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BRAIN RESEARCH

Research Report

Sex-specific and region-specific changes in BDNF–TrkB signalling in the hippocampus of 5-HT1A receptor and BDNF single and double mutant mice

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ARTICLE INFO

Article history:
Accepted 2 March 2012
Available online 10 March 2012

Keywords:
Sex difference
BDNF
5-HT1A
Hippocampus
Depression

ABSTRACT

Brain-derived neurotrophic factor (BDNF) and serotonin 5-HT1A receptors are implicated in the pathophysiology of depression and the mechanism of action of antidepressant drugs. Here, we explore possible reciprocal interactions of 5-HT1A receptor knockout and the expression of BDNF, its receptor TrkB and downstream mitogen-activated protein kinase (MAPK) in the ventral (VHP) and dorsal hippocampus (DHP). We compared female and male double mutant mice (5-HT1A$^{-/-}$/BDNF$^{+/-}$) with single mutant mice (5-HT1A$^{-/-}$, BDNF$^{+/-}$) and wildtype (WT) controls. Protein expression of BDNF, TrkB, phosphorylation of TrkB (pTrkB) and MAPKs (ERK1, ERK2) was examined using Western blot analysis (n=5–7). As expected, the BDNF$^{+/-}$ mice showed ~50% BDNF reduction. Loss of 5-HT1A receptors induced a significant decrease in BDNF levels in the VHP in female mice. The pTrkB/TrkB ratio was also significantly decreased in female 5-HT1A$^{-/-}$ mice and 5-HT1A$^{-/-}$/BDNF$^{+/-}$ mice but not in males. Despite markedly reduced BDNF levels in BDNF$^{+/-}$ mice and double mutants, ERK1 activation was unchanged in the female mice. In contrast, ERK2 activation was significantly elevated in the VHP of female BDNF$^{+/-}$ mice and double mutants. Given the greater vulnerability of women to develop depression and the role of the VHP in stress responses and anxiety-related behaviours, our results may shed more light on sex differences in depression and other psychiatric disorders with BDNF and 5-HT1A receptor dysfunction.

1. Introduction

Depression is a major public health concern worldwide with a lifetime prevalence of approximately one in five (Kessler et al., 2003). Currently, selective serotonin reuptake inhibitors (SSRIs) are the first line of pharmacotherapy for treating depression. However, more than 65% of patients fail to reach full remission (Trivedi et al., 2008) and weeks of treatment are required before full therapeutic efficacy may be achieved (Blier and de Montigny, 1994). This delay may represent time

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doi:10.1016/j.brainres.2012.03.011

required to desensitize inhibitory 5-HT1A autoreceptors (Castro et al., 2003) and to up-regulate pro-survival factors such as brain-derived neurotrophic factor (BDNF) (Molteni et al., 2006). BDNF levels increase with chronic but not acute antidepressant treatment which corresponds to the time course necessary for full clinical efficacy (Castren and Rantamaki, 2010). Genetic polymorphisms in the 5-HT1A (C1019G) and BDNF (Val66Met) gene have been linked to an increased susceptibility to depression (Anttila et al., 2007).

BDNF is part of the neurotrophin family and plays an important role in the regeneration, survival, and synaptic plasticity of neurons. These cellular effects of BDNF are mediated through its high affinity tropomyosin-related kinase B (TrkB) receptor. Binding of BDNF to TrkB phosphorylates its tyrosine kinase domain, leading to the activation of survival pathways including the mitogen-activated protein kinase (MAPK) and other pathways (Yoshii and Constantine-Paton, 2010). Depression is associated with neuronal atrophy, notably in the hippocampus (Campbell et al., 2004) and this has been correlated with reduced BDNF mRNA (Dwivedi et al., 2003) and protein levels (Karege et al., 2005). Specific BDNF knockdown in subregions of the hippocampus of rats adversely affected their performance in the forced swim test (FST) and induced anhedonic-like behaviour (Taliaz et al., 2010). Abnormally low serum BDNF levels in patients with depression were reversed by chronic treatment with SSRIs (Sen et al., 2008), other antidepressant drug classes or electroconvulsive therapy (ECT), further suggesting links between increased BDNF expression and antidepressant action (Dias et al., 2003).

Patients with depression also show significant reductions in postsynaptic 5-HT1A receptor protein level (Szewczyk et al., 2009) as well as binding potential (Drevets et al., 2007) in cortical subregions. Interestingly, however, the significant reduction in 5-HT1A receptor protein expression was only found in female depressed subjects and unchanged in male depressed subjects compared to gender-matched controls (Szewczyk et al., 2009). The 5-HT1A receptor furthermore serves as an autoreceptor in the raphe nuclei to reduce the firing rate of these neurons. Rodent and human studies have found modest antidepressant-like effects following chronic administration of buspirone, a 5-HT1A receptor agonist (Kreiss and Lucki, 1997). This is potentially through desensitization of these 5-HT1A autoreceptors and stimulation of postsynaptic 5-HT1A receptors in the hippocampus. This activation of postsynaptic 5-HT1A receptors has been found to increase hippocampal neurogenesis via stimulating the MAPK pathway (Banerjee et al., 2007) while activation of 5-HT1A receptors in raphe-derived cell lines inhibited basal MAPK activity (Kushwaha and Albert, 2005).

Several studies suggest that BDNF can alter the activation and expression of 5-HT1A receptors. For instance, inducible BDNF knockout (KO) mice exposed to stress show attenuated hippocampal 5-HT1A receptor signalling (Hensler et al., 2007) and unilateral infusion of BDNF into the dentate gyrus (DG) of rats induced a bilateral increase of 5-HT1A mRNA levels (Pinnock and Herbert, 2008). However, despite several reports on an interaction of BDNF and 5-HT1A receptors in depression, the exact interplay of these factors remains unclear.

The present study aimed to examine how altered levels of 5-HT1A receptors influence BDNF–TrkB signalling in the hippocampus of control mice and under impaired BDNF expression. We crossed 5-HT1A KO (5-HT1A$^{-/-}$) mice with BDNF$^{+/-}$ mice, generating a double mutant mouse line (5-HT1A$^{-/-}$/BDNF$^{+/-}$). Protein changes in BDNF and TrkB activation were assessed in these 5-HT1A$^{-/-}$/BDNF$^{+/-}$ mice, their single mutant counterparts (BDNF$^{+/-}$, 5-HT1A$^{-/-}$) and wildtype (WT) controls. Sex differences in affective disorders have not been widely addressed in preclinical research and most studies include male animals only or use a mixed male/female population. In addition, accumulating evidence suggest that the hippocampus can be functionally subdivided along its ventral/dorsal axis. Therefore, in this study, ventral (VHP) and dorsal (DHP) hippocampus of female and male mice were separately analysed. Because an interaction between BDNF–TrkB and the 5-HT1A receptor may occur through their converging downstream mitogenic signalling, we further examined protein levels of the MAPKs, ERK 1 and 2. The current study provides *in vivo* insight into the interaction between BDNF and the 5-HT1A receptor.

2. Results

2.1. *BDNF in the ventral and dorsal hippocampus of female and male mice*

Analysis of variance (ANOVA) revealed a significant main effect of the BDNF$^{+/-}$ genotype on BDNF expression in the VHP of female (Fig. 1A, $F(1,16)=17.5$, $p=0.001$) and male mice (Fig. 1C, $F(1,18)=19.4$, $p<0.001$) and in the DHP of female (Fig. 1B, $F(1,15)=10.2$, $p=0.006$) and male mice (Fig. 1D, $F(1,18)=14.3$, $p=0.001$). Further pair-wise comparison using a planned Bonferroni-corrected *t*-test confirmed that both female and male BDNF$^{+/-}$ mice and BDNF$^{+/-}$/5-HT1A$^{-/-}$ double mutants showed an approximate 50% reduction in BDNF levels compared to WT controls. A significant main effect of the 5-HT1A$^{-/-}$ genotype was also detected in the female VHP (Fig. 1A, $F(1,16)=4.8$, $p=0.043$) without an effect in the female DHP or in the male hippocampal subregions. Pair-wise comparison revealed that BDNF expression in the VHP of female 5-HT1A$^{-/-}$ mice was significantly reduced by 30–40% compared to their WT counterparts (Fig. 1A). There was no significant statistical interaction found between the BDNF$^{+/-}$ genotype and the 5-HT1A$^{-/-}$ genotype in any of the groups.

2.2. *TrkB phosphorylation in the ventral and dorsal hippocampus of female and male mice*

Despite an approximate 50% reduction in BDNF expression in both female and male BDNF$^{+/-}$ mice, no significant changes in TrkB phosphorylation (pTrkB/TrkB) were found throughout the hippocampus (Figs. 2A–D). However, TrkB phosphorylation was significantly reduced down to 50% of WT controls in the VHP of female 5-HT1A$^{-/-}$ mice and BDNF$^{+/-}$/5-HT1A$^{-/-}$ double mutants (Fig. 2A, main effect of 5-HT1A$^{-/-}$ genotype, $F(1,23)=8.5$, $p=0.008$). In the female