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Outward current produced by somatostatin (SRIF) in rat anterior cingulate pyramidal cells *in vitro*

[1]G.A. Hicks, W. Feniuk & P.P.A. Humphrey

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

1 A high density of receptors for somatostatin (SRIF) exists in the anterior cingulate cortex but their function is unknown. Whole-cell patch clamp recordings were made from visualized deep layer pyramidal cells of the rat anterior cingulate cortex contained in isolated brain slices to investigate the putative effects of SRIF and to identify the receptor subtype(s) involved.

2 SRIF (1–1000 nM) produced a concentration-dependent outward current which was associated with an increased membrane conductance, was sensitive to Ba^{2+} (300 μM–1 mM), and was absent in the presence of a maximal concentration of the $GABA_B$ receptor agonist, baclofen (100 μM). These observations suggest the outward current was carried by K^+ ions.

3 SRIF analogues also elicited outward currents with a rank potency order of (EC_{50}, nM): octreotide (1.8) > BIM-23027 (3.7) > SRIF (20) = L-362,855 (20). BIM-23056 was without agonist or antagonist activity. Responses to L-362,855 were unlike those to the other agonists since they were sustained for the duration of the application.

4 The sst_2 receptor antagonist, L-Tyr[8]Cyanamid 154806 (1 μM), had no effect alone but partially reversed responses to submaximal concentrations of SRIF (100 nM, 44±6% reversal) and L-362,855 (100 nM, 70±6% reversal) and fully reversed the response to BIM-23027 (10 nM). In contrast, L-Tyr[8]Cyanamid 154806 did not antagonize the response to baclofen (10 μM).

5 We conclude that SRIF activates a K^+ conductance in anterior cingulate pyramidal neurones via an action predominantly at sst_2 receptors.

Keywords: Somatostatin; L-362,855; sst_2 receptor; sst receptor antagonist; Cyanamid 154806; potassium current; brain slice; anterior cingulate cortex; analgesia

Introduction

Receptors for the cyclic tetradecapeptide neurotransmitter somatostain-14 (SRIF) have been identified in many central nuclei (Epelbaum *et al*., 1982; Tran *et al*., 1984; Reubi & Maurer, 1985; Uhl *et al*., 1985; Katayama *et al*., 1990; Martin *et al*., 1991), including several areas involved in the processing of noxious sensory information. Many studies have shown analgesic activity of SRIF receptor ligands, but the mechanisms by which such analgesia is produced is not understood (Humphrey, 1996). The anterior cingulate cortex contains neurones which are activated by peripheral noxious stimuli (Sikes & Vogt, 1992; Yamamura *et al*., 1996; Hsu & Shyu, 1997) and functional imaging studies in man have strongly implicated this area in the perception of pain (Jones *et al*., 1991; Talbot *et al*., 1991; Casey *et al*., 1994; Craig *et al*., 1996; Rainville *et al*., 1997). These observations suggest that inhibitors of pyramidal cell activity in the anterior cingulate cortex may be useful, novel analgesic agents.

The anterior cingulate cortex contains a high density of SRIF receptors (Uhl *et al*., 1985; Martin *et al*., 1991). Early findings suggested a preferential location in the deeper cortical layers (Uhl *et al*., 1985; Martin *et al*., 1991) but also the possible presence of more than one receptor subtype in the area (Reubi, 1984; 1985; Reubi *et al*., 1987; Martin *et al*., 1991). Radioligand binding studies with a selective sst_2 receptor agonist, c[N-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe] ([^{125}I]-BIM-23027; Holloway *et al*., 1996), confirmed the presence of this subtype in the deeper cortical layers. Furthermore, using a selective antibody, Schindler and colleagues (Schindler *et al*., 1997) localized the sst_{2a} isoform to pyramidal neurone cell bodies of the deeper layer but also found strong staining of the ascending dendrites of these cells.

SRIF receptors couple to and activate K^+ channels to produce neuronal inhibition in many tissues. Probably the best characterized effects of SRIF in the CNS are those in the locus coeruleus where sst_2 receptor activation increases an inwardly rectifying K^+ conductance and inhibits neuronal firing (Inoue *et al*., 1988; Chessell *et al*., 1996). A similar current is activated in submucous plexus (Mihara *et al*., 1987) and in AtT-20 pituitary cells (Pennefather *et al*., 1988). SRIF also activates the M-current in hippocampus (Moore *et al*., 1988), adenosine 5′-triphosphate (ATP)-sensitive K^+ channels in insulinoma cells (Fosset *et al*., 1988) and Ca^{2+}-activated K^+ channels in GH4C1 pituitary cells (White *et al*., 1991) and insulin secreting cells (Ribalet & Eddlestone, 1995). In cultured neurones of the neocortex there is evidence for multiple SRIF receptors coupled to delayed rectifier K^+ channels (Wang *et al*., 1989; 1990). To date, no study has been made of the action of SRIF upon anterior cingulate cortical neurones in brain slices. We have determined the effect of SRIF on visualized rat anterior cingulate cortical pyramidal neurones using whole-cell voltage clamp recording and have employed a range of receptor-selective agonists and the L-Tyr[8] isomer of a novel sst_2 receptor antagonist (Bass *et al*., 1996), in an attempt to identify the SRIF receptor subtype which we now show mediates the production of an outward current in these cells.

[1] Author for correspondence.

Methods

Preparation and maintenace of brain slices

Slices of anterior cingulate cortex (350 μm) were prepared from young male Sprague-Dawley rats (7–21 days; no age-dependent differences in responses to somatostatin receptor ligands were observed), by use of methods described previously for the preparation of midbrain slices (Hicks & Henderson, 1992). Briefly, rats were killed by cervical dislocation followed by a blow to the thorax to ensure permanent cessation of circulation. Coronal slices of forebrain were prepared with a vibrotome and were maintained in artificial cerebrospinal fluid (ACSF) at 33–35°C. Slices were transferred to a tissue bath as required and superfused (2 ml min^{-1}) with ACSF at 35°C. Only slices anterior to the most rostral extent of the caudate putamen were used. The ACSF contained (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.24, $MgCl_2$ 1.3, $CaCl_2$ 2.4 $NaHCO_3$ 26, glucose 10, and was gassed with 95% O_2/5% CO_2, pH 7.3–7.4, (300–310 mOsm). Drugs were applied by superfusion with a solution that differed only in its content of the drug.

Identification of pyramidal neurones

Previous studies have distinguished anterior cingulate pyramidal cells from non-pyramidal cells by their lower input resistance, longer action potential duration and the presence of action potential accommodation (McCormick *et al*., 1985; Tanaka & North, 1994). In addition, fast spiking non-pyramidal neurones are smaller and have significantly shorter action potentials (McCormick *et al*., 1985). However it has recently been shown that non-pyramidal neurones (in the sensory-motor cortex) also display action potential accommodation (Cauli *et al*., 1997). Therefore, in the present study, neurones visualized in the deep layers of the cortex were identified as pyramidal by their pyramidal shape, large size (maximal horizontal × vertical diameter greater than 25×15 μm) and the presence of a large, medially-directed apical dendrite. Morphologically identified pyramidal neurones were used only if the resting membrane potential exceeded −60 mV and action potential duration was greater than 1 ms. Neurones were tested for action potential accommodation (500 ms step direct current injection to a membrane potential just above threshold for action potential firing) and were discarded if it was not present.

Electrophysiological recording

Whole-cell patch clamp recordings (Hamill *et al*., 1981) were made by use of patch pipettes made from borosilicate glass (Clark Electromedical, U.K.) having resistances of 2–4 MΩ. Electrodes were filled with a solution (296 mOsm) containing (mM): Kgluconate 150, HEPES (free acid) 10, $MgCl_2$ 1, $CaCl_2$ 0.7, EGTA 1, MgATP 4, Tris-GTP 0.3. The pH of the solution was adjusted to 7.3 with KOH. A junction potential of −13.8 mV was calculated (Axoscope 1.1) and was taken into account during analysis. Measurement of current was made with an Axopatch 1D patch clamp amplifier controlled with pClamp 6 software (Axon Instruments, U.S.A.). Amplified signals were filtered at 2 or 5 kHz with an 8 pole Bessel filter (Frequency Devices, MA), and plotted on a Gould WindoGraf chart recorder. Unfiltered signals were simultaneously digitized and recorded onto digital audio tape (sampling frequency 48 kHz; Biologic DTR 1204, supplied by Intracell, U.K.). For presentation in figures recorded signals were re-acquired by use of Axoscope 1.1 (Axon Instruments, U.S.A). Series resistance was measured and monitored intermittently by use of voltage steps during the course of experiments but was not compensated. Typically, series resistance was between 5 and 12 MΩ. Data were included in the analysis as long as series resistance did not rise by more than 20% during the recording.

Experimental protocol and data analysis

Outward currents in response to application of the $GABA_B$ receptor agonist, baclofen, and SRIF were non-additive which suggests that the same population of channels was activated by these ligands (see North, 1989). Therefore, the response to SRIF (and other SRIF receptor ligands, see below) was expressed as a percentage of the response to either a prior or subsequent application of 10 μM (+)-baclofen, in order to control for the variation of absolute current amplitude between cells. Thus, for determination of concentration-effect relationships, the current evoked by each concentration of agonist was expressed as a percentage of the current response to 10 μM baclofen. Due to desensitization of responses to SRIF and the analogues, only one concentration was tested in each slice. Concentration-effect curves were obtained by pooling data from individual experiments. Curves were fitted by non-linear regression with GraphPad Prism and EC_{50} values were determined as the concentration producing 50% of each agonist maximum, as estimated from the best single site fit of the whole data set. Each data point is the arithmetic mean ± s.e.mean of at least 3 separate experiments except where stated.

Drugs and solutions

SRIF was from Peninsula Laboratories (St. Helens, U.K.) and octreotide (Sandostatin®) was purchased from a pharmaceutical supplier. Peptides were custom synthesized by Neosystem Laboratoire (Strasbourg, France; BIM 23027 (c[N-Me-Ala-Try-D-Trp-Lys-Abu-Phe]), L-362,855 (c[Ala-Phe-Trp-D-Trp-Lys-Val-Phe]), L-Tyr^{8}Cyanamid 154806 (AcNH-4-NO_2-Phe-c[D-Cys-Tyr-D-Trp-Lys-Thr-Cys]-L-Tyr-NH_2) or by Washington Singer Laboratories (University of Exeter; BIM-23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2). (+)-Baclofen was from RBI (supplied by Semat Technical (UK) Ltd) and (**RS**)-baclofen was from Tocris (Langford, U.K.). All other drugs and chemicals were from Sigma Chemical Co. Ltd. (Poole, U.K.) or Fisher Scientific (Leicester, U.K.) except $CaCl_2$ (1 M stock solution) which was from Fluka BioChemika (Dorset, U.K.).

The original description of the sst_2 receptor antagonist Cyanamid 154806 contained an error in that the amino acid at position 8 was shown to be L-Tyr rather than D-Tyr isomer (Bass *et al*., 1996; erratum, Bass *et al*., 1997). The L-Tyr^8 isomer of Cyanamid 154806 was used in the present study. This peptide has been more fully characterized by Feniuk *et al*. (1998) as a potent and selective sst_2 receptor antagonist.

Results

Characterization of SRIF-induced outward current

When neurones were voltage clamped at approximately −74 mV, application of SRIF (1–1000 nM) resulted in the activation of an outward current (Figure 1a); 97% ($n = 34$) of the identified deep layer pyramidal neurones tested responded

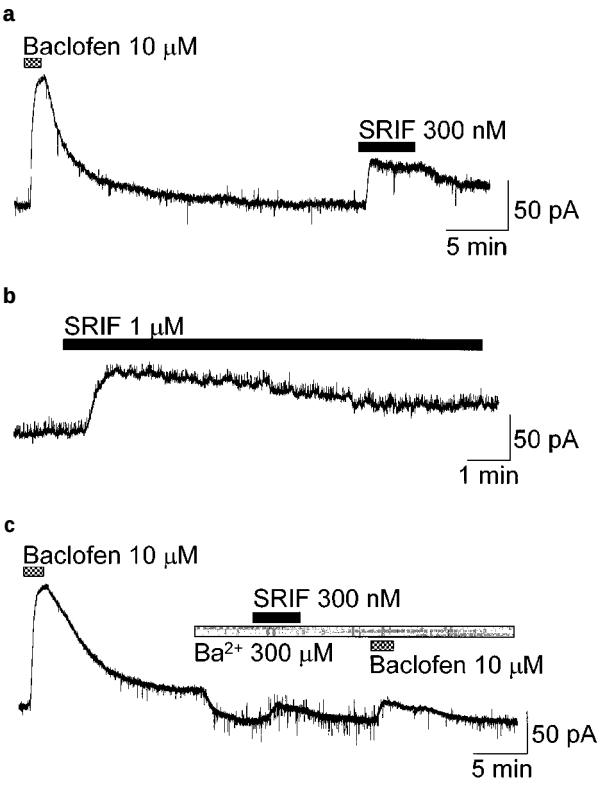

Figure 1 Effect of SRIF on membrane currents of rat anterior cingulate pyramidal neurones. (a) Outward membrane currents produced by baclofen (10 μM) and SRIF (300 nM) in a neurone voltage clamped at -74 mV. Drugs were applied by bath perfusion for the duration shown by the hatched (baclofen) and solid (SRIF) bars. Note the fade of the response in the continued presence of SRIF which was more pronounced with higher agonist concentration (b). (c) Current responses to both agonists were markedly reduced in the presence of Ba^{2+} (300 μM, stippled bar). Note the inward current produced by Ba^{2+}. In this and all subsequent figures holding potential was -74 mV.

to SRIF. All neurones which responded to SRIF also responded to the application of the $GABA_B$ receptor agonist, baclofen (10 μM, Figure 1a), with an activation of an outward current. The mean maximum current, elicited by 300 nM SRIF, was 55 ± 9 pA ($44.6\pm3.9\%$ of the current produced in response to 10 μM baclofen in those neurones, $n=5$). Higher concentrations of SRIF (300–1000 nM), produced outward currents which were not sustained but faded in the continued presence of agonist (Figure 1b). Although holding current returned to the pre-drug level following wash out of agonist, with higher concentrations of SRIF (>100 nM) extended wash times of over 30 min were necessary. Following such a response, it was not possible to elicit a second response of equal amplitude to SRIF even after extended wash of agonist for up to 1 h.

Preincubation with the non-selective K^+ channel blocker, Ba^{2+} (3 mM, $n=3$), abolished responses to both SRIF (30 nM) and baclofen (10 μM). Prolonged washing out of the Ba^{2+} resulted in a partial return of the respone to both ligands (data not shown). A submaximal concentration of Ba^{2+} (300 μM) was also tested which allowed a response to be observed and compared to the control baclofen current observed before Ba^{2+} application. In the presence of 300 μM Ba^{2+}, which produced an inward current, the response to baclofen (10 μM, $n=5$) was $6.1\pm0.8\%$, and that to SRIF (300 nM, $n=3$) was $9.2\pm1.4\%$, of the control baclofen current (Figure 1c). Thus the response to 300 nM SRIF was reduced by $80.8\pm1.7\%$ compared to that of SRIF in control cells. The outward currents elicited by SRIF and baclofen were associated with an increased membrane conductance (Figure 2a). Whole-cell conductance increased by $14.5\pm1.9\%$ and $42.6\pm5.1\%$ at the peaks of responses to SRIF (100 nM, $n=4$, Figure 2b) and baclofen (10 μM, $n=8$), respectively. In the presence of a maximal concentration of baclofen (100 μM), co-application of SRIF (100 nM) produced no further outward current or change in membrane conductance ($n=3$, Figure 2c).

Comparison of agonist potencies

The pharmacological identity of the somatostatin receptor producing the outward current was investigated with a range of SRIF analogues with well characterized agonist profiles at recombinant SRIF receptors. The amplitude of the current evoked by SRIF was concentration-dependent with an EC_{50} of 20 nM (Figure 3). In addition to SRIF itself, the peptide analogues octreotide, BIM-23027 and L-362,855, also produced outward currents (Figure 3). However, BIM-23056 was without agonist (30 or 300 nM, $n=4$) or antagonist (1 μM, $n=3$) effect. Concentration-effect relationships were determined to each agonist and EC_{50} values were calculated. The rank order of agonist potencies estimated from these experiments was (EC_{50}, nM): octreotide (1.8) > BIM-23027 (3.7) > SRIF (22.0) = L-362,855 (22.0) which is compatible with actions at an sst_2 receptor. The maximal responses were similar for all agonists expressed as a percentage of the control baclofen response): L-362,855 ($52.4\pm3\%$), octreotide (43.1 ± 3.1), SRIF ($40.4\pm2.7\%$), BIM-23027 ($39.1\pm1.8\%$).

Effect of L-Tyr^{8}Cyanamid 154806 and BIM-23056

The selective sst_2 receptor antagonist, L-Tyr^{8}Cyanamid 154806 (1 μM, $n=6$), had no effect upon holding current alone or on the response to baclofen (10 μM, $n=4$, Figure 4a), but reversed responses to submaximal concentrations of SRIF receptor agonists (Figure 4a,b). Responses to SRIF (100 nM) were reversed by $44\pm6\%$ ($n=4$) and responses to L-362,855 (100 nM) were reversed by $70\pm6\%$ ($n=5$, Figure 4a,b). Responses to a maximal concentration of L-362,855 (1 μM, $n=4$) were reversed by $45\pm6\%$. In contrast, responses to the sst_2-selective agonist, BIM-23027 (10 nM), were almost fully reversed ($93\pm1\%$, $n=4$) by the antagonist (1 μM). The sst_2 receptor antagonist, BIM-23056, did not produce any reversal of responses to a maximal concentration of L-362,855 (1 μM, $n=3$) or BIM-23027 (30 nM, $n=3$) (data not shown).

Desensitization studies

The outward current induced by high concentrations of SRIF (Figure 1b), BIM-23027 (Figure 5a) and octreotide faded in the continuous presence of agonist. In contrast, responses to L-362,855 at maximal concentration (1 μM) were sustained (Figure 5b) and were maintained even following prolonged washing. Following sst_2 receptor desensitization with BIM-23027 (100 nM), a subsequent application of the same agonist produced no response. However, responses to L-362,855 (1 μM, $n=3$) were still observed, which were $15.6\pm2.1\%$ of the response to baclofen in the same cells (cf 55.1% of the baclofen response in control cells). Furthermore, in the presence of a high concentration of SRIF (1 μM, $n=4$), there was no further response to L-362,855 (100–1000 nM, $n=4$, data not shown).

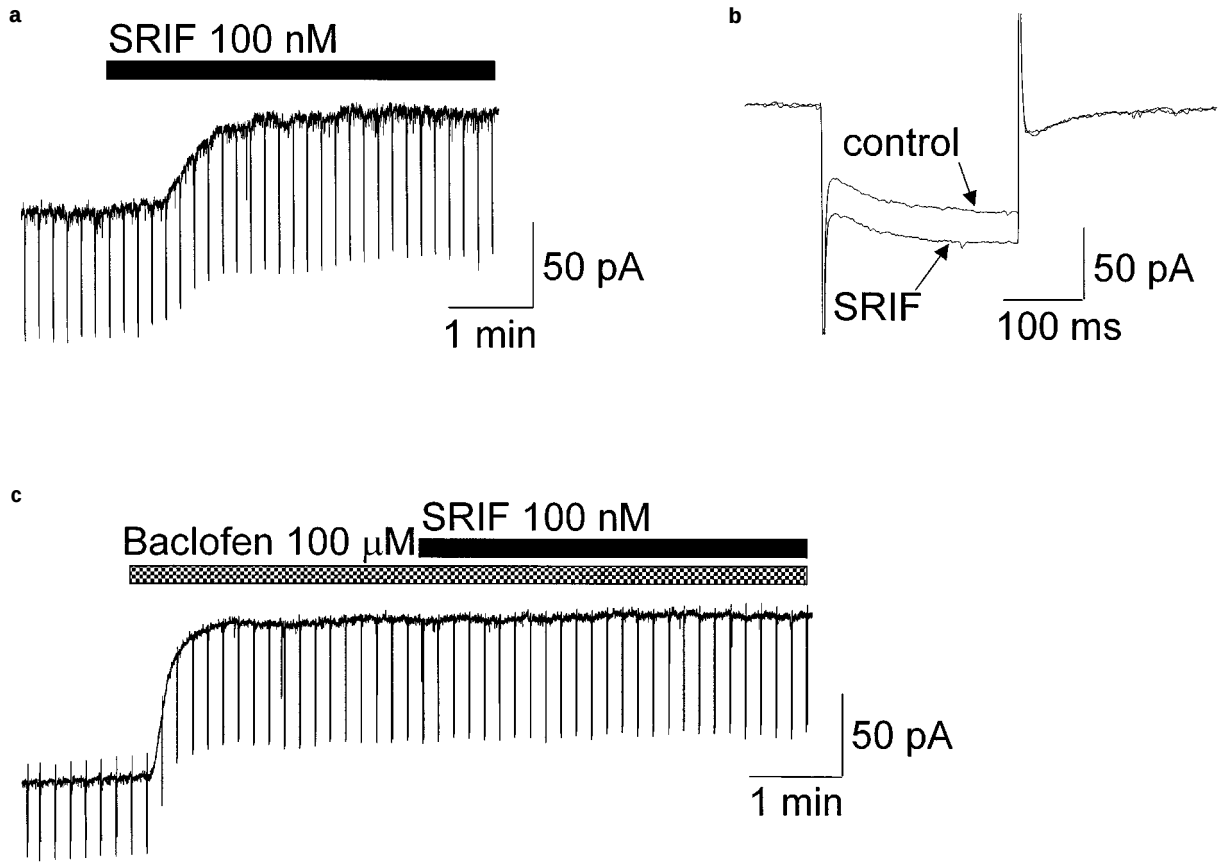

Figure 2 SRIF increased membrane conductance by opening the same population of channels as baclofen. (a) Outward current in response to SRIF (100 nM, solid bar) was sustained at the concentration applied. Downward deflections show the membrane current response to 500 ms long 10 mV hyperpolarizing steps. The traces in (b) show the average of 10 such steps before drug application ('control'), and during the peak of the response ('SRIF'). An 18% increase in membrane conductance was produced by SRIF (100 nM) in this experiment. (c) A maximal concentration of baclofen (100 μM, hatched bar) produced an outward current concomitant with an increased membrane conductance (by 120%). Application of SRIF (100 nM, solid bar) in the continued presence of baclofen produced no further outward current or change in conductance.

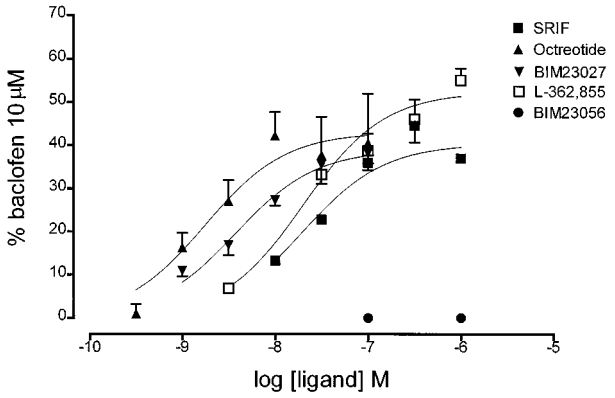

Figure 3 Outward currents were produced by SRIF by an action primarily at sst_2 receptors. Concentration-effect curves for SRIF and related analogues (see legend) for activation of outward currents in rat anterior cingulate cortex neurones. Peak current amplitudes are expressed as the percentage of the response to baclofen (10 μM) in the same neurone. Each data point is the mean from 3–5 experiments; vertical lines show s.e.mean.

Discussion

In our recordings from pyramidal neurones of the rat anterior cingulate cortex, we observed an outward current in response to the application of SRIF receptor ligands. The current was associated with an increased membrane conductance and was blocked by Ba^{2+} ions. In the presence of a maximal response to baclofen, which via an activation of the $GABA_B$ receptor opens an inwardly rectifying K^+ channel in anterior cingulate neurones (Higashi *et al*., 1991; Tanaka & North, 1993), SRIF produced no further outward current. These observations strongly suggest that the outward current activated by SRIF was carried by K^+ ions.

More pertinent to the present study was the elucidation of the receptor subtype(s) producing the outward current. The cloning and expression of at least five receptors for SRIF has helped to explain its diverse range of effects in both the periphery and CNS (see Bell & Reisine, 1993; Hoyer *et al*., 1994, for review). Good evidence for the presence of sst_2 receptors in the anterior cingulate has been provided by autoradiographical and immunocytochemical studies, but other SRIF receptor subtypes may also be present (see Introduction). Recently, peptide analogues of SRIF having selectivity for recombinant sst_2 receptors have been identified (Raynor *et al*., 1993). In the present study the rank order of agonist potencies strongly suggested the involvement of sst_2 receptors. In radioligand binding studies with transfected recombinant receptors, BIM-23027 shows marked selectivity for the sst_2 subtype and octreotide has similar affinity for sst_2 and sst_5 receptors (Raynor *et al*., 1993). In functional studies, with a mouse fibroblast cell line transfected with human recombinant sst_2 receptors (Castro *et al*., 1996), and in studies

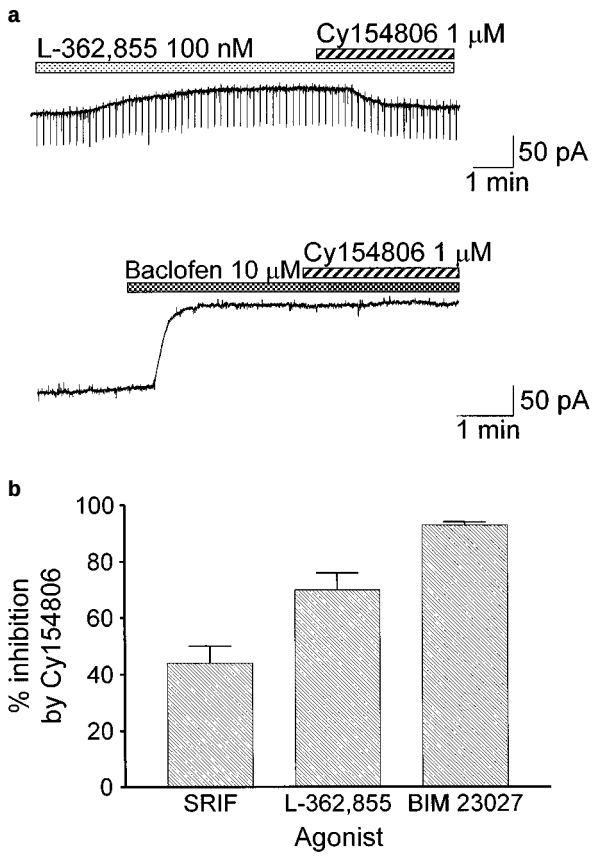

Figure 4 Effect of the sst_2 antagonist L-Tyr^{8}Cyanamid 154806. (a) The antagonist L-Tyr^{8}Cyanamid 154806 (Cy154806, 1 μM) reversed responses to SRIF receptor ligands. The example shown in the upper trace is the reversal of 100 nM L-362,855 (stippled bar, $n=4$). The lower trace shows the lack of effect of L-Tyr^{8}Cyanamid 154806 upon the outward current evoked by baclofen (hatched bar, $n=4$). (b) Histogram showing the percentage reversal of outward current responses to sst_2 receptor ligands and baclofen by the sst_2 receptor antagonist L-Tyr^{8}Cyanamid 154806 (Cy154806, 1 μM). The antagonist reversed responses to the SRIF receptor ligands; SRIF (100 nM), L-362,855 (100 nM) and BIM-23027 (10 nM). Each column shows the mean $\pm$ s.e.mean of 4 experiments in each case.

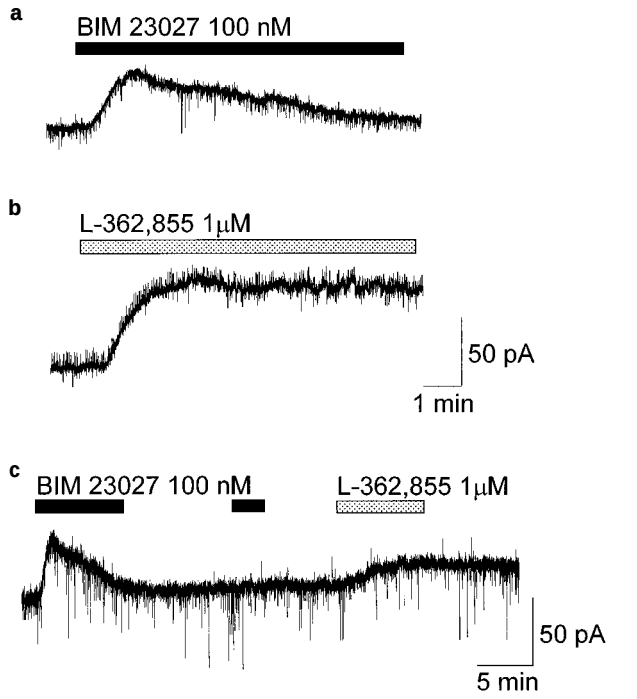

Figure 5 Responses to L-362,855 were sustained in the presence of agonist. (a) Outward currents produced by the sst_2 receptor-selective agonist BIM-23027 (100 nM, solid bar) faded in the continued presence of agonist at high concentration. Responses to SRIF (see Figure 1b) and octreotide (not shown) were also observed to fade during long applications. (b) In contrast, outward currents produced by L-362,855 were sustained for the duration of the application (stippled bar). (c) Following sst_2 receptor desensitization with BIM-23027 (100 nM) a subsequent application of the same agonist produced no response. However, a response to L-362,855 was still observed, which was smaller than those observed in control cells.

of SRIF-induced inhibition of secretion from rat colonic mucosa (McKeen *et al*., 1995), BIM-23027 and octreotide were more potent than SRIF itself. Thus the greater potency of octreotide (12 times more potent) and BIM-23027 (6 times more potent) over SRIF in the present study is consistent with an action at sst_2 receptors and argues strongly against an involvement of sst_1 or sst_4 receptors for which these ligands have binding affinities in the micromolar range (Raynor *et al*., 1993). The similar potency of L-362,855 and SRIF is also consistent with an action at sst_2 receptors, since in functional studies (Castro *et al*., 1996; Chessell *et al*., 1996) this agonist displayed only slightly less potency than SRIF.

Recently, a cyclic peptide with sst_2-receptor antagonist properties was identified (Bass *et al*., 1996). Cyanamid 154806 binds to rat sst_2 receptors expressed in Chinese hamster ovary cells with an affinity of 0.3 nM and has an ED_{50} of 15 nM for antagonism of sst_2 receptor-mediated inhibition of forskolin-stimulated adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation in GH_4C_1 cells. The L-Tyr^8 isomer of the antagonist binds selectively to human sst_2 receptors and antagonizes the effects of SRIF in isolated tissue preparations known to contain sst_2 receptors (Feniuk *et al*., 1998). In the locus coeruleus, where SRIF increases K^+ conductance by an action at sst_2 receptors (Inoue *et al*., 1988; Chessell *et al*., 1996) responses to these three ligands were fully blocked by L-Tyr^{8}Cyanamid 154806 (unpublished observations, G.A. Hicks). In contrast, in the present study, although the effect of the sst_2-selective agonist BIM-23027 was fully reversed by L-Tyr^{8}Cyanamid 154806, responses to SRIF and L-362,855 were only partially reversed suggesting that more than one subtype of receptor may be involved in their action in the anterior cingulate cortex. We investigated this possibility with the sst_5 receptor antagonist BIM-23056 (Wilkinson *et al*., 1996; 1997; Williams *et al*., 1997). BIM-23056 had no direct effect and did not reverse responses to L-362,855 or BIM-23027. These findings suggest that sst_5 receptors are not likely to be involved in the production of the outward current in our experiments. Therefore, our experiments provide evidence for an sst_2 receptor coupled to the activation of a K^+ current in pyramidal neurones of the anterior cingulate cortex. However, based upon our observations with the sst_2 receptor antagonist, we cannot rule out the possibility that SRIF may produce an outward current via actions at more than one SRIF receptor.

Although responses to L-362,855 were largely inhibited by the sst_2 receptor antagonist, the response to this ligand was sustained for the duration of its application. This was in contrast to the effects of the other agonists tested and appeared to be reflected in the slightly greater maximal response observed with this ligand. Furthermore, reduced responses to L-362,855 were still observed following desensitization of the sst_2 receptor population by prolonged exposure to a high concentration of BIM-23027. These observations suggest that

the outward current observed in response to L-362,855 may be produced by the activation of other receptor subtypes in addition to sst_2.

The fade of a response in the continuous presence of agonist is commonly used as a measure of receptor desensitization. In the case of SRIF receptors, the mechanism of desensitization is unknown. Agonist-dependent sst_2 receptor internalization (Koenig *et al*., 1996; Hipkin *et al*., 1997) and phosphorylation (Hipkin *et al*.,1997) have been demonstrated and both have been suggested to play a role in desensitization at these receptors (Hipkin *et al*., 1997; Beaumont *et al*., 1997). It is possible that the lack of fade of the response to L-362,855 in the present study occurred because L-362,855 did not promote receptor internalization and/or phosphorylation in the neurones studied. The use of L-362,855 or other agents with a similar kinetic profile may help in the determination of the mechanisms underlying SRIF receptor desensitization. Furthermore, due to their longer duration of action and sustained response, such compounds may be of great benefit as both research and therapeutic agents.

It is tempting to speculate on the significance of the SRIF-mediated increase in K^+ conductance in the anterior cingulate cortex. Nociceptive neurones have been identified in *in vivo* recordings from anterior cingulate cortex. Thermal, mechanical and transcutaneous electrical stimulation, all produce increases of firing rates in a proportion of cingulate neurones located in both deep and superficial layers (Sikes & Vogt, 1992; Yamamura *et al*., 1996; Hsu & Shyu, 1997). Afferents from nuclei in the medial thalamus provide the excitatory input to these cells (Sikes & Vogt, 1992; Hsu *et al*., 1997). In man, PET scan and fMRI studies show an activation of anterior cingulate cortex in response to certain types of painful stimuli (Jones *et al*., 1991; Talbot *et al*., 1991; Casey *et al*., 1994; Rainville *et al*., 1997). Such responses are altered in patients suffering from chronic disorders such as atypical face pain (Derbyshire *et al*., 1994), irritable bowel syndrome (Silverman *et al*., 1997) and rheumatoid arthritis (Jones & Derbyshire, 1996), in which pain is a major symptom.

Afferent pathway conduction block with local anaesthetic in rats (Vaccarino & Melzack, 1989) or ablation of frontal and anterior cingulate cortex in man (Folz & White, 1962) both produce analgesia. In the latter, the pain relief appears to be produced by an elimination of the 'suffering' component of the sensation rather than of the pain itself. Inhibition of anterior cingulate neuronal activity that would be produced by the SRIF-mediated outward current, which we have demonstrated, could therefore also serve to produce analgesia. Development of centrally penetrative agents with high affinity at sst_2 receptors may thus lead to a new class of analgesic drugs.

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