Chapter 21

Neuronal Transcytosis of WGA Conjugated Protein: A New Approach to Amyloid-β In Vivo

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ABSTRACT

Neuronal transcytosis was observed at the stage when no neurotransmitter was released after the injection of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP; WGA = 22 kDa, HRP = 40 kDa) into the vagus nerve. The co-injection of Rab3A-siRNA with WGA-HRP into the vagus nerve was performed to further examine this phenomenon. This co-injection resulted in the transcytosis of WGA-HRP, both of the passing type, by which it crossed the synapses, and of the secretion type followed by endocytosis of postsynaptic membranes. These findings raised the possibility in vivo that WGA plays an important role in the transcytosis of protein. Therefore, WGA may be a valuable tool for therapeutic drug targeting via transcytosis. The ability of WGA-conjugated Amyloid β (WGA-Aβ) to decrease amyloid deposits in Alzheimer’s disease was investigated. The conjugation of WGA to amyloid-β (1-40) (Aβ; 5 kDa) was confirmed. WGA-Aβ was then shown to move to terminals by axonal flow in vivo as well as WGA-HRP. WGA-Aβ was also observed in the nodose ganglion cells and terminals after injections of fluorescent Aβ (FAB) into the vagus nerve and fluorescent WGA (FWGA) into the common carotid artery. These studies suggested that WGA-Aβ could be localized to solitary neurons via transcytosis.

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INTRODUCTION

A biological analysis of functional implications at the cellular and molecular levels is useful for understanding normal and pathological brain conditions. In the nervous system, axonal transport and synaptic transmission are exclusively essential for brain function. Many studies commonly used wheat germ agglutinin (WGA)-conjugated horseradish peroxidase (HRP) as a neuronal tracer. Recently, WGA-HRP has been indicated to undergo non-vesicular synaptic transport at the stage when no neurotransmitter was released (Takeuchi, 2009) in contrast to vesicular synaptic transport (von Bartheld, 2004). These findings seem to be based on the specificity of protein conjugation to WGA (Kaji, 2006). Therefore, the present study was performed to investigate whether WGA conjugates amyloid-β (Aβ) and whether its conjugation undergoes axonal flow or transcytosis in vivo, as a unique method for the treatment of Alzheimer’s disease.

MATERIALS AND METHODS

Male Wistar rats weighing 180-236 g were anesthetized with intraperitoneal injection of chloral hydrate (490 mg/kg) for all surgical procedures. The experimental procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The Kagawa University Animal Care and Use Committee approved the procedures, and all efforts were made to minimize the number of animals used and their suffering.

1) Experiments of WGA-HRP

A 4% solution of WGA-HRP (0.4-2.0 μl) or a working solution of 1 nM Rab3A-siRNA containing 4% WGA-HRP was injected into the vagus nerve on one side using a 10-μl Hamilton microsyringe (Reno, Nevada, U.S.A.). After a survival period of 12-72 h, the animals were sacrificed by perfusion with 0.1 M phosphate buffer (pH 7.4) followed by a fixative of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer. The blocks containing the nucleus of the solitary tract (NST) and dorsal motor nucleus (DMV) of the vagus nerve were processed for visualization of HRP-reaction products (RP) according to the heavy metal-intensified DAB methods. The blocks were then postfixed in buffered 1% osmium tetroxide for 2 h, block-stained in saturated uranyl acetate for 1 h, dehydrated in a graded acetone or an alcohol series and embedded in an epoxy resin mixture. The NST region was identified by the examination of toluidine blue-stained or unstained 1-μm-thick sections. Ultrathin sections of the region were cut and observed without further lead staining using a JEM 200 CX electron microscope.

2) Experiments of WGA-Aβ

A solution of fluorescent (F) WGA (1.5-2.0 μl) (Alexa 594-conjugated WGA; Invitrogen, USA), FAβ (1.5-2.0 μl) (Alexa 488-conjugated Aβ; Invitrogen, USA) or FWGA containing FAβ was injected into the vagus nerve on one side using a Hamilton microsyringe fitted with a 33-gauge needle in a volume of 5 μL. Further experiments involved the injection of FAβ into the vagus nerve and FWGA into the common carotid artery. For tissue cryosection analysis, the brain stem and nodose ganglions of the animals were processed with 0.1 M phosphate buffer perfusion and a 4% PFA fixative solution, dehydrated in a solution of graded sucrose in 0.1 M phosphate buffer and embedded in an O.C.T. compound. The sliced NST region and nodose ganglions were placed onto slide glasses and processed for nuclear counter staining with 50 ng/ml Hoechst 33258 (Sigma-Aldrich) for 10 min. All slides were mounted in fluorescence mounting medium (DAKO); the localization of molecules was then detected by an epi-illumination fluorescence microscope (DP-72, Olympus) or a confocal laser scanning microscope (Radiance...