Research report

Chronic infusion of Wnt7a, Wnt5a and Dkk-1 in the adult hippocampus induces structural synaptic changes and modifies anxiety and memory performance

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ABSTRACT

Wnt signaling plays an important role in the adult brain function and its dysregulation has been implicated in some neurodegenerative pathways. Despite the functional role of the Wnt signaling in adult neural circuits, there is currently no evidence regarding the relationships between exogenously Wnt signaling activation or inhibition and hippocampal structural changes in vivo. Thus, we analyzed the effect of the chronic infusion of Wnt agonists, Wnt7a and Wnt5a, and antagonist, Dkk-1, on different markers of plasticity such as neuronal MAP-2, Tau, synapse number and morphology, and behavioral changes. We observed that Wnt7a and Wnt5a increased the number of perforated synapses and the content of pre- and postsynaptic proteins associated with synapse assembly compared to control and Dkk-1 infusion. These two Wnt agonists also reduced anxiety-like behavior. Conversely, the canonical antagonist, Dkk-1, increased anxiety and inhibited spatial memory recall. Therefore, the present study elucidates the potential participation of Wnt signaling in the remodeling of hippocampal circuits underlying plasticity events in vivo, and provides evidence of the potential benefits of Wnt agonist infusion for the treatment of some neurodegenerative conditions.

1. Introduction

The Wnt pathway plays a role in brain embryonic developmental processes, such as neuronal maturation, migration, synaptic formation, and establishment of neuronal connectivity (Lee et al., 2000; Machon et al., 2003; Salinas 2005; Inestrosa and Varela-Nallar, 2015). Growing evidence has revealed the function of the Wnt pathway in the mature brain, where it is associated with modulation of axonal remodeling, dendrite outgrowth and maintenance, synaptic activity, neurogenesis and behavioral plasticity (Chen et al., 2017; Inestrosa and Arenas, 2010; Oliva et al., 2013a,b; Ortiz-Matamoros et al., 2013). Cellular mechanisms that control structural changes, such as cytoskeletal remodeling, are involved in the abovementioned events (Gogolla et al., 2007). The mature brain undergoes continuous morphological adjustments in response to internal and external stimuli by rearranging synaptic contacts and reorganizing neuronal networks. Different signals are involved in these structural changes, but there is little information about the ability of Wnt signaling to regulate structural plasticity in the adult brain.

Different Wnt ligands are expressed in the postnatal brain (Shimogori et al., 2004), and these ligands are released in a constitutive- and activity-dependent manner (Chen et al., 2006; Oliva and Inestrosa, 2015). Cumulative evidence has emphasized the involvement of Wnt receptors and ligands to regulate neuronal circuit assembly at pre- and postsynaptic levels. Among the different Wnt ligands, Wnt7a increases the clustering of presynaptic proteins, and promotes synaptic vesicle release and recycling at the presynaptic terminal, in cultured hippocampal neurons (Cerpa et al., 2008; Varela-Nallar et al., 2012), facilitates a JNK-dependent increase in postsynaptic PSD-95 clustering (Farias et al., 2009), and plays a role in the maintenance of dendritic architecture in the adult hippocampus (Chen et al., 2017).

These changes at the pre and postsynaptic levels appear to impact cognition, and recent evidence indicates the participation of the Wnt pathway in experience-mediated synaptic remodeling (Gogolla et al., 2009) and memory regulation, both of which involve Wnt7a and Wnt5a
signaling (Chen et al., 2017; Tabatabze et al., 2012). Infusion of the Wnt/β-catenin antagonist Dkk-1 into the amygdala of male rats prior to training impairs fear memory consolidation without affecting learning (Maguschak and Ressler 2011), and a single dose of Dkk-1 into the hippocampus impairs memory consolidation for both object recognition memory and fear conditioning (Fortress et al., 2013; Xu et al., 2015). Infusion of the Wnt agonists WASP-1 and FOXY-5 enhances basal synaptic transmission and reverses memory impairment in a mouse model of Alzheimer’s disease (Vargas et al., 2014a,b). Moreover, inducible expression of Dkk-1 produces hippocampal synapse loss and memory deficits (Marzo et al., 2016). On the other hand, cumulative evidence has proposed that some Wnt agonists that activate both canonical and non-canonical pathways can be beneficial for treating diseases such as Frontotemporal Dementia (Rosen et al., 2011) and Alzheimer’s disease (Inestrosa and Toledo, 2008; Toledo et al., 2008; Vargas et al., 2014a,b).

Despite the functional role of the Wnt signaling pathway in adult neural circuits and its implication for neuroprotection, there is currently no evidence of the relationship between Wnt signaling modulation and hippocampal structural changes in vivo after chronic exposure, which may have implications for therapeutically targeting this pathway in neurodegenerative conditions. Thus, we aimed to analyze the impact of the chronic infusion of Wnt agonists, Wnt7a and Wnt5a, and an antagonist, Dkk-1, on neuronal cytoskeletal proteins, synapse-associated assembly proteins, synapse number and morphology, as well as the connection between these changes and the performance in hippocampal-associated behaviors.

2. Material and methods

2.1. Neuroblastoma cell cultures

To verify the infusion system and the functionality of the recombinant proteins, the human neuroblastoma MSN cell line was used (Reynolds et al., 1986), seeded in culture dishes with coverslips and maintained under an atmosphere of 5% CO2/95% O2 at 37°C. For preservation and growth, the cells were incubated in neurobasal culture medium RPMI 1640 (GIBCO, Life Technologies, Grand Island, NY, USA), supplemented with nonessential amino acids and 10% fetal calf serum. After 24 h, MSN cells were differentiated by the addition of retinoic acid (10 μM) and nerve growth factor (NGF) (50 ng/mL) in order to induce a mature neuronal phenotype. Three days after differentiation started, cells were treated with phosphate buffered saline (PBS), Wnt7a (600 ng, Wnt signaling agonist), Wnt5a (600 ng, Wnt signaling agonist), or Dkk-1 (200 ng, Wnt signaling antagonist). The recombinant proteins were added to the cultures through mini-osmotic pumps (Fig. 1). To verify the activity of Wnt proteins after 11 days of treatment, Wnt ligands were infused to cultures through the mini-osmotic pumps that were recovered at the end of the in vivo experiments (Fig. 1 Supplementary). After four days of exposure, morphological changes were determined by immunofluorescence staining of cytoskeletal proteins. For immunocytochemistry assays, the cells were fixed with 4% paraformaldehyde (PFA) dissolved in 0.1 M phosphate buffer (PB) for 10 min. Then, were washed four times (5 min each) with cold 0.1 M PB/0.3% Triton X-100, permeabilized with 0.1 M PB/0.3% Triton X-100 for 10 min at room temperature and incubated with blocking solution (0.1 M PB/0.3% Triton X-100 and 5% bovine serum albumin (BSA)) for 24 h at 4°C. The cells were then incubated with primary antibodies against α-tubulin (1:500; monoclonal antibody, mouse anti-α-tubulin, Sigma-Aldrich, St. Louis, MO, USA) overnight at 4°C. Next, they were incubated with a secondary antibody coupled to Alexa Fluor 555 (1:700, donkey anti-mouse, Invitrogen, Carlsbad, CA, USA) for 2 h at room temperature. For F-actin staining, cells were incubated with phalloidin (1:50, Alexa Fluor 488 phalloidin, Invitrogen, Eugene, OR, USA). Coverslips with the cells were mounted on silanized slides using Dako fluorescent mounting medium (North America, Inc, Carpinteria, CA, USA). High-resolution images were captured on a confocal microscope (Nikon A1R 70).  

2.2. Implantation of Alzet mini-osmotic pumps

Male Wistar rats (250–300 g) were used throughout the study and handled with all precautions necessary to avoid their suffering in agreement with the ARRIVE guidelines and Regulations for Research in Health Matters (México), with the approval of the local Animal Care Committee. The animals were anesthetized with 1-2% isoflurane in a 95% O2/5% CO2 mixture and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the nose bar positioned at −0.3 mm. Alzet mini-osmotic pumps (Model 2004, Durect Corp., Cupertino, CA) were implanted subcutaneous in the dorsal region with bilateral cannulation into the CA1 region of the hippocampus (from Bregma = AP −3.6, L −3.1 and V +2.0; Paxinos and Watson 1998). The pump was connected via plastic tubing to a 3.5 mm long cannula made with sili-cate capillaries that delivered a continuous flow of 0.25 μL/h for approximately 11 days, releasing either Wnt7a, Wnt5a (300 ng for each hippocampus), or Dkk-1 (200 ng). All compounds were dissolved in phosphate buffered saline (PBS). At the end of the surgery, the skin incision was sutured, and the rat was placed in an acrylic cage. The rats were allowed to recover for 6 days before the behavioral tests.

2.3. Behavioral tests

2.3.1. Open field

Spontaneous motor activity and anxiety were evaluated after the third session of handling and habituation (ninth day post-surgery) in an open field arena. An acrylic area with 80 cm long x 80 cm wide x 30 cm high black floor and walls, which was divided, with white lines, into sixteen squares of 20 cm x 20 cm each was used. For the open field test, subjects were placed individually at the center of the arena. The locomotion of the animals was recorded for 5 min, and the number of crossings to the center and to the corners of the arena were quantified.

2.3.2. Object location memory test (OLM)

This behavioral paradigm is a spatial memory task modulated by the hippocampus that takes advantage of the natural tendency of rodents to explore novelty (Vogel-Ciernia and Wood, 2014). The task consists of three stages: a session of habituation to the empty training arena; an acquisition phase to explore two identical objects; and a testing phase of exploration, previous displacement of one of the objects to a different location.

2.3.3. Apparatus and objects

For the OLM, a black acrylic arena (60 cm long x 60 cm wide x 30 cm high) with a visual clue on the back wall (a strip of paper with vertical white and black lines) was used. The objects were green glass bottles (12 cm high) filled with white cement to make them heavy and therefore preventing their displacement.

2.3.4. Behavioral test

The rats received 10 min handling and 10 min habituation to the apparatus during three consecutive days. On the tenth day, the rats underwent the acquisition phase. The subjects were individually introduced to the south side of the arena with two identical objects placed in opposite corners (25 cm from each corner), and were allowed to explore for 20 min. Subsequently, rats were removed from the arena and placed in their cages. Long-term memory was tested 24 h later, when one object was displaced to a novel location, and the rats were allowed to explore freely for 10 min. The time spent exploring each object was quantified. The discrimination index of OLM was calculated dividing the time spent exploring the new location by the sum of the total time spent exploring both objects, multiplied by 100.