

# Neuronal dopamine internalization and intracellular stimulation of D<sub>5</sub>-receptor-dependent CDP-diacylglycerol biosynthesis for phosphoinositide signaling

Ashiwel Undieh,<sup>1</sup> Chizurum C. Oluigbo,<sup>1</sup> Mercy O. Arijesudade,<sup>1</sup> Eugen Brailoiu,<sup>2</sup> and Arlette D. Siewe<sup>1</sup>

<sup>1</sup>City Univ of New York School of Medicine; and <sup>2</sup>Temple University School of Medicine

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Dopamine receptor stimulation facilitates phosphatidylinositol resynthesis, thus amplifying subsequent responses to activation of phospholipase C-coupled receptors. Phosphatidylinositol synthesis critically depends on the nucleolipid CDP-diacylglycerol. Dopamine robustly increases microsomal CDP-diacylglycerol biosynthesis through stimulation of D<sub>1</sub>-like receptors, particularly the D<sub>5</sub> subtype the majority of which are intracellularly localized. Here, we explored the mechanism by which extracellular dopamine acts to modulate intracellular CDP-diacylglycerol biosynthesis. Dopamine concentration-dependently stimulated CDP-diacylglycerol synthesis in organotypic and primary neuronal cultures devoid of the presynaptic dopamine transporter. Dopamine was saturably transported into cortical primary neurons or B35 neuroblastoma cells expressing wild-type plasmalemmal monoamine transporter (PMAT), which is known to be the principal component of classical Uptake<sub>2</sub> transporters in the forebrain. Dopamine uptake and CDP-diacylglycerol biosynthesis in brain slices or cultured cells were inhibited by microtubule disrupters which block cytoskeletal transport, and by decynium-22 which blocks Uptake<sub>2</sub>-like transporters. Dopamine effects were selectively mimicked by D<sub>1</sub>-like agonists SKF38393 and SKF83959, competitively inhibited by D<sub>1</sub>-like antagonist SCH23390, and unaffected by D<sub>2</sub>-like agonist or antagonist. These observations indicate that dopamine is actively internalized by Uptake<sub>2</sub> into postsynaptic-type cells where the monoamine can stimulate its intracellular D<sub>5</sub>-type receptors to increase CDP-diacylglycerol production. This finding counters the conventional notion that postsynaptic-type cells internalize supra-threshold levels of synaptic dopamine only to inactivate the transmitter. Given the critical involvement of CDP-diacylglycerol in phospholipase C and phosphatidylinositol-3-kinase signaling systems, our findings imply that intracellular dopamine could play an important role in cellular responses and adaptation to high levels of extracellular dopamine.

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