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REVIEW

New factors influencing G protein coupled receptors' system functions

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Abstract New factors such as the G protein coupled receptor (GPCR) surrounding's chemical environment, cell membrane constituents, the existent gap junction, endogenous receptor affinity status and animal species have been shown to influence the GPCR physiology and variations of those factors can modify the functions of the GPCRs, thus highlighting the possibility to exploit these properties in different pharmacological fields which may lead to obtaining new therapeutic methods and applications. Furthermore, it might help in developing new research methods.

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1. Introduction

In addition of the classical factors linked to pharmacodynamics, different studies have led to the discovery of new factors that may influence the activity of molecules acting on GPCR systems. Such factors will be added to those already known including those related to structure/activity relationship (some aspects have been described by Spedding[1]), physicochemical properties of the agents, patient population and pharmacological interactions. Within the coming examples we focus on one element: ''As a factor has been shown to influence at least one of the elements of the GPCRs system, we suppose that the same factor or another factor that has the same properties may influence one or more GPCR-related systems, and this because of the analogies that exist between GPCRs. On the others hand the factors have been illustrated by selected examples to clarify the concept and introduce the implications.

2. Chemical environment

A recent research pointed out that receptor density, ionic environment and the cell type may influence the pharmacological properties of aripiprazole (an antipsychotic dopamine D2 receptor partial agonist used in the treatment of psychosis) and other partial agonists of the D2.[2] Furthermore, previous researches have described that sodium ions decrease the G protein coupling of dopamine receptors[3,4] and that the surrounding environment (including sodium ions and depending on the brain area) in addition to receptor density modify both properties of functional selective ligands and the affinity of agonists.[2] Indeed, the interaction of the conserved aspartate residue within the transmembrane core of several GPCRs with sodium ions may change the receptor conformation, thus influencing the related pathways activation.[3,5] Furthermore the potential of intra- or extracellular sodium concentrations to modify GPCRs binding and signaling has also been pointed out .[5]

Such data make researches essential to be carried out to clarify the role sodium ions may play in the ability of GPCRs to signal *in vivo*[2] especially that such differences in ionic environment may exist between brain structures supposing that the same ligand may act on the same kind of receptors in different brain areas and produce non similar effects because the impact of sodium ions, or probably other ions, on the ability of GPCR to signal.

2.1. Manganese influence serotonergic receptor 1 properties

Another toxicological aspect about GPCR–manganese (Mn^{2+}) interactions illustrates the influences that ions can have on GPCR properties. Mn^{2+} accumulates in the CNS[6–8] within regions such as basal ganglia, cortical and hippocampal regions of the brain[9–11] and therefore may explain the different symptoms related to the overexposure to manganese. Indeed symptoms of Mn^{2+} poisoning (named manganism) have similarities with Parkinson's disease (PD) so the dopaminerigic pathway was pointed at to explain its toxicodynamics, whereas the serotonergic system has been shown to be probably implicated.[12] Two papers[13,14] pointed out that manganese constitutes an inducer of agonist high-affinity binding to 5-HT1A receptors. The manganism-associated symptoms include impulsiveness, psychosis with euphoria, mental confusion,[15] cognitive disturbances,[16] memory impairment,[9] psychiatric and motoric disturbances,[6] in addition of other neuropsychological and neurological symptoms[17] such as anxiety and irritability.[18,19] On the other hand and in addition to both the activation of oxidative stress pathways and changing neurotransmitters levels in the brain,[6,20] a recent study published in 2011[21] pointed out that agonist binding and signal transduction are enhanced by Mn^{2+} through blocking guanosine nucleotide binding to G proteins in complex with 5-HT1A receptors. This may clarify more the relationship between manganism and the alterations it causes.

Mn^{2+} effects on 5-HT1A receptors in addition of Na^{+} effects, previously described, show that chemical ions may modify the GPCR properties and functions, thus may be used as therapeutical adjutants (local usage) or as chemical additives in experiments when preparing mediums (or media) for cell cultures. More important such a finding will be helpful to develop new conditions for *in vitro* studies and cell culture in neuropharmacology and other aspects that are related to GPCRs by taking into account the influence the surrounding area may have on the pharmacological profile and thus on the obtained data and the final interpretations of the experimental results.

3. Cholesterol and membrane influence

The membrane molecular structures have been shown to have an influence on GPCRs physiology. In addition to other signaling molecules, trimeric G proteins and GPCR were suggested to be entertained by cholesterol, saturated phospholipids, glycolipids and sphingomyelin.[22] Furthermore, many papers have pointed to the important role cholesterol and sphingolipid-enriched membrane domains play in GPCR pathways' signaling.[23–32] Cholesterol of cell membranes is distributed in domains.[33,34] In addition to the role of keeping membranes structure,[35] those domains were pointed out to be involved in signals' transduction.[36]

Indeed, several GPCRs functions have been pointed to as modified by membrane cholesterol[37,38] probably via interacting directly with GPCR or/and by modulating the physical properties of the plasma membrane (PM).[39] A recent study[40] brought out new elements about the possible link between the GPCR signaling pathway and the influence of cell membrane cholesterol content on GPCR mechanism, the results supposed that cholesterol depletion affects the ability of δ opioid receptor (DOR) to transmit the signal rather than affecting the receptor agonist binding site. The paper has highlighted

also that cholesterol depletion has an impact on PM arrangement and, thus deteriorates the coupling of DOR to covalently bound Gi1α[40] which disturbs signal transmission.

These findings point more to the need of further investigations about the role of GPCRs interaction with membrane lipids, which will provide further data to improve the existent therapeutics by taking into account the molecular interactions of GPCRs within the plasma membrane.[41] Thus, it will directly have effect on the pharmacodynamic aspects of such novel drugs.

4. Gap junction's electrical synapses role in neuropathologies

Generally, the neuronal network has two fundamentally different types of synapses. In addition to the chemical synapses, we have Gap junctions (GJ) electrical synapses. GJ are intercellular channels which directly connect the cytoplasm of adjacent cells; they are faster in information transfer compared to chemical synapses.[42] On the other hand, between neurons; GJ play a role in the exchange of second messengers, including those implicated in the GPCRs pathway like cAMP, IP3, Ca^{2+} and other small molecules.[43]

In neurosciences the connexin36 (Cx36) protein constitutes an illustrative example, it is found in GJ of the hippocampus, cerebral cortex, striatum, amygdala, the inferior olive, the cerebellum and the olfactory bulb.[44] Thus, it has a role in the activities that are related to these brain structures. For that matter, the passage of these second messengers through neuronal GJ serves to coordinate activities between coupled neurons. A recent study[44] has pointed that Cx36 deficiency in the mouse leads to behavioral changes in open field activity, anxiety-related behavior in the light–dark box and one-trial object-place recognition. Thus, influence of the neural network therefore; modifies the cell response after the interaction of the cerebral GPCRs with either endogenous ligands or exogenous ligands (agonists, antagonists, inverse agonists...) according to how Cx36 is or is not deficient. Furthermore, the discoveries suggest that the synchronization of neural network activity in the hippocampus and neocortex via neuronal GJ plays a role in the acquisition and/or consolidation of novel object information.

By extrapolating, we can suppose that other neuronal junctions may influence anxiety and other cerebral-related activities such as memory, locomotion and behavior-related activities. Such activities are also mediated via GPCR systems, thus the existence of junction plays an important role in both physiology and pathology of pathways that have been linked with GPCRs. On the other hand treatments targeting GPCR or one of its pathway molecules may not be efficient if the origin of pathogenesis is coming from the gap junctions rather than from one of the GPCRs system molecules.

5. High and low-affinity receptor states *in vivo*

A recent publication has pointed out the possible existence, according to the affinity state, of two populations of GPCRs *in vivo*.[45] It has suggested that for GPCRs two states may exist, high affinity state and low-affinity state for agonist binding. The method that could be used to evaluate and quantify it may face a dynamic problem; In fact the kinetic nature of pharmacodynamic phenomena can make the detection of that high and low affinity agonist binding impossible.[46–50] Methods such as imaging with agonist radioligands and the use of genetically modified mice may provide new tools to further study this phenomenon.[45]

Understanding such differences and how the existence of states of different affinities can provide new elements in therapeutics that will be able to influence directly the affinity of GPCRs to their ligands, thus open more possibilities in both drug development (improve drug properties) and drug research (new targets) especially if it requires an unusual large consumption of the drug or if the therapeutic window of the drug is narrow. In fact some adjuvant may influence the affinity and thus modify the agonists' pharmacodynamics. On the other hand a possible genetic explanation of the high or the low affinities could indicate that gene therapy is also a potential way to modify GPCR properties.[45] However, if the therapeutic index is acceptable, we can enhance the dose if either the affinity or the efficacy is low rather than influencing the affinity of GPCRs to their ligands.

6. Animal species influence on ligand–receptor interaction

One of the most important factors in both animal experiment and cell culture researches are the choice of animal species and the cell strain origin. A ligand may interact differently with the same kind of receptor but coming from two different species. Indeed, comparative studies between human H1R and guinea pig H1R, have shown species-differences in affinity, ligand binding kinetics and rate constants for association and dissociation between human and guinea pig histamine H1-receptors (hH1R and gpH1R) when interacting with histamine H1-receptors (H1R) antagonist mepyramine and partial (H1R) agonist phenoprodifen (a histaprodifen), and this because the exchange of N-terminus and E2-loop influence on the affinity of phenoprodifen to H1R[51]; it influences also the binding kinetics of the H1R antagonist mepyramine. Differences in amino acid sequences of the transmembrane domains exist in some positions within these domains of the H1R.[52] The studies were based on thermodynamic calculations, radioligand binding studies and the numerous new active- and inactive states and GPCR crystal structures.[52–54] Many biogenic amines such as serotonin and dopamine have a chemical analogy and share some physical and chemical properties with histamine, additionally they interact with the GPCR also, therefore, this result may also be relevant for other biogenic amine receptors.

This finding highlights the importance of taking into account the differences that may exist between different species regarding the ligand–receptor interactions, therefore suppose that results which have been or that will be obtained in animal experiments or cell cultures may not be valid for humans because of the interspecies possible differences. Importantly this finding points more the importance of both species and cell culture choices in researches, more importantly, in human clinical trials in drug effect validations, interspecies differences may also exist. The study of the role of serotonergic and serotonergic pathways in decision making process[55] supposed that polymorphism in both of the dopamine transporter (DAT1) and serotonin transporter (STin2) are implicated in individual differences in striatal and amygdala responses during the decision making process which illustrates the influence genetic polymorphism may have on GPCR related functions. In addi-

tion, some serotonin receptors types are GPCRs which have been linked to some neuropsychiatric functions[56] supposing the influence of the mentioned factors on different neuropsychiatric disorders include the serotonergic pathway in the process.

7. Conclusions and perspectives

Studying new elements about GPCR systems and pathways will surely lead to finding out not only new therapies but also explanations for numerous pathogenic phenomena, in addition to the possibility of designing new research protocols and eventually provide data to other research areas including molecular biology and physiology.

The implications of GPCRs *in vivo* functions and processes predict numerous side effects of drugs that interact with GPCRs systems. Thus, GPCR-related system constitutes a pharmacological target that needs particular pharmacovigilance.

Conflict of interest

The authors declare no conflict of interest.

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