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Research report

# Antidepressants promote formation of heterocomplexes of dopamine D2 and somatostatin subtype 5 receptors in the mouse striatum

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ABSTRACT

The interaction between the dopaminergic and somatostatinergic systems is considered to play a potential role in mood regulation. Chronic administration of antidepressants influences release of both neurotransmitters. The molecular basis of the functional cooperation may stem from the physical interaction of somatostatin receptor subtypes and dopamine D2 receptors since they colocalize in striatal interneurons and were shown to undergo ligand-dependent heterodimerization in heterologous expression systems. In present study we adapted *in situ* proximity ligation assay to investigate the occurrence of D2-Sst5 receptor heterocomplexes, and their possible alterations in the striatum of mice treated acutely and repeatedly (21 days) with antidepressant drugs of different pharmacological profiles (escitalopram and desipramine). Additionally we analysed number of heterocomplexes in primary striatal neuronal cultures incubated with both antidepressant drugs for 1 h and 6 days. The studies revealed that antidepressants increase formation of D2-Sst5 receptors heterodimers. These findings provide interesting evidence that dopamine D2 and somatostatin Sst5 heterodimers may be considered as potential mediators of antidepressant effects, since the heterodimerization of these receptors occurs in native brain tissue as well as in primary striatal neuronal cultures where receptors are expressed at physiological levels.

## 1. Introduction

The main hypothesis explaining the etiology of depression is focused on the monoamine neurotransmitters (serotonin and noradrenaline) deficiency, based on the primary effect of antidepressants action (Elhwuegi, 2004). However, increasing evidence suggests that possible significance in the mechanism of action of these drugs may involve two other neuromodulators – dopamine and somatostatin. They belong to the major neurotransmitter systems that are widely distributed in the brain and share a number of structural and functional characteristics. Their action is mediated by receptors that belong to the GPCR superfamily and colocalize in neuronal subgroups (Rocheville et al., 2000).

Somatostatin and dopamine interaction is well described in the literature and their potential role in mood regulation has been considered. It has been reported that dopamine administration regulates somatostatin release (Rodriguez-Sanchez et al., 1997) and that selective dopamine receptors agonists increase somatostatin receptor density in the striatum (Izquierdo-Claros et al., 1997). Likewise, somatostatin positively modulates dopamine release in the striatum (Chesselet and Reisine, 1983; Thermos et al., 1996). Some studies have linked depression with the activity of somatostatin and dopamine systems. Reduced levels of somatostatin and dopamine metabolites were shown in the cerebrospinal fluid of depressive patients (Rubinow, 1986; Molchan et al., 1991; Lin and Sibille, 2013). Intracerebroventricular administration of somatostatin results in antidepressant-like effects in the forced swim test in rats (Engin et al., 2008). More recent studies demonstrate transmitter switching between dopamine and somatostatin receptors expression induced by exposure to short and long-day photoperiods which can affect depressive-like behaviours (Dulcis et al., 2013).

Antidepressants interact with the dopamine and somatostatin pathways: chronic desipramine treatment selectively potentiates somatostatin-induced dopamine release in the nucleus accumbens and the striatum in rats (Pallis et al., 2001). It has been also shown that chronic SSRI treatment results in the increase in somatostatin levels (Pallis et al., 2009).

The molecular basis of this functional interaction between the somatostatinergic and dopaminergic systems may result from the physical interaction of somatostatin and dopamine receptors. Number of studies have indicated that GPCRs can associate and form heteromers that exhibit unique pharmacological and functional properties (Franco et al., 2007; Ferré and Franco, 2010). Immunocytochemical techniques

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established that dopamine D2 receptors colocalize with somatostatin Sst2 (Baragli et al., 2007) and Sst5 (Rocheville et al., 2000) receptors in medium-sized aspiny interneurons in the striatum what suggests the possibility of their direct functional interactions. Heterodimerization of these receptors was investigated in transfected CHO-K1 and HEK293 cell lines (Rocheville et al., 2000; Baragli et al., 2007; Szafran et al., 2012). It has been shown that, when coexpressed in the same cell, these receptors undergo ligand-dependent heterodimerization. D2-Sst5 heterodimers are pharmacologically distinct from its receptors monomers. They modify the ligand affinities and exert a synergistic effect on the transduction pathway, since both receptors signal via inhibition of adenylyl cyclase (Rocheville et al., 2000). Our recent studies using heterologous system revealed that antidepressants promote heterodimerization of somatostatin Sst5 and dopamine D2 receptors (Szafran et al., 2012). These results may be considered as an explanation of previous reports showing that interaction between dopamine and somatostatin systems may induce an antidepressant-like effect.

The purpose of the present work was to investigate effects of acute and chronic administration of two antidepressants − desipramine (a tricyclic antidepressant) and escitalopram (a selective serotonin reuptake inhibitor) on formation of heterocomplexes of dopamine D2 and somatostatin Sst5 receptors in the striatum of mouse as well as in the striatal neuronal cultures, where these receptors are expressed at physiological levels. Since techniques for exploring proteins interactions in native tissue are limited, we adapted a novel method for protein-protein interaction detection – *in situ* Proximity Ligation Assay technique, which has been recently used to detect the presence of endogenous heteromers in tissues and cell cultures (Söderberg et al., 2006; Trifilieff et al., 2011; Borroto-Escuela et al., 2013; Gruszczynska-Biegala and Kuznicki, 2013). With the use of *in situ* PLA, we demonstrated that the number of endogenous D2-Sst5 heterocomplexes is altered by chronic treatment with antidepressant drugs.

## 2. Materials and methods

### 2.1. Animals

Male C57BL/6J mice (N = 4), weighting 25–30 g were used. Animals were kept at a constant room temperature (24 °C), under 12-h light/dark cycle and had free access to food and water. All animal procedures used in this study were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences (Krakow, Poland).

#### 2.1.1. Drug administration

Desipramine and escitalopram (Sigma Aldrich, Germany) were dissolved in saline and given in doses of 20 mg/kg and 10 mg/kg respectively, intraperitoneally (i.p.) either acutely (single dose) or repeatedly (once daily for 21 days). Control animals received saline. Doses were chosen on the basis of previous reports (Dziedzicka-Wasylewska et al., 2006a).

#### 2.1.2. Brain tissue preparation

Mice were decapitated 1 h after administration of the last dose of the drug (or saline), brains were quickly removed, frozen on dry ice and kept at −80 °C. Coronal sections (thickness: 5 μm) were generated using cryostat and mounted on gelatin-covered microscope slides (Menzel Gläser, Germany). Sections were air dried at room temperature and fixed in 4% PFA in PBS (Sigma-Aldrich, Germany), rinsed thrice in PBS and proceed for the *in situ* PLA procedure.

### 2.2. Primary striatal neuronal culture

Primary mouse striatal neurons were purchased from Lonza (Lonza, Switzerland). Primary embryonic (E14, 15) brain neuronal cells were cultured on cover slips (Menzel Gläser, Germany) following the manufacturer's instructions. Neurons were plated at density $3 \times 10^5$ cells/cm$^2$ on cover slips covered with laminin (2 ug/mL; Sigma-Aldrich, Germany) and poly-D-lysine (30 ug/mL; Sigma-Aldrich, Germany). Neurons were grown in Primary Neuron Basal Medium (Lonza, Switzerland) supplemented with 2 mM L-Glutamine, 50ug/mL Gentamicin/37 ng/ml Amphotercin and 2% NSF-1 (Lonza, Switzerland). The cultures were maintained at 37 °C, 5% $CO_2$. The cells were grown on cover slips for 10 days, then antidepressant – desipramine or escitalopram (Sigma Aldrich, Germany) were added to the culture medium (final concentration 1 μM). After incubation either for 1 h or 6 days, the neurons were fixed with a 4% paraformaldehyde solution in PBS (Sigma-Aldrich, Germany) for 10 min at room temperature, washed three times in PBS, and proceeded for the *in situ* PLA procedure.

### 2.3. Culture of neurons from adult mouse brain

For the control experiments of *in situ* Proximity Ligation Assay technique, neurons were isolated from adult mouse brain and cultured according to Brewer and Torricelli, (2007) with minor modifications. In details, adult mouse was decapitated and brain was removed from the skull. The striatum was dissected with the cerebral cortex and transferred to cold Hibernate A/B27 medium containing Hibernate A medium, 1X B27 supplement (Thermo Fisher) and 0.5 mM Glutamax (Thermo Fisher). Brain tissue was cut on small slices in laminar chamber using sterile razor blade. Slices were transferred to 6 ml of filtered Hibernate A minus calcium (BrainBits) with 2 mg/ml papain (Sigma Aldrich) and incubated in water bath at 30 °C for 30 min with shaking (approx. 220 rpm). After enzymatic treatment tissue slices were transferred to 2 ml of fresh Hibernate A/B27 medium and triturated ten times using sialinized 9-in Pasteur pipette with the fire-polished tip (BrainBits). Trituration step was performed three times. After each trituration step supernatant containing cells was collected to a new tube and another 2 ml of fresh Hibernate A/B27 medium was added to the remaining tissue. After trituration, 6 ml of cell suspension was gently applied to the top of the prepared OptiPrep (Sigma Aldrich) density gradient in 15 ml Falcon tube. For detailed description of the preparation of OptiPrep density gradient see Brewer et al. (2007). Cells were centrifuged for 15 min at 800 × *g* at 22 °C. After centrifugation, top 6 ml of medium containing cell debris was aspirated and the lower layer of density gradient (1.5 ml) was collected for neuronal cell culture. Collected fraction was diluted with 5 ml of Hibernate A/B27 medium and centrifuged at 200 x g for 2 min. Supernatant was aspirated and the cell pellet was resuspended in complete Neurobasal A medium containing Neurobasal A (Thermo Fisher), 1 x B27 supplement (Thermo Fisher), 0.5 mM Glutamax (Thermo Fisher), 1ug/ml Gentamycin (Thermo Fisher) and 10 ng/ml recombinant FGFb (Thermo Fisher). Cells were seeded on 16-well glass chamber slides (Nunc Lab-Tek Chamber Slide System) coated with poly-D-lysine (100 ug/mL; Merck Millipore) and laminin (10 ug/mL; Sigma-Aldrich, Germany). Cells were incubated in at 37 °C in 5% CO2 atmosphere for 12 h and after that rinsed three times with Hibernate A/B27 medium and then cultured in complete Neurobasal A medium. Neuronal cell culture was further maintained with replacing 50% of the growth media every 3 days. After 10 days of cultivation neurons were incubated for 1 or 3 h with 10 μM dopamine hydrochloride (Sigma Aldrich). Afterwards cells were fixed with a 4% paraformaldehyde solution in PBS (Sigma-Aldrich, Germany) for 10 min at room temperature, washed three times in PBS, and proceeded for the *in situ* PLA procedure.

### 2.4. In situ PLA

The receptor–receptor interactions in the mouse striatal sections and in neuronal cultures were detected using the Duolink II *in situ* Proximity Ligation Assay detection kit (Olink Bioscience, Sweden). *In situ* PLA was performed according to the manufacturer's protocol. After