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PERSPECTIVE

Protein-Protein Interactions and Dopamine D_2 Receptor Signaling: A Calcium Connection

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ABSTRACT

The third cytoplasmic loop is a crucial site of physical contact between some G protein-coupled receptors (GPCRs) and their respective G proteins. However, interactions not only occur among these proteins but also involve a number of additional protein binding partners. Modulation of these protein-protein interactions may represent an important new avenue of pharmacotherapy through which signaling of GPCRs can be modulated. In the current issue of *Molecular Pharmacology*, Liu et al. (p. 371) report that dopamine D_2 receptors interact with the Ca^{2+} binding protein S100B. Using the third intracellular loop of the dopamine D_2 receptor as bait in a bacterial two-hybrid system, S100B was determined to be a potential binding partner. They used pull-down assays both in vitro and in vivo to confirm the interaction and define its specificity. Neither the D_3 nor the D_4 receptor expresses the motif conferring the interaction, and peptides based on the third intracellular loop of the D_3 receptor did not pull down S100B. Some groups might stop there, but Liu and colleagues moved on to demonstrate colocalization of the D_2 receptor and S100B by immunostaining. Functional assays were then used to show that coexpression of S100B with the D_2 receptor increases the ability of D_2 receptors to activate ERK and to inhibit adenylyl cyclase. These data suggest that S100B coexpression may serve as an important mediator of D_2 receptor signaling efficacy in the brain. These interactions contribute to cellular, regional, and developmental differences in D_2 receptor activation.

Dopamine (DA) is widely distributed in the central nervous system and serves a variety of functions in the mature brain, including control of movement, cognition, endocrine responses, and reward. Dopaminergic abnormalities contribute to many neurological and psychiatric disorders, including schizophrenia, Parkinson's disease, attention-deficit hyperactivity disorder, and drug addiction (Kiyatkin, 1995; Goldman-Rakic, 1998; Nestler, 2001; Girault and Greengard, 2004; Arnsten and Li, 2005; Biederman and Faraone, 2005; Kalivas and Volkow, 2005).

DA receptors are G protein-coupled receptors (GPCRs), characterized by an extracellular N-terminal region, intracellular C-terminal region, and seven membrane-spanning regions. There are two subfamilies of DA receptors, D_1-like receptors and D_2-like receptors, based on their pharmacological profiles and sequence homology (Lachowicz and Sibley, 1997; Missale et al., 1998). D_1-like receptors, including the D_1 and D_5 receptor subtypes, catalyze synthesis of cAMP. D_2-like receptors, including the D_2, D_3, and D_4 receptor subtypes, inhibit cAMP synthesis. The receptors also affect activation of potassium channels, mitogen-activated protein kinases, and Akt (Neve et al., 2004; Beaulieu et al., 2005).

A "holy grail" in understanding GPCR structure-function relationships is identification of the mechanism(s) responsible for control of the specificity and efficacy of receptor signaling properties. The same GPCR can couple to multiple G proteins in regionally and temporally specific ways and can also activate G protein-independent signal cascades. It is hypothesized that protein-protein interactions with GPCR-binding partners are central in conferring specificity to these signals (Bockaert et al., 2004; Kabbani and Levenson, 2007). In D_2-like receptors, the third intracellular loop seems to be particularly crucial in mediating these interactions.

The study by Liu et al. reported in this issue of *Molecular Pharmacology* (Liu et al., 2008) adds one more piece of the puzzle to the study of DA D_2 receptor signaling. Several

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ABBREVIATIONS: DA, dopamine; GPCR, G protein-coupled receptor.

previous studies have identified binding partners for the D_2 receptor including coreceptors, signaling molecules, and scaffolding proteins (see Fig. 1) (Smith et al., 1999; Binda et al., 2002; Macey et al., 2004; Beaulieu et al., 2005; Fuxe et al., 2005; Negyessy and Goldman-Rakic, 2005; So et al., 2005; Liu et al., 2006, 2007; Beaulieu et al., 2007; Free et al., 2007; Rashid et al., 2007; Kim et al., 2008).

In the new study by Liu et al. (2008), the authors used the third intracellular loop of the DA D_2 receptor as bait in a bacterial two-hybrid system and discovered a novel interacting partner, the Ca^{2+} binding protein S100B. They then used His-tagged fusion proteins based on sequences derived from DA receptors to confirm interactions between S100B and the D_{2S} and D_{2L} receptors in bacteria and then documented the interaction using full-length receptor protein expressed in human embryonic kidney 293 cells. In contrast, the DA D_3 and D_4 receptors do not express the motif that seems to confer the interaction, and in fact, D_3 receptor did not pull down S100B in the His assay. It is noteworthy that the D_2 receptor and S100B proteins were also coimmunoprecipitated from a native tissue, adult rat striatum.

Immunohistochemistry in primary neuronal cultures of the striatum was used to demonstrate colocalization of the D_2 receptor and S100B and provided further evidence that the interaction may be important in native brain tissue. Functional assays of MAPK activation and inhibition of foskolin-induced cAMP accumulation were used to impressively demonstrate that coexpression of S100B with the D_2 receptor increases the ability of D_2 receptors to activate ERK and to inhibit adenylate cyclase. Taken together, the data suggest that S100B coexpression may serve as an important mediator of D_2 receptor signaling efficacy in the brain and may contribute to differences in cellular, regional, and developmental differences in D_2 receptor activation. It is noteworthy that previous work by these authors defined calmodulin, also a Ca^{2+} binding protein, as another promoter of D_2 receptor signaling (Liu et al., 2007), suggesting that there may be a more generalized mechanism at work here.

So why does D_2 receptor bind S100B? Relatively little is actually known about S100 proteins within neurons. These small proteins (9–13 kDa) bind Ca^{2+}, are highly enriched in glia, and can be released into the extracellular fluid. S100B is a homodimeric protein and has been associated with modulation of diverse processes including Ca^{2+} flux, cell growth, cell survival, energy metabolism, and neurite outgrowth. Overexpression of S100B in vivo enhances pathological response to cerebral hypoxic-ischemic injury (Wainwright et al., 2004). S100B freely passes the blood-brain barrier and is increased in serum after traumatic brain injury (Kleindienst et al., 2007). Increases in S100B have also been associated with schizophrenia and depression (Rothermundt et al., 2004; Sen and Belli, 2007), disorders with links to altered biogenic amine neurotransmission. Direct connections between S100B and neurodevelopmental functions of serotonin receptors have also been documented (Azmitia, 2001), suggesting now that S100B might also modulate GPCR signaling during development. Another S100 protein, S100A10 (p11), was recently shown to interact with 5-HT_{1b} receptors (Svenningsson et al., 2006).

The identification of this interaction therefore raises a number of intriguing questions for future study. Is the interaction between D_2 receptors and S100B developmentally and/or regionally regulated? Does Ca^{2+} binding to S100B alter its ability to interact with the D_2 receptor or the ability of D_2 receptors to interact with other proteins? Do Ca^{2+} signals produced by receptor activation contribute to such regulation, perhaps as some type of feedback or feed-forward mechanism? What are the functional consequences of the interaction on D_2 receptor function in native tissues? Does the interaction contribute to subcellular localization of either protein? Is this interaction specifically altered in disease states such as schizophrenia? Thus, as is often the case with defining studies, this novel finding presents far more new questions than any one study can answer.

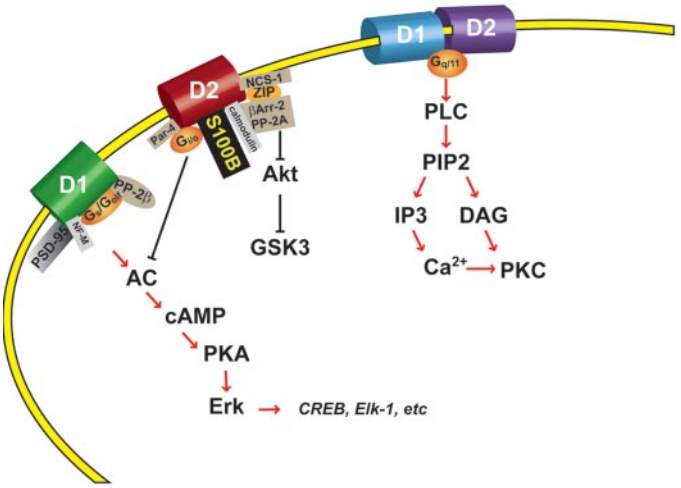

Fig. 1. Representation of interactions between several known interacting partners of dopamine D_1 and D_2 receptors. Not all known interacting proteins have been depicted, for simplicity. In addition, D_1 and D_2 receptors are capable of hetero-oligomerization, producing even further regulatory complexity.

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