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MEETING ABSTRACT Open Access

Calmodulin kinase II regulates amphetamine-induced reverse transport in the dopamine transporter: implications for the importance of the dopamine transporter in Angelman syndrome

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Background

The dopamine transporter (DAT) mediates dopamine (DA) reuptake once DA gets released into the synaptic cleft; thereby, the DAT regulates DA content available for synaptic transmission. Psychostimulants like amphetamines can induce the reverse operation and induce outward transport, thereby increasing extracellular dopamine concentrations. Increases of DA in the synaptic cleft are associated with psychosis and drug addiction. Influx and efflux of substrate via the DAT are thought to be asymmetrical and were shown to possess consensus sites for the regulation by intracellular kinases. It was demonstrated that the loss of N-terminal serines ablates amphetamine-induced reverse transport in the DAT and that Ca^{2+}/calmodulin-dependent protein kinase IIa (CaMKIIa) can physically bind the DAT C-terminus and phosphorylate N-terminal serines. Pharmacological inhibition of CaMKIIa with KN93 dramatically reduces amphetamine-induced efflux in both cells stably transfected with the human DAT and in rat striatal slices. Here we show that amphetamine-induced DAT-mediated efflux is reduced in CamKIIa mutants and in a mouse model of Angelman syndrome (AS).

Methods

Mouse striatal synaptosomes preloaded with [^{3}H]MPP^+, a substrate for the DAT, were superfused using a superfusion system. [^{3}H]MPP^+ release was induced by the addition of 3 μM D-amphetamine. Superfusates were collected and quantified using a szintillation counter. Immunoblots and [^{3}H]CFT binding experiments were used to assess DAT protein expression levels. Uptake assays were performed in striatal synaptosomes using [^{3}H]MPP^+ as substrate. For non-specific uptake we used the DAT-inhibitor mazindol.

Results

Both pharmacological inhibition of CamKIIa or genetic ablation of CaMKIIa function (in CaMKIIa knock-out and AS mice) reduces amphetamine-induced reverse transport in the DAT. As CaMKIIa is one of the brain's most abundant proteins involved in a plethora of regulatory processes it is not possible to pharmacologically target it in human AS patients. However, the DAT would be a possible target also for these patients and it might be promising to further investigate potential DAT influencing medications to treat Angelman syndrome.

Conclusions

Pharmacologic inhibition of CamKIIa as well as genetic knock-out or activity-downregulation of CamKIIa reduce amphetamine-induced reverse transport in the DAT.

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