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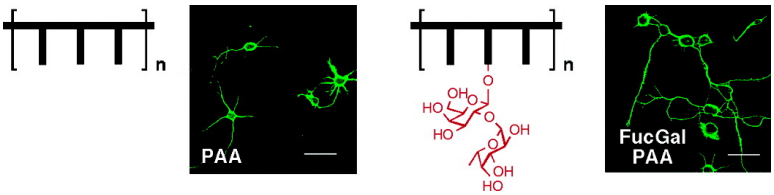
J. Am. Chem. Soc., **2005**, 127 (5), 1340-1341• DOI: 10.1021/ja044631v • Publication Date (Web): 15 January 2005



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Published on Web 01/15/2005

A Role for Fucose α(1−2) Galactose Carbohydrates in Neuronal Growth

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Fucose α(1−2) galactose carbohydrates have been implicated in neuronal communication that underlies long-term memory storage. For instance, 2-deoxy-D-galactose (2-dGal), which incorporates into glycan chains and precludes the formation of Fucα-(1−2)Gal termini,[1] has been reported to cause reversible amnesia in animals and prevent the maintenance of long-term potentiation (LTP).[2] Furthermore, administration of L-fucose or 2′-fucosyllactose increased memory consolidation in animals and enhanced LTP.[3] Despite these intriguing observations, the molecular mechanisms by which Fucα(1−2)Gal carbohydrates influence neuronal communication are not well understood. Here we use chemical approaches to understand the biological effects of Fucα(1−2)Gal on neurons. Our studies provide the first demonstration that Fucα(1−2)Gal and its associated proteins promote the outgrowth of hippocampal neurons, and they identify a novel, carbohydrate-mediated pathway for the modulation of neuronal growth and morphology.

We hypothesized that the Fucα(1−2)Gal moiety might be interacting with a lectin receptor in the brain. As no such proteins have been reported, we designed and synthesized compound **1** to probe for the presence of potential lectin receptors in neurons (Figure 1). Embryonic hippocampal neurons were treated for 1 h with **1** or a biotin control. To prevent intracellular uptake of the compounds, neurons were co-incubated with the endocytosis inhibitor phenylarsine oxide (PAO).[4] Following fixation and staining with streptavidin conjugated to AlexaFluor 488 dye, cells were imaged by confocal fluorescence microscopy. As shown in Figure 1, probe **1** binds to the cell body and neurite processes. Lipid extraction[5] of cellular membranes using MeOH/$CHCl_3$ prior to treatment with **1** did not diminish the labeling (Supporting Information), consistent with a carbohydrate−protein interaction.

We next investigated whether the association of Fucα(1−2)Gal with potential lectins would stimulate a neuronal response. As carbohydrates have weak binding affinities for lectins (K_{assoc}=10^3−10^6 M),[6] we used polyacrylamide polymers bearing multiple Fucα(1−2)Gal epitopes (FucGal-PAA; MW ≈ 30 kDa, 20% disaccharide density) to enhance the interaction. Remarkably, neurons treated with FucGal-PAA for 24 h exhibited striking changes in cellular morphology: the length of the major neurite extension was increased by 50 ± 6% relative to the untreated control (Figure 2A). A polymer lacking the disaccharide (PAA) had no significant effect, indicating that Fucα(1−2)Gal was responsible for the growth-inducing activity.

We examined the specificity of the effects by treating neurons with polymers bearing different carbohydrate epitopes. Polymers containing *N*-acetylglucosamine (GlcNAc-PAA) or D-galactose (Gal-PAA) failed to promote neuronal outgrowth. Interestingly, other L-fucose-bearing polymers, such as L-fucose PAA (Fuc-PAA) and Fucα(1−3)GlcNAc PAA (FucGlcNAc-PAA), displayed neuronal processes similar to those of untreated cells, suggesting that the galactose moiety of Fucα(1−2)Gal is an important contributor to lectin binding and/or activation. Together, these findings

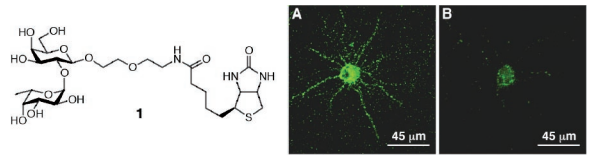

Figure 1. Fucα(1−2)Gal−biotin probe **1** binds to embryonic day 18 hippocampal neurons. Immunofluorescence images of neurons (23 days *in vitro*) after treatment with 3 mM of (A) **1** or (B) biotin in the presence of 10 μM PAO. See Supporting Information for the synthesis of **1** and conditions.

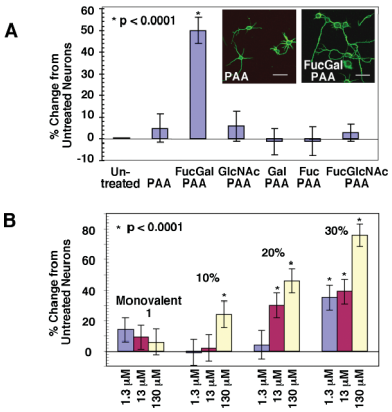

Figure 2. (A) Fucα(1−2)Gal promotes neuronal growth. Neurite outgrowth was quantified by measuring the longest neurite per cell after treatment with 130 μM of the indicated compounds. Error bars represent SEM from 100 total neurons in two separate experiments. (B) Neurite outgrowth activity increases with the Fucα(1−2)Gal concentration and polymer valency. Disaccharide residue concentrations are reported. *p* values are relative to the untreated neurons.

demonstrate that the observed neuritogenic activity is specific for Fucα(1−2)Gal.

To test whether the cellular response was dependent on the concentration of disaccharide, polymers of different Fucα(1−2)-Gal density (10, 20, and 30%) were examined at various concentrations. The growth-promoting activity of each polymer increased as the concentration of disaccharide increased (Figure 2B). In addition, the potency of the compounds was dramatically enhanced with increasing carbohydrate valency, with the 30% density polymer stimulating growth by 76 ± 8%. In contrast, monovalent ligand **1** showed only modest activity even though it was capable of binding to neurons. These results suggest that the multivalent PAA scaffold promotes the interaction of Fucα(1−2)Gal with lectin receptors and may facilitate their assembly into higher-order complexes. The ability of multivalent ligands to enhance binding affinity and cluster lectins has been observed in other systems.[7]

The presence of potential lectins specific for Fucα(1−2)Gal implies the existence of glycoproteins covalently modified by the disaccharide epitope. To examine whether such glycoproteins are present in hippocampal neurons, we treated cells with *Ulex*

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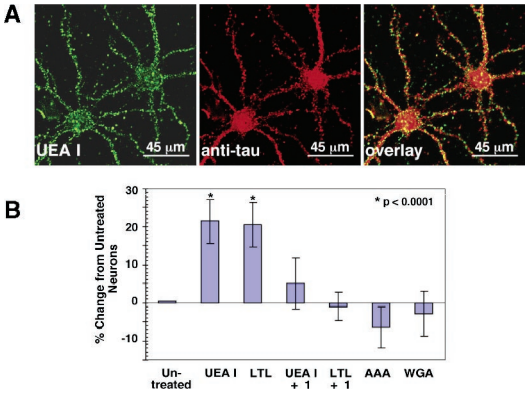

Figure 3. (A) Co-staining of hippocampal neurons (23 days *in vitro*) with UEA I lectin (green) and an anti-tau antibody (red) in the presence of 10 μM PAO. Lipid extraction using MeOH/$CHCl_3$ did not diminish the labeling (Supporting Information). (B) Only Fucα(1−2)Gal-selective lectins stimulate neurite outgrowth. Quantitative analysis of neurite length after treatment with the indicated lectins (3.7 μM). Error bars represent SEM from 100 total neurons in two separate experiments.

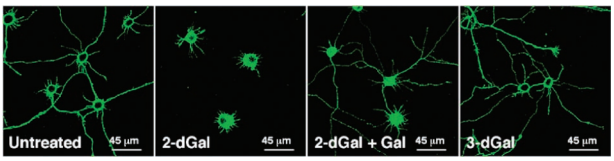

Figure 4. Treatment of neurons with 2-dGal (15 mM), but not 3-dGal (15 mM), for 2 days inhibits neuronal growth. The effects of 2-dGal are rescued by subsequent treatment with Gal (75 mM) for an additional 2 days. Neurons were immunostained with an anti-tau antibody. See Supporting Information for additional concentrations of 2-dGal.

europeaus agglutinin I lectin (UEA I) conjugated to fluorescein. UEA I has been used previously to detect Fucα(1−2)Gal glycoproteins in cells and tissues.[8] As shown in Figure 3A, Fucα-(1−2)Gal glycoproteins are indeed present and display a punctate staining consistent with localization to synapses.

We next used exogenous lectins to probe whether the Fucα(1−2)Gal glycoproteins are associated with neuronal growth pathways. Previous studies have shown that lectins promote the clustering of glycoproteins at the cell surface.[9] Treatment of hippocampal neurons with the Fucα(1−2)Gal-specific lectins UEA I or *Lotus tetragonolobus* (LTL)[8] stimulated neurite outgrowth by 21 ± 6% and 20 ± 6%, respectively, relative to the untreated control (Figure 3B). As expected, competition with 400-fold excess **1** abolished the effects of UEA I and LTL. Moreover, lectins selective for GlcNAc (wheat germ agglutinin, WGA) and Fucα(1−3)Gal (*Anguilla anguilla* agglutinin, AAA)[8] failed to enhance neurite outgrowth, indicating that the growth-promoting activity is specific for Fucα(1−2)Gal carbohydrates.

Finally, we investigated the effects of the amnesic compound 2-dGal on neuronal morphology. Consistent with earlier studies,[1] treatment of neurons with 2-dGal disrupted synthesis of the Fucα-(1−2)Gal epitope on glycoproteins (Supporting Information). Interestingly, the neurons also exhibited severely stunted neurites and failed to form synapses (Figure 4). The effects were fully reversible: subsequent addition of D-galactose led to regeneration of the neuronal processes. In contrast, 3-deoxy-D-galactose (3-dGal) had no impact on neurite outgrowth. These results are consistent with a stimulatory role for Fucα(1−2)Gal glycoproteins and highlight the striking influence of Fucα(1−2)Gal carbohydrates on neuronal growth.

Together, our studies demonstrate that Fucα(1−2)Gal carbohydrates are capable of modulating neuronal outgrowth and morphology. We provide strong evidence for the presence of Fucα(1−2)Gal glycoproteins and lectin receptors in hippocampal neurons. Furthermore, we show that manipulation of Fucα(1−2)Gal-associated proteins using small-molecule and lectin probes induces dramatic changes in neuronal morphology. This represents a new carbohydrate-mediated pathway for the regulation of neuronal cell growth.

Our findings may also shed light on behavioral and electrophysiological studies implicating Fucα(1−2)Gal in long-term memory storage. Alterations in neuronal morphology, such as dynamic changes in dendritic spine number and shape, occur during memory consolidation and LTP.[10] One possibility is that Fucα-(1−2)Gal and its associated proteins are involved in structural remodeling events that contribute to synaptic plasticity. Efforts are currently underway to identify Fucα(1−2)Gal-associated proteins using affinity-based and genomics tools. Finally, this work illustrates the potential of small molecules to modulate processes such as neuronal growth and regeneration.

Acknowledgment. We thank the Biological Imaging Center at Caltech for instrumentation. This research was supported by an NIH Training Grant (T32 GM08501), NIH (RO1 NS045061), and the Alfred P. Sloan Foundation.

Supporting Information Available: Syntheses and complete experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA044631V