



# Somatostatin receptor-mediated arachidonic acid mobilization: evidence for partial agonism of synthetic peptides

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**1** Somatostatin and the stable octapeptide analogues, octreotide and angiopeptin, were examined for their ability to stimulate the release of tritium from [<sup>3</sup>H]-arachidonic acid pre-loaded CHO-K1 cells expressing human recombinant sst<sub>2</sub>, sst<sub>3</sub> or sst<sub>5</sub> receptors.

**2** Somatostatin stimulated tritium release (pEC<sub>50</sub>) through the sst<sub>2</sub> (7.8 ± 0.1) and sst<sub>5</sub> (7.3 ± 0.2), but not the sst<sub>3</sub> receptor. Octreotide behaved as a full (sst<sub>2</sub> receptor) or partial agonist (sst<sub>5</sub> receptor), whereas angiopeptin behaved as a weak partial agonist at both receptor types.

**3** Maximum responses to somatostatin through both receptor types were significantly reduced by pertussis toxin, whereas pEC<sub>50</sub> estimates were unaffected.

**4** Inhibition of MEK1 or Src, but not PKA, PI 3-kinases or tyrosine kinases, by reportedly selective inhibitors reduced sst<sub>2</sub>-mediated responses by somatostatin, but not angiopeptin. A selective inhibitor of PKC (Ro-31-8220) reduced both somatostatin and angiopeptin responses.

**5** These data provide further evidence for partial agonist activity of synthetic peptides of somatostatin. Furthermore, the somatostatin receptor signalling mechanisms which mediate arachidonic acid mobilization appear to be multiple and complex.

*British Journal of Pharmacology* (2001) **132**, 760–766

**Keywords:** Somatostatin; angiopeptin, octreotide, arachidonic acid; sst<sub>2</sub> receptor; sst<sub>5</sub> receptor

**Abbreviations:** AA, arachidonic acid; CHO-K1, Chinese hamster ovary cell; cyclic AMP, adenosine 3', 5' cyclic monophosphate; IP, inositol phosphate; MAP, mitogen activated protein; MEK1, mitogen activated kinase 1; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PKA, protein kinase A; PKC, protein kinase C; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; SRIF, somatotrophin release inhibiting factor; sst, somatostatin receptor

## Introduction

Somatostatin (SRIF; somatotrophin release inhibiting factor) receptors (sst<sub>1</sub>–sst<sub>5</sub>) couple to multiple transduction pathways, including the inhibition of adenylate cyclase (Patel *et al.*, 1994; Carruthers *et al.*, 1999), the stimulation of Ca<sup>2+</sup> mobilization and inositol phosphate (IP) production (Akbar *et al.*, 1994; Wilkinson *et al.*, 1997a,b), and the activation of protein tyrosine phosphatases (Buscail *et al.*, 1994; Florio *et al.*, 1994; Reardon *et al.*, 1997). Additionally, there are reports that somatostatin receptors couple to the mobilization of arachidonic acid. Thus studies have demonstrated that activation of recombinant somatostatin receptors, such as the rat sst<sub>4</sub> (Sakanaka *et al.*, 1994) and the human sst<sub>5</sub> receptor (Carruthers *et al.*, 1999), can lead to arachidonic acid release. Somatostatin-stimulated increases in neuronal M-currents have been suggested to be mediated through the generation of an arachidonic acid metabolite(s), such as LTC<sub>4</sub>, in CA1 pyramidal cells (Schweitzer *et al.*, 1990) and in the hippocampus (Lammers *et al.*, 1996). Similarly, the stimulation by somatostatin of BK<sub>Ca</sub> channel activity in rat pituitary tumour cells, possibly through sst<sub>2</sub> receptor activation, is reportedly *via* the production of a leukotriene, possibly also LTC<sub>4</sub> (Duerson *et al.*,

1996). However, the mobilization of arachidonic acid through the sst<sub>5</sub> receptor by somatostatin occurs at agonist concentrations a thousand fold higher than required for inhibition of adenylate cyclase, and similar to those for the stimulation of cyclic AMP (adenosine 3', 5' cyclic monophosphate) accumulation (Carruthers *et al.*, 1999), Ca<sup>2+</sup> mobilization (Wilkinson *et al.*, 1996), inositol phosphate production (Wilkinson *et al.*, 1997a) and GTPγS binding (Williams *et al.*, 1997).

On the basis of structural and pharmacological similarities, somatostatin receptors (sst<sub>1</sub>–sst<sub>5</sub>) can be sub-divided into two groups, the SRIF<sub>1</sub> group, comprising the sst<sub>2</sub>, sst<sub>3</sub> and sst<sub>5</sub> receptors, and the SRIF<sub>2</sub> group, comprising the sst<sub>1</sub> and sst<sub>4</sub> receptor (see Hoyer *et al.*, 1995; Humphrey *et al.*, 1998). Angiopeptin and octreotide, both stable octapeptide somatostatin receptor analogues, are selective for the SRIF<sub>1</sub> group of receptors (Patel & Srikant, 1994). Both of these analogues have generated recent interest concerning their potential use therapeutically for the prevention of angiogenesis (Woltering *et al.*, 1997; Albini *et al.*, 1999) and restenosis following balloon angioplasty (Emanuelsson *et al.*, 1995; von Essen *et al.*, 1997). Although angiopeptin and octreotide both behave as potent and full agonists to inhibit adenylate cyclase or stimulate extracellular acidification through the sst<sub>2</sub> or sst<sub>5</sub> receptor (Patel *et al.*, 1994; Taylor *et al.*, 1996), the ability of these peptides to mobilize arachidonic acid has not been reported. The aims of this study were to characterize the effects of somatostatin, angiopeptin and octreotide on

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arachidonic acid mobilization through human recombinant receptors, comprising the SRIF<sub>1</sub> receptor group, and to examine some of the potential transduction mechanisms involved.

## Methods

### Cell culture

Chinese hamster ovary cells (CHO-K1 cells) stably expressing either recombinant human sst<sub>2</sub> (CHO h sst<sub>2</sub>), sst<sub>3</sub> (CHO h sst<sub>3</sub>) or sst<sub>5</sub> (CHO h sst<sub>5</sub>) receptors were used as previously described (see Wilkinson *et al.*, 1996). Following single cell dilution cloning, levels of receptor expression in all the CHO-K1 cell lines used were of the same order (mean B<sub>max</sub> estimates ( $n=3$ ) in pmol mg<sup>-1</sup> protein, h sst<sub>2</sub> 6.2 ± 0.5; h sst<sub>3</sub> 12.7 ± 2.2; h sst<sub>5</sub> 5.6 ± 0.9). All recombinant CHO-K1 cell lines were maintained in Dulbecco's modified Eagles Medium/Hams F-12 nutrient (1:1) mix supplemented with Glutamax I, 10% foetal calf serum (FCS) and 0.5 mg ml<sup>-1</sup> G418 sulphate (Geneticin) as the selection agent (Life Technologies, Paisley, Scotland). Cells were maintained in the appropriate medium at 37°C in humidified air containing 5% carbon dioxide.

### [<sup>3</sup>H]-arachidonic acid assay

Cells were seeded into 24-well plates at a density of 5 × 10<sup>4</sup> and were incubated with 0.5 µCi ml<sup>-1</sup> of [5,6,8,9,11,12,14,15-<sup>3</sup>H]-arachidonic acid (215 Ci mmol<sup>-1</sup>; Amersham International Ltd) in normal cell culture medium 18 h prior to experimental use. Arachidonic acid is rapidly taken up and almost completely stored in phospholipids (see Washizaki *et al.*, 1994). Cells were then washed four times with 20 mM HEPES-buffered Krebs (mM: NaCl 125; KCl 5.4; NaHCO<sub>3</sub> 16.2; D-glucose 5.5; HEPES 20; NaH<sub>2</sub>PO<sub>4</sub> 1 and CaCl<sub>2</sub> 1.3, pH 7.4), supplemented with 0.1% protease-free BSA and left to equilibrate at 37°C for 10 min. The wash buffer was removed and replaced with fresh buffer (1 ml) containing varying agonist concentrations. An aliquot (600 µl) of the buffer was then removed and counted on a Canberra Packard 2500TR Liquid Scintillation Analyser following the addition of 2 ml of Ultima Gold XR scintillant (Packard). In experiments where the effects of pertussis toxin were to be determined, the toxin was added at the same time as the [<sup>3</sup>H]-arachidonic acid. Experiments to investigate the susceptibility of tritium release to a variety of enzyme inhibitors were modified to include a 120 min pre-incubation of the cells in the presence or absence (control) of the relevant enzyme inhibitor at 37°C. After pre-incubation, the buffer was removed and replaced with fresh buffer containing enzyme inhibitor ± 1 µM somatostatin (basal and agonist-stimulated tritium release for each enzyme concentration).

### Chemicals

Somatostatin was obtained from Peninsula Laboratories Europe Ltd (St. Helens, Merseyside, U.K.). Pertussis toxin, genistein, forskolin, PD 98059, Ro-31-8220, PP1 and LY-294002 were purchased from Calbiochem-Novabiochem Ltd (Beeston, Nottingham, U.K.). Angiopeptin, indomethacin,

eicosatriynoic acid (ETI), arachidonic acid, PGE<sub>2</sub>, quinacrine and the PKA inhibitor amide 14–22 were obtained from Sigma-Aldrich Company Ltd (Poole, Dorset, U.K.). Octreotide was obtained from Novartis Pharma AG (Basel, Switzerland).

### Data analysis

All drug responses were initially measured as disintegrations per minute (d.p.m.) well<sup>-1</sup> and have been expressed either as a per cent over the basal d.p.m. well<sup>-1</sup> or as a percentage of the response to 1 µM somatostatin. For all concentration-effect curves, pEC<sub>50</sub> values (negative log of the concentration of each individual agonist to produce half its own maximum stimulation) were calculated for the curves by non-linear regression analysis of the data and fitting to a four parameter logistic function, using the curve-fitting programme Graph-Pad Prism. Statistical significance was determined (where appropriate) using Student's unpaired *t*-test with  $P < 0.05$  as the level of significance.

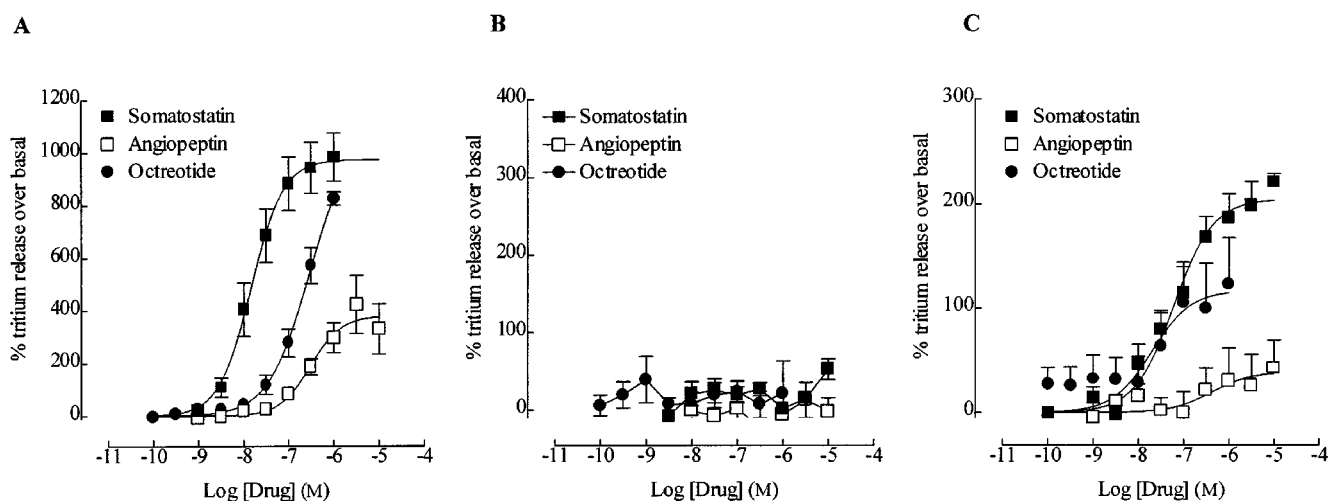
## Results

### Effects of somatostatin peptide analogues on tritium release from [<sup>3</sup>H]-arachidonic acid preloaded CHO-K1 cells

In CHO h sst<sub>2</sub> cells, somatostatin stimulated the release of tritium in a concentration-dependent manner with a pEC<sub>50</sub> of 7.8 ± 0.13 and maximum stimulation of 985 ± 91% over basal (Figure 1A). Octreotide did not reach a maximum response at 1 µM, although the response at this concentration (827 ± 25%; estimated pEC<sub>50</sub> value, 6.33 ± 0.23) did not differ significantly ( $P > 0.05$ ) from that of somatostatin. In contrast, angiopeptin (pEC<sub>50</sub>, 6.57 ± 0.15) acted as a partial agonist, with a maximum response of 423 ± 111% at 3 µM (Figure 1A). Neither somatostatin (31 ± 13%), angiopeptin (13 ± 22%) or octreotide (22 ± 41%) were able to elicit the release of tritium significantly above that of basal (0%) from CHO h sst<sub>3</sub> cells at concentrations up to 10 µM (Figure 1B). In CHO h sst<sub>5</sub> cells, somatostatin stimulated tritium release (pEC<sub>50</sub>, 7.29 ± 0.17; maximum response 221 ± 8%). Octreotide also stimulated the release of tritium (pEC<sub>50</sub>, 7.44 ± 0.29) but produced a lower ( $P < 0.05$ ) maximum stimulation than that of somatostatin (123 ± 44%). Angiopeptin did not significantly stimulate the release of tritium above that of basal (42 ± 27% at 10 µM; Figure 1C). The estimated pEC<sub>50</sub> values, maximum stimulation and Hill slopes obtained with CHO h sst<sub>2</sub> or CHO h sst<sub>5</sub> cells are summarized in Table 1.

### Effects of pertussis toxin

Compared to the responses without pertussis toxin pre-treatment, the maximum response to somatostatin (percentage over basal) after pre-incubation of cells with pertussis toxin (100 ng ml<sup>-1</sup> for 18 h) was reduced in both CHO h sst<sub>2</sub> (1127 ± 96% and 613 ± 120%, respectively; Figure 2A) and CHO h sst<sub>5</sub> cells (199 ± 23 and 108 ± 8%, respectively; Figure 2C) by approximately 50%. However, there was no significant change between the pEC<sub>50</sub> values for somatostatin before and after pertussis toxin pre-treatment of CHO h sst<sub>2</sub>



**Figure 1** The effect of somatostatin, angiopeptin and octreotide on the release of tritium on (A) CHO h *sst*<sub>2</sub> cells, (B) CHO h *sst*<sub>3</sub> cells and (C) CHO h *sst*<sub>5</sub> cells. All cells were pre-loaded with [<sup>3</sup>H]-arachidonic acid for 18 h before experimentation and were incubated with drug or vehicle for 1 h. All values are normalized as a percentage over the basal response (0%) and are the mean from 5–7 experiments. Vertical lines represent the s.e.mean. Where no error bar is shown, the s.e.mean lies within the symbol.

**Table 1** A summary of pEC<sub>50</sub> values, the Hill slopes (n<sub>H</sub>) and the maximum responses to somatostatin, angiopeptin or octreotide in CHO h *sst*<sub>2</sub> or CHO h *sst*<sub>5</sub> cells pre-loaded with [<sup>3</sup>H]-arachidonic acid

	pEC <sub>50</sub>	Human <i>sst</i> <sub>2</sub> receptor Hill slope (n <sub>H</sub> )	Maximum	pEC <sub>50</sub>	Human <i>sst</i> <sub>5</sub> receptor Hill slope (n <sub>H</sub> )	Maximum
Somatostatin	7.8 ± 0.13	1.21 ± 11	985 ± 91	7.29 ± 0.17	0.76 ± 0.17	221 ± 8
Angiopeptin	6.57 ± 0.15	1.26 ± 19	423 ± 111	<5	–	43 ± 27
Octreotide	6.33 ± 0.23	0.94 ± 18	827 ± 25	7.44 ± 0.29	0.76 ± 0.26	123 ± 44

cells ( $7.65 \pm 0.08$  and  $7.53 \pm 0.06$ , respectively), or CHO h *sst*<sub>5</sub> cells ( $7.15 \pm 0.17$  and  $7.02 \pm 0.28$ , respectively). Pre-treatment of CHO h *sst*<sub>3</sub> cells with pertussis toxin did not alter the inability of somatostatin to stimulate tritium release (maximum response  $33 \pm 20\%$  over basal with  $10 \mu\text{M}$  somatostatin after pertussis toxin; Figure 2B).

In order to determine whether the partial agonism of angiopeptin in CHO h *sst*<sub>2</sub> cells was due to its inability to stimulate coupling to specific G proteins, the effect of pertussis toxin on the ability of angiopeptin to release tritium was examined. The potency (pEC<sub>50</sub> values) of angiopeptin was similar before and after pertussis toxin treatment ( $6.40 \pm 0.18$  and  $6.01 \pm 0.36$ , respectively), whereas the maximum response was significantly reduced ( $474 \pm 104\%$  and  $230 \pm 26\%$ , respectively;  $P < 0.05$ ; Figure 2A).

#### Mechanism of tritium release through the *sst*<sub>2</sub> receptor

The mechanism of somatostatin-stimulated tritium release via the *sst*<sub>2</sub> receptor was further investigated. Quinacrine ( $1$  or  $10 \mu\text{M}$ ), a non-selective inhibitor of PLA<sub>2</sub>, or PGE<sub>2</sub> ( $1$  nM to  $10 \mu\text{M}$ ) had no effect on the basal or somatostatin ( $1 \mu\text{M}$ )-stimulated release of tritium (see Table 2 for values). The selective MEK1 inhibitor, PD 98059 ( $40 \mu\text{M}$ ), had no effect on the basal tritium release ( $8.5 \pm 1.1\%$  and  $9.3 \pm 1.2\%$ , respectively; values are expressed as a per cent of the  $1 \mu\text{M}$  somatostatin response), but reduced the somatostatin ( $1 \mu\text{M}$ )-stimulated release of tritium to  $61.9 \pm 3.0\%$  (Figure 3A). A higher concentration of PD 98059 ( $60 \mu\text{M}$ ) had no further

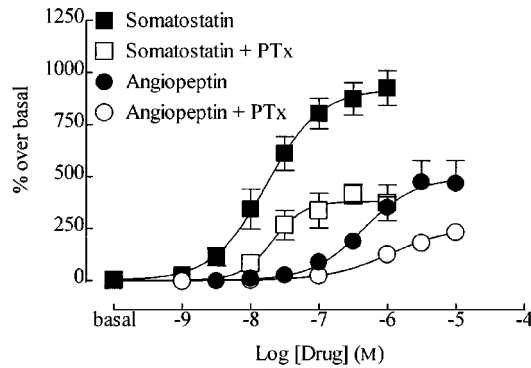
effect (data not shown). Surprisingly, the response to the partial agonist angiopeptin in CHO h *sst*<sub>2</sub> cells was unaffected by PD 98059 ( $42.5 \pm 10.4\%$  and  $50.1 \pm 3.4\%$ , respectively). After pre-treatment of CHO h *sst*<sub>2</sub> cells with pertussis toxin, the ability of somatostatin ( $34.1 \pm 2.5\%$ ) or angiopeptin ( $20.4 \pm 1.9\%$ ) to stimulate tritium release was unaffected by the PD 98059 compound ( $33.1 \pm 1.0\%$  and  $25.2 \pm 0.8\%$ , respectively; Figure 3A).

Similar to PD 98059, a selective inhibitor of Src, PP1 ( $200$  nM), had no effect on the basal release of tritium ( $7.1 \pm 0.9\%$  and  $11.0 \pm 3.7\%$  before and after PP1, respectively), but inhibited somatostatin ( $1 \mu\text{M}$ )-stimulated release ( $100\%$  and  $64.0 \pm 1.7\%$ , respectively; Figure 3B). In contrast, the responses to angiopeptin ( $1 \mu\text{M}$ ) were unaffected by PP1 ( $42.5 \pm 10.4\%$  and  $51.7 \pm 10.8\%$ , respectively). In the presence of pertussis toxin, somatostatin- and angiopeptin-stimulated tritium release ( $26.8 \pm 3.7\%$  and  $17.7 \pm 2.2\%$ , respectively) was unaffected by PP1 ( $34.0 \pm 1.7\%$  and  $18.4 \pm 0.8\%$ , respectively; Figure 3B).

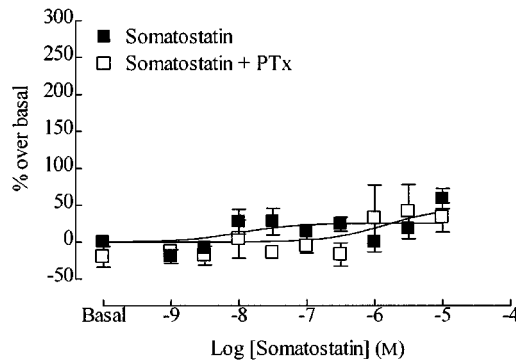
In the presence of PD 98059 and PP1 combined, somatostatin-stimulated tritium release was reduced to  $65.6 \pm 3.4\%$ , no different to the effect produced by either inhibitor alone on the somatostatin response (see above). As expected, the inhibitory effect of both inhibitors combined upon somatostatin was abolished after pertussis toxin pre-treatment (data not shown).

Genistein ( $10 \mu\text{M}$ ), a non-selective inhibitor of protein tyrosine kinases, LY 294004 ( $1 \mu\text{M}$ ), a selective PI 3-kinase inhibitor, the adenylate cyclase activator, forskolin ( $10 \mu\text{M}$ ),

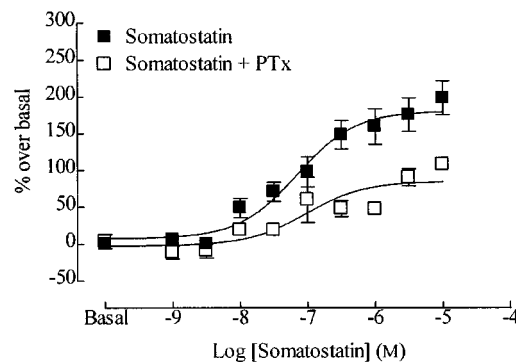
A



B



C



**Figure 2** The effect of pertussis toxin (PTx; 100 ng.ml<sup>-1</sup>, 18 h) upon somatostatin-stimulated release of tritium. (A) CHO h sst<sub>2</sub>, (B) CHO h sst<sub>3</sub>, and (C) CHO h sst<sub>5</sub> cells pre-loaded with [<sup>3</sup>H]-arachidonic acid. Cells were pre-incubated with pertussis toxin for 18 h. Vertical lines represent the s.e.mean ( $n=4-6$ ). Where no error bar is shown, s.e.mean lies within the symbol.

and a PKA inhibitor amide 14–22 (1  $\mu$ M), all had no effect on responses to either somatostatin or angiopeptin (values shown in Table 2).

Basal tritium release before ( $5.6 \pm 0.4\%$ ) or after ( $6.8 \pm 0.2\%$ ) pertussis toxin pre-treatment was unaffected by the protein kinase C (PKC) inhibitor, Ro-31-8220 (1  $\mu$ M;  $5.1 \pm 0.1\%$  and  $4.3 \pm 0.2\%$ , respectively). In both the absence and presence of pertussis toxin pre-treatment, the response to somatostatin ( $100\%$  and  $28.3 \pm 0.6$ , respectively) or angiopeptin ( $43.2 \pm 1.0\%$  and  $17.1 \pm 1.3$ , respectively) were reduced

**Table 2** Effects of inhibitors on the release of tritium from CHO h sst<sub>2</sub> cells

	Somatostatin (1 $\mu$ M)	Angiopeptin (1 $\mu$ M)
Basal	100%	50.9 $\pm$ 3.7%
Pertussis toxin (100 ng ml <sup>-1</sup> )	34.1 $\pm$ 2.5*	20.4 $\pm$ 1.9*
Quinacrine (10 $\mu$ M)	106.8 $\pm$ 2.9	n.d.
PGE <sub>2</sub> (10 $\mu$ M)	97.5 $\pm$ 5.8	n.d.
LY-294002 (1 $\mu$ M)	97.4 $\pm$ 8.7	44.1 $\pm$ 1.3
Genistein (10 $\mu$ M)	99.2 $\pm$ 5.0	52.2 $\pm$ 4.9
Forskolin (10 $\mu$ M)	101.9 $\pm$ 2.6	50.9 $\pm$ 4.3
PKA inhibitor (1 $\mu$ M)	95.9 $\pm$ 9.2	49.7 $\pm$ 2.7
Ro-31-8220 (1 $\mu$ M)	65.9 $\pm$ 1.3*	31.6 $\pm$ 1.0*
PD 98059 (40 $\mu$ M)	61.9 $\pm$ 3.0*	50.1 $\pm$ 3.4
PP1 (200 nM)	64.0 $\pm$ 1.7*	51.7 $\pm$ 10.8

\*Represents  $P < 0.05$  compared to basal for somatostatin or angiopeptin. n.d. represents data not determined.

by Ro-31-8220 ( $65.9 \pm 1.3\%$  and  $13.8 \pm 0.5\%$  for somatostatin;  $31.6 \pm 1.0\%$  and  $9.5 \pm 1.1\%$  for angiopeptin, respectively; Figure 4).

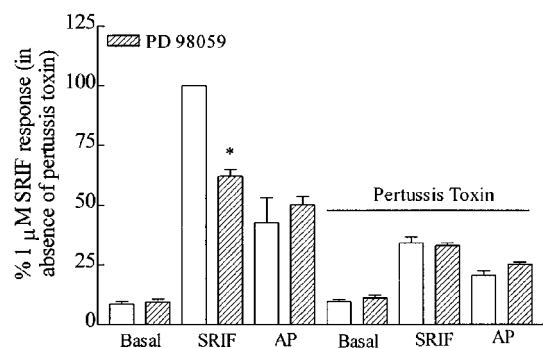
## Discussion

We have characterized agonist-stimulated tritium release from various CHO-K1 cell lines expressing recombinant receptor types of the SRIF<sub>1</sub> group and pre-loaded with [<sup>3</sup>H]-arachidonic acid. Although few studies to date have examined recombinant somatostatin receptor activation and effects on arachidonic acid mobilization, several groups have used a similar assay to study other recombinant G protein-coupled receptors, such as substance P (Garcia *et al.*, 1994), dopamine (Nilsson *et al.*, 1998), 5-hydroxytryptamine (Berg *et al.*, 1998) and adrenoceptors (Xing & Insel, 1996).

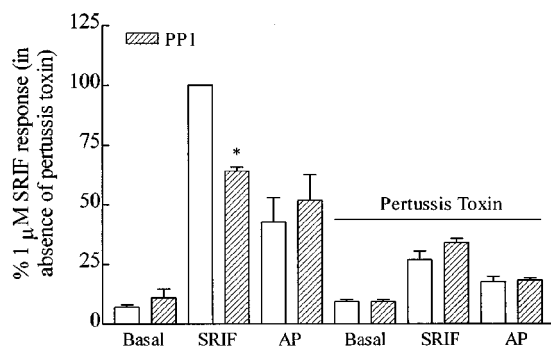
Using recombinant somatostatin receptor systems, the rat sst<sub>4</sub> and the human sst<sub>5</sub> receptor have been identified as coupling to arachidonic acid mobilization (Bito *et al.*, 1994; Carruthers *et al.*, 1999). These observations have been extended in this study by demonstrating that, under similar experimental conditions, agonist-stimulated tritium release occurs through the recombinant sst<sub>2</sub> and sst<sub>5</sub> receptor, but not the sst<sub>3</sub> receptor. Somatostatin receptor density ( $B_{\max}$ ) was similar for CHO h sst<sub>2</sub> (6.2) and CHO h sst<sub>5</sub> cells (5.6) and only slightly higher in CHO h sst<sub>3</sub> cells (12.7). Although variation in receptor density can dramatically alter agonist activity (see Kenakin, 1996; Hermans *et al.*, 1999), the distinct differences seen in the present study within the SRIF<sub>1</sub> receptor group to mediate arachidonic acid release do not appear to be attributable to differences in receptor expression levels.

The tritiated product released by somatostatin receptor activation in the current study was not identified. Prostaglandin E<sub>2</sub> represents only a relatively small proportion of the released product through recombinant sst<sub>5</sub> receptor activation (Carruthers *et al.*, 1999). Nilsson *et al.* (1998) report that 90–99% of the tritiated product measured from CHO cells expressing D<sub>2</sub> receptors is attributable to free arachidonic acid release. It would be of great interest to confirm whether or not arachidonic acid itself represents the major releasable product upon somatostatin receptor activation since the

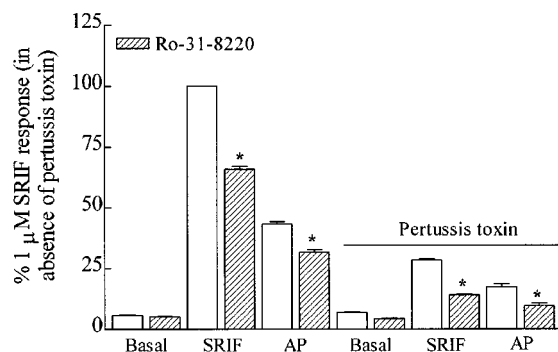
A



B



**Figure 3** The inhibition of MEK and Src. The effect of (A) the selective MEK1 inhibitor, PD 98059 (40  $\mu$ M), and (B) the Src inhibitor, PP1 (200 nM), on the release of tritium from CHO h sst<sub>2</sub> cells stimulated by somatostatin (SRIF) and angiotensin (AP; both 1  $\mu$ M) in the presence and absence of pertussis toxin (100 ng ml<sup>-1</sup>; 18 h). Results are expressed as a percentage of the somatostatin response in the absence of pertussis toxin. \*Significantly different from somatostatin alone ( $P < 0.05$ ). Vertical lines represent the s.e.mean ( $n = 5$ ).



**Figure 4** The inhibition of PKC. The effect of a PKC inhibitor, Ro-31-8220 (1  $\mu$ M), on basal, somatostatin- or angiotensin (both 1  $\mu$ M)-stimulated tritium release from CHO h sst<sub>2</sub> cells pre-loaded with [<sup>3</sup>H]-arachidonic acid. The effects of Ro-31-8220 to block tritium release were examined in the absence and presence of pertussis toxin (100 ng ml<sup>-1</sup>; 18 h). Results are expressed as a percentage of the somatostatin response in the absence of pertussis toxin. \*Significantly different from peptide in absence of Ro-31-8220 ( $P < 0.05$ ). Vertical lines represent the s.e.mean ( $n = 5$ ).

arachidonic acid cascade (see Shimizu & Wolfe, 1990; Piomelli, 1993). Although experiments using the same CHO h sst<sub>5</sub> cell line have demonstrated the existence of functional prostanoic receptors positively linked to adenylate cyclase (Carruthers *et al.*, 1999), exogenous PGE<sub>2</sub> had no effect on the ability of somatostatin to stimulate arachidonic acid release through sst<sub>2</sub> or sst<sub>5</sub> receptors (this study). This contrasts with the findings of Di Marzo & Piomelli (1992), who report that in CHO-K1 cells expressing dopamine D<sub>2</sub> receptors, PGE<sub>2</sub> increased D<sub>2</sub> receptor-mediated arachidonic acid release.

In this study, both the sst<sub>2</sub> and sst<sub>5</sub> receptor coupled to pertussis toxin-sensitive and -insensitive G proteins to stimulate tritium release. In contrast, the rat sst<sub>4</sub> receptor reportedly mediates arachidonic acid release exclusively through G<sub>i/o</sub> proteins (Bito *et al.*, 1994). Although the maximum response to somatostatin through the sst<sub>2</sub> and sst<sub>5</sub> receptors was markedly reduced by pertussis toxin, there was no change in the estimated pEC<sub>50</sub> values, implying that both receptor-types couple equally efficiently to pertussis toxin-sensitive and -insensitive G proteins to stimulate the release of tritium. The particular G proteins involved in the mobilization of arachidonic acid by somatostatin have yet to be determined. Previous work in our laboratory suggests that the pertussis toxin-insensitive release of arachidonic acid through the sst<sub>5</sub> receptor is not mediated through a G $\alpha_s$  protein (Carruthers *et al.*, 1999), indicating the possibility of the involvement of G $\alpha_q$  or G $\alpha_{12}$  proteins.

Several studies have employed inhibition of forskolin-stimulated cyclic AMP as a functional assay to determine agonist activity of octreotide and angiotensin, where both peptides act as full and potent agonists (for example, Patel *et al.*, 1994). This is in agreement with unpublished data from our own laboratory using these cell lines. However, because of the efficient coupling of somatostatin receptors to the inhibition of adenylate cyclase, even a very weak partial agonist may display full agonist activity (Carruthers *et al.*, 1999). We have now shown that octreotide and angiotensin are actually considerably weaker than somatostatin at mobilizing arachidonic acid even although both agonists bind with high affinity at the sst<sub>2</sub> receptor (IC<sub>50</sub> values of 0.36 nM and 0.26 nM, respectively) and sst<sub>5</sub> receptor (pIC<sub>50</sub> values of 20.5 nM and 6.92 nM, respectively). In particular, angiotensin had low intrinsic activity at both the sst<sub>2</sub> and the sst<sub>5</sub> receptor when employing arachidonic acid mobilization as an index of activity. The hypothesis that the partial agonism of angiotensin at the sst<sub>2</sub> receptor was due to differential stimulation of pertussis toxin-sensitive and -insensitive G proteins was discounted since angiotensin behaved as a partial agonist with similar pEC<sub>50</sub> values both before and after pertussis toxin treatment, although the maximum response was reduced with pertussis toxin treatment.

In light of the ability of a receptor to couple to multiple G proteins with differing affinities for the receptor, it has been postulated that the intrinsic efficacy of a drug/receptor is neither constant nor independent of downstream G proteins, but rather may have multiple estimated values (see Kenakin, 1996; Berg *et al.*, 1998). Since it is apparent that agonist-activity is dependent in part upon the downstream response measured (see Kenakin, 1996 for review) it becomes necessary to know the particular intracellular mechanisms which couple

particular eicosanoid(s) released may have dramatic consequences for the physiological relevance of activation of the

to the receptor and are involved in mediating the response measured. Thus, theoretically, the development of a clinically useful agonist ought not to be targeted against the receptor type alone, but the receptor-G protein complex implicated in mediating the response considered a target for therapeutic intervention. Although it is yet uncertain whether somatostatin receptors differentially couple to multiple G proteins *in vivo*, there are important implications clinically if this is found to be so. For example, the inhibition of proliferation in rat vascular smooth muscle cells is reported to be through the sst<sub>5</sub> receptor (Lauder *et al.*, 1997). Supposing this effect were mediated *in vivo* through the inhibition of adenylate cyclase (efficiently-coupled), octreotide or angiopeptin (both full agonists), may be prove to be effective antiproliferative agents. However, were the antiproliferative effect mediated through an sst<sub>5</sub>-stimulated release of arachidonic acid (poorly-coupled), it would be predicted that both peptides would be ineffective therapeutic agents.

The selective MEK1 inhibitor, PD98059, and the Src inhibitor, PP1, reduced somatostatin sst<sub>2</sub> receptor-mediated responses, although these effects were observed only in the absence of pertussis toxin. This suggests that both p42/44 MAP kinase and Src are involved exclusively in the Gi/o protein-mediated release of tritium. Intriguingly, the MEK and Src inhibitors reduced the responses to somatostatin but not those to angiopeptin. A possible explanation for this may be that somatostatin (a full agonist) is able to recruit a comparatively greater number of different downstream signalling molecules to mobilize arachidonic acid release than angiopeptin (a partial agonist). Assuming this to be the case, the effects of specific inhibitory compounds, such as PD 98059 or PP1, would be more readily observed against a somatostatin rather than an angiopeptin response, which was indeed found to be so.

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- Somatostatin-induced tritium release *via* the sst<sub>2</sub> receptor was insensitive to the non-selective PLA<sub>2</sub> inhibitor, quina- crine, even at high concentrations (10 μM), or the selective PI 3-kinase inhibitor, LY-294002. In contrast, sst<sub>4</sub> receptor-mediated mobilization of AA is reportedly dependent upon both PLA<sub>2</sub> and PI 3-kinase (Bito *et al.*, 1994; Sakanaka *et al.*, 1994). This suggests that there are discrete differences between somatostatin receptor types to mobilize AA release. The lack of effect of quina- crine in our study may reflect a situation where arachidonic acid is not synthesized *de novo*, but rather exogenous [<sup>3</sup>H]-arachidonic acid is loosely stored in intracellular/plasma membrane 'pools' and is easily releasable upon receptor activation (see Chilton, 1989). Somatostatin sst<sub>2</sub> receptor-mediated AA release was independent of cyclic AMP since neither forskolin nor a selective PKA inhibitor affected responses. Conversely, the ability of the PKC inhibitor, Ro-31-8220, to reduce responses to somatostatin or angiopeptin in the presence or absence of pertussis toxin, indicates a possible role for PKC in the mobilization of arachidonic acid *via* the sst<sub>2</sub> receptor.
- In conclusion, this study has characterized the ability of the somatostatin receptor types comprising the SRIF<sub>1</sub> group to mobilize tritium from CHO-K1 cells pre-loaded with [<sup>3</sup>H]-arachidonic acid. The signalling pathways utilized by the sst<sub>2</sub> receptor to release arachidonic acid and/or its metabolites remain to be further characterized, but appear to involve PKC and p42/44 MAP kinase. Perhaps most notably, the somatostatin receptor peptide analogues, octreotide and angiopeptin, have low intrinsic activity at the sst<sub>2</sub> and sst<sub>5</sub> receptors which may have important implications for their potential as therapeutic agents, and highlights the need for rigorous analyses of agonist activity.

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(Received October 5, 2000

Accepted October 5, 2000)