Activation of Adenosine A_{2A} Receptors Enhances Phosphorylation of the Src Family Tyrosine Kinases in the Rat Dorsal Striatum

UMKC HEALTH SCIENCES DISTRICT

Shannon S. Demehri, Li-Min Mao, and John Q. Wang

Department of Biomedical Sciences, University of Missouri-Kansas City School of Medicine

UMKC School of Medicine

Introduction

Src family kinases (SFK) are a subfamily of nonreceptor tyrosine kinases consisting of nine members, five of which are expressed in the mammalian brain. These SFKs are activated by a phosphorylation-dependent mechanism. Specifically, SFKs are phosphorylated at a conserved residue, tyrosine 416 (Y416), leading to activation of the kinase[1]. Notably, Y416 phosphorylation is an inducible step which is highly sensitive to a variety of stimuli. As such, SFKs play a vital role in regulating various neuronal and synaptic activities.

The striatum, a key structure in the basal ganglia of the brain, is characterized by abundant expression of the two members of SFKs: Src and Fyn[2]. Adenosine A_{2A} receptors are $G_{s/olf}$-coupled receptors that are predominantly expressed in this brain region and are thought to be important for regulating striatal neuronal activity, although detailed molecular mechanisms are poorly understood. As a mostly postsynaptic receptor, A_{2A} receptors are believed to be actively involved in the regulation of a variety of intracellular signaling pathways in striatopallidal output neurons and are linked to the pathogenesis of drugs of abuse as well as various neuropsychiatric and neurological disorders such as Parkinson's disease. This study investigated the possible role of A_{2A} receptors in the modulation of phosphorylation of SFKs in the adult rat striatum.

Methodology

- Adult male rats were used for preparing acute striatal slices. Animal use and care were approved by IACUC.
- Striatal slices were treated with the A_{2A} agonist CGS-21680 (0.1, 1, or 3 μM) or the A_{2A} antagonist KW-6002 (0.1 or 1 μM) for 15 min (n = 4 - 6 per group).
- The dorsal striatum, i.e., the caudate putamen, was collected. Western blot was conducted using rabbit antibodies against phosphorylated SFK at Y416 (pY416), Fyn, or Src.
- One-way ANOVA statistical analysis was performed. $P < 0.05$ was considered significant difference.

Results

Figure 1. The A_{2A} agonist CGS-21680 increased Y416 phosphorylation in the rat dorsal striatum.

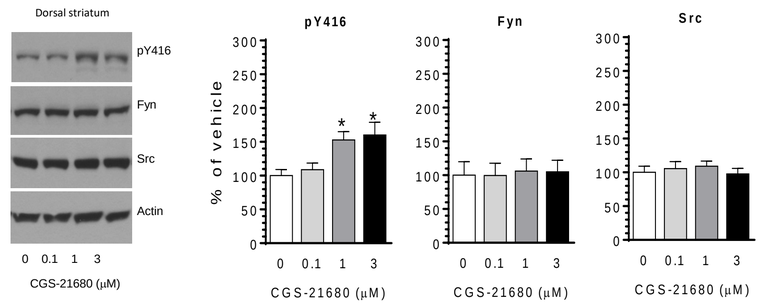
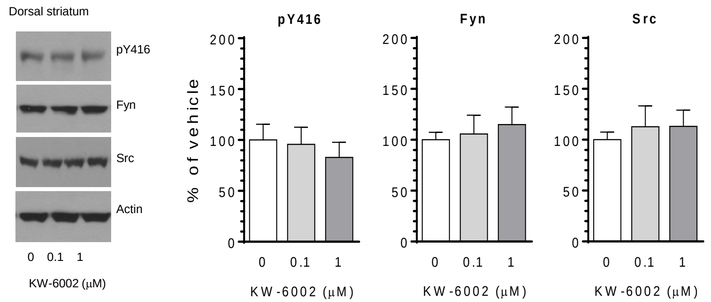


Figure 2. The A_{2A} antagonist KW-6002 had no effect on Y416 phosphorylation in the rat dorsal striatum.



Summary

- Addition of the A_{2A} agonist CGS-21680 induced an increase in phosphorylation of SFKs at a conserved autophosphorylation site (Y416) in the dorsal striatum.
- Addition of the A_{2A} antagonist KW-6002 had no significant effect on SFK Y416 phosphorylation in the dorsal striatum.
- Both CGS-21680 and KW-6002 did not alter total protein levels of the two key SFK members, Fyn and Src, which are enriched in the dorsal striatum.

Conclusion

- These data demonstrates that the SFK is a substrate of A_{2A} receptors in striatal neurons.
- Activation of A_{2A} receptors leads to phosphorylation (activation) of SFKs.
- The A_{2A}-SFK pathway may be implicated in neuropsychiatric and neurological disorders derived from the dysfunctional striatum.

References

1. Roskoski R. Jr., 2005. Src kinase regulation by phosphorylation and dephosphorylation. Biochem. Biophys. Res. Commun. 331, 1-14.
2. Pascoli V., Besnard A., Herve D., Pages C., Heck N., Girault J.A., Caboche J., Vanhoutte P., 2011. Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. Biol. Psychiatry 69, 218-227.

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