Knockout of alpha(1D) – adrenergic receptor affects the BDNF–ERK pathway in prefrontal cortex of mice brain: modulation by antidepressant drugs

I. Nalepa, K. Chorążka, J. Kuśmierczyk, J. Śmiałek, M. Kowalska

Maj Institute of Pharmacology, Polish Academy of Sciences, Dept. of Brain Biochemistry, Kraków, Poland

Objectives

To determine whether alpha(1D)-adrenergic receptor is involved in mechanism of action of antidepressant drugs and to assess its role in regulation of the brain-derived neurotrophic factor (BDNF) – the extracellular signal-regulated kinase (ERK) pathway.

Background

Neurotrophic factors are critical for the etiology and treatment of depression. The brain-derived neurotrophic factor (BDNF) production may be stimulated by noradrenaline what can be mediated by alpha(1)-adrenergic receptor ($\alpha1$ -AR). There are three subtypes of $\alpha1$ -AR family named $\alpha1A$ -, $\alpha1B$ - and $\alpha1D$ -AR which are expressed in brain. Chronically given antidepressant were shown to induce changes in density of various receptors including $\alpha1$ -ARs. We aimed to evaluate the effect of knockout of $\alpha1D$ -AR and chronic treatment with antidepressant drugs on expression of selected proteins: BDNF and signaling proteins – the extracellular signal-regulated kinases (ERK1/2) and the cAMP response element-binding protein (CREB), which are downstream to the BDNF-activated TrkB receptor (Fig. 1).

Materials and Methods

The study was performed in male mice devoid of the $\alpha1D$ -AR ($\alpha1D$ -KO) and in wild type (*wt*) controls. The $\alpha1D$ -KO mouse colony was derived from triple $\alpha1ABD$ -KO line which was then crossed with C57Bl6/J mice and bred to receive a single $\alpha1D$ -KO colony. Mice were chronically treated (21 days) with desipramine (20 mg/kg) or milnacipran (30 mg/kg, i.p.) or saline. The level of the signaling proteins in basal and phosphorylated forms were analyzed by Western blot in the cerebral prefrontal cortex (PFC).

Fig 1. Signaling cascades of $\alpha1$ -ARs and of BDNF with levels of their interaction. Antidepressant drugs act through serotonergic and adrenergic transmission. Asterisks denote the proteins investigated in this experiment; KO indicates $\alpha1D$ knockout.

Fig 2. The expression of BDNF in PFC of wt and $\alpha1D$ -KO mice. Data are expressed as mean $\pm$ SEM relative to wt male sal group that were set as 1. Number of animals in groups N=7-8. * p <0.05 vs wt sal.

Fig 3. The phosphorylation of ERK1 (Fig 3A) and ERK2 (Fig 3B) in PFC of wt and $\alpha1D$ -KO mice. Data are expressed as mean $\pm$ SEM relative to wt male sal group that were set as 1. Number of animals in groups N=7-8. * p <0.05 vs wt sal. Fig. 3C: representative Western blot.

Results and Conclusions

The most important finding was that the $\alpha1D$ -KO mice display decreased BDNF expression level (**Fig. 2**) [$F(1,41)=8.205$, $p<0.01$] and the reduction in ERK1 and ERK2 phosphorylation (**Figs. 3A and 3B**) [$F(1,41)=19.575$, $p<0.001$] and [$F(1,41)=25.001$, $p<0.001$]. The effect of drugs were seen in the case of BDNF which was slightly decreased by desipramine in *wt* and in the ERK2 phosphorylation level reduced by chronic milnacipran.

❖ **We conclude that the $\alpha1D$ -AR participates in regulation of BDNF expression and of ERKs activation but this receptor is less important (if at all) for the effect of investigated antidepressant drugs.**

Fig 4. The phosphorylation of CREB (Fig 4) in PFC of wt and $\alpha1D$ -KO mice. Data are expressed as mean $\pm$ SEM relative to wt male sal group that were set as 1. Number of animals in groups N=7-8.