British Journal of Pharmacology (2004) 142, 150–160
www.nature.com/bjp

Comparison of functional profiles at human recombinant somatostatin sst_2 receptor: simultaneous determination of intracellular Ca^{2+} and luciferase expression in CHO-K1 cells

[1,3]Caroline Nunn, [1,4]Davide Cervia, [1]Daniel Langenegger, [2]Laurent Tenaillon, [2]Rochdi Bouhelal & *,[1]Daniel Hoyer

[1]Neuroscience Research, Novartis Institutes for Biomedical Research, Novartis Pharma AG, CH-4002, Basel, Switzerland and [2]Novartis Lead Discovery Centre, Novartis Institutes for Biomedical Research, Novartis Pharma AG, CH-4002, Basel, Switzerland

1 Somatostatin (somatotropin release inhibiting factor; SRIF) acts *via* five G protein-coupled receptors (sst_1–sst_5) that modulate multiple cellular effectors. The aim of this study was to compare two functional effects of the human sst_2 receptor stably expressed in CHO-K1 cells in a single experiment using a duplex assay for intracellular calcium and serum response element (SRE)-driven luciferase expression.

2 Intracellular calcium was measured using a fluorometric imaging plate reader II (FLIPR II). SRIF-14 rapidly and transiently increased intracellular calcium with a pEC_{50} of 8.74 ± 0.03 ($n = 52$). At 5 h after FLIPR II measurements, luciferase expression was determined. SRIF-14 concentration-dependently increased luciferase expression ($pEC_{50} = 9.06 \pm 0.03$, $n = 52$).

3 Natural and synthetic agonist/antagonist ligands for SRIF receptors were tested in the duplex assay. Correlation of agonist potencies and efficacies between the two responses were significant ($r^2 = 0.83$ and 0.90, pEC_{50} and E_{max}, respectively).

4 Pertussis toxin pretreatment reduced SRIF-14/octreotide-mediated intracellular calcium increases by 45–47% and luciferase expression by 95–98%.

5 Thapsigargin pretreatment abolished the SRIF-14/octreotide-mediated intracellular calcium increase but had no effect on luciferase expression.

6 In conclusion, SRIF stimulates an increase in intracellular calcium and SRE-luciferase expression *via* human sst_2 receptors in CHO-K1 cells. The increase in luciferase is mediated *via* G_i/G_o while intracellular calcium increase is mediated by both G_i/G_o proteins and pertussis toxin-insensitive G proteins, and is mainly *via* release of calcium from intracellular stores. SRIF ligands display a similar recognition profile suggesting that the ligand/receptor/G protein/effector interaction is similar for the two parameters.

British Journal of Pharmacology (2004) **142,** 150–160. doi:10.1038/sj.bjp.0705735

Keywords: Somatostatin receptor subtype 2; CHO-K1 cells; FLIPR II; SRE-luciferase; duplex assay; pertussis toxin; thapsigargin

Abbreviations: CHO-K1, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle's Medium; FLIPR II, fluorometric imaging plate reader II; FLU, fluorescence light units; GppNHp, 5′guanylyl-imidodiphosphate; MAP kinase, mitogen-activated protein kinase; RLU, relative luminescence units; SRE, serum response element; SRF, serum response factor; SRIF, somatostatin/somatotropin release inhibiting factor; sst, somatostatin receptor; TCF, ternary complex factor

Introduction

The neuropeptide somatostatin (somatotropin release inhibiting factor; SRIF) acts *via* five G protein-coupled receptors (sst_1–sst_5; Hoyer *et al*., 1995). According to structural and pharmacological similarities, the five receptors can be classified in two groups – $SRIF_1$ which consists of sst_2, sst_3 and sst_5, and $SRIF_2$ which consists of sst_1 and sst_4 (Hoyer *et al*., 1995; Hannon *et al*., 2002).

All five receptors are coupled to adenylyl cyclase *via* G_i/G_o proteins, leading to inhibition of cyclic AMP (cAMP) accumulation (Patel *et al*., 1994; Carruthers *et al*., 1999; Siehler & Hoyer, 1999b). In addition, the receptors have been shown to modulate multiple cellular effectors including phospholipase C, Ca^{2+} channels, K^+ channels, mitogen-activated protein kinases (MAP kinases) and phosphotyrosine phosphatases (Patel & Srikant, 1997; Meyerhof, 1998).

*Author for correspondence at: Neuroscience Research, WSJ 386/745, Novartis Institutes for Biomedical Research, Novartis Pharma AG, CH-4002, Basel, Switzerland;
E-mail address: daniel1.hoyer@pharma.novartis.com
[3]Current address: Department of Pharmacology, Rikshospitalet University Hospital, University of Oslo, PO Box 1057, N-0316 Oslo, Norway
[4]Current address: Dipartimento di Fisiologia e Biochimica 'G Moruzzi', Università di Pisa, 56127 Pisa, Italy
Advance online publication: 22 March 2004