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SPECIAL REPORT

# Rat somatostatin $sst_{2(a)}$ and $sst_{2(b)}$ receptor isoforms mediate opposite effects on cell proliferation

*,[1]Forbes Alderton, [2]Tai-Ping D. Fan, [1]Marcus Schindler & [1]Patrick P.A. Humphrey

[1]Glaxo Institute of Applied Pharmacology and [2]Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QJ

We have investigated the actions of somatostatin (SRIF) and angiopeptin on cell proliferation of CHO-K1 cells expressing the recently cloned rat $sst_{2(b)}$ receptor ($CHOsst_{2(b)}$) and compared these to their effects in cells expressing the $sst_{2(a)}$ receptor ($CHOsst_{2(a)}$). In contrast to the $sst_{2(a)}$ receptor, the $sst_{2(b)}$ receptor did not mediate inhibition of bFGF (10 ng $ml^{-1}$)-stimulated re-growth and cell proliferation. Rather, SRIF (0.1–1000 nM) and angiopeptin (0.1–1000 nM) stimulated basal re-growth and proliferation of $CHOsst_{2(b)}$ cells in a concentration-dependent manner (estimated $pEC_{50}$ values of 7.8 and 7.9, respectively). The opposite effects of SRIF on cell proliferation mediated through the two $sst_2$ receptor isoforms were both abolished by 18 h pre-treatment with pertussis toxin. The proliferative effect *via* the $sst_{2(b)}$ receptor was also abolished by the tyrosine kinase inhibitor, genistein. In conclusion, the present study shows that the rat $sst_{2(a)}$ and $sst_{2(b)}$ receptor splice variants mediate opposite effects on cell proliferation.

**Keywords:** Somatostatin; $sst_{2(a)}$ receptor; $sst_{2(b)}$ receptor; proliferation; angiopeptin; CHO-K1 cell

**Introduction** Somatostatin (SRIF)-induced effects on cell growth are mediated through specific cell surface receptors (see Leszczynski *et al*., 1993; Lauder *et al*., 1997) of which the genes for five different types have been cloned and the recombinant receptors termed $sst_1$–$sst_5$ (see Hoyer *et al*., 1995). In addition, two splice variants of the $sst_2$ receptor, the $sst_{2(a)}$ and $sst_{2(b)}$ have been identified in the mouse (Vanetti *et al*., 1992) and more recently in the rat (Schindler *et al*., 1998c).

It is known that the $sst_{2(a)}$ and $sst_{2(b)}$ receptor splice variants are differentially distributed in the cells of the central nervous system (CNS; Schindler *et al*., 1998b) and gastrointestinal (GI) tract (Kidd *et al*., 1998). However, studies performed in our laboratory have shown that the two isoforms display similar ligand binding profiles and transduction characteristics, functionally coupling to the inhibition of adenylate cyclase and activation of increased extracellular acidification (Schindler *et al*., 1998c). Although it is well known that SRIF can inhibit cell proliferation through the rat recombinant $sst_{2(a)}$ receptor (Alderton *et al*., 1998), the effect upon cell growth and proliferation of the rat $sst_{2(b)}$ splice variant has not yet been examined. In the present study we describe the unexpected effects of SRIF, as well as the synthetic peptide angiopeptin (BIM-23014; under clinical investigation for the treatment of acromegaly and inhibition of tumour growth; see Gillespie *et al*., 1998), on cell proliferation mediated through the rat recombinant $sst_{2(b)}$ receptor expressed in CHO-K1 cells.

**Methods** CHO-K1 cells stably expressing either the rat $sst_{2(a)}$ ($CHOsst_{2(a)}$) or $sst_{2(b)}$ receptor ($CHOsst_{2(b)}$; see Schindler *et al*., 1998c) were maintained in Dulbecco's Modified Eagles Medium (DMEM)/Ham's F-12 (1 : 1) nutrient mix supplemented with 10% foetal calf serum (FCS) and G418 sulphate (Life Technologies, Paisley, Scotland). Cells were seeded at a density of $2 \times 10^5$ on to 13-mm Thermanox™ coverslips (NUNC, Life Technologies) in 24-well plates and grown to confluence.

Using the mechanical 'wounder' described by Lauder *et al*. (1998), eleven parallel areas (400 $\mu$m wide) of the confluent monolayer were denuded of cells. Coverslips were washed three times in Dulbecco's phosphate buffered saline (PBS; Life Technologies, Paisley), and placed in a fresh well containing drug or vehicle in appropriate media. Experiments were terminated after 24 h by washing the coverslips three times in PBS and either fixed in absolute ethanol for 5 min, and allowed to air dry before image analysis, or harvested by adding 0.05% trypsin/0.02% EDTA solution (Life Technologies, Paisley) for 2–5 min. The digestion process was terminated by adding complete media and the single cell suspension counted using a Coulter Counter™ (Coulter Euro Diagnostics GmbH, Krefeld, Germany). Image analysis was carried out with a Seescan™ semi-automated, image analysis machine (Seescan, Cambridge, U.K.). For each coverslip five fields of view selected at random were analysed and data expressed as the mean percentage of the area recovered following the period of re-growth as previously described (Alderton *et al*., 1998). All values are means ± s.e.mean from a minimum of three experiments with four replicates per test group.

SRIF-14 was obtained from Peninsula Laboratories Europe Ltd (St. Helens, Merseyside, U.K.). Angiopeptin (BIM-23014), pertussis toxin (PTx) and basic fibroblast growth factor (bFGF) were obtained from Sigma-Aldrich Company Ltd (Poole, Dorset, U.K.). Genistein was purchased from Calbiochem-Novabiochem Ltd (Beeston, Nottingham, U.K.). SRIF-14, angiopeptin and pertussis toxin were initially dissolved in distilled water and bFGF was reconstituted using a 0.2 $\mu$m-filtered solution containing 1% FCS in buffered saline solution. Genistein was initially dissolved in DMSO. Further dilutions of all drugs were made using Dulbecco's modified Eagles Medium/Hams F-12 nutrient (1 : 1) mix supplemented with Glutamax but without FCS.

**Results** We have previously shown in $CHOsst_{2(a)}$ cells that SRIF has no effect on basal re-growth but can inhibit bFGF-stimulated re-growth after partial denudation of a confluent cell monolayer (see Figure 1a,b; Alderton *et al*., 1998). In

* Author for correspondence.

marked contrast to these effects, basal re-growth in CHOsst$_{2(b)}$ cells (10.6±0.4%) was stimulated by SRIF (0.1–1000 nM; Figure 1a) and angiopeptin (0.1–1000 nM) in a concentration-dependent manner with the percentage re-growth at 1 $\mu$M not reaching a fully defined maximum (pEC$_{50}$ values of 7.79±0.20 and 7.94±0.29 estimated by curve fitting; maxima of 20.2±0.7% and 18.8±0.8%, respectively). Basic FGF (10 ng ml$^{-1}$) potently stimulated CHOsst$_{2(b)}$ cell re-growth (23.3±1.0%), however both SRIF (Figure 1b) and angiopeptin were unable to modify this re-growth (23.6±0.7% and 23.3±1.9% at 1 $\mu$M, respectively). Somatostatin and angiopeptin (both 1 $\mu$M) increased basal cell numbers from 177831±7978 to 241581±14075 ($P<0.05$) and 242023±13470 ($P<0.05$), respectively. SRIF had no effect on bFGF-stimulated increase in cell counts (296498±5690). In addition, SRIF (0.1–1000 nM) was unable to affect either

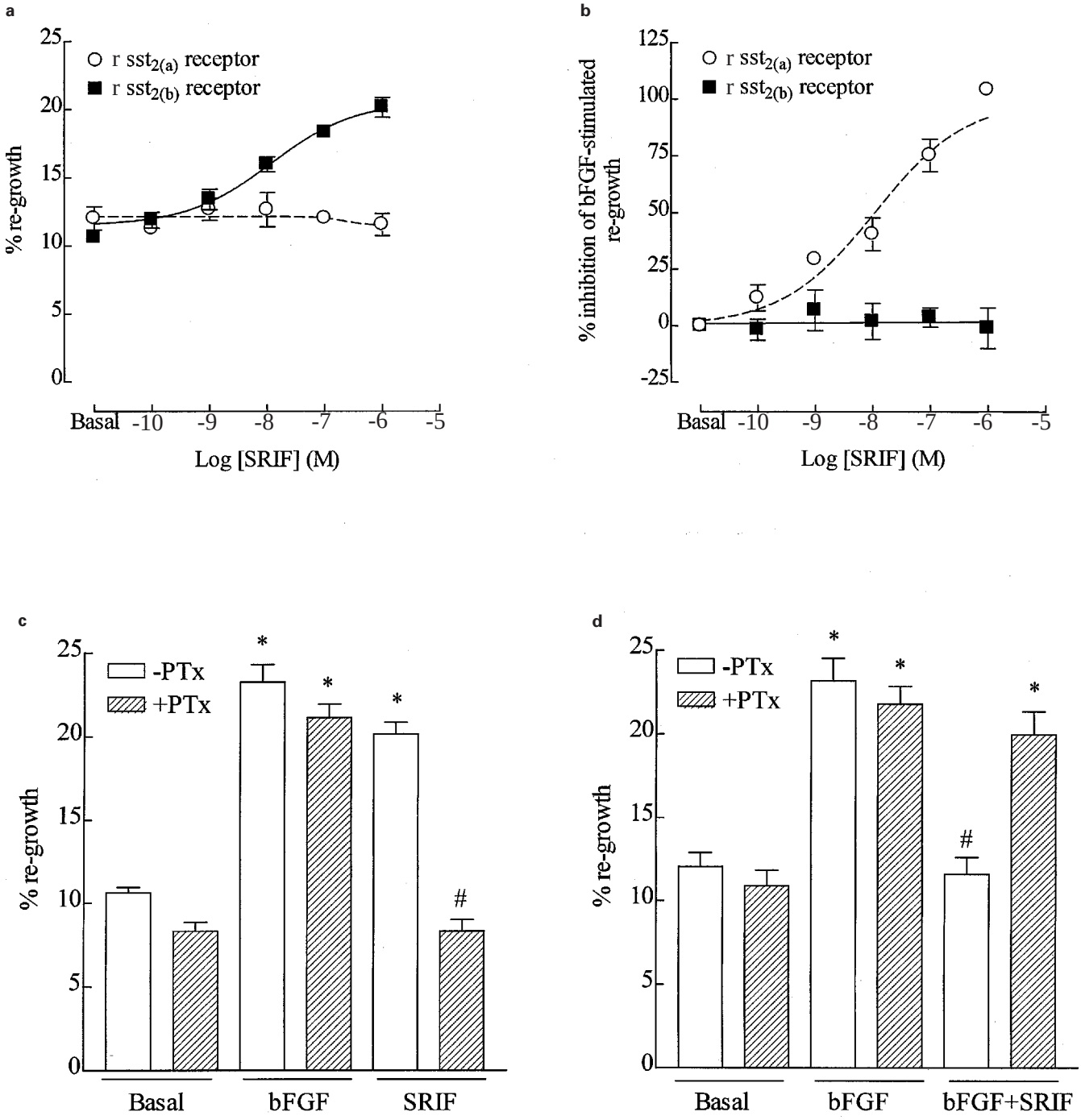


**Figure 1** Concentration-effect curves to SRIF (0.1–1000 nM) were constructed for (a) its effects on basal re-growth and (b) on bFGF (10 ng ml$^{-1}$)-stimulated re-growth in CHOsst$_{2(a)}$ and CHOsst$_{2(b)}$ cells. Values are expressed as the mean percentage re-growth and the mean percentage inhibition of bFGF(10 ng ml$^{-1}$)-stimulated re-growth at 24 h, respectively. Data for the effects of SRIF on basal and bFGF-stimulated re-growth in CHOsst$_{2(a)}$ cells are from Alderton *et al.* (1998) and are provided for comparison with equivalent data in CHOsst$_{2(b)}$ cells. In addition, the effects of pertussis-toxin pretreatment (PTx; 100 ng ml$^{-1}$; 18 h) were determined on basal, bFGF- and SRIF-mediated inhibition or stimulation of re-growth in (c) CHOsst$_{2(b)}$ cells and (d) CHOsst$_{2(a)}$ cells. Pertussis toxin had no effect on basal or bFGF-stimulated re-growth but abolished the SRIF-mediated effects in both recombinant cell lines. All values are the means±s.e.mean from three experiments performed in quadruplicate. * and # represent $P<0.05$ significance to basal and bFGF-stimulated values, respectively.

basal or bFGF-stimulated re-growth in wild-type CHO-K1 cells which do not express SRIF receptors (data not shown).

The effect of pretreatment with pertussis toxin (100 ng $ml^{-1}$) for 18 h on SRIF-induced re-growth into the denuded area was determined in CHO$sst_{2(b)}$ cells. Pertussis toxin did not significantly inhibit basal ($8.3 \pm 0.6\%$) or bFGF-stimulated ($21.1 \pm 0.8\%$) re-growth but abolished the proliferative effects of SRIF upon basal re-growth ($8.3 \pm 0.7\%$ re-growth at 1 $\mu$M SRIF in the presence of pertussis toxin; Figure 1c). Moreover, the proliferative effect of SRIF could also be abolished by the tyrosine kinase inhibitor, genistein (50 $\mu$M; data not shown).

In CHO$sst_{2(a)}$ cells, pretreatment with pertussis toxin had no significant effect on either basal re-growth ($10.9 \pm 0.9\%$ and $12.0 \pm 0.9\%$, respectively) or bFGF-stimulated re-growth ($21.8 \pm 1.0\%$ and $23.2 \pm 1.4\%$, respectively), but abolished the SRIF-mediated inhibition of bFGF re-growth ($11.6 \pm 1.0\%$ to $20.0 \pm 1.4\%$; Figure 1d).

**Discussion** Somatostatin is known to be antiproliferative in many different cell types, including tumour cells, vascular smooth muscle cells and thymocytes (see Lamberts *et al*., 1987; Lauder *et al*., 1997; Mascardo *et al*., 1984). However, in contrast to the known ability of SRIF to inhibit cell growth, there have been some reports that SRIF and its analogues are able to stimulate cell proliferation, for example, in rat mesangial cells (Ruiz-Torres *et al*., 1993), human parietal HGT-1 cells (Wyatt *et al*., 1997) and human meningioma cells (Koper *et al*., 1992), although the mechanism(s) through which SRIF acts to stimulate cell growth is uncertain. Using an *in vitro* model of re-growth and cell proliferation we have examined the ability of the rat $sst_{2(b)}$ receptor, recently identified by Schindler *et al*. (1998c), to modulate cell growth when expressed in CHO-K1 cells. The results from the present study demonstrate that the rat $sst_{2(b)}$ receptor mediates opposite effects on cell growth to that of the rat $sst_{2(a)}$ receptor, previously reported to mediate antiproliferative effects of SRIF (Alderton *et al*., 1998).

Preliminary studies have shown that in the CNS and GI tract there is both overlapping and differential localization of the $sst_{2(a)}$ and $sst_{2(b)}$ receptor isoforms (see Kidd *et al*., 1998; Schindler *et al*., 1998b). Interestingly, the studies by Ruiz-Torres *et al*. (1993) and Wyatt *et al*. (1997) have shown that SRIF can both inhibit and stimulate proliferation in the same cell type and it is tempting to suggest that activation of the somatostatin $sst_{2(b)}$ receptor splice variant may account for some of the seemingly contradictory reports that SRIF can stimulate, as well as inhibit, cellular proliferation. Moreover, since angiopeptin can similarly inhibit or stimulate growth through the rat $sst_{2(a)}$ or $sst_{2(b)}$ receptor, respectively (Alderton *et al*., 1998; this study), the value of using angiopeptin and other $sst_2$ receptor-selective analogues clinically as antiproliferative agents may be negated if $sst_{2(a)}$ and $sst_{2(b)}$ receptors are co-localized. However, such extrapolation must be cautioned by the caveat that splice variants of the human $sst_2$ receptor have yet to be definitively identified (see Schindler *et al*., 1996).

The ability of the rat $sst_{2(b)}$ receptor to mediate stimulation of cell proliferation involves the activation both of pertussis toxin-sensitive G-proteins and tyrosine kinases since the effect is abolished by either pertussis toxin or genistein. So far, studies examining the signal transduction pathways of the rat $sst_{2(a)}$ and $sst_{2(b)}$ splice variants have shown that they can both functionally couple to $G_{i/o}$ and other G-proteins, with little differences in their ability to couple to adenylate cyclase, or activate increases in extracellular acidification or MAP kinase (Schindler *et al*., 1998a,c), all of which may be linked to cell growth. Further work is warranted to ascertain the exact G-protein subunits involved on activation of the two receptor isoforms, since this may provide insight into subtle differences in intracellular signalling.

In conclusion, we show that, in marked contrast to the rat $sst_{2(a)}$ receptor, the recently identified rat $sst_{2(b)}$ isoform, when recombinantly expressed in CHO-K1 cells, mediates stimulation of cell proliferation. However, the transduction mechanisms activated by the rat $sst_{2(a)}$ and $sst_{2(b)}$ receptors which result in opposite effects on cell growth remain to be determined.

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