On the role of GPCR homo and heteroreceptor complexes balance on Parkinson´s disease
Dasiel O. Borroto-Escuela, Julia Oflijan, Thorsten Schäfer Luca Pinton, Ismel Brito, Manuel Narváez, Kristina Friedland, Luigi F. Agnati, Rafael Franco, Kjell Fuxe
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Stockholm – Sweden
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... a little of history and background
Where and how the concept of GPCR receptor-receptor started?

Fuxe/Agnati introduced the hypothesis of receptor-receptor interactions in 1980-1981. In membrane preparations of various CNS regions they found that neuropeptides could modulate the binding characteristics, especially the affinity of the monoamine receptors in a receptor subtype specific way.

Fuxe et al. 1982, Fuxe et al. 1983, Agnati et al., 1985
Receptor–Receptor Interactions as an Integrative Mechanism in Nerve Cells

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Receptor–Receptor Interactions

MONOMERS

HOMODIMERS

HETERODIMERS

NO EFFECT

EFFECT A

EFFECT B

EFFECT C

TA ➔ EFFECT A

TB ➔ EFFECT B

TA + TB ➔ EFFECTS A + B + C

Fig. 4. Homo- and heterodimerization of membrane receptors. Heterodimers induce a cellular effect different from homodimers. The relative proportion of the two transmitters, the concentration of the two receptor populations, and the characteristics of receptor–receptor interactions will determine the amount of homo- vs heterodimers and thus the overall effect on the target cell.
The concept of GPCR heterodimer was later confirmed in an excellent way in 1998-99 by studies reporting that two non-functional GPCR monomers, GABAB1 and GABAB2, can assemble in a signaling heterodimer at the cell surface.

The GABAB receptor belongs to the class C of GPCR with dimerization taking place between the venus flytrap modules and the C terminal coiled-coil domains.

GABA_B receptors – the first 7TM heterodimers

Fiona H. Marshall, Kenneth A. Jones, Klemens Kaupmann and Bernhard Bettler
Phylogenetic tree representation of the human GPCR superfamily.
The GPCR Network: a large-scale collaboration to determine human GPCR structure and function.

Fig. 3 — Structures of the CXCR4 dimer and the CXCL12 monomer and dimer (PDB ID: 2P72) colored according to their electrostatic potential from red (negative) to blue (positive), in order to highlight the charge complementarity of these proteins. On the left, the CXCR4 structure is shown in two orientations — on the top looking into the ligand binding pocket and on the bottom, from the side, of the dimer. The top right shows the monomer and dimer of CXCL12; the bottom right shows the structure of the CXCR4 dimer, clipped, in order to illustrate the binding pocket and that multiple stoichiometries and orientations of the CXCL12/CXCR4 seem feasible, as described in the text (no orientations are implied in the figure). Figures were prepared using ICM software (source: Molsoft.com).

Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists.

Wu et al. (2010) Science 330: 1066-1071
RECEPTOR-RECEPTOR INTERACTIONS: A NEW INTEGRATIVE MECHANISM AT MEMBRANE LEVEL

MONOMERS

HOMOMERS

NEGATIVE COOPERATIVITY IN β-ADRENERGIC RECEPTORS

LIMBIRD, LEFKOWITZ 1975

HETEROMERS

NEUROPEPTIDE-MONOAMINE REC-REC INTERACTIONS

REC-REC INTERACTIONS

ADAPTER PROTEIN-MEDIATED INTERACTIONS

AGNATI, FUXE 1980; FUXE, AGNATI 1981

Fuxe et al. 2010
... diversity
Fuxe K. et al 2013
... promiscuity and specificity
The whole picture…the GPCR heterodimer network

http://www.gpcr-hetnet.com
... on the balance of homo and heteroreceptor complexes
A1-D1 heteroreceptor complexes

✓ Heteroreceptor complexes of A1Rs and D1Rs were demonstrated with coimmunoprecipitation in cotransfected Ltk-fibroblast cells and later on in striatum using also this technique. With BRET and FRET, further evidence was later on obtained for their existence in A1R an D1R cotransfected cell lines.

✓ Antagonistic allosteric A1R-D1R receptor--receptor interactions were found in these complexes as seen from the substantial reduction of D1Rs in the high-affinity state induced by A1R agonists in cellular models and in striatal membrane preparations.

✓ the Gi/o coupled A1R antagonistically also interact with the Gs/olf coupled D1R at the AC level.

✓ A1R agonists in rabbits can counteract D1R agonist-induced oral dyskinesias

The balance of A1-D1 homo and heteroreceptor complexes

Antonelli et al. 2006; Fuxe et al. 2007, 2010, Borroto-Escuela et al. 2015 a and b
... relevance for Parkinson's disease??
<table>
<thead>
<tr>
<th>Heteroreceptor complex</th>
<th>Location</th>
<th>Signaling</th>
<th>Potential relevance for PD</th>
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<tbody>
<tr>
<td>D1R-D2R</td>
<td>Subsets of MSNs in accumbens (DYN, ENK, GABA/glutamate) [58]</td>
<td>$G_{q/11}$, Ca$^{2+}$ release via IP$_3$, recruitment of BDNF via CaMK-II and MeCP2 are blocked by D1R and D2R antagonist. SKF83959 D1R-D2R heteromer agonist</td>
<td>Hyperdopaminergia increases function of D1R-D2R heteromer, mental side effects with l-DOPA and D2R agonist including addiction [60,179]</td>
</tr>
<tr>
<td>D1R-D3R</td>
<td>Certain striato-nigral GABA neurons [72]</td>
<td>D3R enhancement of D1R affinity and postsynaptic signaling</td>
<td>D3R enhances D1R-induced locomotion and dyskinesias [68,69]</td>
</tr>
<tr>
<td>D1R-D3R-A1R</td>
<td>Hippocampus, striatum (synapse) [145,149]</td>
<td>D1R reduced NMDA currents and excitotoxicity. NMDA increased D1R signaling. D1R activation upregulates NMDA-dependent LTP and promotes working memory and NR1-CaMK-II coupling</td>
<td>Postulated [110,132] Cognitive dysfunction after uncoupling of the receptor complex [180]</td>
</tr>
</tbody>
</table>

BDNF: Brain-derived neurotrophic factor; LTP: Long-term potentiation; PD: Parkinson’s disease.
Table 2. Heteroreceptor complexes containing dopamine D2R subtype.

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<td>D2R-D1R</td>
<td>Subsets of MSNs in accumbens (DYN, ENK, GABA/glutamate) [58]</td>
<td>$G_{O1}$, $Ca^{2+}$ release via IP$_3$, recruitment of BDNF via CaMK-II and MeCP2, blocked by D1R and D2R antagonist, inactivation of GSK-3b. SKF83959 D1R-D2R heteromer agonist</td>
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<td>D2R-D3R</td>
<td>Ventral striatum [83]</td>
<td>Partial D2R agonists turn into D2R antagonists at the D2R-D3R heteromer</td>
<td>Partial D2R agonists in PD treatment have reduced mental side-effects [41,84]</td>
</tr>
<tr>
<td>D2R-D4R</td>
<td>Striatum [87,88]</td>
<td>Combined D2R and D4R agonist treatment resulted in potentiating effects on ERK1/2 phosphorylation for D4.2R, D4.4R but not for D4.7R containing heteromers [87]</td>
<td>The synergistic effects on ERK signaling may increase plasticity responses to L-DOPA treatment. The D4.7 variant may be linked to ADHD [87,88]</td>
</tr>
<tr>
<td>D2R-NMDAR (NR2B)</td>
<td>Striatal glutamate synapses [133]</td>
<td>This complex blocks CaMK-II-NR2B interaction with reduction of NR2B phosphorylation and NMDAR currents. Disruption of the D2R-NR2B complex reduced cocaine-induced locomotion. The composite is increased by cocaine.</td>
<td>Reduction of dopamine VT in PD can reduce the formation of the D2R-NMDAR complex increasing the NMDA-mediated synaptic glutamate drive [133]</td>
</tr>
<tr>
<td>D2R-A2AR</td>
<td>Striato-pallidal GABA neurons, striatal cholinergic interneurons [44,46,47,103]</td>
<td>Antagonistic A2AR-D2R receptor-receptor interactions in the heteroreceptor complex and at the AC level. D2R recognition, Gi/o coupling and signaling reduced [45,181]. A2A agonist blocked D2R-induced LTD and restored LTP [109]</td>
<td>A2AR antagonists may significantly target the A2A protomer. They increase locomotion, contralateral turning behavior after subthreshold doses of L-DOPA and D2 like agonists. No worsening of dyskinesia. Antidepressant activity [107,110]</td>
</tr>
<tr>
<td>D2R-A2AR-mGluR5</td>
<td>Striato-pallidal GABA neurons.</td>
<td>A2AR-mGluR5 synergize to reduce D2R recognition and Gi/o coupling and signaling [45,120,124]. Interactions also at the level of the signaling cascades: MAPK and CREB-P. A2AR and mGluR5 agonists synergistically increase GABA release in ventral pallidum [125]</td>
<td>MGlur5 antagonists and negative allosteric modulators may significantly target the mGluR5 protomer. They increase locomotion and exert antiparkisonian actions and antidyskinetic actions specially combined with A2A antagonists [126,127,129,130]</td>
</tr>
</tbody>
</table>

AC: Adenylate cyclase; BDNF: Brain-derived neurotrophic factor; LTD: Long term depression; LTP: Long term potentiation; PD: Parkinson’s disease; VT: Volume transmission.
Examples of GPCR heteroreceptor complexes relevance for PD.

Fuxe et al. 2015, Borroto-Escuela et al. 2015
Clinical pharmacology

Disease progression

... can be defined in terms of changes in disease status as a function of time

In degenerative disorders such as PD, natural disease progression is caused by a continuous degeneration of dopaminergic neurons, which is reflected in such disease status measured as the UPDRS.

Drug action

... reflects the effect of a drug on disease status

Drug may provide symptomatic benefit without influencing the underlying progression of the disease (relieve clinical symptoms)

Or

Drug may influence the underlying time course of progression (slow disease progression)

We should consider to find out how the time course of levodopa effects might be modified as PD progresses and how the different relevant dopamine homo and heteroreceptor complexes are altered during each specific disease status.
GPCR homo and heteroreceptor complexes balance on Parkinson’s disease progression and treatment
... are we technological ready?
Light Resonance Energy Transfer-based methods

- Single cell BRET imaging
- Intra-molecular FRET/BRE
- TR-FRET
- BiFC
- New FRET- and BRET-based second messenger sensors

Borroto-Escuela et al. 2013
METHODS

- Radioligand binding assay
- Coimmunoprecipitation
- Split-ubiquitin Membrane Yeast Two Hybrid system (MYTHS)
- Fluorescence Cross Correlation Spectroscopy
  - Receptor activation by biased ligands and small interface interfering peptides (SIIP)
  - Phosphoproteomics
  - NanoScan PET/MRI scanner
  - Generation and phenotypical analysis of knock-in rats
- In situ Proximity Ligation Assays (PLA)
Fluorescence Cross Correlation Spectroscopy (FCCS)

CONFOCAL VOLUME

COMPUTER

LASER

APD DETECTORS

OBJECTIVE

OUTPUT

INTENSITY VS TIME

FCCS COMBINED WITH FIDA

ABSOLUTE CONCENTRATIONS OF MONOMERS, HETEROMERS AND HOMOMERS ON EACH SINGLE LIVE CELL
In situ PLA

Conjugation. A pair of primary antibodies bind to the proteins to be detected.

A pair of PLA probes (PLUS and MINUS) bind their respective primary antibody.

Two connector oligonucleotides are joined to form a circular molecule by a ligase.

A polymerase replicates the circle, producing an concatemeric product.

Detection of hybridization by oligonucleotide tagged with fluorescent compound.
Examples
A2AR-mGluR5
Bregma 1.00 mm
A2AR-A2AR heteroreceptor complexes in the rat cortex and hippocampus
[preliminary results]

Borroto-Escuela et al. 2014

[A2AR-A2AR heteroreceptor complexes WT]
CA2

[A1-A2A]
A1R-A2AR heteroreceptor complexes in the rat cortex and hippocampus

[preliminary results]

Borroto-Escuela et al. 2014

[A1R-A2AR heteroreceptor complexes WT]
Usually four important parameters should be kept in mind for a proper analysis and in situ PLA result interpretation:

- the number of DAPI nuclei in the sample field.
- the number of positive PLA/dots per sample field
- the total number of positive PLA cells/nuclei per sample field.
- the diameter sizes of the individual PLA blobs (the diameter may indicate if aggregates (higher order) of receptor complexes exists).

Within these four values it will be possible to get an overall view of the expression/enrichment of GPCR homo/heteroreceptor complexes in the different brain areas analyzed and extract relevant conclusions from the comparisons between brain areas. Certainly, we cannot compare or determine directly a balance between the homo- and heteroreceptor complexes populations in the same tissue using the in situ PLA approach, because of a technical limitation of the procedure itself. But the method could help us determine each population independently and compare their relative expression levels after an appropriate numerical analysis.
We have proposed the molecular phenomenon of receptor-receptor interactions as a fruitful way to understand how brain function can increase through molecular integration of signals.

An alteration in specific receptor-receptor interactions or their balance/equilibrium (with the corresponding monomers-homomers) are indeed considered to have a role in the pathogenic mechanisms that lead to various brain diseases.

Therefore, targeting protomer-protomer interactions in heteroreceptor complexes or changing the balance with their corresponding homoreceptor complexes in discrete brain regions may become an important field for developing novel drugs, including hetero-bivalent drugs and optimal types of combined treatments.

The analysis of animal or human brain material with in situ PLA can reveal if the relative abundance of specific homo-and heteroreceptor complexes in discrete brain regions is altered in brain diseases or under certain drug treatments, for instance, chronic L-dopa treatment in Parkinson’s disease.