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Title: Amphetamine action at dopamine and serotonin transporters is modulated by aCaMKII

Abstract in English:

The cocaine- and antidepressant-sensitive dopamine and serotonin (5HT) transporters (DAT and SERT) mediate the reuptake of the neurotransmitters dopamine and 5HT from the synaptic cleft, respectively. They hence regulate the dopamine and 5HT content available for synaptic transmission. Certain stimuli, such as changes in the ionic composition of the extracellular fluid or psychostimulants (e.g. amphetamines) are able to induce outward transport via both, DAT and SERT and thus increase extracellular dopamine and 5HT concentrations.

Influx and efflux of substrates via these transporters are thought to be asymmetrical processes that are not only regulated by ion gradients but also modulated by intracellular kinases. It has been demonstrated that removal of N-terminal serines ablates amphetamine-induced reverse transport of DAT. Similarly, truncation of the SERT-N terminus abolished amphetamine-induced SERT-mediated 5HT efflux. Hence, the N-terminus of DAT and SERT seem to be important for amphetamine action. Interestingly, the Ca2+/calmodulin-dependent protein kinase II a (aCaMKII) has been demonstrated to bind to the DAT C-terminus and to phosphorylate N-terminal serines. Pharmacological inhibition of aCaMKII dramatically reduced amphetamine-induced efflux both, in cells stably transfected with the human DAT as well as in rat striatal slices. Based on these findings, I wanted to investigate the role of aCaMKII on the modulation of amphetamine-induced DAT-mediated reverse transport in mice deficient of functional aCaMKII by using a combination of in vitro, ex vivo and in vivo experiments. Besides, I wanted to find out, whether SERT was also regulated by aCaMKII and whether they interacted biochemically.

Additionally, the phosphoinositide PIP2 was investigated for its role in reverse transport at SERT. My thesis shows that aCaMKII regulates amphetamine-induced DAT-mediated efflux in mice with different mutations in the aCaMKII gene. Mice lacking aCaMKII (aCaMKII-KO) or mice with a permanently self-inhibited version of aCaMKII (aCaMKII T305D) display significantly reduced amphetamine-induced substrate efflux. A similar finding was observed in a mouse model of Angelman Syndrome, a neurogenetic disease characterized by motor impairments and autism spectrum disorders. Angelman Syndrome mice have a reduced aCaMKII activity and show comparable impairments in DAT function as aCaMKII mutants. This suggests that DAT-mediated dopaminergic signalling is affected in Angelman Syndrome. In vivo experiments revealed that aCaMKII-deficient mice have elevated extracellular dopamine levels but still display a decreased amphetamine-induced dopamine efflux. The increased dopamine levels of aCaMKII-KO mice are not caused by a decrease in DAT-mediated dopamine reuptake but are most likely due to an increase in vesicular dopamine release. Behaviourally, the increased dopaminergic tone of aCaMKII-KO mice translates into a profound hyperactivity and severe deficits in acute and chronic locomotor responses to both, amphetamine and cocaine.

I further show that aCaMKII regulates the closely-related SERT: both pharmacological inhibition and genetic disruption of aCaMKII function significantly attenuate amphetamine-induced SERT-mediated substrate efflux in transiently transfected cells and mouse brain preparations. Similarly, depletion of PIP2 impairs reverse transport at SERT in both, cells and rat slices. In conclusion, aCaMKII exerts an important modulatory role in amphetamine-induced DAT- and SERT-mediated subtstrate efflux. The

finding that DAT-mediated efflux is affected in Angelman Syndrome mice might help in the understanding of the underlying pathophysiology.