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Protein-Protein Interactions and Dopamine D₂ Receptor Signaling: a Calcium Connection

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Invited **Perspective** Article for *Molecular Pharmacology*

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Running head: S100B binds to and modulates dopamine D₂ receptors

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Abbreviations: dopamine (DA), G protein-coupled receptor (GPCR)

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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 occur not just between these proteins, but also involve a number of additional protein binding partners. It is thought that 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. report that dopamine D₂ receptors interact with the Ca²⁺ binding protein S100B. Using the third intracellular loop of the dopamine D₂ 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 D₃ nor D₄ receptor expresses the motif conferring the interaction, and peptides based on the third intracellular loop of the D₃ receptor did not pull down S100B. Some groups might stop there, but Liu and colleagues moved on to demonstrate co-localization of the D₂ receptor and S100B by immunostaining. Functional assays were then used to show that co-expression of S100B with the D₂ receptor increases the ability of D₂ receptors to activate ERK and to inhibit adenylyl cyclase. These data suggest that S100B co-expression may serve as an important mediator of D₂ receptor signaling efficacy in the brain. These interactions contribute to cellular, regional, and developmental differences in D₂ receptor activation.

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Dopamine (DA) is widely distributed in the central nervous system (CNS) 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 (Arnsten and Li, 2005; Biederman and Faraone, 2005; Girault and Greengard, 2004; Goldman-Rakic, 1998; Kalivas and Volkow, 2005; Kiyatkin, 1995; Nestler, 2001).

DA receptors are G protein-coupled receptors (GPCRs), characterized by an extracellular N-terminus region, intracellular C-terminus region, and seven membrane spanning regions. There are two subfamilies of DA receptors, D₁-like receptors and D₂-like receptors, based on their pharmacological profiles and sequence homology (Lachowicz and Sibley, 1997; Missale et al., 1998). D₁-like receptors, including the D₁ and D₅ receptor sub-types, catalyze synthesis of cAMP. D₂-like receptors, including the D₂, D₃, and D₄ receptor subtypes, inhibit cAMP synthesis. The receptors also affect activation of potassium channels, mitogen-activated protein kinases, and Akt (Beaulieu et al., 2005; Neve et al., 2004).

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 D₂-like receptors, the third intracellular loop seems to be particularly crucial in mediating these interactions.

The study by Liu et al. reported in the current issue of *Molecular Pharmacology* (Liu et al., 2008) adds one more piece of the puzzle to the study of DA D₂ receptor signaling. Several previous studies have identified binding partners for the D₂ receptor including co-receptors, signaling molecules, and scaffolding proteins (see **Fig. 1**) (Beaulieu et al., 2005; Beaulieu et al., 2007; Binda et al., 2002; Free et al., 2007; Fuxe et al., 2005; Kim et al., 2008; Liu et al., 2006; Liu et al., 2007; Macey et al., 2004; Negyessy and Goldman-Rakic, 2005; Rashid et al., 2007; Smith et al., 1999; So et al., 2005).

In the new study by Liu and colleagues (Liu et al., 2008), the authors used the third intracellular loop of the DA D₂ receptor as bait in a bacterial two-hybrid system and discovered a novel interacting partner, the Ca²⁺ 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 HEK293 cells. In contrast, the DA D₃ and D₄ receptors do not express the motif that appears to confer the interaction, and in fact, D₃ receptor did not pull down S100B in the His assay. Importantly, the D₂ receptor and S100B proteins were also co-immunoprecipitated from a native tissue, adult rat striatum.

Immunohistochemistry in primary neuronal cultures of the striatum were used to demonstrate co-localization of the D₂ receptor and S100B, and provided further evidence that the interaction may be important in native brain tissue. Impressively,

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functional assays of MAPK activation and inhibition of forskolin-induced cAMP accumulation were used to demonstrate that co-expression of S100B with the D₂ receptor increases the ability of D₂ receptors to activate ERK and to inhibit adenylate cyclase. Taken together, the data suggest that S100B co-expression may serve as an important mediator of D₂ receptor signaling efficacy in the brain, and may contribute to differences in cellular, regional, and developmental differences in D₂ receptor activation. Interestingly, previous work by the authors defined calmodulin, also a Ca²⁺ binding protein, as another promoter of D₂ receptor signaling (Liu et al., 2007), suggesting that there may be a more generalized mechanism at work here.

So why does D₂ receptor bind S100B? Relatively little is actually known about S100 proteins within neurons. These small proteins (9-13kD) bind Ca²⁺, 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 Ca²⁺ 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 following 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.

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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 D₂ receptors and S100B developmentally and/or regionally regulated? Does Ca²⁺ binding to S100B alter its ability to interact with the D₂ receptor or the ability of D₂ receptors to interact with other proteins? Do Ca²⁺ 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 D₂ 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.

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Acknowledgements

Graphic assistance was provided by the Vanderbilt Kennedy Center Graphics Center, which is supported by P30-HD15052. I also thank Rachel Fogg for excellent copy-editing.

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Legend for Figure

Fig. 1. Representation of interactions between several known interacting partners of dopamine D₁ and D₂ receptors. Not all known interacting proteins have been depicted, for simplicity. Additionally, D₁ and D₂ receptors are capable of hetero-oligomerization, producing even further regulatory complexity.

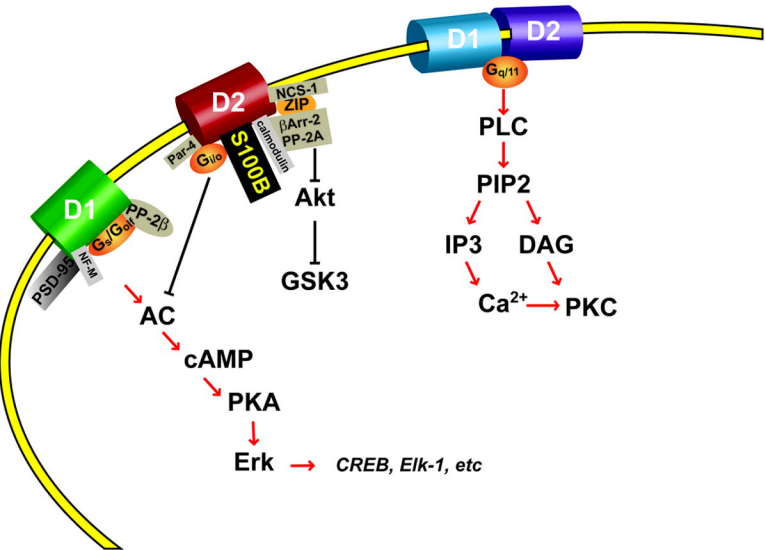


Figure 1