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Golo Kronenberg[1*], Valentina Mosienko[2,4*], Karen Gertz[3], Natalia Alenina[2], Rainer Hellweg[1], and Friederike Klempin[2#]

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[1]Department of Psychiatry and Psychotherapy, Campus Charité Mitte, Charité - University Medicine Berlin, Berlin, Germany

[2]Max-Delbruck-Center for Molecular Medicine (MDC), 13125 Berlin, Germany

[3]Department of Neurology, Charité - University Medicine Berlin, Berlin, Germany

[4]*current address:* University of Bristol, School of Physiology and Pharmacology, United Kingdom

[*]denotes equal contribution

[#]Please address correspondence to Friederike Klempin, PhD, Research Team 'Cardiovascular Hormones and Peptides', Max-Delbruck-Center for Molecular Medicine, Robert-Roessle-Str. 10, 13125 Berlin, Germany. Email: friederike.klempin@mdc-berlin.de

Abstract

The interplay between BDNF signaling and the serotonergic system remains incompletely understood. Using a highly sensitive enzyme-linked immunosorbent assay, we studied BDNF concentrations in hippocampus and cortex of two mouse models of altered serotonin signaling: tryptophan hydroxylase (*Tph*)*2*-deficient (*Tph2*$^{-/-}$) mice lacking brain serotonin and serotonin transporter (SERT) deficient (SERT$^{-/-}$) mice lacking serotonin re-uptake. Surprisingly, hippocampal BDNF was significantly elevated in *Tph2*$^{-/-}$ mice, whereas no significant changes were observed in SERT$^{-/-}$ mice. Furthermore, BDNF levels were increased in the prefrontal cortex of *Tph2*$^{-/-}$ but not of SERT$^{-/-}$ mice. Our results emphasize the interaction between serotonin signaling and BDNF. Complete lack of brain serotonin induces BDNF expression.

Keywords

BDNF, SERT, TPH2, serotonin, depression, antidepressant

Introduction

5-hydroxytryptamine (serotonin) is synthesized in the brain stem raphe nuclei by the neuron-specific enzyme tryptophan hydroxylase (TPH) 2 [1]. Upon release, extracellular concentrations of serotonin are regulated by plasmalemmal re-uptake into the pre-synaptic neuron/boutons via SERT (5-HTT, SLC6A4), which is widely expressed in the brain and constitutes a chief target for antidepressant psychopharmacotherapy [2, 3]. BDNF is the most abundant neurotrophin in the adult hippocampus and cortex of rodents [4]. Apart from local biosynthesis, BDNF protein is also supplied by anterograde neuronal trafficking [5]. Both central serotonin and BDNF signaling are crucially involved in shaping important aspects of brain plasticity such as synaptic plasticity and the generation and survival of new neurons in the hippocampus [6, 7]. Importantly, chronic treatment with a selective serotonin re-uptake inhibitor (SSRI) has been shown to increase the expression of cAMP response element binding protein (CREB) and of CREB target gene BDNF in the hippocampus [8-10]. Conversely, serotonin neurotransmission is under the influence of BDNF [11, 12].

Mouse models genetically modified for altered serotonin signaling open an exciting new window on brain plasticity and may shed new light on the neurobiology of depression and mechanisms of antidepressant actions [13]. Here, we measured BDNF protein levels in *Tph2*$^{-/-}$ mice that lack brain serotonin (and show certain features of depression-like behaviors such as impaired maternal care and increased immobility in the Porsolt forced swim test; reviewed in [14]), and SERT$^{-/-}$ mice, which exhibit increased extracellular serotonin levels [15, 16] despite an overall reduction (60 to 80%) in serotonin tissue concentrations [17].

In the adult hippocampus, chronic SSRI treatment increases precursor cell proliferation and neurogenesis [18]. It might therefore be speculated that impaired serotonergic signaling is also accompanied by reduced neurogenesis. However, we and others have recently shown that in adult mice modified for no (*Tph2*$^{-/-}$, *VMAT2*$^{SERT\text{-}Cre}$) or reduced brain serotonin (*Tph2KI, Pet1*$^{-/-}$) baseline levels of cell proliferation in the dentate gyrus are unexpectedly normal (reviewed in [19]). We speculate that sustained cell proliferation under conditions of serotonin deficiency most probably is the result of developmental compensation by other factors in the niche, e.g., BDNF.

Methods

Animals

The generation of *Tph2*$^{-/-}$ and SERT$^{-/-}$ mice has been described in detail elsewhere [15, 20]. Both *Tph2*$^{-/-}$ and SERT$^{-/-}$ mice have been established on a pure C57BL/6 genetic background [21]. Animals were 10 to 12 weeks old at the time of sacrifice. Age and background-matched wild type (WT) mice were used in all experiments. All mice were kept in the same room under a controlled environment (12/12 h light-dark cycle, free access to food and water). All procedures were approved by the respective official committees (LaGeSo) and carried out in accordance with the Animal Welfare Act and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Tissue preparation and enzyme-linked immunosorbent assay (ELISA)

After sacrifice, brains were quickly removed. Prefrontal cortex and hippocampus were dissected, snap-frozen in liquid nitrogen, and then stored at -80 °C until further use. Endogenous levels of BDNF were measured in the rethawed homogenates using commercial ELISA kits in principle according to the manufacturer's instructions (Promega) but adapted to a highly sensitive fluorometric technique as described in detail elsewhere [22]. BDNF content was expressed as equivalents of recombinant human BDNF. The detection limit of the assay was 1 pg/ml. Determinations of recovery and specific and unspecific neurotrophin binding (the latter against mouse IgG1 obtained from MOPC 21) involved triplicate fluorescence determinations for each tissue sample. Using this improved fluorometric ELISA, it was feasible to quantify BDNF in brain tissue with a minimal wet weight of approximately 5 pg [23, 24]. BDNF levels were expressed as picograms per milligram of tissue (wet weight).

Statistics

Statistical differences between group means were evaluated by ANOVA followed by Tukey's post hoc test when appropriate (GraphPad PRISM 5.01 software). For individual comparisons, a Student's *t* test was used. All values are expressed as mean ± SEM. P values of <0.05 were considered statistically significant.

Results

The results of ELISA measurements are summarized in Figure 1. BDNF protein concentrations in hippocampus were generally higher than in cortex. A genotype effect was observed for *Tph2*$^{-/-}$ mice (ANOVA $F(5,50) = 15.23$, $p < 0.0001$) with significantly increased BDNF levels in the hippocampus of female and male *Tph2*$^{-/-}$ mice relative to WT (Student's t test $p = 0.0442$, and $p = 0.0280$, respectively; **Figure 1A**). Furthermore, cortical BDNF concentrations were significantly increased in *Tph2*$^{-/-}$ mice compared with WT (Student's t test $p = 0.0165$). We did not detect significant effects of SERT$^{-/-}$ genotype on BDNF expression (**Figure 1B**).

Discussion

In this study, we assessed BDNF levels in the adult hippocampus and cortex of two mouse models with altered brain serotonin signaling. Using an improved fluorometric ELISA, BDNF protein content in brain tissue was quantitatively evaluated. As described in the methods section, the ELISA procedure employed here is characterized by a very high sensitivity and is far superior to alternative methods described for the quantification of the BDNF protein such as immunohistochemistry or immunoblotting [23, 24]. We would also like to point out that earlier studies in experimental mice yielded very similar absolute BDNF concentrations to the values reported here. Moreover, a generally similar pattern of sex-specific differences in BDNF levels has been described [25]. Furthermore, it should be noted that the concentration of the bioactive BDNF protein in a given brain structure cannot simply be extrapolated from BDNF mRNA expression in that structure. Indeed, discrepancies between BDNF mRNA and protein have been described in the literature [26]. These discrepancies may relate to protein trafficking and changes in BDNF mRNA stability [27, 28].

The major finding of our study is that BDNF concentrations are significantly increased in the brains of *Tph2*$^{-/-}$ mice in all regions investigated. We argue that increased BNDF levels in these animals may reflect a compensatory mechanism of the brain in the absence of serotonin. Our study confirms and extends to the protein level the results by Migliarini and co-workers [29]. They reported increased BDNF gene transcription in the hippocampus of a knock-in mouse line in which the *Tph2* gene is replaced by enhanced green fluorescent protein (eGFP). However, in that study, BDNF mRNA expression changes were rather subtle and did not yield statistically significant differences in the cortex [29]. Our results are also directly relevant to the situation in the human brain, where a loss-of-function mutation in the *Tph2* gene has been described resulting in an approximately 80% decrease in serotonin production [30]. We speculate that similar changes may also accompany the severe serotonergic damage that has been described with 3,4-methylenedioxymethamphetamine (MDMA) use [31].

The literature on SERT and BDNF signaling yields a somewhat complicated picture. Inhibition of SERT function that leads to increased serotonin levels in the synaptic cleft is thought to be the main therapeutic target of SSRIs. In turn, chronic administration of sertraline increases hippocampal BDNF mRNA levels in rats [9]. Similarly, SSRI-induced increases in serum BDNF levels have been detected in depressed patients [32]. Furthermore, SERT heterozygous null mice subjected to poor maternal care show elevated hippocampal BDNF mRNA levels relative to wild type controls undergoing identical experimental procedures [33]. Our data of slightly increased BDNF protein in the hippocampus of SERT-deficient mice seems to fit with these findings. Conceptually, mild increases in BDNF protein content in $SERT^{-/-}$ mice with reduced serotonin concentrations in brain tissue also fit well with more robust BDNF increases in *Tph2*$^{-/-}$ mice that lack brain serotonin completely. However, our results differ somewhat from other studies that found reduced BDNF signaling in the ventral hippocampus and prefrontal cortex of SERT deficient rats [34, 35]. Finally, another study of BDNF conducted in $SERT^{+/+}$, $SERT^{+/-}$ and $SERT^{-/-}$ mice did not yield any significant differences in BDNF protein levels between genotypes in hippocampus, frontal cortex, and brain stem [36]. In line with this report, we also did not detect altered BDNF levels in the prefrontal cortex of the $SERT^{-/-}$ mouse strain used in our study. Regarding the hippocampus, it also has to be noted that a recent study in SERT mutant rats adds a new level of complexity by suggesting that BDNF expression may differ in different hippocampal subfields [34].

Taken together, our study adds to a growing body of evidence showing that the serotonergic system and the BDNF system interact. Strikingly, loss of central serotonin promotes BDNF protein expression.

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Legends

Figure 1.

BDNF protein levels in the hippocampus and prefrontal cortex of *Tph2*$^{-/-}$ and SERT$^{-/-}$ mice. (A) BDNF protein levels were increased in female (n = 8) and male (n = 7) hippocampi, and prefrontal cortex of female *Tph2*$^{-/-}$ mice in comparison to WT. (B) No statistically significant changes in BDNF concentrations were measured in the adult hippocampus and prefrontal cortex of female SERT$^{-/-}$ mice (n = 6). Notably, BDNF levels were generally lower in the prefrontal cortex than in the hippocampus. *P < 0.05 indicates statistical significance between genotypes, and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ to hippocampus of female mice of the same genotype, Student's t test; data are presented as mean ± SEM