Davide Cervia · Dominique Fehlmann · Daniel Hoyer

Native somatostatin sst_2 and sst_5 receptors functionally coupled to $G_{i/o}$ -protein, but not to the serum response element in AtT-20 mouse tumour corticotrophs

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Abstract Of the five cloned somatostatin (SRIF: somatotropin release inhibitory factor) receptors (sst_{1-5}), only sst_2 and sst_5 receptors appear to be endogenously expressed and functionally active in AtT-20 mouse anterior pituitary tumour cells. In this study, the presence and the functional coupling of SRIF receptors to G-protein in AtT-20 cells was evaluated by receptor autoradiography and guanosine-5'-O-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTP γ S) binding, respectively. In addition, transcriptional effects via the serum response element (SRE) were assessed in AtT-20-SRE-luci cells, engineered to express constitutively SRE upstream of the luciferase reporter gene.

[^{125}I]LTT-SRIF-28, [^{125}I]CGP 23996 and [^{125}I]Tyr³-octreotide binding illustrates the high level of $\text{sst}_{2/5}$ receptor in AtT-20 cell membranes. SRIF-14 and SRIF-28 produced a concentration-dependent increase in [^{35}S]GTP γ S binding ($\text{pEC}_{50} = 6.72$ and 7.45 ; $E_{\text{max}} = 79$ and 74.9 , respectively) which was completely abolished by pertussis toxin. $\text{sst}_{2/5}$ receptor-selective ligands caused a concentration-dependent increase in [^{35}S]GTP γ S binding ($\text{pEC}_{50} = 7.74$ – 5.84 ; $E_{\text{max}} = 76.6$ – 20.2) while $\text{sst}_{1/3/4}$ receptor-selective ligands were devoid of activity. The binding profiles of [^{125}I]LTT-SRIF-28 and the inhibition of cAMP accumulation correlated highly significantly with their corresponding [^{35}S]GTP γ S binding profiles ($r=0.862$ and 0.874 , respectively). The effects of the sst_2 receptor-preferring agonists Tyr³-octreotide and BIM 23027 on [^{35}S]GTP γ S binding, but not those of SRIF-14 and the $\text{sst}_{5/1}$ receptor selective-agonist L-817,818, were competitively antagonised by the sst_2 receptor antagonist D-Tyr⁸-CYN 154806 ($\text{pK}_B = 7.36$ and 7.72 , respectively; slope

factors not significantly different from unity). In AtT-20-SRE-luci cells, which carry a SRE-luciferase construct functioning in a very efficient manner, SRIF and its analogues did not affect luciferase activity.

Taken together, these results demonstrate that in AtT-20 cells the expression of sst_2 and sst_5 receptors fit with their functional coupling to $G_{i/o}$ -proteins. The pharmacological implications of the existence of different ligand/receptor complexes are discussed. However, the intracellular pathways coupled to the activation of sst_2 and sst_5 receptors appear not to modulate the SRE-mediated transcriptional activity, suggesting that SRIF effects on gene expression coupled to mechanisms that have promoters other than SRE.

Keywords Somatostatin analogues · Receptor autoradiography · [^{35}S]GTP γ S binding · Serum response element (SRE) · Luciferase assay · AtT-20 cells

Introduction

The cyclic neuropeptide somatostatin (SRIF: somatotropin release inhibitory factor) exhibits multiple biological effects, which include the inhibition of several pituitary hormones (Patel 1999). The effects of SRIF are mediated via its interaction with specific membrane bound receptors, five subtypes of which have been characterized by molecular cloning from mammalian species, and termed sst_{1-5} ; these receptors belong to the seven transmembrane spanning superfamily of G-protein-coupled receptors (Hannon et al. 2002). To date, although numerous studies have explored SRIF receptor function when expressed in recombinant systems, specific physiological responses of native receptor subtypes are generally unknown. This is particularly true in cells that express several subtypes of SRIF receptors, which tend to show partially overlapping pharmacological and transductional features that confuse the interpretation of mechanistic studies. Another reason is the lack of subtype specific agonists/antagonists with adequate pharmacokinetic proper-

D. Cervia (✉)
Dipartimento di Fisiologia e Biochimica "G. Moruzzi",
Università di Pisa, 56127 Pisa, Italy
Tel.: +39-050-2213459, Fax: +39-50-2213527,
e-mail: d.cervia@dfb.unipi.it

D. Fehlmann · D. Hoyer
Nervous System Research, WSJ-386/745, Novartis Pharma AG,
4002 Basel, Switzerland