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INTRODUCTION :

It has been previously reported that dopamine and somatostatin may play role in the pathophysiology of depression:

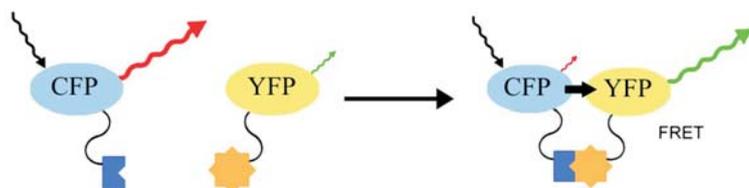
- ▶ somatostatin regulates dopamine release, which suggests its potential role in mood regulation [1],
- ▶ chronic desipramine treatment selectively potentiates somatostatin-induced dopamine release in the nucleus accumbens and the striatum [2],
- ▶ dopamine D2 and somatostatin Sst5 receptors were shown to colocalise in rat brain cortex and striatum and undergo ligand-dependent heterodimerization with enhanced functional activity [3].

AIM OF THE STUDY :

To investigate the formation of D2 and Sst5 receptors heterodimers upon treatment with antidepressant drugs (desipramine and citalopram), using *in vitro* model.

METHODS

The studies of dopamine D2 and somatostatin Sst5 receptors interaction were based on Förster Resonance Energy Transfer (FRET) phenomenon.



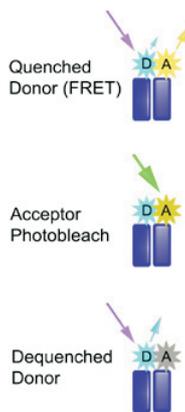
- ▶ The human receptor proteins were tagged with fluorescent proteins, cyan (CFP, fluorescence donor) and yellow (YFP, fluorescence acceptor) and expressed in the HEK 293 cells.
- ▶ Desipramine and citalopram were present in the culture medium at concentrations of 0.1 μM, 1 μM and 10 μM for 24, 48 and 72 hours prior to the fixation.
- ▶ The acceptor photobleaching technique with the use of confocal microscopy was used to determine the FRET efficiency.

FRET Acceptor Photobleaching

In the event of FRET the donor encounters a quenching of fluorescence due to its energy transfer to the acceptor. The donor fluorescence will be unquenched after photobleaching of the acceptor. The difference of fluorescence intensity of the donor before and after photobleaching gives a direct indication to the FRET efficiency and can be quantified as:

$$FRET\ eff = (D_{post} - D_{pre}) / D_{post}$$

where D_{post} is the fluorescence intensity of the donor after acceptor photobleaching, and D_{pre} the fluorescence intensity of the donor before acceptor photobleaching.



RESULTS

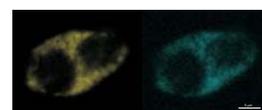


Fig 1. HEK 293 cells were transiently transfected with the plasmid DNA for sst5-CFP and D2-YFP constructs.

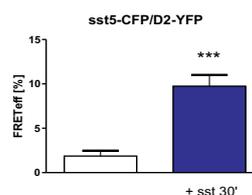


Fig 2. Somatostatin stimulation of HEK293 cells expressing sst5-CFP (fluorescence donor) and D2-YFP (fluorescence acceptor) receptors. Somatostatin 14 (1 μM) was present in the incubation medium for 30 minutes. FRET efficiency was measured by acceptor photobleaching. Data are presented as mean ± S.E.M. (***) p<0.001

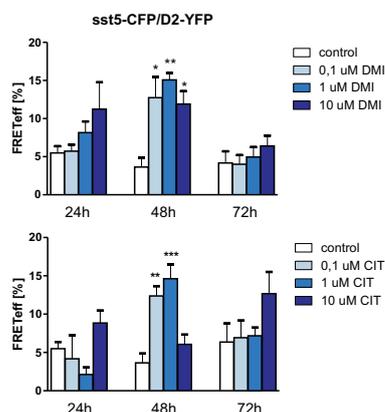


Fig 3. FRET efficiency of the sst5-CFP and D2-YFP pairs by acceptor photobleaching. Antidepressant drugs (DMI-desipramine, CIT-citalopram) at concentrations 0.1 μM, 1 μM, 10 μM were present in the incubation medium for 24, 48 and 72 hours prior to cell fixation. Data are presented as mean ± S.E.M. (* p<0.05; ** p<0.01; *** p<0.001).

CONCLUSIONS :

- ▶ In the present study we provide physical evidence, based on FRET analysis, that **antidepressants increase Sst5 and D2 receptors dimerization**.
- ▶ Desipramine and citalopram presence for 48 hours in culture medium affects ability of Sst5 and D2 receptors to form heterodimers, despite of lack of affinity of these drugs to dopamine or somatostatin receptors.
- ▶ Since it has been reported that dopamine and somatostatin may play a role in the pathophysiology of depression, D2-Sst5 heterodimers with enhanced functional activity can be proposed as a new drug discovery target in research of improvement in antidepressant therapy.

References:

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2. Pallis, E., Thermos, K., Spyrali, C., 2001 Chronic desipramine treatment selectively potentiates somatostatin-induced dopamine release in the nucleus accumbens. *European Journal of Neuroscience* 14, 763-767.
3. Rocheville, M., Lange, D.C., Kumar, U., Patel, S.C., Patel, R.C., Patel, Y.C., 2000 Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. *Science* 7;288(5463):154-7.