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Short communication

Antidepressant drugs promote the heterodimerization of the dopamine D2 and somatostatin Sst5 receptors – fluorescence *in vitro* studies

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Abstract:
Background: The interaction between the dopaminergic and somatostatinergic systems and their role in mood regulation have been well-documented. Therefore, we decided to investigate the effect of antidepressant drugs on the heterodimerization of the dopamine D2 and somatostatin Sst5 receptors.
Methods: The human receptor proteins were tagged with fluorescent proteins, expressed in the HEK 293 cells and incubated with antidepressant drugs: desipramine and citalopram. To determine the FRET efficiency, the fluorescence resonance energy transfer (FRET) and photobleaching confocal microscopy techniques were used.
Results: We found that the efficiency of FRET is markedly increased in cells coexpressing the somatostatin Sst5 and dopamine D2 receptors after 48 h of incubation with desipramine and citalopram.
Conclusions: In the present study we provide physical evidence, based on FRET analysis, that antidepressants increase Sst5 and D2 receptors heterodimerization. The effect is specific because desipramine in the incubation medium uncouples other pairs of receptors, such as the dopamine D1-D2 receptors.

Key words:
somatostatin receptor 5, dopamine receptor 2, antidepressants, FRET, heterodimerization

Abbreviations: CFP – cyan fluorescent protein, CIT – citalopram, D2 – dopamine type 2 receptor, DMI – desipramine, FRET – Förster (Fluorescence) Resonance Energy Transfer, GPCR – G-protein-coupled receptors, HEK 293 – Human Embryonic Kidney 293 cell line, pbFRET – photobleaching FRET, Sst5 – somatostatin type 5 receptor, YFP – yellow fluorescent protein

Introduction

Dopaminergic and somatostatinergic system interactions have been suggested for many years based on anatomical, behavioral and biochemical studies. It has

been reported that dopamine administration regulates somatostatin release in the rat striatum and hippocampus [17] and that selective dopamine D1 and D2 receptor agonists increase somatostatin receptor density in the striatum [8]. Likewise, somatostatin positively modulates dopamine release in the striatum [3, 19]. Dopamine and somatostatin have also been implicated in the pathophysiology of depression because of their potential roles in mood regulation. Reduced levels of somatostatin have been observed in the cerebrospinal fluid [10, 18] and recently in the subgenual anterior cingulate cortex [20] of depressive patients. Intracerebroventricular administration of somatostatin results in an antidepressant-like effect in rats, as shown by a forced swim test [6]. Furthermore, chronic desipramine treatment selectively potentiates somatostatin-induced dopamine release in the nucleus accumbens and the striatum [12]. It has also been shown that chronic administration of antidepressants influences somatostatin levels and somatostatin receptor density in rat brains [13]. More recent studies show that imipramine upregulates somatostatin release in the mouse hypothalamus, eliciting antidepressant-like effects in tail suspension test [11].

The molecular basis of this functional interaction between the somatostatinergic and dopaminergic systems may stem from the interaction of the somatostatin and dopamine receptors. These receptors were found to share some similarities: they are members of the G protein-coupled receptors (GPCRs) family, show sequence homology and appear to be structurally related [6]. It has been demonstrated that members of both superfamilies, when coexpressed in the same cell and in the presence of specific ligands, may interact at the membrane level, forming homo- and heterodimers. These dimers may constitute a novel receptor that can activate alternative pathways, possibly enhancing ligand affinity and signal transduction [7].

It has been established by immunocytochemical studies that the dopamine D2 and somatostatin Sst5 receptors colocalize in medium-sized aspiny interneurons in the striatum and pyramidal neurons in the cerebral cortex, an observation that suggests the possibility of functional interactions between these receptors. It has been shown that the dopamine D2 and somatostatin Sst5 receptors, when coexpressed in the same cell, undergo ligand-dependent heterodimerization with enhanced functional activity. D2-Sst5 heterodimerization is associated with a modification in ligand binding and a synergistic effect on the activation of the transduction pathway because both receptors signal through the inhibition of adenylyl cyclase *via* Gi proteins [16].

In the present study, we investigated the heterodimerization of the human D2 and Sst5 receptors upon treatment with antidepressant drugs (desipramine and citalopram) in the proposed *in vitro* model by employing biophysical approaches, such as photobleaching fluorescence resonance energy transfer microscopy. Förster (Fluorescence) Resonance Energy Transfer (FRET) is a physical phenomenon that is being used more and more in biomedical research and drug discovery, as it allows the study of interactions between proteins in a single cell. FRET is a process that relies on the distance-dependent transfer of energy between an excited donor molecule and an acceptor molecule in a non-radiative way. This technique provides insight into the interactions between proteins that are in close proximity (less than 10 nm) to each other, which makes it an excellent method for measuring the extent to which receptors dimerize. One of the established and commonly used techniques for measuring FRET is acceptor photobleaching [14]. It is based on the fact that FRET reduces the amount of fluorescence released by the donor fluorophore. This method involves measuring the donor fluorescence intensity in the same sample before and after destroying the acceptor by photobleaching. If FRET is initially present, an increase in unquenched donor fluorescence occurs.

We used two antidepressant drugs with different pharmacological profiles: desipramine a tricyclic antidepressant that inhibits the continued uptake of noradrenaline and, to a lesser extent, serotonin; and citalopram, a serotonin uptake inhibitor. Although none of the antidepressants used in this study have any affinity for the dopaminergic or somatostatinergic receptors [2], 48 h of incubation has been found to affect their ability to form heterodimers.

Materials and Methods

Materials

HEK293 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell culture reagents were purchased from Gibco (Carlsbad, CA, USA), Sigma-Aldrich (Poznań, Poland) and PAA Laboratories GmbH. Molecular biology reagents were