HIGHLIGHTS IN PURINERGIC SIGNALLING

New evidences in the characterization of receptor oligomers: novel operational versatile models for receptor function in vivo?

Renata Ciccarelli

Navarro G., Ferré S., Cordomi A., Moreno E., Mallol J., Casadó V., Cortés A., Hoffmann H., Ortiz J., Canela E.I., Lluís C., Pardo L., Franco R., and Woods A.S. (2010) Interactions between intracellular domains as key determinants of the quaternary structure and function of receptor heteromers. J Biol Chem. 285(35): 27346–59.

Article summary

In this article, authors, who have a long-lasting and qualified experience in G-protein-coupled receptor (GPCR) oligomerization, identified new molecular features dictating the structure and function of the receptor heteromers formed by adenosine A_{2A}, cannabinoid CB_1, and dopamine D_2 receptors. Using recent and sophisticated techniques, based on resonance energy transfer (RET) such as bioluminescence, fluorescence, and sequential RET, as well as on the analysis of computational models coupled to classic molecular biology methods (cell transfection with receptor sequences mutated for specific amino acids and Western blot analysis to determine variations in the receptor heteromer signal transduction), they demonstrated the existence of electrostatic interactions between selected intracellular domains in one of the examined receptor and the intracellular domains of the other two receptors, allowing the formation of the quaternary structure not only of the trimeric receptor (A_{2A}–CB_1–D_2) but also of each possible receptor

R. Ciccarelli (✉)
Department of Biomedical Sciences, "Gabriele D'Annunzio",
University of Chieti-Pescara,
Chieti, Italy
e-mail: r.ciccarelli@dsb.unich.it

dimer (i.e., A_{2A}–CB_1, A_{2A}–D_2, and CB_1–D_2). In particular, they demonstrated a key role for two different casein kinase (CK)1/2-dependent phosphorylatable Ser and Thr residues, one in cytoplasmic C terminus (CT) and the other one in the third intracellular loop (IL3) of the CB_1 receptor for establishing electrostatic interactions with adjacent evolutionary-conserved Arg residues in the IL3 of the D_2 receptor and an Arg-rich cytoplasmic domain at the end of transmembrane helix 5 (TM5) of the A_{2A} receptor, respectively. They also showed the occurrence of similar electrostatic interactions between phosphorylated Ser in CT of the A_{2A} receptor and an Arg-rich cytoplasmic domain at the end of the TM5 of the D_2 receptor. These particular electrostatic interactions may constitute a general mechanism for receptor heteromerization; studies using synthetic peptides confirmed that these electrostatic interactions are particularly stable, mainly the noncovalent Arg–phosphate interaction. Additionally, Navarro and colleagues demonstrated that some transmembrane domains (TM4 and TM5), which are important for the oligomerization of family A GPCRs, are primarily involved also in A_{2A}–CB_1–D_2 heterodimerization. Overall, these studies support a triangular rather than a linear arrangement of this heteromer that would allow the simultaneous homodimerization of each receptor unit, using the interface TM1. Thus, the proposed model might favor the formation of a higher-order oligomer with new possible functions.

Further experiments were aimed to demonstrate that the interactions of the intracellular domains of the CB_1 receptor with those of A_{2A} and D_2 receptors are fundamental not only for the correct formation of the quaternary structure but also to assure a specific function of the A_{2A}–CB_1–D_2 receptor heteromers, which seems to be different from those played by each receptor as a unit or as an heterodimer. Indeed, results show that phosphorylation of ERK1/2 is significantly increased when A_{2A} and D_2 are coactivated in the presence

of CB_1. Therefore, the specific qualitative pattern of mitogen-activated protein kinase (MAPK) activation can be considered as a biochemical fingerprint of the formation of functional A_{2A}–CB_1–D_2 receptor heteromers; authors indeed use this fingerprint to demonstrate the presence/function of the examined A_{2A}–CB_1–D_2 receptor heteromers in a native tissue, i.e., the striatum of rodent brain.

Commentary

Receptor oligomerization is currently stimulating intense research to the ultimate goal of understanding how receptors work in vivo and discovering novel targets for drug development that may, in turn, uncover new strategies for disease therapy. The first debates on the possible existence and significance of receptor oligomers started in the 1980s; since then, and especially in the last few years, tens of papers and reviews have been published, indicating the great ferment existing in this area. Advancement in this field has been also favored by new technologies, allowing a better characterization of the structure and function of oligomers. To make order in the large body of experimental findings, in 2007, the International Union of Basic and Clinical Pharmacology (IUPHAR) gave a definition of receptor oligomer as the aggregation of two or more single receptors resulting in a new entity with novel specific function and distinguished it from oligomeric receptors that refer to those receptors in which two or more different subunits are not functional on their own, but concur to receptor function (i.e., the $GABA_B$ receptor composed by $GABA_{B1}$ and $GABA_{B2}$ units, concurring to ligand recognition and activation of signal transduction pathways, respectively). A database for Oligomerization Knowledge Base (www.gpcr-okb.org) has been also created. Moreover, there is now general agreement in the scientific community on the recommendations and guidelines that researchers should follow to demonstrate the identification of a new oligomer. Thus, it is necessary to (1) identify the physical association of the subunits composing the new receptor oligomer in living cells and also in native tissues to avoid technical artifacts; (2) provide evidence that the formation of the oligomer leads to a specific functional property, which is different from that consequent to the activation of single receptor units and may be used as a fingerprint; (3) evaluate if this new activity, consequent to the activation of the newly identified oligomer, occurs either in transfected cells or in native tissues.

The Navarro et al. article reported above reports new structural, biophysical, and biochemical information on an oligomer that also includes the A_{2A} receptor (one of the most intensively studied purinoceptors) that seems to fulfill the criteria requested by IUPHAR.

So far, most studies concerning the assemblage of GPCRs have emphasized that, in the formation and function of an oligomer, a particularly important role is played by the interactions between the TM helical regions of each receptor monomer (in particular, of the lipid-exposed surfaces of TM1, TM4, and/or TM5 at the dimerization/oligomerization interfaces of several GPCRs). On this basis, Navarro et al. also initially studied the interaction between these TM residues, highlighting the importance of binding occurring at the interfaces of TM4/TM5. Interestingly, this kind of interaction allows the TM1 domain to remain available to undergo further oligomerization with additional receptors. This possibility supports the new concept that heteromer formation does not preclude the possible enlargement of the receptor complex, with a positive or negative control of the activity of the established heteromer. This concept, in turn, leads to the definition of a high-order oligomer, regarded as a receptor network operating within the cell membrane, that enhances the diversity and performance by which extracellular signals are transferred to G proteins and could assure a greater plasticity for cell response upon multiple messages from the extracellular environment.

However, in high-order oligomers, the specific contribution of individual receptor amino acids to TM interfaces is more difficult to determine or to generalize. As a consequence, it may be harder to find specific tools (e.g., peptides or small molecules), that, by interfering with the receptor oligomer, may improve or disrupt its structure or function. Such tools are needed to gain more information about the pathophysiological significance of each specific oligomer. In this respect, the different experimental approaches used by Navarro and colleagues in their paper are particularly interesting. A first emerging key finding is the identification of electrostatic and quite stable interactions among individual amino acids in the receptor's intracellular regions, such as the third loop and C terminus. These regions are believed to contain important domains for coupling to G proteins and have been previously demonstrated to be involved in the formation of oligomers (in particular of the A_{2A}–D_2 dimer) by other authors, including researchers of this same group. This information reinforces the concept that the aggregation of more receptor entities to form an oligomer does not occur randomly but is a quite specific event, so that the disruption of intradomain bonds does not allow the formation and activity of oligomers. It is also compatible with the suggestion that the domains involved in the interactions play a key role in oligomer/G protein coupling. Secondly, while for a number of conventional single receptors it is possible to identify their respective crystal structures (that are crucial for the design of new more specific ligands), at the current state of the art, the same does not seem to be possible for oligomers, which are more functional rather than structural entities. So, the identification of the molecular key

determinants for a quaternary structure becomes crucial to uncover fundamental mechanisms and dynamics governing GPCR dimerization/oligomerization. Besides selective electrostatic interactions between intracellular domains, a key role in constituting the oligomer at the cell membrane is also played by the possible triangular arrangement of the single-receptor units. Indeed, the integrative activity within the single oligomer's units depends on both receptor stoichiometry (number of monomers) and topology (spatial arrangement). Third, the existence of these interactions may be useful also to demonstrate the real presence of oligomers in native tissues, thus supporting a relevant role for these entities in living tissues. Finally, of extreme value, the identification of specific determinant regions for oligomer formation represents a new target for molecules able to interfere with such sites, thus potentiating or turning down oligomer activity.

Of additional interest, in this specific study, the activation of the A_{2A}–CB_1–D_2 heteromers leads to an increase of MAPK activation, which is taken by the authors as a specific biochemical fingerprint of heteromer function. This specific fingerprint just represents one of the many possible functional properties of an oligomer, which may include both receptor subunit cooperativity (as in this case) and receptor downregulation via internalization/desensitization of specific receptor's subunits. As affirmed by the same authors, this specific consequence of heteromer formation is not important "per se" but indeed represents one of the first examples in this field of research; what matters is the demonstration that oligomer formation does indeed occur in native tissues, which may represent a new operation model of value also for other GPCRs. Since results emerging from RET techniques are indicative for GPCR oligomers, but none of these techniques univocally demonstrates their existence in living animals, the identification of this functional counterpart of receptor oligomerization holds considerable importance.

In conclusion, there is growing evidence that GPCR dimers/oligomers are formed in living cells and could be implicated in clinically relevant diseases of both the central nervous system (Parkinson's disease, schizophrenia) and the periphery (asthma, hypertension). Nevertheless, a lot of work still has to be done in the future to achieve a better comprehension as to why oligomers are formed, what inputs causes receptor aggregation/disaggregation, how much they are stable in the membrane, and what is their real function in vivo. A partial answer to these questions comes from the literature, where a role for high-order oligomers, mainly located at the perisynaptic level of specific neurons, has been suggested in the control of the signals and activity delivered by traditional presynaptic and postsynaptic single receptors. These oligomers, receiving different inputs from the extracellular environment and working as a "hub," might integrate these different signals, resulting in a final activity that interferes with/modulates the transduction pathway(s) activated by single operating receptors. Just to exemplify, I will cite the oligomer formed by $mGlu_5$–D_2–A_{2A} receptors, the major location of which appears to be in the perisynaptic region of corticostriatal glutamatergic synapses interacting with dendritic spines of striatopallidal γ-aminobutyric acid (GABA) neurons. The synergism of $mGlu_5$ and A_{2A} activation in reducing D_2 inhibitory signaling may be the major integrative event in the heteromer. In this way, the glutamate input to activate the striatopallidal GABA neurons can be finely controlled, producing discrete motor inhibition. Again, in the striatum, the integrative activity in the trimeric receptor can lead to dynamic changes of two forms of glutamate synaptic plasticity, i.e., long-term potentiation (when A_{2A}–$mGlu_5$ signal dominates) and long-term depression (when D_2 activity is preponderant), thus remarkably affecting striatal-based motor learning and memory processes.

These and many other examples from the literature suggest oligomers as new targets for drug development. Specific druggable targets may be represented by the binding sites of one or more of the receptor oligomer's units, as well as by the key determinants acting at the TM interfaces or at specific intracellular loops. New potential drugs, binding to only one of the receptor's sites within the oligomer, could make the binding sites of the other receptor units more or less available for ligand coupling, thus acting as a "super" agonist or antagonist. In this respect, an interesting example is represented by multivalent GPCR ligand–dendrimer conjugates, recently synthesized by Ken Jacobson and colleagues, which have increased potency or selectivity in comparison to monomeric ligands (Kim Y et al., *Purinergic Signalling* (2009) (1):39–50). However, the greatest interest seems to be for molecules able to reinforce or disrupt specific interactions between receptor units within an oligomer. It is expected that such agents may be useful not only as new therapeutic entities for diseases but also to limit the side effects produced by current therapies that target the whole receptor oligomer. The screening of small molecules that may act on receptor–receptor interactions has already started, and, in this respect, a congress entitled "Arrowhead's Allosteric Modulator Drug Discovery Congress" (www.allostericmodulatorcongress.com) that will be held in San Diego, California, on November 11–12, 2010, has been announced.

At the end, although it is unusual for a commentary, I would like to suggest a very limited list of references that helped me in writing this highlight article and might be useful to readers naïve for this topic:

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About the author

Renata Ciccarelli is Professor of Pharmacology at the Faculty of Medicine, University of Chieti-Pescara (Italy). She has been interested in the characterization of the role of adenine- and guanine-based purines in the central nervous system, as modulators of either neurotransmission or the glial cell function. At present, her research is focused also on features modulating the growth and differentiation of mesenchymal (normal and tumoral) stem cells, likely involving purines and their receptors.