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Characterization of human recombinant somatostatin sst_5 receptors mediating activation of phosphoinositide metabolism

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1 We have functionally characterized the human recombinant somatostatin (SRIF) sst_5 receptor in Chinese hamster ovary-K1 ($CHOsst_5$) cells by measuring total [^{3}H]-inositol phosphate ([^{3}H]-InsPx) accumulation, in the presence of 10 mM LiCl, in cells labelled with [^{3}H]-*myo*-inositol.

2 In $CHOsst_5$ cells, SRIF, SRIF-28 and the cyclic hexapeptide, L-362,855, produced time- and concentration-related increases in [^{3}H]-InsPx accumulation, with similar potency (pEC_{50} values of 6.5, 6.8 and 7.2, respectively). L-362,855 behaved as a partial agonist, producing approximately 30% of the SRIF maximum response. The other peptide analogues of SRIF, BIM-23027 and BIM-23056, were inactive as agonists.

3 Increasing concentrations of L-362,855 increased [^{3}H]-InsPx accumulation and simultaneously produced rightward shifts of SRIF concentration-effect curves, with an estimated pK_p value of 7.6, confirming that it was acting as a partial agonist.

4 BIM-23056, but not BIM-23027, potently antagonized SRIF-induced [^{3}H]-InsPx accumulation, with an estimated pK_B value of 7.4. BIM-23056 did not antagonize [^{3}H]-InsPx accumulation induced by uridine 5′-triphosphate (UTP).

5 SRIF- but not UTP-induced [^{3}H]-InsPx accumulation was inhibited by increasing concentrations of pertussis toxin (0.01–100 ng ml^{-1}), indicating the involvement of pertussis toxin-sensitive G-proteins.

6 These findings show that the human recombinant sst_5 receptor, when stably expressed in CHO-K1 cells, is able to mediate activation of phosphoinositide metabolism in a pertussis toxin-sensitive manner. In this system L-362,855 behaved as a partial agonist while BIM-23056 was a specific antagonist. These agents should provide useful tools for functionally characterizing endogenous SRIF receptors.

Keywords: Somatostatin; sst_5 receptor; inositol phosphates; BIM-23056; L-362,855; CHO-K1 cells

Introduction

Somatostatin (SRIF; somatotropin release inhibitory factor) is a tetradecapeptide which has widespread effects in both the peripheral and central nervous systems (see Schindler *et al*., 1996). The genes for five distinct somatostatin receptors have now been cloned and the receptor proteins have been named sst_1–sst_5. Each receptor type has the putative seven-transmembrane spanning structure characteristic of G-protein coupled receptors and purportedly receptor-selective ligands are available (see Hoyer *et al*., 1995). However, it remains to be determined which endogenous receptors mediate the many effects of SRIF, although work is currently in progress with selective ligands in whole tissues (e.g. McKeen *et al*., 1996; Chessell *et al*., 1996).

All five recombinant somatostatin receptors have been shown to negatively couple to adenylyl cyclase but, in studies of endogenous somatostatin receptors in tissues, numerous transduction mechanisms have been described. These include not only inhibition of adenylyl cyclase activation (Jakobs *et al*., 1983) but also inhibition of voltage dependent-calcium channel opening (Lewis *et al*., 1986), stimulation of protein phosphatase activity (Liebow *et al*., 1989) and the activation of voltage-dependent K^+ channels (Yamashita *et al*., 1987). All these responses have been shown to be sensitive to pertussis toxin (PTX), implicating $G_{i/o}$ G-proteins. In contrast, other SRIF mediated responses, such as inhibition of Na^+/H^+ exchange in enteric endocrine cells (Barber *et al*., 1989) and proliferation of AR4-2J cell growth (Viguerier *et al*., 1989), are insensitive to PTX.

More recently, recombinant sst_2 and sst_5 somatostatin receptors have been shown to couple to PTX-sensitive transduction mechanisms linked to inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) formation and increases in intracellular calcium ion concentration ($[Ca^{2+}]_i$) (Akbar *et al*., 1994). In view of the ability of many receptors to couple promiscuously to G-proteins and the recent availability of selective agonists and antagonists at the sst_5 receptor (Wilkinson *et al*., 1996b; Williams *et al*., 1996a; Thurlow *et al*., 1996), we have examined the functional characteristics of this receptor to mediate phosphoinositide metabolism, as an index of phospholipase C (PLC) activity, for comparison with its other transduction mechanisms, including increases in extracellular acidification (Thurlow *et al*., 1996), GTPγS binding (Williams *et al*., 1996a) and inhibition/activation of adenylyl cyclase (Williams *et al*., 1996b).

Methods

Cell culture

Chinese hamster ovary-K1 (CHO-K1) cells stably expressing the human recombinant sst_5 receptor ($CHOsst_5$) (Wilkinson *et al*., 1996b) at 3.01 ± 0.3 pmol mg^{-1} protein ($n=3$), determined from binding of [^{125}I]-Tyr^{11}-SRIF to membranes prepared from these cells (A.J. Williams, unpublished observations), were grown in Dulbecco's modified Eagles Medium/Hams F-12 nutrient (1 : 1) mix supplemented with Glutamax, 10% foetal calf serum and G418 (0.5 mg ml^{-1}). Cultures were maintained at 37°C in a 5% CO_2/humidified air atmosphere. For measurements of [^{3}H]-

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InsPx accumulation, cells were subcultured into 24-well multiplates and used when they had reached confluence.

Measurement of total [^{3}H]-inositol phosphate accumulation

$CHOsst_5$ cells grown to confluence in 24-well multiplates were incubated with 0.074 MBq ml^{-1} (1.0 μCi ml^{-1}) of D-*myo*-[2-^{3}H]-inositol in 0.25 ml of medium M199, supplemented with 1% foetal calf serum and 0.5 mg ml^{-1} G418, for 24 h. Cells were washed twice with 250 μl of a balanced salt solution (BSS) buffer (composition (mM): NaCl 125, KCl 5.4, $NaHCO_3$ 16.2, D-glucose 5.5, HEPES 20, NaH_2PO_4 1, $CaCl_2$ 1.3, buffered to pH 7.4 with NaOH (5 M) and gassed with 95% O_2/5% CO_2) containing 10 mM LiCl (Li^+) and preincubated for 10 min at 37°C. To control for the final concentration of Cl^- ions in the buffer, the amount of NaCl added was proportionately reduced when Li^+ was present. When the effects of antagonists were determined, cells were washed once in BSS without Li^+ and then incubated in this buffer for 60 min with the required antagonist concentration; Li^+ (10 mM) was added to this preincubate for the final 10 min. When the effects of pertussis toxin were determined, the toxin, at the required concentration, was added directly to the well for the last 18 h of loading with D-*myo*-[2-^{3}H]-inositol. Experiments were initiated by removal of the preincubate and addition of 250 μl Li^+ containing buffer, with the required treatment at 37°C. Incubations were terminated with 250 μl of ice-cold 1.0 M trichloroacetic acid (TCA) and the multiplate was put on ice for 15 min. The TCA was extracted from a 250 μl aliquot with 250 μl of a 1:1 (v:v) mixture of tri-n-octylamine:1,1,2-trichlorotrifluro-ethane by vortexing the samples for 15 s. The samples were left at room temperature for 15 min and subsequently centrifuged at 13000 x *g* for 3 min. A 200 μl aliquot of the upper aqueous layer was taken and 100 μl of 25 mM $NaHCO_3$ was added, this was subsequently applied to Dowex AG1X8-400 (200–400 mesh) minicolumns. After washing with 20 ml water, followed by 10 ml of 60 mM ammonium formate, inositol phosphates were eluted with 5 ml of 2.0 M ammonium formate. A 1 ml aliquot was taken and 4 ml of scintillation cocktail added and [^{3}H]-InsPx determined by liquid scintillation spectrometry. Dowex columns were regenerated with 5 ml 1.0 M formic acid followed by 20 ml water.

Analyses

pK_B estimates pK_B estimates (negative logarithm of the estimated antagonist equilibrium dissociation constant) were calculated from the agonist concentration-ratios determined at individual antagonist concentrations, from the Gaddum-Schild equation. In addition, an Arunlakshana-Schild plot was used to determine the slope (as a test for competitive antagonism) and the intercept (pK_B) of the line of unit slope (see Jenkinson *et al*., 1995).

pK_P estimates pK_P values were determined as estimates of the equilibrium dissociation constant for the partial agonist, L-362,855, by use of the Black-Leff operational model (see Leff *et al*., 1993). Each pair of concentration-effect curves for SRIF, one in the absence and one in the presence of a given concentration of L-362,855, was fitted simultaneously to the logistic equation:-

$$E = \frac{E_m([A]K_p + \tau_P[P][EC_{50}])^n}{[EC_{50}]^n(K_p + [P])^n + ([A]K_p + \tau_P[P][EC_{50}])^n}$$

where, [A] is the concentration of SRIF and [P] is the concentration of L-362,855, so as to calculate the shared parameters of K_P, EC_{50}, E_m and n as well as τ_P, an efficacy parameter for L-362,855. The EC_{50} and E_m values are fitted estimates of the concentration of SRIF required to produce 50% of its own maximum effect in the absence of the partial agonist and associated computed maximum, respectively. The data were fitted by use of the programme, Uridian (Torac, Harlow, Essex, U.K.). The derived estimates for each parameter were meaned from all the data by use of three concentrations of L-362,855 (30 nM, 300 nM and 3 μM) and the K_P and EC_{50} values expressed as their negative logarithms (pK_P and pEC_{50} respectively).

Other agonist concentration-effect curves were analysed as described previously (Wilkinson *et al*., 1996b).

Statistical analysis

All data are expressed as the mean ± s.e.mean of *n* separate determinations (experiments). Differences between values were tested for statistical significance by Student's *t* test at $P = 0.05$.

Materials

Unless otherwise stated, all reagents were purchased from Sigma. Tissue culture media were from Life Technologies (Paisley, U.K.) and tissue culture ware was from Costar. SRIF was obtained from Peninsula Laboratories, BIM-23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2), BIM-23027 (c[N-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe]), L-362,855 (c[Aha-Phe-D-Trp-Lys-Thr-Phe]) were custom synthesized by Peptide and Protein Research Consultants, University of Exeter, U.K. D-*myo*-[2-^{3}H]-inositol was from RBI (Semat, St Albans, U.K.).

Results

Time and concentration-related increases in total [^{3}H]-inositol phosphate accumulation

SRIF, SRIF-28 and L-362,855 (all at 1 μM) induced rapid increases in [^{3}H]-InsPx accumulation, which were not significantly different from one another within the first 60 s of agonist addition (Figure 1). Subsequently, the increase in [^{3}H]-InsPx accumulation in response to SRIF and SRIF-28 continued for up to 30 min. However, there was no further increase in response to L-362,855 after 5 min. BIM-23027 and BIM-23056 (both at 1 μM) did not stimulate increases in [^{3}H]-InsPx accumulation above those produced by vehicle (data not shown).

The increases in [^{3}H]-InsPx accumulation produced by SRIF, SRIF-28 and L-362,855 were concentration-dependent (Figure 2). When cells were incubated with agonist for 30 min, both SRIF and SRIF-28 produced maximal increases in [^{3}H]-InsPx accumulation which were not significantly different from

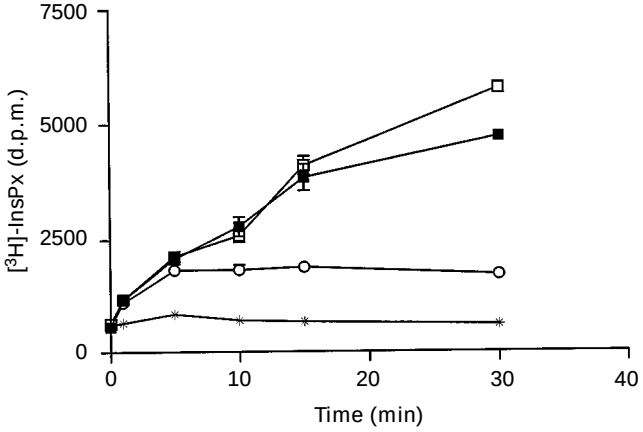

Figure 1 Time course of total [^{3}H]-inositol phosphate accumulation in $CHOsst_5$ cells in response to somatostatin (SRIF, 1 μM) (■), SRIF-28 (1 μM) (□), L-362,855 (1 μM) (○) and vehicle (*). Cells were incubated with the agonist for the times indicated in the presence of 10 mM Li^+. Data are the mean of a single triplicate experiment and are representative of three individual experiments; vertical lines show s.e.mean.

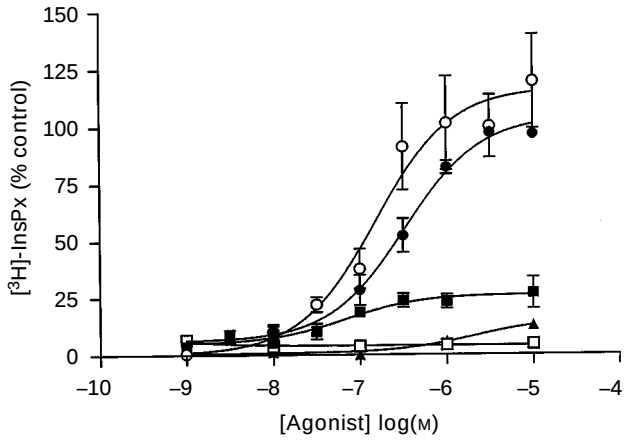

Figure 2 Concentration-effect curves for accumulation of total [^{3}H]-inositol phosphates in CHOsst_5 cells in response to somatostatin (SRIF) and a number of analogues. Cells were preincubated with LiCl (10 mM) for 10 min and concentration-effect curves were then constructed by addition of the indicated concentrations of SRIF (●), SRIF-28 (○), L-362,855 (■), BIM-23056 (□) and BIM-23027 (▲). Incubations were carried out for 30 min. Data for each curve are presented as a percentage of the maximum response to the control SRIF curve after subtraction of the corresponding basal values and are the mean of 3 to 7 experiments performed in triplicate, vertical lines show s.e.mean.

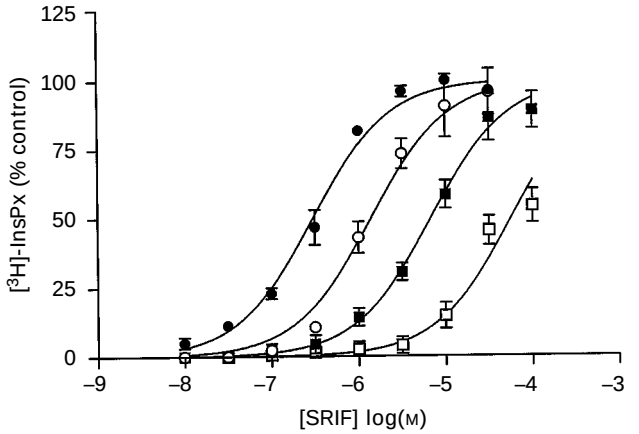

Figure 3 Effects of increasing concentrations of BIM-23056 on concentration-effect curves to somatostatin (SRIF). Cells were preincubated with either zero (●), 1×10^{-7} M (○), 1×10^{-6} M (■) or 1×10^{-5} M (□) BIM-23056 and concentration-effect curves to SRIF were subsequently constructed in the continued presence of the preincubate. Preincubations with either vehicle or BIM-23056 were for one hour; LiCl (10 mM) was added during the last 10 min of this preincubation. Agonist concentration-effect curves were subsequently constructed in the continued presence of the preincubate for 30 min. Data for each curve are presented as a percentage of the maximum response to the control SRIF curve, after subtraction of the corresponding basal values, and are the mean of 3 to 4 experiments carried out in triplicate; vertical lines show s.e.mean.

each other, with pEC_{50} values of 6.48 ± 0.10 ($n=9$) and 6.83 ± 0.25 ($n=5$), respectively. The cyclic hexapeptide, L-362,855, produced a maximal response which was $31.2\pm5.0\%$ of that produced by SRIF, with a pEC_{50} of 7.16 ± 0.26 ($n=6$).

Inhibition of SRIF concentration-effect curves

Increasing concentrations of BIM-23056 (100 nM, 1 μM and 10 μM, Figure 3) produced rightward shifts of the concentration-effect curve to SRIF yielding agonist concentration ratios of 5.8 ± 2.2, 26.4 ± 6.5 and 200.9 ± 39.0 respectively ($n=3$). By Gaddum-Schild analysis of these data, corresponding pK_B values for BIM-23056 at the sst_5 receptor were estimated to be 7.58 ± 0.21, 7.37 ± 0.13 and 7.28 ± 0.09, respectively. Arunlakshana-Schild analysis of the collective data provided a slope (0.85 ± 0.10) which was not significantly different from unity and a mean pK_B of 7.41 ± 0.09, when the slope was constrained to unity ($n=9$). In two further experiments, the highest concentration of BIM-23056 (10 μM) had little effect on UTP-induced increases in [^{3}H]-InsPx accumulation giving concentration-ratios of 0.41 and 2.37, respectively.

L-362,855 was also able to antagonize responses to SRIF. Increasing concentrations of L-362,855 (30 nM, 300 nM and 3 μM) produced small increases in [^{3}H]-InsPx accumulation and also rightward shifts of the concentration-effect curve for SRIF (Figure 4). The fitting of these data to the Black-Leff operational model provided an estimated pK_P for L-362,855 of 7.58 ± 0.15, with τ_P and n values of 0.37 ± 0.04 and 0.97 ± 0.06, respectively ($n=12$ for each). The computed pEC_{50} and E_m values of SRIF, respectively, were 6.67 ± 0.07 and $109\pm4.0\%$.

BIM-23027 (10 μM) had no effect on SRIF-induced increases in [^{3}H]-InsPx accumulation (concentration-ratio, 1.3 ± 0.4, $n=3$).

Effects of pertussis toxin (PTX)

In order to establish whether or not the responses to SRIF were PTX-sensitive, cells were incubated for 18 h with increasing concentrations of PTX (0.01 to 100 ng ml^{-1}) and [^{3}H]-InsPx accumulation measured. Incubation with PTX, produced a concentration-related reduction in the magnitude of accumulation of [^{3}H]-InsPx by SRIF (1 μM) (Figure 5). After incubation with 100 ng ml^{-1} PTX and after deduction of

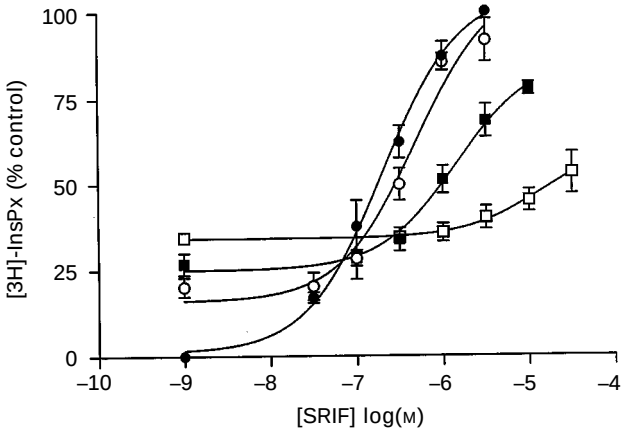

Figure 4 Concentration-effect curves to SRIF were constructed in the absence and presence of increasing concentrations of L-362,855. Cells were preincubated with either zero (●), 3×10^{-8} M (○), 3×10^{-7} M (■) or 3×10^{-6} M (□) L-362,855 and concentration-effect curves to SRIF were constructed in the continued presence of the preincubate. Preincubations with either vehicle or L-362,855 were for one hour; LiCl (10 mM) was added during the last 10 min of this preincubation. SRIF concentration-effect curves were subsequently constructed in the continued presence of the preincubate for 30 min. Data for each curve are presented as a percentage of the maximum response to the control SRIF curve after subtraction of the corresponding basal values and are the mean of 3 experiments performed in triplicate; vertical lines show s.e.mean.

the corresponding basal values for each experiment, responses to SRIF were reduced by $91.9\pm3.2\%$ ($n=5$), from 3302 ± 259 to 282 ± 113 d.p.m. ($n=5$) respectively. There was no significant effect of PTX (100 ng ml^{-1}) on basal accumulation; 737 ± 66 d.p.m. in the absence and 638 ± 162 d.p.m. in the presence of PTX ($n=5$).

Pretreatment of cells with the highest concentration of PTX used (100 ng ml^{-1}), had no effect on [^{3}H]-InsPx accumulation in response to UTP (100 μM), 3396 ± 177 d.p.m. in the absence and 3524 ± 223 d.p.m. in the presence of PTX ($n=3$).

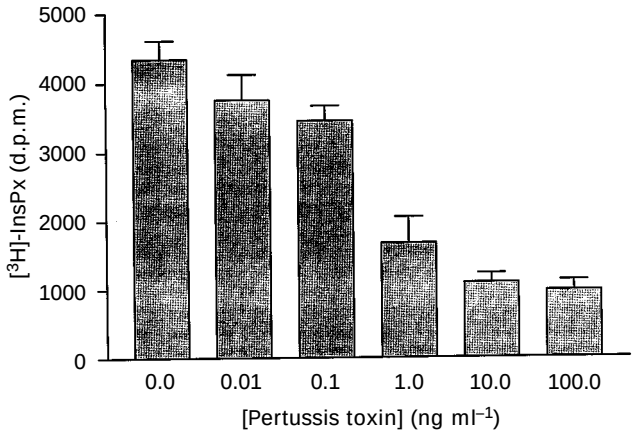

Figure 5 Effect of preincubation with pertussis toxin (PTX) for 18 h on total [^{3}H]-inositol phosphate accumulation in response to SRIF (1 μM) in CHOsst$_5$ cells. Cells were pre-incubated with 10 mM LiCl and then stimulated with SRIF for 30 min after they had been exposed to the indicated concentrations of PTX. Data are the mean $\pm$ s.e.mean of 5 experiments performed in triplicate and are presented without deduction of control values for the effects of PTX on basal [^{3}H]-inositol phosphate accumulation. The effects of preincubation, for 18 h in the absence or presence of 100 ng ml^{-1} PTX on basal [^{3}H]-inositol phosphate accumulation over a 30 min time period, are detailed in the main text.

Discussion

In this study we have investigated some of the operational characteristics of the human recombinant somatostatin sst$_5$ receptor which, in addition to inhibition of adenylyl cyclase activity (Williams *et al*., 1996b), mediates increases in phosphoinositide metabolism, when stably expressed in CHO-K1 cells. In addition, we have also provided data indicating that this receptor is coupled to activation of this transduction pathway almost entirely via pertussis toxin sensitive G-proteins. Comparisons with responses elicited by UTP, which acts at endogenous P2Y$_2$ purinoceptors on these cells (Iredale & Hill, 1993; Wilkinson *et al*., 1996a), have been made.

Receptors for SRIF have been subdivided into two groups, SRIF$_1$ and SRIF$_2$, on the basis of the higher affinity of MK-678 (seglitide) and SMS-201995 (octreotide) for SRIF$_1$ receptors, with the sst$_5$ receptor being a subtype of the SRIF$_1$ group (Hoyer *et al*., 1995). Selective peptide ligands have been shown to be able to distinguish between this and the other receptor subtypes, sst$_2$ and sst$_3$, in the SRIF$_1$ group (Raynor *et al*., 1993). These include BIM-23027, which is an sst$_2$ receptor selective ligand (McKeen *et al*., 1996; Chessell *et al*., 1996) and the purportedly sst$_3$ receptor selective ligand, BIM-23056 (O'Carroll *et al*., 1994), which we have shown to be a potent antagonist at the sst$_5$ receptor (Wilkinson *et al*., 1996b). L-362,855 has been shown to be an sst$_5$ selective ligand and we have confirmed that it has high affinity for the human sst$_5$ receptor (Williams *et al*., 1996a). Furthermore, in microphysiometer studies in which the rate of extracellular acidification was measured, we have confirmed that it is a potent agonist, albeit with lower intrinsic activity than SRIF itself (Thurlow *et al*., 1996). This partial agonist profile of L-362,855 has also been corroborated in studies involving sst$_5$ receptor-mediated stimulation of radiolabelled GTPγS binding where it displayed similar potency to SRIF (Williams *et al*., 1996a).

In the present study, a rank order of agonist potencies similar to those described when measuring extracellular acidification rates (Thurlow *et al*., 1996), was observed when [^{3}H]-InsPx accumulation was measured in CHOsst$_5$ cells. SRIF and SRIF-28 were full agonists, while L-362,855 produced only 30% of the maximal response to SRIF. BIM-23027 and BIM-23056 were inactive as agonists. Interaction studies carried out between SRIF and L-362,855 were consistent with the concept that L-362,855 is a partial agonist at the sst$_5$ receptor with a computed affinity estimate (pK_P of 7.58), which is close to the experimental pEC$_{50}$ value of 7.16. This is to be expected of a compound with low efficacy which exhibits low intrinsic activity, such that the agonist concentration-effect curve approximates to its receptor occupancy-concentration relationship. The higher pEC$_{50}$ value for L-362,855 than that of SRIF itself is indicative of a somewhat higher affinity at the receptor. Indeed radioligand binding studies have confirmed that L-362,855 has some three to ten fold greater affinity than SRIF for the human sst$_5$ receptor (O'Carroll *et al*., 1993; Williams *et al*., 1996a).

The antagonist action of BIM-23056 was not unexpected on the basis of previous data from our laboratory, where it has been shown to antagonize selectively the rise in intracellular calcium ion concentration ([Ca^{2+}]$_i$) produced by SRIF, with an estimated pK_B value of 8.0 (Wilkinson *et al*., 1996b). In this study we have shown surmountable antagonism of SRIF induced increases in [^{3}H]-InsPx accumulation over a one hundred fold concentration range of BIM-23056. The Arunlakshana-Schild analysis provided a slope which was not significantly different from unity suggesting that BIM-23056 was acting as a competitive antagonist. The calculated pK_B values were in the range 7.28 – 7.58, which were lower than the value obtained when measuring [Ca^{2+}]$_i$ responses, but almost identical to the value of 7.28 obtained for antagonism of SRIF-induced [^{35}S]-GTPγS binding mediated by the human recombinant sst$_5$ receptor (A.J. Williams, unpublished observations). It may be that the pK_B value obtained from measurement of [Ca^{2+}]$_i$ is artificially high because of hemiequilibrium kinetics due to the short agonist contact time, resulting from the transient nature of the response. The lack of effect of BIM-23056 on UTP-induced [^{3}H]-InsPx accumulation (present study) as well as increases in [Ca^{2+}]$_i$ (Wilkinson *et al*., 1996b) suggests that BIM-23056 is a specific sst$_5$ receptor blocking drug.

BIM-23056 also has high affinity at sst$_3$ and sst$_4$ receptors (Raynor *et al*., 1993; O'Carroll *et al*., 1994) and studies in our own laboratories are under way to determine whether it behaves as an agonist or an antagonist at these and other recombinant SRIF receptors. Although BIM-23056 was devoid of agonist activity at the sst$_5$ receptor when [^{3}H]-InsPx accumulation was measured, it behaved as a partial agonist when inhibition of forskolin stimulated adenylyl cyclase activity was studied, (Williams *et al*., 1996b). This suggests that activation of phospholipase C (PLC) following sst$_5$ receptor activation is less efficiently coupled than inhibition of adenylyl cyclase. In keeping with this concept, the absolute potencies of SRIF (pEC$_{50}$ 6.5), SRIF-28 (pEC$_{50}$ 6.8), L-362,855 (pEC$_{50}$ 7.2) and BIM-23027 (pEC$_{50}$ $<$5.0) at stimulating increases in [^{3}H]-InsPx accumulation were at least 100 fold lower than we have previously obtained in other functional assays in the same CHOsst$_5$ cell line (Williams *et al*., 1996a; Thurlow *et al*., 1996). Whilst the rank order of agonist potencies of these compounds is compatible with the rank order of affinity as determined in binding studies on recombinant sst$_5$ receptors (O'Carroll *et al*., 1994; Williams *et al*., 1996a), the absolute potency in the binding studies is much higher and is a measure of high affinity agonist binding in membrane preparations in the absence of G proteins.

Increases in [^{3}H]-InsPx accumulation in response to SRIF but not to UTP were markedly reduced after preincubating the cells with increasing concentrations of PTX, indicating that the sst$_5$ receptor specifically mediates activation of PLC via G-proteins of the G$_i$ and/or G$_o$ family. The sensitivity to low concentrations of PTX is also consistent with poor receptor-effector coupling or possibly a low G-protein reserve. The effects of PTX on [^{3}H]-InsPx accumulation to SRIF were different from those we have observed when measuring extracellular acidification rates from these cells, where only a portion of the SRIF response was inhibited by PTX (Thurlow *et al*., 1996), indicating that when expressed in CHO-K1 cells,

the sst_5 receptor is able to couple to a number of transduction pathways, involving both PTX-sensitive and -insensitive G-proteins.

It is evident that multiple signal transduction cascades can be activated by a single receptor population and the potency of agonists to activate these pathways can differ markedly (see Introduction). However, there does not appear to be a common pattern for the relative differences in the potency of an agonist at a given receptor to mediate different intracellular responses. As has already been mentioned, SRIF is much more potent at inhibiting forskolin stimulated cyclic AMP accumulation than at stimulating phosphoinositide metabolism or increases in $[Ca^{2+}]_i$ (see above), whilst in CHO cells expressing recombinant adenosine A_1 receptors, the potency of N_6-cyclopentyl adenosine at inhibiting adenylyl cyclase and increasing $[Ca^{2+}]_i$ was similar (Townsend-Nicholson & Shine, 1992; Iredale *et al*., 1994). In contrast, in CHO cells endogenously expressing a very low density of 5-HT_{1B} receptors, 5-hydroxytryptamine is more potent at increasing $[Ca^{2+}]_i$ than inhibiting forskolin-stimulated adenylyl cyclase (Dickenson & Hill, 1995; Giles *et al*., 1996). With respect to SRIF receptors, Akbar *et al*. (1994) have shown that all five recombinantly expressed receptors couple negatively to adenylyl cyclase, but only the sst_2 and sst_5 receptors stimulate phosphoinositide metabolism, which is consistent with unpublished data from our laboratory. This suggests that even when expressed at high concentrations, it is a receptor specific characteristic which determines the efficiency of the coupling to the various G-proteins. It is now well recognised that G-protein $\beta\gamma$ subunits, as well as the α subunits, are involved in a range of receptor-mediated transduction processes which include not only PLC activation (Camps *et al*., 1992; Wu *et al*., 1993) but also ion channels (Kleuss *et al*., 1992) and tyrosine kinase signalling cascades (Luttrell *et al*., 1995; Van Biesen *et al*., 1995). It is possible, that the so-called promiscuous coupling of PTX-sensitive receptors to multimeric G-proteins may involve the release of different α, $\beta\gamma$ subunit permutations on activation, thus explaining the differences for different receptors in the position of concentration-effect curves for the various transduction pathways.

There are only a few studies which describe the coupling of endogenous somatostatin receptors to stimulation of the inositol phosphate-calcium signalling pathway in native tissues (Miyoshi *et al*., 1989; Munoz-Acedo *et al*., 1995). However, the agonist responses they describe were small and the receptor types involved were not identified. This, together with the poor coupling of the recombinant sst_5 receptor we have demonstrated here, might suggest that such coupling may only occur with SRIF receptors when they are expressed at high density. However, we have shown that sst_2 receptors mediate contraction of the human saphenous vein by a mechanism involving both intracellular and extracellular Ca^{2+} (Dimech *et al*., 1995). Since phosphoinositide metabolism may well be involved in this response, the transduction characteristics of the SRIF receptor in this tissue deserves attention.

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