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Pharmacological characterisation of native somatostatin receptors in AtT-20 mouse tumour corticotrophs

[1,2]Davide Cervia, [2]Caroline Nunn, [2]Dominique Fehlmann, [2]Daniel Langenegger, [2]Edi Schuepbach & *,[2]Daniel Hoyer

[1]Dipartimento di Fisiologia e Biochimica 'G. Moruzzi', Università di Pisa, 56127 Pisa, Italy and [2]Nervous System Research, Novartis Pharma AG, CH-4002 Basel, Switzerland

1 The mouse corticotroph tumour cell line AtT-20 is a useful model to investigate the physiological role of native somatostatin (SRIF, Somatotropin release inhibitory factor) receptor subtypes (sst_1 – sst_5). The objective of this study was to characterise the pharmacological features and the functional effects of SRIF receptors expressed by AtT-20 cells using radioligand binding and cAMP accumulation.

2 [^{125}I]LTT-SRIF-28, [^{125}I]CGP 23996, [^{125}I]Tyr^{10}-cortistatin-14 and [^{125}I]Tyr^3-octreotide labelled SRIF receptor binding sites with high affinity and in a saturable manner ($B_{max} = 315$, 274, 239 and 206 fmol mg^{-1}, respectively). [^{125}I]LTT-SRIF-28 labels significantly more sites than [^{125}I]Tyr^{10} -cortistatin-14 and [^{125}I]Tyr^3 -octreotide as seen previously in cells expressing pure populations of sst_2 or sst_5 receptors.

3 SRIF analogues displaced the binding of the four radioligands. $sst_{2/5}$ receptor-selective ligands showed much higher affinity than $sst_{1/3/4}$ receptor-selective ligands. The binding profile of [^{125}I]Tyr^3-octreotide was different from that of [^{125}I]LTT-SRIF-28, [^{125}I]CGP 23996 and [^{125}I]Tyr^{10}-cortistatin-14. The $sst_{5/1}$ receptor-selective ligand L-817,818 identified two binding sites, one with subnanomolar affinity (sst_5 receptors) and one with micromolar affinity (sst_2 receptors); however, the proportions were different: 70 – 80% high affinity with [^{125}I]LTT-SRIF-28, [^{125}I]CGP 23996, [^{125}I]Tyr^{10}-cortistatin-14, but only 20% with [^{125}I]Tyr^3-octreotide.

4 SRIF analogues inhibited the forskolin-stimulated cAMP levels depending on concentration. $sst_{2/5}$ receptor-selective ligands were highly potent, whereas $sst_{1/3/4}$ receptor-selective ligands had no significant effects. The sst_2 receptor antagonist D-Tyr^8-CYN 154806 competitively antagonised the effects of SRIF-14 and sst_2 receptor-preferring agonists, but not those of L-817,818.

5 The complex binding properties of SRIF receptor analogues indicate that sst_2 and sst_5 receptors are the predominant SRIF receptors expressed on AtT-20 cell membranes with no or only negligible presence of sst_1, sst_3 and sst_4 receptors. In the functional studies using cAMP accumulation, only sst_2 and sst_5 receptors appear to play a role. However, the 'predominant' receptor appears to be the sst_2 receptor, although sst_5 receptors can also mediate the effect, when the ligand is not able to activate sst_2 receptors. This clearly adds flexibility to SRIF-mediated functional effects and suggests that the physiological role of SRIF and its analogues may be mediated preferentially via one subtype over another.

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Abbreviations: ACTH, adrenocorticotropin; DMEM, Dulbecco's MEM with Glutamax-I; EDTA, ethylenediaminetetraacetic acid; FRSK, forskolin; GH, growth hormone; HEPES, *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulphonic acid]; IBMX, isobutylmethylxanthine; SRIF, somatostatin, somatotropin release inhibitory factor; sst_1 – sst_5 receptors, SRIF receptor subtypes 1 – 5

Introduction

Somatostatin (SRIF; somatotropin release inhibitory factor) is known to be a potent regulator of endocrine secretion (Hannon *et al*., 2002a). In particular, in the anterior pituitary, SRIF acts as a neurohormone, secreted from neurones into the blood, and inhibiting the secretion of growth hormone (GH), adrenocorticotropin (ACTH) as well as other pituitary hormones. Biological actions of SRIF are exerted through the activation of multiple heptahelical, G-protein-linked SRIF receptor subtypes (sst_1 – sst_5) which are generally coupled to an inhibition of cAMP levels (Patel, 1999). SRIF receptors have a broad and overlapping tissue distribution; in particular, in the rat pituitary, sst_2 and sst_5 receptors are the principal subtype expressed (Mezey *et al*., 1998). To date, although numerous studies have explored the SRIF receptor function when expressed in transfected systems, assigning a pharmacological and functional profile to an individual native SRIF receptor remains difficult. This is due to the limited availability of

*Author for correspondence;
E-mail: daniel1.hoyer@pharma.novartis.com