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Operational characteristics of somatostatin receptors mediating inhibitory actions on rat locus coeruleus neurones

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1 In order to characterize somatostatin (SRIF) receptor inhibiting spontaneous firing of rat locus coeruleus neurones, and their transduction mechanism(S), extracellular recordings were obtained from a pontine slice preparation of rat brain containing the locus coeruleus (LC). LC neurones were identified by electrophysiological and pharmacological properties; spontaneous firing (characteristically 0.5-5 Hz) was reversibly and concentration-dependently inhibited by exogenously applied noradrenaline.

2 Spontaneous firing of LC neurones was reversibly and concentration-dependently inhibited by SRIF and the *N*-terminally extended form, somatostatin-28 (SRIF-28), with EC_{50} values of 15.1 and 19.4 nM, respectively. The synthetic SRIF analogues (octreotide, MK-678, BIM-23027 and L-362,855) also caused concentration-dependent inhibition of LC neurone firing with a rank order of agonist potencies compatible with actions at a receptor resembling the recombinant sst_2 receptor. The putative sst_3 selective agonist, BIM-23056, was without agonist or antagonist effect.

3 Addition of 100 nM desipramine significantly increased the efficacy of exogenously applied noradrenaline (EC_{50} values, 2.96 and 0.13 μM, absence and presence of desipramine, respectively) but did not significantly affect SRIF-induced inhibition (EC_{50} values, 15.6 and 8.0 nM, respectively). Furthermore, application of phenoxybenzamine (3 μM) abolished responses to NA, but did not affect responses to SRIF (EC_{50} = 14.1 nM).

4 Application of the cyclic AMP analogue, 8-bromoadenosine-cyclic monophosphate (8-Br-cyclic AMP; 500 μM), significantly increased the spontaneous firing rate of all neurones tested (223 ± 24% over basal rate). Concentration-effect curves for SRIF constructed in the absence and presence of 8-Br-cyclic AMP had similar threshold concentrations, maxima and EC_{50} values.

5 Incubation of pontine slices in a modified artificial CSF containing 500 ng ml^{-1} pertussis toxin (PTX) for 18 h prior to extracellular recording affected neither the spontaneous firing of LC neurones, nor the inhibitory responses to muscimol (EC_{50} 2.2 and 1.2 μM, absence and presence of PTX). However, inhibitory responses to SRIF were markedly attenuated.

6 We conclude that the inhibitory actions of SRIF on spontaneous firing of LC neurones are mediated directly by activation of somatodendritic SRIF receptors, and not indirectly by release of noradrenaline. The SRIF receptors involved appear to couple via a pertussis toxin sensitive G-protein, and elicit their response by a mechanism apparently independent of inhibition of cyclic AMP formation. The agonist profile of several selective and novel SRIF analogues suggests the identity of this receptor to be similar to the recombinant sst_2 receptor.

Keywords: Locus coeruleus; somatostatin; extracellular recording; pertussis toxin; noradrenaline

Introduction

Somatostatin (SRIF) has a wide distribution and a variety of actions within the central nervous system (Epelbaum, 1986; Kiyama & Emson, 1994). Binding sites for this peptide are abundant in the rat locus coeruleus (Moyse *et al.*, 1992), and previous studies have described inhibitory actions of SRIF on spontaneous firing of neurones within this nucleus (Chiu *et al.*, 1994), involving the opening of an inwardly rectifying K^+ channel (Inoue *et al.*, 1988). However, the receptor type(s) mediating these effects, and their mechanism of transduction is largely unexplored.

Heterogeneity of SRIF receptors was originally suggested from radioligand binding studies in rat brain (for review, see Raynor & Reisine, 1992). More recently, molecular cloning studies have revealed five distinct types, termed sst_1-sst_5, belonging to the seven transmembrane domain superfamily of G-protein coupled receptors (see Hoyer *et al.*, 1995). Pharmacological characterization of these receptor types has been impeded by the lack of selective compounds. More recently, however, several structurally related SRIF analogues have been identified which display selectivity for the recombinant receptors, including BIM-23027, BIM-23056 and L-362,855, which exhibit high affinities for sst_2, sst_3, and sst_5 receptors, respectively (Raynor *et al.*, 1993 a,b).

In order to characterize the receptor involved in inhibition of rat locus coeruleus neurones, extracellular recordings using a single carbon fibre electrode were made from these neurones, and the actions of several SRIF related analogues with distinct agonist profiles at the different recombinant receptors were investigated. Activation of SRIF receptors has been reported to release noradrenaline (NA) in at least two separate systems (Tsujimoto & Tanaka, 1981; Panconesi *et al.*, 1987). Thus, the possibility of an indirect action of SRIF, inhibiting locus coeuleus (LC) neurones by release of NA, was also investigated. In addition, SRIF receptors have been reported to inhibit formation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) in both 'isolated cells' (Patel *et al.*, 1994) and brain (Markstein *et al.*, 1989), and SRIF-induced neuronal hyperpolarization has been found to be insensitive to pertussis

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toxin treatment in some neuronal systems (Twery *et al.*, 1991). Therefore, in this study, we have examined these operational mechanisms to determine the manner by which SRIF receptors mediate inhibition of LC neurone activity. Functional studies of this type should help to elucidate operational characteristics of neuronal SRIF receptors, as part of an attempt to provide functional correlates between endogenous receptors in whole tissues and recombinant SRIF receptors. In addition, it is hoped that an understanding of the receptor types involved in neuronal SRIF function will help to identify potential therapeutic targets in the future.

A preliminary account of some of this work has been presented at a meeting of the British Pharmacological Society (Black *et al.*, 1995).

Methods

Preparation of brain slices

Male AHA Sprague-Dawley rats (150–200 g) were humanely killed by cervical dislocation. The brain was rapidly removed and irrigated with ice-cold modified artificial cerebral spinal fluid (ACSF) buffer (containing, in mM, NaCl 125, $NaHCO_3$ 25, KCl 1.9, KH_2PO_4 1.3, $MgSO_4$ 2, D-glucose 11 and $CaCl_2$ 2). The brain stem was excised and mounted caudal side down onto a vibrotome block with cyanoacrylate glue. Coronal sections (350 μm) were collected from approximately bregma −9.0 mm to bregma −10.3 mm (Paxinos & Watson, 1982). During these procedures, the brain stem was maintained in ice-cold oxygenated ACSF. Two of the sections were transferred onto a platinum grid and placed in a recording chamber maintained at 32°C and perfused at a rate of 2 ml min^{-1} with oxygenated buffer. A nylon mesh was used to hold the slices in place and no further manipulations were made for 45 min.

Extracellular recording

Extracellular unit recordings were made using a single carbon-fibre electrode placed into the LC, which was visible as a translucent area on the ventrolateral border of the 4th ventricle. Precise adjustment of electrode placement was performed using a motorised micro-manipulator (PM-10; Microinstruments, U.K.). LC neurones were identified by their characteristic regular firing (frequency of 0.5–4 Hz; Figure 1) and depression of firing frequency by exogenously added NA. Spikes were discriminated using standard Neurolog units, or by Spike2 software (Cambridge Electronic Design, Cambridge, U.K.; Figure 1). Single unit recording was confirmed by analysis of the stimulus interval histogram (Figure 1). The integrated rate of firing was determined using standard Neurolog modules (Digitimer Ltd., Hertford, U.K.) or Spike2 software.

Experimental procedures

Following a 45 min equilibration period, neurones were identified by application of 30 μM NA, which abolished spontaneous firing (Figure 1). After a further 45 min, during which basal rate recordings were made, cumulative concentration-effect curves, in separate tissue preparations, for inhibition of

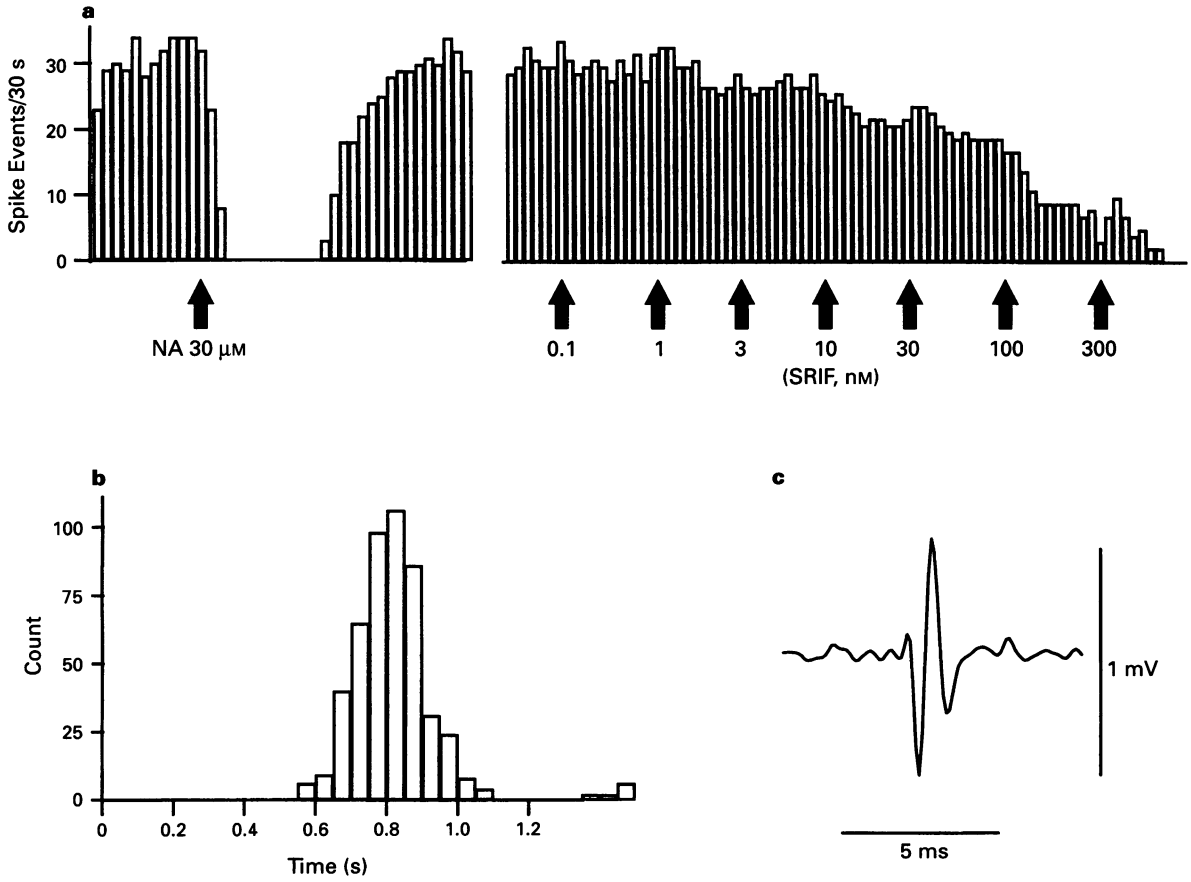

Figure 1 (a) Discontinuous records of integrated spontaneous firing rate of a locus coeruleus neurone. Application of noradrenaline (NA) 30 μM abolished spontaneous firing, and following a 45 min recovery period (interval not shown), a cumulative concentration-effect curve to somatostatin (SRIF) was constructed. Each bar represents 30 s. (b) Spike interval histogram for the same neurone confirming single unit recording. The abscissa scale shows the interval(s) between successive discriminated spikes, and the ordinate the number of spikes per time bin. (c) Representative discriminated spike.

spontaneous firing induced by SRIF or NA was determined. Each concentration of agonist was applied for 5 min, during which peak effects were observed. Following a 45 min washout period, a second concentration-effect curve was determined for SRIF, NA or one of the SRIF analogues. In some cases, first and second concentration-effect curves to NA or SRIF were determined in the absence and presence of 100 nM desipramine, respectively.

In experiments where the effects of phenoxybenzamine (PBZ) were investigated, a test concentration of NA (10 μM) was applied to the slice preparation, and following a washout period (30 min), PBZ (3 μM) was superfused for 45 min. Following a second dose of NA, a concentration-effect curve to SRIF was determined after a 30 min washout period, and finally, a third test dose of NA was applied to the tissue.

To investigate the effects of the cyclic AMP analogue, 8-bromoadenosine-cyclic monophosphate (8-Br-cyclic AMP), on SRIF-induced inhibition of spontaneous firing, a concentration-effect curve was determined for SRIF, and following a 45 min washout period, 8-Br-cyclic AMP (500 μM) was added to the buffer and perfused during and for at least 30 min prior to construction of a second concentration-effect curve to SRIF. The effects of forskolin (3 μM) on basal firing rate were examined by comparison of mean firing rates before and during a 30 min application of this compound.

For experiments to investigate the effects of pertussis toxin (PTX) pre-treatment on the effect of SRIF, a modified protocol was adopted. Slices were obtained as described above, but maintained in the recording chamber in a modified oxygenated buffer, containing (in mM): NaCl 116, $NaHCO_3$ 26.2, NaH_2PO_4 1.0, KCl 5.4, $MgSO_4$ 1, D-glucose 24.6, $CaCl_2$ 1 and gentamicin 0.005%. Slices were incubated for 18 h at 32°C in the presence or absence of 500 ng ml^{-1} PTX in a re-circulating stock (100 ml) of this buffer. Following this incubation, cumulative concentration-effect curves to SRIF and the γ-aminobutyric acid receptor ($GABA_A$) agonist, muscimol (as a positive control), were determined, using fresh buffer of the same composition and in the continued presence of PTX. A further series of experiments were performed using the same protocol, but in the absence of PTX.

Analysis of results

For each concentration-effect curve, a basal spike frequency was determined just prior to application of the first drug concentration. The depression of spontaneous firing rate was measured as a decrease in the integrated spike frequency over periods of 30 s, and expressed as a percentage of the measured basal rate. Where appropriate, concentration-effect curves were fitted using a four parameter logistic equation (GraphPad Prism, GraphPad Software Inc., San Diego, U.S.A.).

For SRIF and its analogues, equi-effective molar ratios (EMRs) were calculated between first and second agonist concentration-effect curves, constructed from the same tissue, at a level of 50% of the maximum of the first curve to SRIF. The EMR calculated between first and second curves to SRIF was used as a correction factor to normalise all EMR values for the related analogues, in order to allow for any spontaneous change in sensitivity to SRIF over the time course of the experiment.

For effects of 8-Br-cyclic AMP and desipramine on NA or SRIF-induced inhibition of spontaneous firing, EC_{50} values were calculated and compared for the first and second agonist concentration-effect curves in the absence or presence of 8-Br-cyclic AMP or desipramine. The effects of phenoxybenzamine (PBZ) were investigated by comparison of EC_{50} values for first agonist concentration-effect curves to SRIF from separate experiments with or without addition of PBZ.

Data are expressed as arithmetic mean $\pm$ s.e.mean or geometric mean with 95% confidence intervals where appropriate. Differences within experimental groups were assessed (where appropriate) using Student's paired t test, and between groups using an unpaired t test.

Chemicals

SRIF and SRIF-28 were obtained from Peninsula Laboratories Inc. (California, U.S.A.). BIM-23027 (c[N-Me-Ala-Try-D-Trp-Lys-Abu-Phe]), BIM-23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2), MK-678 (Seglitide; cyclo [N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe]) and L-362,855 (c[Ala-Phe-Trp-D-Trp-Lys-Thr-Phe]) were synthesized by Medicinal Chemistry, Glaxo Research and Development.

Octreotide (Sandostatin) was purchased from a pharmaceutical retailer. Muscimol was purchased from RBI (Herts, U.K.). Phenoxybenzamine (PBZ) was obtained from Calbiochem-Novabiochem (Nottingham, U.K.). Gentamicin was purchased from GibcoBRL (Paisley, U.K.). All other chemicals and compounds were purchased from Sigma Chemicals (Poole, U.K.) or BDH Laboratory Supplies (Leicester, U.K.).

Results

Effects of SRIF and related analogues on locus coeruleus neurone firing rate

Cumulative application of SRIF to the slice preparation caused a concentration-dependent decrease in the spontaneous firing rate (see Figure 1) with an EC_{50} of 11.8 nM (geometric mean; 95% confidence intervals, 8.4 – 16.6 nM, $n = 30$) and a mean Hill slope of 1.4 ± 0.07. The Gaussian distribution of the interspike interval was shifted rightwards, but no alteration in spike am-

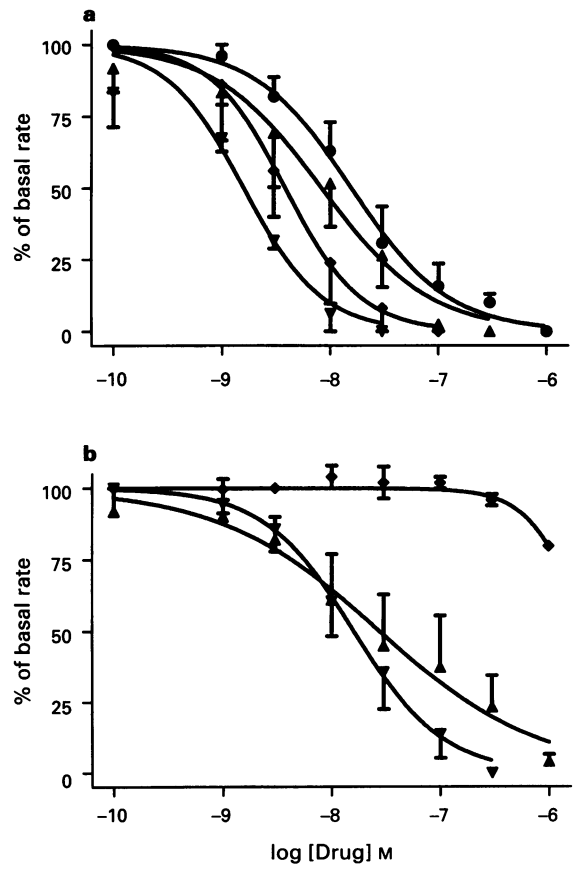

Figure 2 Concentration-effect curves for somatostatin (SRIF) and related analogues for inhibition of spontaneous firing of rat LC neurones. All data are second concentration-effect curves following first curves to SRIF. Values are mean $\pm$ s.e.mean from 3–5 experiments for (a) (●) SRIF, (▲) BIM-23027, (▼) octreotide, (◆) MK-678 and (b) (▲) SRIF-28, (▼) L-362,855, and (◆) BIM-23056.

plitude or shape was observed. Following a 45 min washout period, a second concentration-effect curve was constructed to SRIF, yielding similar EC_{50} and Hill slope values (EC_{50} 15.1 [5.0–45.2] nM, Hill slope 1.6±0.5, $n=4$; Figure 2). As this second concentration-effect curve to SRIF was highly reproducible, EMRs to SRIF-related analogues were determined by substitution of an analogue for SRIF for the second concentration-effect curve (Table 1). The analogues exhibiting the greatest potency were octreotide, BIM-23027 and MK-678 (Figure 2). BIM-23056 was virtually devoid of agonist activity (Figure 2) and displayed no antagonist activity against SRIF (data not shown).

Effects of noradrenaline, desipramine and phenoxybenzamine

Addition of a low concentration of the monoamine uptake inhibitor, desipramine (100 nM), which itself did not affect the rate of spontaneous firing at this concentration, caused a significant leftward shift of the NA concentration-effect curve (EC_{50} values, 2.96 [0.54–16.39] and 0.13 [0.03–0.48] μM, absence and presence of desipramine, respectively $P<0.05$, $n=4$; Figure 3), while first and second concentration-effect curves for NA in the absence of desipramine were similar (EC_{50} values 3.4 [0.7–18.0] and 3.3 [0.7–15.8], $n=3$). Concentration-effect curves to SRIF were not significantly affected by the addition of 100 nM desipramine (EC_{50} values, 15.6 [4.0–60.9] and 8.0 [2.0–32.4] nM, absence and presence of desipramine, respectively, $n=4$; see Figure 3).

In experiments to investigate the effects of PBZ, the first test concentration of NA (10 μM) abolished spontaneous firing in all cases. Following addition and washout of PBZ, the second test dose of NA was without effect. Agonist concentration-effect curves to SRIF were similar to those observed in experiments performed in the absence of PBZ ($EC_{50}=14.1$ [1.9–101.7] nM, $n=3$). The third test concentration of NA was also without effect.

Effect of 8-bromoadenosine-cyclic monophosphate and forskolin

Application of the non-hydrolysable cyclic AMP analogue, 8-Br-cyclic AMP (500 μM), significantly increased the spontaneous firing rate of all neurones investigated to 223±24% over basal firing rate ($P<0.001$, $n=4$). However, application of 8-Br-cyclic AMP 30 min prior to construction of a second concentration-effect curve to SRIF did not reveal any alteration in the apparent potency of SRIF to inhibit completely spontaneous firing (EC_{50}, 7.6 [4.1–13.9] and 10.2 [2.7–38.2] nM, absence and presence of 8-Br-cyclic AMP, respectively). In common with 8-Br-cyclic AMP, application of forskolin (3 μM) also significantly increased the firing rate to 231.5±3.6% of basal rate.

Effect of pretreatment with pertussis toxin on SRIF-induced responses

Pretreatment of pontine slices with pertussis toxin (PTX) required an overnight incubation of the slice in modified buffer, and it was therefore necessary to control for any tissue degeneration associated with this protocol. Therefore, experiments were performed with prior overnight incubation of the slice in either the absence and presence of PTX (500 ng ml^{-1}). Overnight incubation of the slice did not affect the generation of spontaneous action potentials, or the ability of SRIF to abolish spontaneous firing (EC_{50}, 7.7 [0.6–97.1] nM, Hill

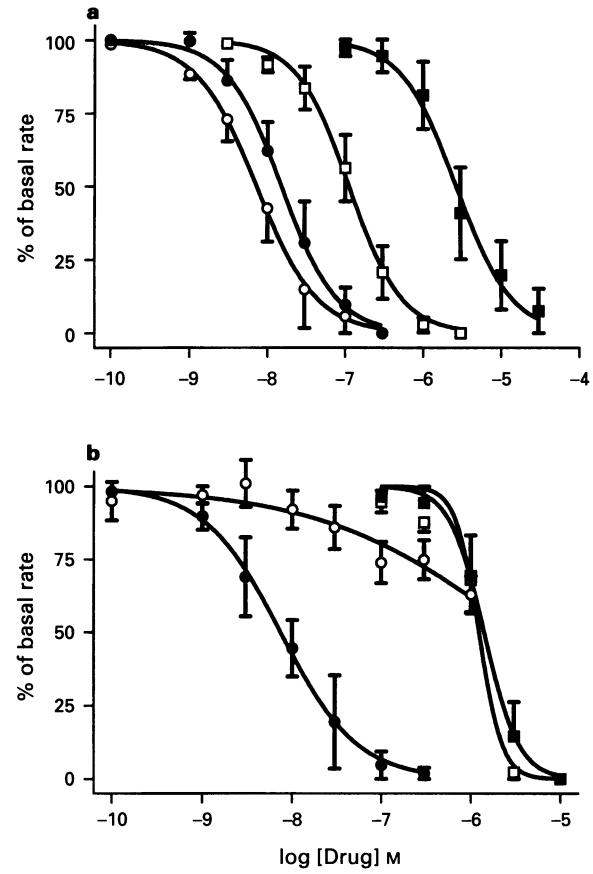

Figure 3 (a) Concentration-effect curves for noradrenaline (NA) and somatostatin (SRIF) in the absence (■ and ●, NA and SRIF, respectively) and presence (□ and ○) of 100 nM desipramine, first and second curves, respectively. Values are mean±s.e.mean from 4 experiments for each drug. No significant differences were observed between EC_{50} or Hill slope values for SRIF in the absence or presence of desipramine, and each data point from respective curves was not significantly different, with the exception of data for 1 nM SRIF ($P=0.01$, Student's paired t test). (b) Concentration-effect curves for SRIF and muscimol constructed from slices following an 18 h pretreatment in the absence (● and ■, SRIF and muscimol, respectively) or presence (○ and □) of 500 ng ml^{-1} PTX. Data are curves obtained following incubation as described in Methods section, mean±s.e.mean from 3 experiments per drug.

Table 1 Relative potencies of somatostatin (SRIF) and related analogues for inhibition of spontaneous firing of rat LC neurones

	EC_{50} (nM)	*EMR*	*Hill slope*	*n*
SRIF	15.1 [5.0–45.2]	1.0 [0.5–2.6]	1.6±0.5	4
SRIF-28	19.4 [1.3–293.5]	1.2 [0.1–10.6]	1.4±0.4	5
BIM-23027	6.2 [0.2–16.1]	0.15 [0.1–1.1]	1.3±0.2	4
MK-678	4.2 [0.8–20.5]	0.33 [0.1–0.8]	1.9±0.2	4
Octreotide	1.3 [0.3–6.1]	0.2 [0.1–2.6]	1.3±0.3	3
L-362,855	16.7 [3.8–73.1]	2.6 [1.0–6.8]	1.5±0.4	4
BIM-23056	inactive	–	–	3

EC_{50} and equipotent molar ratio (EMR) values are geometric means with 95% confidence intervals. Hill slope values are mean±s.e.mean.

slope, 1.15±0.11, $n=3$). Pre-incubation with PTX substantially reduced the inhibitory effects of SRIF (see Figure 3), such that inhibitory responses to SRIF at concentrations greater than 3 nM were significantly ($P<0.05$, Student's t test) smaller than those observed in the absence of PTX (e.g. reduction of 24.7±6.7 and 94.2±4.7% of basal firing rate at 300 nM SRIF, presence and absence of PTX, respectively). However, no significant difference in the ability of muscimol to abolish spontaneous firing was observed between slices pre-incubated in the absence or presence of PTX (EC_{50}, 2.2 [0.7–6.8] and 1.2 [0.7–1.9] μM, respectively, $n=3$, Figure 3).

Discussion

Somatostatin (SRIF) and several of the related analogues tested, produced a concentration-dependent inhibition in spontaneous firing of rat LC neurones. In addition, this inhibition of firing was not dependent upon release of endogenous NA, and appeared to be independent of mechanisms involving inhibition of cyclic AMP formation. A novel methodology was employed to pretreat pontine slices with pertussis toxin; this pretreatment revealed that the effects of SRIF receptor activation are mediated via a pertussis-sensitive G-protein.

Recently, a number of receptor-selective peptides have been identified using various recombinant SRIF receptor types stably transfected into different cell lines (Raynor *et al.*, 1993a, b). These peptides include BIM-23027, BIM-23056 and L-362,855, which show selective affinity for recombinant sst_2, sst_3, and sst_5 receptors, respectively. However, the functional effects of these peptides in tissue preparations, particularly of the CNS, remain largely unexplored. In the present study, the sst_2 selective ligand, BIM-23027, was found to be a potent agonist in the pontine slice preparation, being more potent than SRIF itself. The sst_5 selective peptide, L-362,855, was less potent than SRIF, and the sst_3 selective peptide, BIM-23056, was without effect. In addition, MK-678 has been reported to display some selectivity for the recombinant sst_2 receptor (Hoyer *et al.*, 1994), and in the present study was approximately three times more potent than SRIF itself.

In binding studies with transfected recombinant receptors, the reported affinity of the putative sst_5 receptor ligand L-362,855, was approximately 90 fold lower than SRIF itself at sst_2 receptors, but 150–200 fold greater than SRIF at sst_5 receptors (Hoyer *et al.*, 1994). However, other recent data suggest that functional selectivity of L-362,855 is less marked than that found in binding studies. In a mouse fibroblast cell line transfected with the human sst_2 receptor, this compound was approximately 30 fold less potent than SRIF (Castro *et al.*, 1995), while in studies of inhibition of basal secretion in rat distal colonic mucosa, L-362,855 was only 5 times less potent that SRIF at a putative sst_2 receptor (McKeen *et al.*, 1995). Thus, the high potency of L-362,855 in the present study may be consistent with its actions at sst_2, rather than sst_5, receptors. We conclude, therefore, that in the present study, the preparation contains a receptor similar to the recombinant sst_2 receptor, but admittedly, the additional involvement of an sst_5 receptor cannot be excluded definitively.

The inhibitory effects of SRIF and its analogues on the firing of LC neurones did not exhibit desensitization (i.e. no fade of responses was observed during agonist application, and successive identical doses yielded similar responses). This is in contrast to other studies (e.g. Feniuk *et al.*, 1995), where profound desensitization was observed. It is tempting to speculate that this reflects activation of different isoforms of the sst_2 receptor, as it has been suggested that of the alternative splice variants of the sst_2 receptor, the longer form is more prone to desensitization (Vanetti *et al.*, 1993). However, at the present time, the two isoforms of the sst_2 receptor cannot be distinguished pharmacologically.

It has been claimed that SRIF can cause release of endogenous amine transmitters from various brain regions (Tsujimoto & Tanaka, 1981; Tanaka & Tsujimoto, 1981; Chesselet & Reisine, 1983). Thus, in order to determine whether the inhibitory effects of SRIF on the spontaneous firing of LC neurones was attributable to release of endogenous NA, experiments were performed to manipulate the effects of NA. Under conditions where uptake of NA was blocked using a low concentration of desipramine, which alone had no effect on the spontaneous firing of LC neurones, the potency of NA for inhibition of spontaneous firing was increased by approximately 20 fold, while the potency of SRIF was unaffected. In addition, phenoxybenzamine abolished inhibitory responses to NA, but did not affect inhibitory responses to SRIF. Thus we were able to demonstrate that SRIF-induced inhibition of spontaneous firing of LC neurones was independent of release of endogenous NA.

Somatostatin receptors have been reported to couple to a diversity of second messenger transducing systems, including inhibition (Patel *et al.*, 1994) or stimulation (Markstein *et al.*, 1989) of cyclic AMP formation, or modulation of the concentration of inositol-1,4,5-triphosphate (IP_3) (Lachowicz *et al.*, 1994). In addition, there is evidence that SRIF receptors may couple, via a G-protein, to modulate directly the activity of Ca^{2+} (Wang *et al.*, 1990) or K^+ (Shefner & Chiu, 1986; Tatsumi *et al.*, 1990) channels. In the present study, we have used the cyclic AMP analogue, 8-Br-cyclic AMP, to investigate whether the inhibitory effects of SRIF in the LC are mediated by alterations in cyclic AMP concentrations. In contrast to previous studies (Andrade & Aghajanian, 1985), exogenous application of 8-Br-cyclic AMP significantly increased the basal firing rate of all neurones tested, as did the adenylyl cyclase activator, forskolin. The possibility that 8-Br-cyclic AMP was hydrolysed to yield adenosine is unlikely, as adenosine has been shown to have inhibitory effects at LC neurone receptors (Shefner & Chiu, 1986). Taken together, these data indicate the increases in basal firing rate observed during application of 8-Br-cyclic AMP are likely to be due to its mimicking the actions of intracellular cyclic AMP. Under these circumstances, no alterations in the agonist profile of SRIF were observed, with similar thresholds, maxima, and EC_{50} values, suggesting that, in this case, the actions of SRIF are mediated by a mechanism which is independent of inhibition of adenylyl cyclase, lowering intracellular cyclic AMP concentrations.

In order to investigate the effects of pretreatment with PTX, we employed a novel methodology whereby the slices were incubated overnight in a modified buffer containing PTX. Somatostatin was able to abolish spontaneous firing in similarly incubated slices in the absence of PTX, while its effects were significantly attenuated in slices pretreated with PTX. Under identical conditions, reponses to muscimol were unaffected, consistent with the action of an agonist which acts via a ligand gated ion channel ($GABA_A$ receptor), which does not depend on a PTX-sensitive G-protein to elicit a response (for review, see McDonald & Olsen, 1994). These effects are consistent with other studies (Inoue *et al.*, 1988; Tatsumi *et al.*, 1990) where PTX inhibited a SRIF-induced K^+ conductance, although SRIF-induced hyperpolarization of septal neurones was not inhibited by PTX pretreatment (Twery *et al.*, 1991). In the LC, however, it is likely that the receptor mediating inhibition of spontaneous firing couples via a PTX sensitive G_i/G_o protein to a K^+ channel, and it is tempting to speculate that this is the same K^+ channel which mediates the inhibitory effects of α_2-adrenoceptors and μ-opioid receptors in this brain region (North *et al.*, 1987).

In summary, the results from this study, using some recently identified SRIF receptor selective ligands, suggest that inhibition of spontaneous firing of rat LC neurones is mediated via a SRIF receptor which is similar to the recombinant sst_2 receptor. Furthermore, evidence has been presented that suggests that this inhibition is mediated by a direct receptor effect, and is independent of release of noradrenaline. This receptor

mediates its effects by interaction with a pertussis toxin sensitive G-protein, whose transduction mechanism appears to be independent of alterations in intracellular cyclic AMP concentration. Studies such as these, which explore the operational characteristics of SRIF receptors in functional systems, will aid the understanding of the diverse actions of SRIF as a central neurotransmitter.

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