thr-Phe]) and seglitide (MK 678) were synthesized by Dr J. Kitchin's team, Chemistry Division, Glaxo Group Research Ltd. Octreotide (Sandostatin) was purchased from a pharmaceutical supplier.

All peptides were initially dissolved in distilled water, divided into aliquots and stored at −20°C. Fresh aliquots were used on each experimental day and samples were kept on ice during the experiment. There was no loss of biological activity of these samples during the course of an experiment and stored samples showed similar activity to samples prepared from solid stock material and used immediately.

Results

Effect of SRIF

Preliminary studies showed that the rat distal colon was contracted by SRIF (1 μM). If a concentration of SRIF (1 μM) was repeated 5 min after a similar application, the contractile response of the peptide was greatly diminished. In

subsequent experiments pronounced desensitization was overcome by having only a 1 min agonist contact time and by leaving a period of 15 min between application. With this protocol it was possible to obtain reproducible responses over several hours.

SRIF (1 nM–1 μM) produced concentration-dependent contractions in the rat isolated distal colon (Figure 1a). The contractile response to each concentration of SRIF reached a peak within 30–50 s, and even in the continuous presence of the drug, returned rapidly towards baseline. First and second concentration-effect curves to SRIF were reproducible with mean EC_{50} values of 12.3 (8.4–15) and 9.2 (6.5–12)nM respectively, there being only a 0.53 (0.38–0.73) fold leftward shift between the first and second concentration-effect curve ($n = 20$) (Figure 2a). The maximum contractile responses (at 1 μM) for the first and second concentration-effect curve to SRIF were 1.83 ± 0.23 g and 2.28 ± 0.28 g respectively, and these were not significantly different ($P < 0.05$).

Effects of atropine and tetrodotoxin on contractile responses to SRIF

Contractile responses to SRIF (1 nM–1 μM) remained unchanged following a 30 min exposure to atropine (1 μM) (concentration ratio 1.6 (0.4–7.6), $n = 4$). Treatment of preparations with tetrodotoxin (1 μM, 20 min contact time) produced pronounced spontaneous activity (Figure 1b). In the continuous presence of tetrodotoxin (1 μM), contractile responses to a maximally effective concentration of SRIF (1 μM) were abolished. In contrast contractile responses produced by acetylcholine (30 nM) remained unchanged following tetrodotoxin (1 μM) pretreatment (Figure 1b).

Effect of analogues of SRIF

A range of SRIF analogues were tested for activity on the rat distal colon, $SRIF_{28}$, octreotide, angiopeptin, seglitide, CGP 23996, $SRIF_{25}$, BIM23027, and D-Trp^8-SRIF mimicked contractions induced by SRIF. The maximum contractile effect produced by each of these analogues was not significantly different from that produced by SRIF (Table 1). $SRIF_{28}$, octreotide, angiopeptin, CGP23996 and $SRIF_{25}$ were of similar potency to SRIF (Table 1). Seglitide was also equipotent to SRIF and produced a similar maximum contractile response (Table 1). However, at high concentrations (>0.1 μM) seglitide caused smaller contractile responses than were observed at lower concentrations, thus producing a bell shaped concentration-effect curve (Figure 2b). BIM23027 and D-Trp^8-SRIF were the most potent agonists tested being approximately 12 and 7 times more potent than SRIF, respectively (Table 1). In contrast, L362,855 was devoid of contractile activity up to concentrations of 0.3 μM (Figure 2b). Higher concentrations of L362,855 produced contractile responses, although a maximum response was not achieved at a concentration of 3 μM. Assessment of the relative agonist potency of L362,855 at the level of 50% of the SRIF maximum produced an EMR of 120(58–248), $n = 4$. BIM23056 was inactive at concentrations up to 3 μM (Figure 2b). In view of the lack of intrinsic activity of BIM23056, it was tested as a potential SRIF receptor blocking drug. However, BIM23056 (3 μM, 30 min contact time) had no significant effect on either the potency or maximum amplitude of contraction produced by SRIF (concentration ratio 1.0 (0.4–2.6), $n = 4$).

Effect of peptidase inhibitors

The influence of a combination of phosphoramidon (1 μM) and amastatin (10 μM) on the potency of SRIF and $SRIF_{28}$ was examined in the rat distal colon. Phosphoramidon and amastatin did not increase the potency or maximum amplitude of contraction produced by any of the peptides examined (concentration-ratios were 1.2(0.2–6.3) and 3.2 (1.2–8.8) respectively, $n = 4$, $P > 0.05$). BIM23056 was inac-

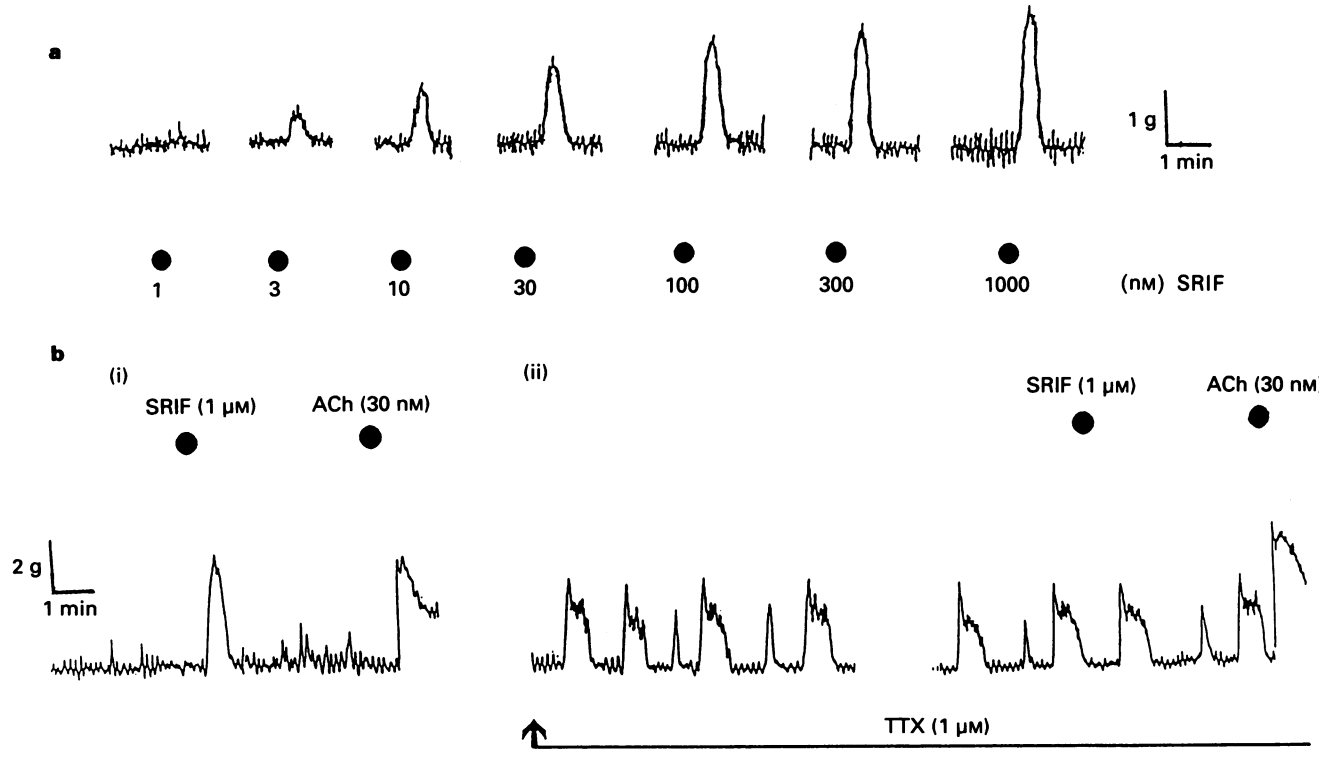

Figure 1 (a) Experimental recordings demonstrating the contractile effect of SRIF (1 nM–1 μM) in the rat isolated distal colon. Each concentration of SRIF was applied for approximately 1 min during which time the peak contractile response was reached, and returned rapidly towards baseline. A washout period of 15 min was used between each sequential dose of SRIF. (b) Contractile action of SRIF (1 μM) and acetylcholine (ACh, 30 nM), in the rat distal colon in the absence (i) and in the presence of tetrodotoxin (TTX) (1 μM) (ii). Note that TTX (1 μM) produced pronounced spontaneous activity and that the contractile responses to SRIF were abolished while the contractions to acetylcholine remained unchanged.

Table 1 A comparison of the potencies of several SRIF analogues in producing contraction in the rat isolated distal colon

Analogue	EC_{50} (nM)	*EMR*	*Maximum (% original SRIF max)*
SRIF	11.5 (8.7–15.6)	1	126 ± 4*
BIM23027	0.9 (0.1–8.3)	0.08 (0.21–0.34)	130 ± 7
[D-Trp^8]-SRIF$_{14}$	2.0 (0.2–17.2)	0.14 (0.02–0.94)	126 ± 8
MK678	2.6 (1.5–4.5)	0.45 (0.15–1.33)	106 ± 7
Octreotide	6.7 (3.0–15.0)	0.31 (0.04–2.37)	120 ± 7
CGP23996	14.9 (2.90–11.3)	0.58 (0.19–1.80)	141 ± 15
Angiopeptin	18.5 (4.9–70.1)	0.94 (0.15–5.90)	103 ± 12
SRIF$_{25}$	14.3 (11.3–17.2)	1.03 (0.53–2.00)	113 ± 7
SRIF$_{28}$	20.0 (8.0–49.2)	1.58 (0.55–4.59)	102 ± 5
L362,855	–	120 (58–248)	NA
BIM23056	>3000	>300	NA

All values are expressed as geometric mean (95% confidence limits), except values for % original SRIF max which are expressed as mean ± s.e.mean, where $n = 4–5$ observations. EMR (equieffective molar ratio) values of less than unity indicate greater potency than that of SRIF. *Values for SRIF are given for the second concentration-effect curves in the control preparations ($n = 34$). NA, maximum not achieved.

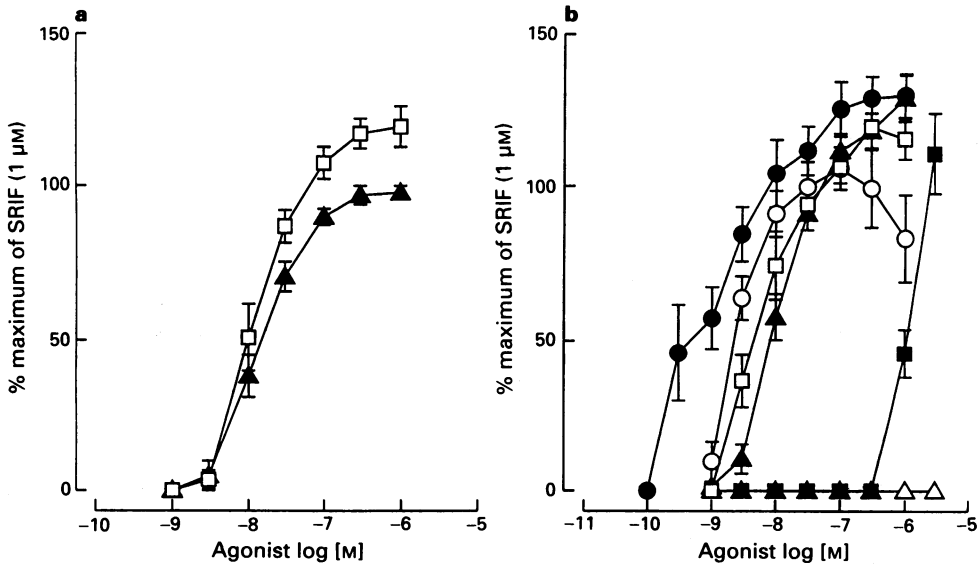

Figure 2 (a) Reproducibility of the first and second concentration-effect curves to SRIF in the rat isolated distal colon. First concentration-effect curve (▲), second concentration-effect curve (□). Each point represents the mean ± s.e.mean ($n = 20$). The abscissa scale shows the log molar concentration of drug and the ordinate scale the contractile response expressed as % of the maximum contractile response to SRIF (1 μM). (b) Comparison of the contractile responses produced by SRIF (▲), BIM23027 (●), BIM23056 (△), L362,855 (■), seglitide (○), and octreotide (□). Each point represents the mean and s.e.mean ($n = 4$, except SRIF $n = 20$). The abscissa scale shows the log molar concentration of drug and the ordinate scale the contractile response expressed as % of the maximum contractile response to SRIF (1 μM).

tive at concentrations up to 3 μM even in the presence of phosphoramidon (1 μM) and amastatin (10 μM) ($n = 4$).

Effect of BIM23027 desensitization

In view of the pronounced susceptibility of BIM23027-induced contractions to desensitization, it was used in cross-desensitization studies with SRIF and SRIF$_{28}$. Pre-exposure of preparations to BIM23027 (0.3 μM) for 4 min period followed by washout (see methods), abolished the contractile responses produced by SRIF and SRIF$_{28}$ (Figure 3a,b), whilst contractions produced by acetylcholine were unchanged (Figure 3c).

Effects of putative antagonists on contractions to SRIF

The putative SRIF receptor blocking drug, CPP (1 μM, 1 h contact time), was inactive at 1 μM, and had no significant effects on either the potency or maximum amplitude of contraction produced by SRIF (concentration ratio 0.8(0.5–1.3); $n = 4$).

Naloxone (1 μM, 30 min contact time) had no antagonistic effect on the contractile action of SRIF (concentration-ratio 0.4(0.04–3.3), $n = 4$).

Discussion

In the present study we have demonstrated that SRIF and several SRIF analogues produce concentration-dependent contractions in the rat isolated distal colon. The contractile response to SRIF appears to be neurogenic, and does not involve cholinergic stimulation since the effects of SRIF were abolished by tetrodotoxin and unaffected by atropine. In general SRIF has been reported to be a potent inhibitor of gastrointestinal motility. For example, SRIF inhibits neurogenically mediated contractile responses in the guinea-pig isolated ileum (Guillemin, 1976; Feniuk *et al.*, 1993), while in

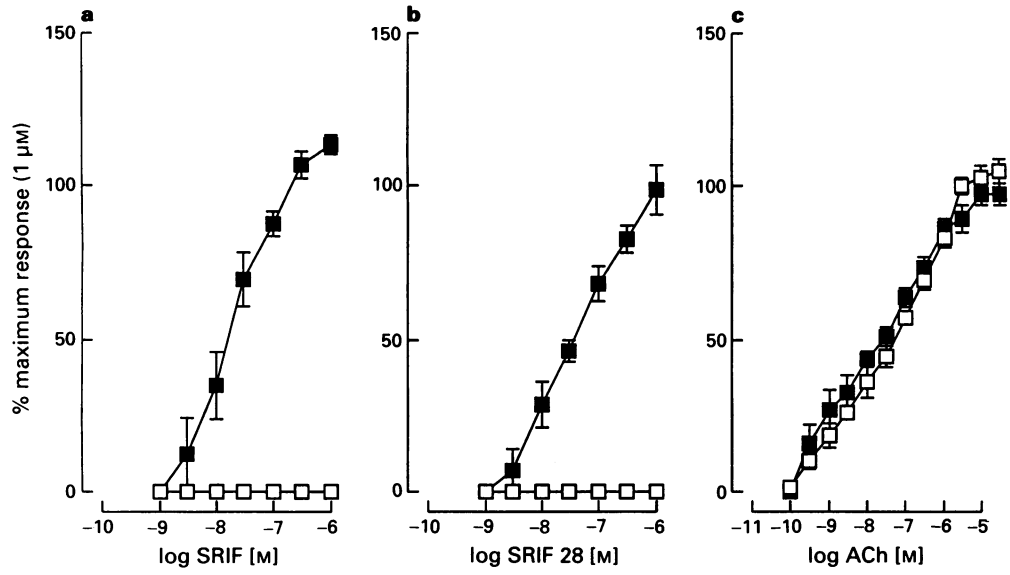

Figure 3 Effect of (a) SRIF, (b) $SRIF_{28}$ and (c) acetylcholine in the rat isolated distal colon in the absence (■) and following desensitization of preparations by BIM23027 (0.3 μM) (□). Desensitization was achieved by applying BIM23027 (0.3 μM) with a contact time of 4 min, followed by a 1 min washout period before administration of SRIF or $SRIF_{28}$. BIM23027 was applied before each concentration of the test agonist, and a recovery period of 10 min was allowed between successive concentrations. Each point represents the mean ± s.e.mean ($n = 4$). The abscissa scale shows the log molar concentration of drug and the ordinate scale the contractile response expressed as % of the maximum contractile response to SRIF (1 μM).

the conscious dogs SRIF injected intravenously inhibits both spontaneous and CCK-induced colonic motility (Atanassova *et al.*, 1993). Thus, as far as we know this is the first evidence of a stimulatory effect of SRIF on colonic motility. The main objective of this study was to study the SRIF receptor involved in this action by comparing the relative agonist potencies of some SRIF analogues that have been claimed to be selective for various SRIF receptor types.

The SRIF analogues, $SRIF_{28}$, $SRIF_{25}$, angiopeptin, and CGP23996 all induced contractile responses in the rat distal colon, each being of similar potency to SRIF. Interestingly, all of these SRIF agonists also showed similar potency to SRIF at inhibiting neurogenically mediated contractions in the guinea-pig isolated ileum (Feniuk *et al.*, 1993), suggesting that the SRIF receptor in the rat colon and the guinea-pig ileum may be similar. Endogenous SRIF and $SRIF_{28}$ are known to be substrates for the catabolic activity of endopeptidase (Marks *et al.*, 1976). Indeed, in tissues such as the guinea-pig isolated vas deferens, pretreatment with peptidase inhibitors (phosphoramidon and amastatin) has been shown to enhance the potency of SRIF by about five fold (Feniuk *et al.*, 1993). However, since the potency of SRIF or $SRIF_{28}$ was not increased by phosphoramidon and amastatin in this study, it seems unlikely that these peptides are degraded by endopeptidases in the rat distal colon.

The characteristics of the SRIF receptor mediating contraction in the rat colon obviously differs from that found in the guinea-pig vas deferens and right atrium. For example, in the guinea-pig vas deferens and right atrium, $SRIF_{28}$ has been shown to be approximately 30 times more potent than SRIF at inhibiting neurogenically mediated contractions and the negative inotropic effect of SRIF, respectively (Feniuk *et al.*, 1993). Furthermore, in the guinea-pig right atrium, the synthetic analogues octreotide and angiopeptin exhibited low intrinsic activity. Indeed, angiopeptin acted as a competitive SRIF receptor blocking drug by specifically antagonizing the negative inotropic action of both SRIF and $SRIF_{28}$ with estimated pK_B values 7.4 and 7.2, respectively. Seglitide has been shown also to act as a SRIF receptor antagonist in the guinea-pig atrium but was approximately 20 times weaker than angiopeptin in this respect (Dimech *et al.*, 1993). In the present study, both angiopeptin and seglitide were agonists with similar potency and maximal activity relative to SRIF. Interestingly, at high concentrations (>0.3 μM), seglitide produced smaller contractions, suggesting that at these concentrations, responses to seglitide may be susceptible to desensitization even with a 30 min washout period between each concentration of drug.

In the rat distal colon, the putative SRIF receptor blocking drug CPP, had no contractile action *per se* and did not antagonize the contractile action of SRIF. Interestingly, CPP has also been reported to have no effect on the inhibitory action of SRIF in the guinea-pig isolated ileum or vas deferens, nor did it antagonize the negative inotropic action of SRIF in the right atrium (Feniuk *et al.*, 1993). This is in contrast to the findings of Fries *et al.* (1982), who found that CPP antagonized SRIF-induced inhibitory effects upon GH, insulin and glucagon release in anaesthetized rats. Araujo *et al.* (1990) have also shown CPP to block the ability of SRIF to enhance acetylcholine release from hippocampal slices. These differences in the antagonistic profile of CPP may be a reflection of SRIF receptor heterogeneity. Alternatively, they may reflect a non-specific action of CPP. Further studies are needed to establish the usefulness of CPP as a tool for classifying SRIF receptors.

It has been suggested that SRIF exerts its effects by interacting with opiate μ-receptors (Terenius, 1976). However, in the rat distal colon such an interaction seems unlikely since naloxone had no effect on the contractile action of SRIF. This observation is consistent with similar findings in the guinea-pig ileum, vas deferens and atrium (Feniuk *et al.*, 1993). In the rat colon the lack of effect of naloxone would also suggest that enkephalins are unlikely to be involved in the mechanism underlying the contractile action of SRIF. Previous studies have suggested that γ-aminobutyric acid, tachykinins and vasointestinal peptide may mediate SRIF-induced contractions in the guinea-pig isolated ileum (Grider *et al.*, 1987; Roberts *et al.*, 1993). However, our unpublished studies with specific antagonists have excluded these neurotransmitters as mediators of SRIF-induced contraction in the rat colon. Further studies are needed to determine the site and mechanism of action of SRIF in the colon.

The plethora of actions exerted by SRIF in the gastrointes-

tinal tract are likely to be mediated by distinct SRIF receptor types. The earliest evidence for the existence of distinct types of SRIF receptor was based on the differential sensitivity to the native peptides SRIF and $SRIF_{28}$ (Meyers *et al.*, 1980; Brazeau *et al.*, 1981; Hirst *et al.*, 1982). The relative binding affinities of more metabolically stable analogues of SRIF such as seglitide and octreotide has been used as the basis for the subclassification of SRIF receptors into $SRIF_1$ and $SRIF_2$ in the rat brain (Tran *et al.*, 1985; Raynor & Reisine, 1992a). The $SRIF_1$ receptor has high affinity for seglitide and octreotide, whereas $SRIF_2$ receptors are insensitive to these analogues. On this basis, the SRIF receptor mediating contraction in the rat distal colon belongs to the $SRIF_1$ group, since seglitide and octreotide displayed high potency relative to SRIF. As previously mentioned (see introduction), the recent cloning of genes encoding multiple SRIF receptors has revealed a SRIF receptor family, composed of at least five distinct SRIF receptor types termed $SSTR_1$-$SSTR_5$. Pharmacological and structural comparisons suggest that there may be subgroups within the SRIF receptor family, with $SSTR_1$ and $SSTR_4$ receptors comprising one group, and $SSTR_2$, $SSTR_3$ and $SSTR_5$ receptors comprising a second group (Raynor *et al.*, 1993b). In view of the high affinity of the latter group for seglitide (Raynor *et al.*, 1993b), this group may represent members of the $SRIF_1$ receptor group, whilst, $SSTR_1$ and $SSTR_4$ represent members of the $SRIF_2$ receptor group.

Radioligand binding studies on several mammalian cell lines expressing the different recombinant SRIF receptors have identified several peptide analogues that are highly potent and selective for certain subtypes of SRIF receptors (Raynor *et al.*, 1993a,b). Such compounds include BIM 23027, BIM23056 and L362,855 which selectively bind with high affinity to $SSTR_2$, $SSTR_3$ and $SSTR_5$ receptors, respectively (Raynor *et al.*, 1993a,b). However, to date, the functional characteristics of these analogues in preparations naturally expressing SRIF receptors is unknown. In the rat distal colon, the contractile effect of SRIF would not appear to be mediated by a SRIF receptor similar to the cloned $SSTR_3$ and $SSTR_5$ receptors for several reasons. First, BIM 23056 which has high affinity and selectivity for the $SSTR_3$ receptor (Raynor *et al.*, 1993a), was inactive up to concentrations of 1 μM in the rat distal colon. Secondly, the $SSTR_5$ receptor selective analogue, L362,855, (Raynor *et al.*, 1993b) was approximately 120 times weaker than SRIF in the present study. In contrast, BIM23027, which has high affinity and selectivity for the cloned $SSTR_2$ receptor, was the most potent agonist (approximately 12 times more potent than SRIF) examined in the rat distal colon. Our contention that a SRIF-receptor similar to the cloned $SSTR_2$ receptor mediates the contractile effect of SRIF in the rat colon is clearly based on the high agonist potency of BIM23027 and its apparent selectivity for the recombinant $SSTR_2$ receptor (Raynor *et al.*, 1993a,b). Studies in our laboratories (Castro, unpublished) with BIM23027 on recombinant $SSTR_1$ and $SSTR_2$ receptors expressed in LTK^- cells, have confirmed a greater than 10,000 fold selectivity of BIM23027 for the $SSTR_2$ receptor, although the affinity estimate of BIM23027 (IC_{50} of 0.035 nM) for the $SSTR_2$ receptor was somewhat lower than that described by Raynor *et al.*, 1993a. A recent study (Bruno *et al.*, 1993) using solution hybridisation/nuclease protection analysis with sequence specific SRIF-receptor cRNA probes have identified the existence of $SSTR_2$ mRNA in several peripheral rat tissues such as the spleen, pancreas and stomach, however the colon was not examined. Further studies using either *in situ* hybridisation and/or PCR analyses should confirm the presence of $SSTR_2$ receptors in the rat colon. Recently, two splice variants of the $SSTR_2$ receptor have been cloned, the recombinant receptors being termed $SSTR_{2A}$ and $SSTR_{2B}$ (Vanetti *et al.*, 1992). These receptors exhibit similar affinities for $SSTR_2$ receptor-selective agonists such as BIM23027, both are regulated by agonist pretreatment and both mediate their effects through G proteins (Reisine *et al.*, 1993). It was not therefore possible in the present study to determine operationally which of the spliced variants might mediate the contractile effect of SRIF in the rat distal colon.

At present, the exact relationship between recombinant SRIF receptors expressed in cell lines and SRIF receptors expressed naturally in tissues is unclear. For example, there is a large discrepancy between the agonist binding affinity estimates (IC_{50} values) obtained in the radioligand binding studies on the recombinant SRIF receptors (Raynor *et al.*, 1993a,b), and their potencies (EC_{50} values) in the functional study described here and in the guinea-pig (Feniuk *et al.*, 1993). The identification of more potent and selective SRIF receptor antagonists for use in both functional and radioligand binding studies would be invaluable tools in comparing and contrasting operational characteristics of SRIF receptors, both native and recombinant.

The contractile action of all of the SRIF agonists examined in the present study were subject to desensitization, the hexapeptides BIM23027 and MK678 being most susceptible. Studies have also shown that some of the effects of SRIF are susceptible to desensitization. For example, pretreatment with SRIF in anterior pituitary tumour cells has been shown to reduce SRIF receptor coupling to adenylyl cyclase (Reisine, 1985). In contrast, SRIF receptor desensitization selectively abolishes non-adrenergic inhibitory postsynaptic potentials in sympathetically denervated guinea-pig submucosal neurones, and does not appear to involve adenylyl cyclase, protein kinase C or receptor internalization (Shen & Suprenant, 1993). Although the mechanism of desensitization to SRIF was not investigated in the present study, desensitization of preparations by BIM23027 selectively abolished contractile responses to SRIF and $SRIF_{28}$, suggesting that these agonists all act via a common receptor mechanism. Agonist-induced desensitization is a characteristic that has been used to differentiate between types of SRIF receptors. For example, chronic exposure of $SRIF_1$ receptors to SRIF or seglitide, reduces high affinity agonist binding, whilst $SRIF_2$ receptors appear unaffected (Rens-Domiano *et al.*, 1992; Raynor & Reisine, 1992b). It is also noteworthy that the cloned $SSTR_2$ receptor can be readily desensitized by prior exposure to seglitide (Rens-Domiano *et al.*, 1992). The finding that the contractile action of SRIF and $SRIF_{28}$ were readily desensitized by prior exposure to BIM23027 supports further the view that a subtype of the $SRIF_1$ receptor group mediates the contractile action of SRIF in the rat colon.

In summary, we have shown for the first time a contractile action of SRIF and several SRIF analogues in the rat distal colon. Seglitide and octreotide exhibited similar potency and efficacy to SRIF, suggesting that the SRIF receptor mediating the contractile action of SRIF belongs to the $SRIF_1$ receptor group. The high potency of recently identified SRIF receptor-selective ligands such as BIM23027, the weak agonist activity of L362,855 relative to SRIF, and the lack of activity exhibited by BIM23056 suggests that the SRIF receptor subtype mediating the contractile action of SRIF in the rat distal colon in similar to the recombinant $SSTR_2$ receptor. The contractile action of SRIF in the rat distal colon appears to involve activation of non-cholinergic nerves, however the precise site and mechanism involved remains to be elucidated.

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