The Role of the Novel Mutation in the *bdnf* Gene, which may lead to Intellectual Disability

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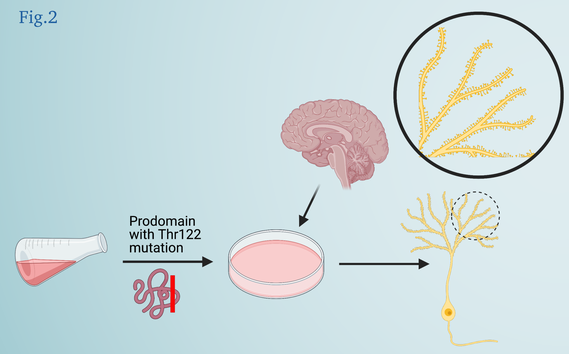
Abstract

Brain-derived neurotrophic factor (BDNF) is synthesized as an inactive precursor proBDNF consisting of a prodomain and mature BDNF (mBDNF). It stays inactive until the prodomain is cleaved off. A mutation was described in the *Bdnf* gene that changes Methionine to Threonine at the position 122. This mutation is associated with intellectual disability in a human patient. Intellectual disability (ID) is characterized by delayed learning and inability to master basic skills and remember acquired information. Since BDNF is a critical molecule for learning and memory, we hypothesized that this mutation may interfere with BDNF function. Another BDNF prodomain containing the single nucleotide polymorphism resulting in a change of Valine to Methionine at the position 66 counteracts the effects of BDNF on dendritic spines. We therefore examined the impact of Thr122 prodomain on neuronal morphology. We did not find significant changes after adding Thr122 prodomain, but we noticed a trend pointing to the increase of thin spines. Using the hemi-synapse assay we examined the impact of Thr122 prodomain on BDNF ability to induce synapses. We hypothesized that Thr122 can bind to BDNF and affect its ability to generate synapses.We found that in fact BDNF's ability to generate synaptic connections is impaired in presence of Thr122 prodomain. Taken together, these results suggest that the novel mutation in Bdnf gene leads to a generation of prodomain that reduces synapse formation by interfering with critical memory protein BDNF. This preliminary data points to further analysis of BDNF signaling associated with memory formation.

Experimental Design and Findings (1)

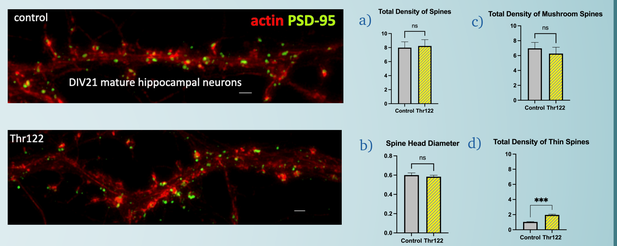
In previous research on a single nucleotide polymorphism (SNP) on the location 66 -- common in about 25% of the population -- of the prodomain we observed a gain-of-function mutation. To test, if Thr122 prodomain can also affect neuronal morphology, we produced Thr122 in bacteria. (Fig.2) Then we collected the prodomain and added it to mature neurons in culture to examine spine morhology. Structural plasticity of dendritic spines is related to learning and memory and such defects can be associated with Intellectual Disability (ID) .

Fig.2



DIV21 hippocampal neurons were visualized with actin and imaged using 3D SIM microscopy (Fig. 3)

Fig.3



We used Nikon Super-structured illumination microscopy (SIM), is a revolutionary imaging technique for doubling the resolution of a widefield microscope in 3D to image the hippocampal neurons. It advances imaging by the collection of data from 3 angles and 5 different phases. This technique improved the detection of thin spines by roughly 50%

***p<0.05; data represented as mean ± SEM

Results

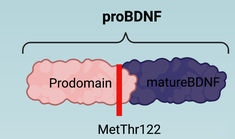
Our goal was to understand the role of the Threonine 122 mutation. In Experiment one we quantified the data of several 3D SIM images and a slight trend can be detected towards the formation of thin spines possibly by preventing BDNF mediated spine expansion. This result (see Fig. 3a and c) is reflected in the density count of both spine head types. In Experiment 2 we explored if the introduction of Thr122 prodomain to i vitro 14 neurons (immature neurons, which have not yet formed spines) will cause a failure of synaptogenesis. Looking at the synapsin 1 (synaptic marker located at the pre-synaptic site) column of Fig.5a there is a clear reduction in BDNF ability to form synapses, when BNDF is added with Thr122 prodomain as oppose to BDNF alone that leads to significant synaptogenesis. One hypothesis may be that the Thr122 prodomain prevents the binding of TrkB at the presynaptic site with postsynaptic Slitrk5, thus preventing synapse formation. These results are consistent with intellectual disability that is characterized by inability to learn represented by ability to form synapses and neuronal connections during learning. Additionally, these results suggest the repetition of experiment 1 with BDNF-Thr122 prodomain complexes as oppose to Thr122 alone to determine its effect on spines.

Introduction

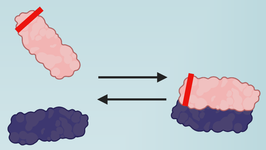
BDNF is synsthesized as proBDNF and the prodomain is cleaved of the mature one.

Fig.1

a)



b)

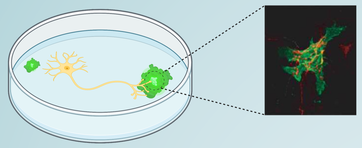


The BDNF precursor-proBDNF (Fig.1a) consists of a prodomain and matureBDNF. A mutation was described near the cleavage site (marked in red). After the cleavage, the prodomain can act on its own or form a complex with mBDNF and interfere with its function. The role of the mutation that leads to change of Methionine to Threonine at the position 122 of the prodomain is is currently unknown.

Experimental Design and Findings (2)

Thr 122 prodomain prevents BDNF induced synapse formation

Fig.4



We know that BDNF is a synaptogenic molecule. In this experiment, we used the hemisynapse (artificial synapse) assay (Fig.4) to test if Thr122 prodomain can interfere with this process. For this experiment we mixed non-neuronal cells expressing synaptogenic molecule Slitrk5 (normally present in the spines and dendrites) that interacts with BDNF receptor TrkB. If put together in culture the non-neuronal cells (presented in green) act as post-synaptic site and the neuronal axon can interact with it, thus forming a synapse. The expression of the synaptogenic molecules in the non-neural cells eg. human embryonic kidney cells (HEK-cells) gives the illusion to the neuron that there is a spine and therefore causes synapsis.

Fig.5

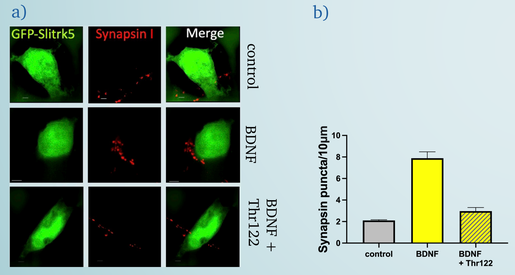
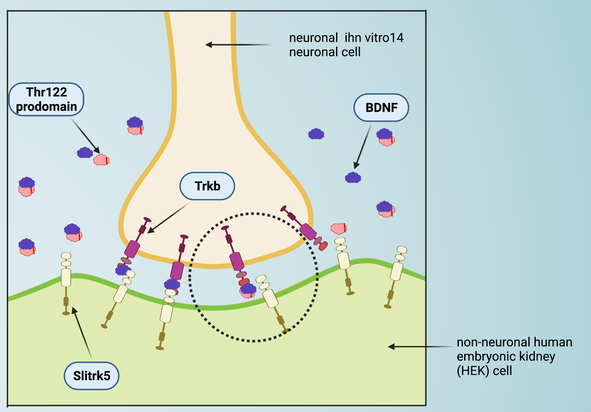


Fig.6



As mentioned previously, after the prodomain has been cleaved off (Fig.1) it has two possible ways of functioning, which is either on its own or in complex with mBDNF(Fig.1b), either of which may lead to interference of BDNF's interaction with the synaptogenic molecules. If we take a closer look at the process of artificially induced synaptogenises itself in our experiment, we can hypothesize that Thr122 prodomain creates a possible barrier for mBDNF to bind to its presynaptic receptor TrkB which induces the binding with Slitrk5. This may lead to a failure in synaptogenesis process.

Conclusion

Our experiments suggests that Thr122 prodomain interferes with the process of synaptic plasticity. The hemisynapse assay is a clear evidence of this failure. It also gives an indication that in this process Thr122 prodomain might be acting by ameliorating the effects of BDNF rather than acting alone. This suggests that we should repeat our Experiment 1 by adding BDNF alone or in complex with Thr122 prodomain to see if BDNF induced spine head expansion critical in learning is affected. Thesa data provides the grounds for further experimental analysis by examining BDNF signaling ion presence of Thr122 prodomain.

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