Neurodegenerative Diseases

Neurodegenerative Dis 2012;10:23–26
DOI: 10.1159/000333360

Received: June 30, 2011
Accepted after revision: September 16, 2011
Published online: January 17, 2012

Wnt-5a Is a Synaptogenic Factor with Neuroprotective Properties against Aβ Toxicity

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Key Words

Wnt signaling pathway · Wnt-5a · Synaptic contacts · Miniature excitatory postsynaptic currents · Alzheimer's disease

Abstract

Background: We have recently found that Wnt-5a regulates the synaptic structure and function in hippocampal neurons. This ligand is expressed in the hippocampus, stimulates dendritic spine morphogenesis and increases glutamatergic neurotransmission. Moreover, we have also shown that Wnt-5a induces the clustering of PSD-95. ***Objective:*** To explore the role of Wnt-5a in the formation of synaptic contacts. ***Methods:*** Primary rat hippocampal neurons were exposed to a formylated hexapeptide (Foxy-5) derived from the sequence of Wnt-5a to study synapse formation and function. ***Results:*** In short-term experiments, Wnt-5a only induced the clustering of PSD-95 but had no effect on the density of presynaptic puncta, while in long-term experiments, it induced both pre- and postsynaptic protein clustering and the number of synaptic contacts, in agreement with electrophysiological studies. In long-term experiments, Foxy-5 increased miniature excitatory postsynaptic current amplitude and frequency. ***Conclusion:*** Our findings indicate that Wnt-5a induces synapse formation in hippocampal neurons. In addition, we discuss recent findings indicating a neuroprotective action of Wnt-5a against Aβ neurotoxicity.

Wnt signaling is activated by the interaction of a member of the Wnt family of secreted proteins with the Frizzled (Fz) family of 7 transmembrane cell surface receptors. Different pathways have been described downstream of Fz receptors: the canonical Wnt/β-catenin pathway that leads to stabilization and nuclear accumulation of β-catenin that acts as a transcriptional coactivator of TCF/LEF transcription factors resulting in the expression of Wnt target genes, and the noncanonical ones which involve intracellular signaling by Ca^{2+} (Wnt/Ca^{2+} pathway) and the Jun N-terminal kinase cascade (the Wnt/Jun N-terminal kinase pathway) [1, 2]. In addition to Fz receptors, Ror2 and Ryk have been identified more recently as alternative Wnt receptors [3].

Wnt signaling controls several processes during development such as specification of cell fate, cell proliferation, migration and morphogenesis [2, 3]. In the nervous

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1660–2854/12/0104–0023$38.00/0

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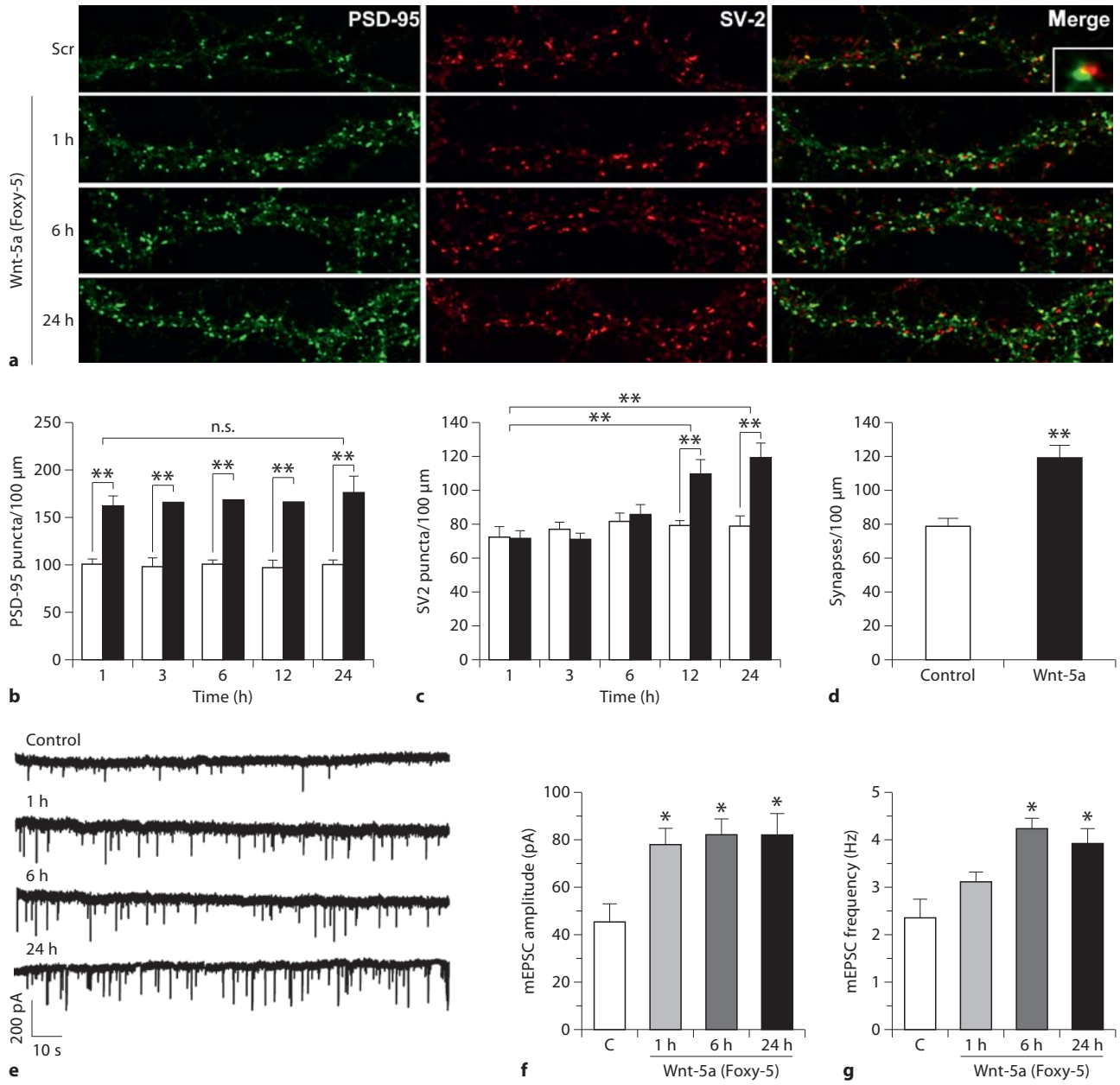

Fig. 1. The formylated hexapeptide Foxy-5 derived from the sequence of Wnt-5a ligand induces synaptic contact density. **a** Hippocampal neurons at 14 days in vitro were incubated with 50 µM Foxy-5 or a control scrambled peptide (Scr) for different periods. PSD-95 and SV2 were detected by immunofluorescence. Scale bar: 10 µm. Quantification of 3 independent experiments of PSD-95 clusters (**b**), SV2 puncta (**c**) and synapse numbers identified by close apposition of both markers (**d**) per neurite length, in neurons treated with 50 µM Foxy-5 (black bars) or Scr (white bars). The number of synapses (**d**) represents values taken at 24 h of treatment. **e** Miniature current traces of whole-cell patch clamp in neurons exposed to Foxy-5 for 1, 3 or 24 h. **f**, **g** Plot of total mEPSC amplitude (**f**) or frequency (**g**) of neurons recorded in **e**. The bars are means ± SE from 12 different cells. * $p < 0.05$; ** $p < 0.01$.

system, it plays key roles in neuronal patterning and differentiation, hippocampal formation, dendritic morphogenesis, axon guidance and synapse formation [3]. Different Wnt ligands have been shown to modulate the presynaptic region [4, 5]. On the other hand, Wnt-5a has shown postsynaptic effects stimulating dendrite spine morphogenesis and synaptic function [6]. In addition, Wnt-5a upregulates NMDA receptor-mediated currents and facilitates induction of long-term potentiation [7]. Recently, a role for Wnt-7a was also determined in dendritic spine morphogenesis through activation of CaMKII [8]. The physiological relevance of the Wnt pathway at the synapse was shown by treatment with Wnt scavengers that decreased spine density, miniature excitatory postsynaptic currents (mEPSC), and the amplitude of field excitatory postsynaptic potentials, supporting that endogenous Wnt signaling plays a relevant role in the normal synaptic structure and function [6].

Our previous findings on Wnt-5a regulating spine morphogenesis and PSD-95 clustering as well as glutamatergic synapses [6, 9] have led us to suggest that Wnt-5a is postsynaptically regulating the synapse; however, an important question remains: is this ligand ultimately involved in synapse formation? To assess this possibility, hippocampal neurons at 14 days in vitro were incubated for different time periods with a formylated hexapeptide (Foxy-5) derived from the sequence of Wnt-5a that in neurons and other systems has shown to mimic the full Wnt-5a molecule action [9, 10]. Treatment with 50 μM Foxy-5 increased the number of PSD-95 clusters compared to control neurons treated with the scrambled version of the hexapeptide (fig. 1a). Previous studies indicate that Wnt-5a induces an increase in PSD-95 clustering starting after 30 min of treatment with a peak effect after 1 h [9]. No significant differences were found after longer exposures (fig. 1b). The number of puncta of the presynaptic protein synaptic vesicle protein 2 (SV2) per neurite length was not increased after 1, 3 and 6 h, indicating a specific postsynaptic effect of Wnt-5a (fig. 1a, c). However, after 12 and 24 h of treatment, a significant increase in the number of SV2 clusters was observed. Indeed, as shown in figure 1d, the number of synaptic contacts significantly increased after 24 h of treatment ($Scr_{1\,h}$: 71.11 ± 5.50, Foxy-$5_{1\,h}$: 71.00 ± 3.55; $Scr_{24\,h}$: 78.39 ± 5.28, Foxy-$5_{24\,h}$: 118.53 ± 8.14, $p < 0.01$). Interestingly, our results suggest that Wnt-5a rapidly increases the number of PSD-95 clusters lacking a presynaptic counterpart that afterwards finds a presynaptic terminal increasing the number of synapses. In agreement with these findings, we determined that neurons incubated for 1 h with 50 μM Foxy-5 showed an increase in mEPSC amplitude (fig. 1e, f), and in neurons treated for 6 and 24 h there was a significant increase in the amplitude and frequency of mEPSC (fig. 1f, g). Immunofluorescence data together with electrophysiological recordings suggest that Foxy-5 rapidly induces synaptic function by modulating preformed synapses. In fact, it was recently shown that Wnt-5a acutely and specifically upregulates synaptic NMDA receptor currents in rat hippocampal slices by increasing the proportion of NR2B containing NMDA receptors at the synapse [7]. In longer treatments, Wnt-5a also increases the number of synaptic contacts and therefore increases the frequency and amplitude of mEPSC.

In addition to its synaptic role, we have studied whether Wnt-5a is able to protect neurons against amyloid-β (Aβ) oligomer synaptotoxicity [11]. Deregulation of the Wnt signaling has been suggested as an etiological cause for Alzheimer's disease [2], which is the most common neurodegenerative disorder, characterized by progressive memory and cognitive impairment and cerebral accumulation of extracellular amyloid plaques composed mainly of Aβ peptide aggregates, and intraneuronal neurofibrillary tangles composed of hyperphosphorylated twisted filaments of the microtubule-associated protein tau [12]. Synaptic pathology is an early event in Alzheimer's disease, and soluble Aβ oligomers are responsible for the synaptic failure that occurs before the plaque deposition and neuronal death [13, 14]. We have recently determined a neuroprotective effect of Wnt-5a against Aβ toxicity [11]. Electrophysiological analysis of Schaffer collaterals-CA1 glutamatergic transmission in hippocampal slices demonstrated that Wnt-5a prevents the decrease in the amplitude of field excitatory postsynaptic potentials and EPSCs, indicating that Wnt-5a prevents the synaptic damage triggered by Aβ oligomers. Moreover, Wnt-5a prevented the decrease in the postsynaptic density scaffold protein PSD-95 and synaptic loss in cultured hippocampal neurons [11]. In conclusion, our findings indicate that Wnt-5a is a synaptic factor that regulates normal brain function as well as improves synaptic function in the presence of Aβ.

Acknowledgements

The Basal Center for Excellence in Science and Technology (PFB12/2007) gave financial support to N.C.I. and the CONICYT Insertion Project to L.V.-N. (79090027).

References

1 Gordon MD, Nusse R: Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem 2006; 281:22429–22433.

2 Inestrosa NC, Arenas E: Emerging roles of Wnts in the adult nervous system. Nat Rev Neurosci 2010;11:77–86.

3 Salinas PC, Zou Y: Wnt signaling in neural circuit assembly. Annu Rev Neurosci 2008; 31:339–358.

4 Hall AC, Lucas FR, Salinas PC: Axonal remodeling and synaptic differentiation in the cerebellum is regulated by Wnt-7a signaling. Cell 2000;100:525–535.

5 Cerpa W, Godoy JA, Alfaro I, Farias GG, Metcalfe MJ, Fuentealba R, Bonansco C, Inestrosa NC: Wnt-7a modulates the synaptic vesicle cycle and synaptic transmission in hippocampal neurons. J Biol Chem 2008; 283:5918–5927.

6 Varela-Nallar L, Alfaro IE, Serrano FG, Parodi J, Inestrosa NC: Wingless-type family member 5a (Wnt-5a) stimulates synaptic differentiation and function of glutamatergic synapses. Proc Natl Acad Sci USA 2010;107: 21164–21169.

7 Cerpa W, Gambrill A, Inestrosa NC, Barria A: Regulation of NMDA-receptor synaptic transmission by Wnt signaling. J Neurosci 2011;31:9466–9471.

8 Ciani L, Boyle KA, Dickins E, Sahores M, Anane D, Lopes DM, Gibb AJ, Salinas PC: Wnt7a signaling promotes dendritic spine growth and synaptic strength through Ca^{2+}/Calmodulin-dependent protein kinase II. Proc Natl Acad Sci USA 2011;108:10732–10737.

9 Farias GG, Alfaro IE, Cerpa W, Grabowski CP, Godoy JA, Bonansco C, Inestrosa NC: Wnt-5a/JNK signaling promotes the clustering of PSD-95 in hippocampal neurons. J Biol Chem 2009;284:15857–15866.

10 Safholm A, Leandersson K, Dejmek J, Nielsen CK, Villoutreix BO, Andersson T: A formylated hexapeptide ligand mimics the ability of Wnt-5a to impair migration of human breast epithelial cells. J Biol Chem 2006; 281:2740–2749.

11 Cerpa W, Farias GG, Godoy JA, Fuenzalida M, Bonansco C, Inestrosa NC: Wnt-5a occludes Abeta oligomer-induced depression of glutamatergic transmission in hippocampal neurons. Mol Neurodegener 2010;5:3.

12 Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E: Alzheimer's disease. Lancet 2011;377:1019–1031.

13 Selkoe DJ: Alzheimer's disease is a synaptic failure. Science 2002;298:789–791.

14 Cerpa W, Dinamarca MC, Inestrosa NC: Structure-function implications in Alzheimer's disease: effect of Abeta oligomers at central synapses. Curr Alzheimer Res 2008; 5:233–243.