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Meeting abstract

The presence of phosphatidylinositol-4,5-bisphosphate directly impacts on amphetamine-induced serotonin transporter-mediated efflux

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Monoaminergic neurotransmitters are released into the synaptic cleft by exocytosis; sodium chloride-dependent monoamine transporters like the serotonin transporter (SERT) efficiently clear the synapse of serotonin to stop neurotransmission. Administration of amphetamines (AMPH), induces the reversal of substrate transport. It has been repeatedly shown that inward and outward transport can be independently regulated; phosphorylation of key residues in the amino-terminus is thought to be the responsible mechanism. In our study, we provide evidence for a novel intracellular regulation mechanism of SERT. Depletion of phosphatidylinositol-4,5-bisphosphate (PIP2) reduced AMPH-induced SERT-mediated substrate release specifically. This effect was limited to SERT-mediated efflux because SERT-mediated reuptake was completely unaffected. PIP2 depletion was achieved by activation of phospholipase C (PLC) upon application of 2,4,6-trimethyl-N-[3-(trifluoromethyl)phenyl]benzenesulfonamide (m-3M3FBS; 25 μM), a direct and potent PLC activator. Efflux was determined in a superfusion system that allows for measurement of AMPH-triggered fractional release rates of substrate efflux. Preincubation (10 min) of SERT wild-type-expressing cells with m-3M3FBS led to a significant and concentration-dependent reduction of AMPH-induced efflux. This effect was largely blocked by co-application of U73122 (5 μM; 2 min), a PLC inhibitor. PLC-mediated PIP2 hydrolysation generates inositol-3-phosphate and diacylglycerol and thereby provides downstream effects like protein kinase C (PKC) activation and intracellular calcium rise. Thus we (i) stimulated PKC with PMA (1.0 μM; 10 min), (ii) inhibited with GF109203X (1.0 μM; 10 min) and (iii) chelated Ca^{2+} with BAPTA-AM (50 μM; 30 min): There was no effect on efflux with or without m-3M3FBS. This strongly supports the notion that the presence of PIP2 in the immediate vicinity of SERT is essential for the AMPH-induced SERT-mediated efflux. HEK cells endogenously express P2Y receptors which activate PLC by $G_q\alpha$. Stimulation of these receptors with ATP and ADP (under suppression of phosphatidylinositol-4-kinase by 30 μM phenylarsineoxide) showed a reduction of release. The trafficking process of SERT was excluded by fluorescence confocal experiments upon administration of m-3M3FBS. Perforated patch-clamp recordings on hSERT-expressing HEK cells using m-3M3FBS and PCA showed a significant reduction of membrane current effects compared to control. Additionally, these results provide evidence for the necessity of the presence of PIP2 in the plasma membrane for the AMPH-induced outward configuration of SERT.