

P015 Inhibition of adenylyl cyclase 9 by cyclin-dependent protein kinase 5 (cdk5)

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AC9 is a membrane-bound enzyme expressed by all CNS neurons. Analysis of the phosphorylation of human AC9 stably over-expressed in human embryonic kidney 293 (HEK293) cells by mass-spectrometry revealed two proline-directed phosphoserine motifs. Immunoprecipitated AC9 was phosphorylated on both sites by cdk5/p35. HEK293 cells were found to express endogenous cdk5/p35 activity and p35 was detected in cellular fractions enriched for AC9. Accordingly, both phosphoserines were detectable in membrane preparations and treatment with roscovitine reduced the levels of phosphorylation by up to 95% in a time- and concentration-dependent manner. In COS7 cells, that lacked endogenous cdk5/p35 activity, transfection of p35 increased AC9 phosphorylation, which was inhibited by dominant negative mutant cdk5. Treatment with roscovitine increased cAMP synthesis 2-3 fold in HEK293 cells expressing human or mouse AC9. The activity of AC9 in membranes of roscovitine treated cells was also enhanced 2-fold. In summary, cdk5/p35 inhibits AC9. Both AC9 and cdk5/p35 are membrane-attached proteins. As AC9 is widely distributed in the body while cdk/p35 is restricted to the CNS and a few select peripheral organs, the data imply cell-specific regulation of AC9 which is likely to be confined to cellular compartments.