Individual cerebellar Purkinje cells express different cGMP phosphodiesterases (PDEs): in vivo phosphorylation of cGMP-specific PDE (PDE5) as an indicator of cGMP-dependent protein kinase (PKG) activation.

Abstract:

The nitric oxide (NO)-cGMP pathway has been implicated as playing a crucial role in the induction of cerebellar long-term depression (LTD). The amplitude and duration of the cGMP signal is controlled by cyclic nucleotide phosphodiesterases (PDEs). Here we identify PDE5 and PDE1B as the two major cGMP-hydrolyzing PDEs specifically and differentially expressed in the Purkinje neurons of mouse cerebellum. PDE5 was found in all Purkinje neurons, whereas PDE1B was detected only in a subset of these cells, suggesting that individual Purkinje cells may differentially regulate cGMP, depending on the PDE isozymes expressed. Although expression of guanylate cyclase and/or cGMP-dependent protein kinase (PKG) in Purkinje cells have been reported, neither cGMP accumulation nor PKG activation in these cells in vivo has been demonstrated. To determine if changes in PKG activation and PDE5 regulation occur in vivo we have examined the phosphorylation of PDE5 in mouse cerebellar Purkinje cells by immunocytochemistry and Western blot analyses using a phosphospecific PDE5 antibody. Injection of sodium nitroprusside or selective PKG activators into the lateral ventricle of mouse brain
induced PDE5 phosphorylation in vivo, but was completely missing in Purkinje cell-specific PKG I knock-out mice. In cerebellar slices, treatment with sildenafil or IBMX led to different levels of phospho-PDE5 accumulation and activation of PDE5. These results suggest that phosphorylation of PDE5 in Purkinje neurons after cGMP-PKG activation performs a critical role in the termination of the cGMP signal during LTD progression; moreover, PDE5 phosphorylation may be used as an in vivo indicator for PKG activation.