Neuropharmacology 56 (2009) 213–225

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Towards a quantitative representation of the cell signaling mechanisms of hallucinogens: Measurement and mathematical modeling of 5-HT1A and 5-HT2A receptor-mediated ERK1/2 activation

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ARTICLE INFO

Article history:
Received 2 May 2008
Received in revised form 23 July 2008
Accepted 25 July 2008

Keywords:
Computational simulations of signaling pathways
G protein coupled receptors
Systems biology
Receptors of hallucinogenic drugs of abuse
Serotonin receptors
MAPK phosphorylation cascade

ABSTRACT

Through a multidisciplinary approach involving experimental and computational studies, we address quantitative aspects of signaling mechanisms triggered in the cell by the receptor targets of hallucinogenic drugs, the serotonin 5-HT2A receptors. To reveal the properties of the signaling pathways, and the way in which responses elicited through these receptors alone and in combination with other serotonin receptors' subtypes (the 5-HT1AR), we developed a detailed mathematical model of receptor-mediated ERK1/2 activation in cells expressing the 5-HT1A and 5-HT2A subtypes individually, and together. In parallel, we measured experimentally the activation of ERK1/2 by the action of selective agonists on these receptors expressed in HEK293 cells. We show here that the 5-HT1AR agonist Xaliproden HCl elicited transient activation of ERK1/2 by phosphorylation, whereas 5-HT2AR activation by TCB-2 led to higher, and more sustained responses. The 5-HT2AR response dominated the MAPK signaling pathway when co-expressed with 5-HT1AR, and diminution of the response by the 5-HT2AR antagonist Ketanserin could not be rescued by the 5-HT1AR agonist. Computational simulations produced qualitative results in good agreement with these experimental data, and parameter optimization made this agreement quantitative. *In silico* simulation experiments suggest that the deletion of the positive regulators PKC in the 5-HT2AR pathway, or PLA2 in the combined 5-HT1A/2AR model greatly decreased the basal level of active ERK1/2. Deletion of negative regulators of MKP and PP2A in 5-HT1AR and 5-HT2AR models was found to have even stronger effects. Under various parameter sets, simulation results implied that the extent of constitutive activity in a particular tissue and the specific drug efficacy properties may determine the distinct dynamics of the 5-HT receptor-mediated ERK1/2 activation pathways. Thus, the mathematical models are useful exploratory tools in the ongoing efforts to establish a mechanistic understanding and an experimentally testable representation of hallucinogen-specific signaling in the cellular machinery, and can be refined with quantitative, function-related information.

1. Introduction

Through a collaborative multidisciplinary approach involving experimental and computational studies at various scales – from molecular to cellular and organismal – we are engaged in an effort to reveal the mechanisms that engender the complex effects of hallucinogenic drugs of abuse (for some reviews see Aghajanian and Marek, 1999; Gresch et al., 2002; Nichols, 2004). Our computational work addresses quantitative and structural aspects of the mechanisms of hallucinogenic drugs in various chemical classes. Using methods of molecular biophysics and computational biology, we simulate the dynamic properties of the ligand–bound receptor systems for hallucinogens compared to non-hallucinogenic congeners (Weinstein, 2006). Proceeding further up in the size and time scale of the relevant processes, we show here how mathematical models of receptor-mediated signaling properties can be used to connect to experimentally determined signaling. We focus on molecular complexes and interactions of these compounds with serotonin receptors in the G protein coupled receptors (GPCRs) family, and follow the mechanisms through ensuing interaction processes with other components of the signaling cascades such as membrane components (e.g., PIP2) and PDZ domains (Madsen et al., 2005; Khelashvili et al., 2008).

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doi:10.1016/j.neuropharm.2008.07.049

The rigorous quantitative approach we apply is made possible by recent advances in many aspects of experimental and computational biology, from the molecular to the integrative level of cell signaling systems. These advances have improved our understanding of GPCR activation as an allosteric mechanism, triggered by ligand binding, that results in conformational changes transduced by the transmembrane domain (Han et al., 2008; Urban et al., 2007; Weinstein, 2006; Deupi and Kobilka, 2007; Kobilka and Deupi, 2007). The advances have also allowed us to characterize the steps of intra-receptor activation mechanism by combining computational modeling and experimentation (Guo et al., 2005). Experimental evidence points to multiple conformations related to the activation of the receptor (Niv et al., 2006; Filizola et al., 2006; Han et al., 2008). Different ligands binding to the same receptor may induce different conformational states, which in turn can result in coupling to different signaling pathways (specifically for the hallucinogens, see Gonzales-Maeso et al., 2007), and functional hetero-oligomerization (Gonzalez-Maeso et al., 2008). We have recently reviewed (Weinstein, 2006) some key aspects of functional understanding achievable from computational modeling of hallucinogen mechanisms at the molecular and cellular level, emphasizing not only the structural context of the mechanisms of the receptor molecules and their interactions, but also the importance of bioinformatics and mathematical modeling tools in revealing the specific consequences of hallucinogen binding to GPCRs. The findings leading to this newly gained understanding include key mechanistic components such as (i) modes of receptor response (conformational rearrangements and stabilization of "activated state(s)") responsible for protein–protein interactions ranging from homo- and hetero-oligomerization to interactions with scaffold proteins (e.g., PDZ domains) and (ii) the role of conformational rearrangements of the receptor due to hallucinogen binding in association/dissociation of specific protein–protein interactions and selective signaling. These developments show why models of the activated forms of GPCRs have become increasingly necessary for the development of a clear understanding of signal propagation into the cell (Niv et al., 2006; Filizola et al., 2006; Han et al., 2008).

Here we summarize briefly the recent progress along these lines, by presenting a topological network and a mathematical model that offer a detailed visual, quantitative and dynamic illustration of the 5-HT receptors-mediated ERK pathways, known to be targeted by hallucinogens in their actions (5-HT2A, and relations to 5-HT1A). The current understanding of detailed signaling mechanisms is still incomplete, and the determinants for the function of these GPCRs in cellular signaling triggered by hallucinogens are only partially delineated. Therefore, in order to gain quantitative presentations of this complicated network, we derive mathematical models by focusing on particular signaling processes activated by hallucinogens through 5-HT receptors. Quantitative understanding of the actions of hallucinogens must include the signaling pathways activated by these ligands after binding to the target GPCRs (for a discussion see Niv et al., 2006; Weinstein, 2006; Kholodenko, 2006; Palsson, 2006). Here we illustrate the use of computational modeling in the quantitative interpretation of currently available data of serotonin receptors-mediated MAPK cascade, and collect all the pieces into function-related, time-dependent information. While network representations shown in Figs. 1 and 2 may be incomplete, and the values of the parameters may carry significant uncertainties, these are likely to be remedied by results from continuing research. However, the integrative representation and quantitative summary of current literature offered by these models are the focus of interest. At the very least, the simulations of these models can aid ongoing efforts to construct an increasingly comprehensive mechanistic understanding by validating or eliminating specific assumptions, answering particular mechanistic questions (see below), and guide the necessary

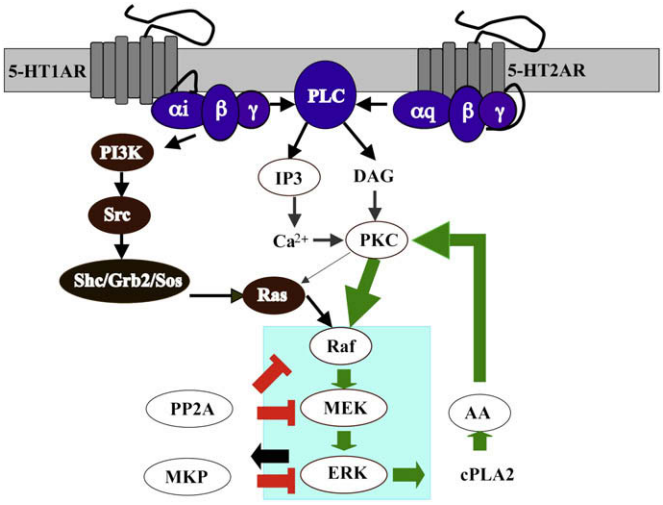

Fig. 1. Signaling pathways of MAP kinase activation mediated by human serotonin receptors 5-HT1A and 5-HT2A.

experimental effort by producing experimentally testable hypotheses based on the representation of hallucinogen-specific signaling in the cellular machinery.

We have focused on the activation of the eukaryotic MAPK cascade via serotonin receptors 5-HT1A and 5-HT2A as it is ubiquitously expressed in diverse biological processes. MAPK signal transduction pathways mediate short-term effects such as modulation of potassium channel (Yuan et al., 2002) and glutamate receptor function (Endo and Launey, 2003) as well as long-term effects such as cell differentiation, long-term potentiation (LTP), learning and memory (Adams and Sweatt, 2002). Signaling through MAPK pathways is known to positively regulate immediate early genes (IEGs). In addition, MAPK cascades in a variety of cells are tightly regulated by multiple feedback loops. Although the basic structure of all MAPK cascades is the same, differences in feedback control enable them to generate a plethora of biological responses, including oscillations, gradual and ultrasensitive responses (Huang and Ferrell, 1996; Chang and Karin, 2001; Bhalla et al., 2002; Heinrich et al., 2002; Kholodenko et al., 1997; Kholodenko, 2000).

The action of hallucinogens on 5-HT receptors is well documented (Nichols, 2004). While drug discrimination experiments have singled out the 5-HT2AR subtype as the important target of hallucinogens, both the 5-HT1A and 5-HT2A receptors have been suggested to be involved. The serotonin receptor 5-HT1A is coupled to G_i/G_o proteins, and stimulates the MAPK growth-signaling pathway possibly through G protein $\beta\gamma$ complex-mediated phosphatidylinositol 3′-kinase (PI-3K) or phospholipase (PLC) β pathways (Della Rocca et al., 1997, 1999; Adayev et al., 1999).

The 5-HT2A serotonin receptor is $G_{q/11}$-coupled and has diverse roles in both the central nervous system (CNS) and peripheral vasculature, and is known to trigger MAPK activation via PKC/Raf-1 pathway (Hershenson et al., 1995; Watts, 1996), and also to stimulation of PLA2 to produce the second messenger arachidonic acid (AA) (Felder et al., 1990; Tournois et al., 1998). While the 5-HT2A receptors have been implicated as direct targets of hallucinogens, the balance of signaling activities that produce the hallucinogenic effect remains unknown. In particular, an essential issue in signal transduction is how the activated receptors are integrated into signaling pathways and how specific conformations of the activated receptor may establish the distinct patterns of signal transduction observed when they bind different ligands; notably, hallucinogens produced entirely different transcriptome fingureprints compared to their non-hallucinogenic congeners (Gonzalez-Maeso et al., 2003). Some key questions then become: Which of the reactions in