BDNF Met Prodomain Changes BDNF Signaling

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Abstract

Our memories are stored in spines, which are small protrusions located on the dendritic branches of the neuronal cells. As we learn, these spines become enlarged as the neurons create more efficient synaptic connections. One of the molecules regulating this process is Brain Derived Neurotrophic Factor (BDNF). BDNF induces spine expansion through binding and subsequent internalization of its receptor tropomyosin receptor kinase B(TrkB). BDNF is synthesized in an immature form called Pro-BDNF composed of mature BDNF and a prodomain. After the prodomain is cleaved off, functional BDNF and an independent prodomain are released. Giza et al., 2018 found that the prodomain with human polymorphism, where Valine (Val) at the position 66 is substituted with Methionine (Met) can shrink spines and eliminate synapses in neurons, an effect opposite to that of BDNF. However, Met prodomain is also found in complexes with BDNF. Our preliminary data suggests that the BDNF in complex with Met prodomain loses its ability to enlarge the spine and instead causes spine shrinkage. We are currently examining the mechanism underlying this switch. Here, we show that the presence of the Met prodomain in complex with BDNF prevents its receptor internalization, necessary for spine enlargement. We are proposing a hypothesis that the BDNF-Met complex retains TrkB on the surface and might instead recruit another co-receptor, which redirects TrkB signaling.

Introduction

BDNF and Met prodomain have opposite effects on synaptic plasticity through their action on dendritic spines. BDNF binds with TrkB receptors located on the surface of the spines. This complex gets internalized and activates signaling promoting the enlarged mushroom spines. On the other hand, the Met prodomain itself is responsible for shrinking the spine. We observed that the Met prodomain while in complex with BDNF, inhibits TrkB internalization, necessary for the spine enlargement.

Since spine size dysregulations is the underlying cause of many neuropsychiatric disorders, our results will help point critical therapeutic targets to develop treatments for many neuropsychiatric disorders including Alzheimer's and PTSD.

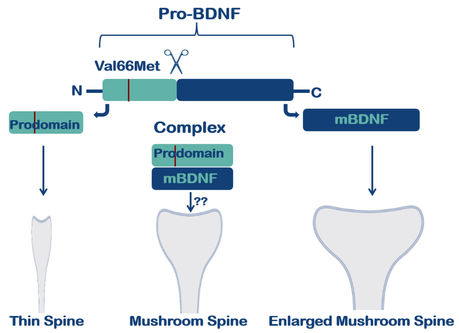

Fig.1 Brain-Derived Neurotrophic Factor (BDNF) is a Growth Factor Regulating Synaptic Plasticity in the Brain

Method

1. Neurons were plated on coverslips allowed to grow for 3 weeks.
2. Prior to the experiment, the Met prodomin and BDNF were combine in the tube to create complexes.
3. This complex was added to neurons on coverslips for 15 minutes.
4. Subsequently, the neurons were fixed
5. Actin Phalloidin 546 was used to visualize the spines.
6. TrkB was stained with antibody against TrkB and secondary antibody conjugated with Fluorescent 488. The staining of TrkB was in the absence of neuronal permeability.
7. Anti-MAP2 antibody was used to visualize the dendrites.

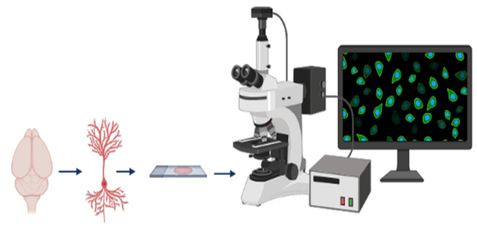

Fig.2 Experimental Design Set-up

Results

3a. BDNF binding to TrkB causes internalization required for BDNF signaling, leading to spine head expansion as previously described.

3b. In the presence of the Met prodomain, TrkB is not internalized.

Dendrites, spines and TrkB receptors were visualized using immunofluorescence. In the figure below, the red color indicates the spines located on the dendrites outlined in white. Green dots represent the TrkB located on the surface. Regular mushroom spines are observed in control image (no treatment) with plenty of TrkB receptors (green dots). These receptors are gone from the surface after internalization when treated with BDNF (very few green dots are visible). Finally, when treated with the BDNF-Met prodomain complex, many TrkB receptors are remaining on the surface similar to the control . This result indicates that the Met prodomain bound to BDNF, inhibits TrkB internalization and signaling. The spine changes are not observed here as the treatment only lasted 15min and the spine changes are observed within one hour of treatment.

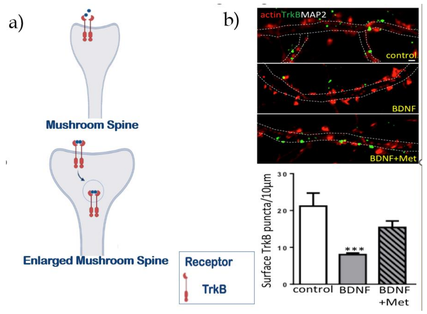

Fig.3 Met Prodomain Binding to BDNF Interferes with BDNF Signaling

Conclusion and Discussion

SorCS2 and p75 are co-receptors necessary for the Met prodomain mediated spine shrinkage. We are proposing a hypothesis that the BDNF-Met complex retains TrkB on the surface and subsequently recruits these co-receptors in order to redirect TrkB signaling.

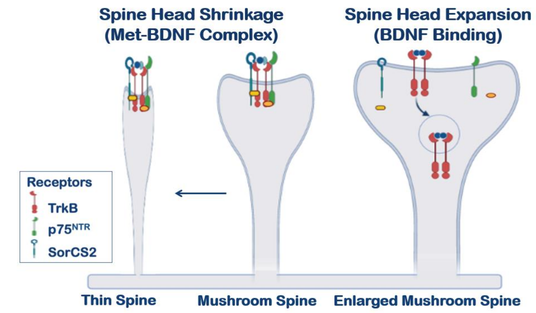

Fig.4 Hypothesis Model of BDNF Signaling in Presence of Met Prodomain

References

Giza et al. Neuron. 2018; https://doi.org/10.1016/j.neuron.2018.05.024

Goncharuk, S.A., Artemieva, L.E., Nadezhdin, K.D. *et al. Sci Rep* **10,** 13686 (2020). https://doi.org/10.1038/s41598-020-70721-8

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