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BRAIN RESEARCH

Research Report

Polysialic acid limits septal neurite outgrowth on laminin

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ABSTRACT

Polysialic acid (PSA) is a large carbohydrate found exclusively on the neural cell adhesion molecule (NCAM). In the adult brain, PSA is re-expressed by septal axons sprouting and regenerating in an environment rich in laminin. Using an *in vitro* model, we tested the possibility that PSA limits septal outgrowth by preventing maximal interactions with a laminin substrate. Our results indicate that PSA removal from primary septal neurons plated on laminin significantly increased neurite outgrowth at 12 h (14%, $p<0.05$) and 24 h (22%, $p<0.01$). In contrast, the removal of PSA had no impact on septal neurite outgrowth on poly-D-lysine. PSA did not influence the plating adhesion of septal neurons on laminin or poly-D-lysine, indicating that the increase in neurite outgrowth caused by PSA removal on laminin is not related to the initial attachment of the neurons to this substrate. Neurite length on laminin was significantly reduced by the function-blocking β1-integrin antibody in the presence of PSA (20% decrease, $p<0.05$), and following PSA removal (34% decrease compared to neurites treated with endoN and without the β1-integrin antibody, $p<0.01$). Importantly, the β1-integrin antibody completely abolished the neurite outgrowth promoting effect of PSA removal on laminin. The β1-integrin antibody had no impact on septal neurite length on poly-D-lysine. Taken together, these results indicate that the removal of PSA from septal neurons increases neurite outgrowth on laminin by promoting interactions between β1-integrin and laminin.

1. Introduction

Polysialic acid (PSA) is a homopolymer of α2,8-linked 5-N-acetylneuraminic acid, present exclusively on the neural cell adhesion molecule (NCAM) (Maness and Schachner, 2007; Rutishauser, 1998). PSA is abundant in the developing brain and it is limited to areas of neural plasticity in adulthood (Seki, 2003; Seki and Arai, 1991).

In vivo, the presence of PSA is crucial to brain development (Weinhold et al., 2005) and for the regeneration of

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Abbreviations: PSA, polysialic acid; NCAM, neural cell adhesion molecule; endoN, endoneuraminidase N; GAP43, growth associated protein 43

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peripheral axons (Franz et al., 2005). Following a lesion of the fimbria-fornix in the adult brain, PSA is abundantly expressed by damaged and regenerating septohippocampal axons, and at the injury site where reactive gliosis occurs (Aubert et al., 1998). It is unknown whether PSA is acting to facilitate or limit the regrowth of septohippocampal axons after injury. The presence of several permissive substrates, including laminin, at the zone of reactive gliosis is not sufficient to induce the regeneration of PSA-positive septohippocampal axons (Aubert et al., 1998; Kawaja et al., 1992). In these cases, the ability of laminin to serve as a supportive substrate may depend on its availability to regrowing neurons (Tardy, 2002). Thus, it is possible that PSA's shielding mode of action (Rutishauser, 1998) limits interactions between regenerating neurites and laminin thereby restricting optimal regeneration of septohippocampal axons following a fimbria-fornix lesion.

The biophysical properties of PSA have been described to cause steric hindrance and shield ligand–receptor interactions (Fujimoto et al., 2001; Johnson et al., 2005; Rutishauser, 1998). *In vitro*, PSA can promote or limit neurite outgrowth, depending on the environment (Acheson et al., 1991; Doherty et al., 1990; Hrynkow et al., 1998; Seidenfaden et al., 2006; Zhang et al., 1992).

We postulated that PSA restricts interactions between β1-integrin on septal neurons and a laminin substrate. We compared the impact of PSA removal on septal neurite outgrowth in the presence of either laminin or poly-D-lysine. Our findings indicate that the removal of PSA from septal neurons significantly increased neurite outgrowth on laminin. This effect was abolished by the use of β1-integrin function-blocking antibodies, suggesting that PSA prevents β1-integrin interactions with laminin.

2. Results

2.1. *Endoneuraminidase N removes PSA from septal neurons without affecting their survival*

In normal conditions, PSA is expressed by all embryonic septal neurons *in vitro* and it is efficiently removed by endoneuraminidase N (endoN), as previously established in these neuronal cultures (Burgess and Aubert, 2006). EndoN is a bacteriophage enzyme which specifically cleaves PSA from NCAM (Hallenbeck et al., 1987; Rutishauser et al., 1985). Here, the complete removal of PSA by endoN is illustrated by western blotting after 24 h in culture, the longest time point of the present study (Fig. 1A). Prior to neurite length analysis, PSA removal was confirmed by immunocytochemistry in each set of experiments (e.g. Fig. 3, and as in Burgess and Aubert, 2006). PSA removal did not significantly alter the total number of septal cells, as presented at 24 h *in vitro* (Table 1).

To evaluate the impact of PSA removal on neurite outgrowth, neurons were labeled for growth associated protein-43 (GAP-43). GAP-43 is abundant throughout the entire neurite length, including the growth cone (Fig 1B). The expression of GAP-43 is similar in control and endoN treated neurons at 12 and 24 h.

2.2. *PSA removal increases neurite outgrowth on laminin but not on poly-D-lysine*

GAP-43 immunoreactive septal cells were selected in a systematic random manner and for each neuron, the length of the longest neurite was measured in normal conditions and in presence of endoN, which removed PSA.

Interestingly, our main finding indicates that the removal of PSA from septal neurons plated on laminin significantly increased neurite outgrowth at 12 h (14%, $^{*}p<0.05$), and 24 h (22%, $^{**}p<0.01$) (Fig. 2A). The effect of PSA removal on neurite length was substrate specific since the same treatment with endoN had no impact on the elongation of neurites on poly-D-lysine (Fig. 2B).

Our results also demonstrate that septal neurites extending on poly-D-lysine or laminin are significantly longer after 24 h in culture compared to 12 h, regardless of the presence of PSA at the cell surface (Fig. 2). The mean neurite length was greater on laminin than on poly-D-lysine at both time points (Fig. 2).

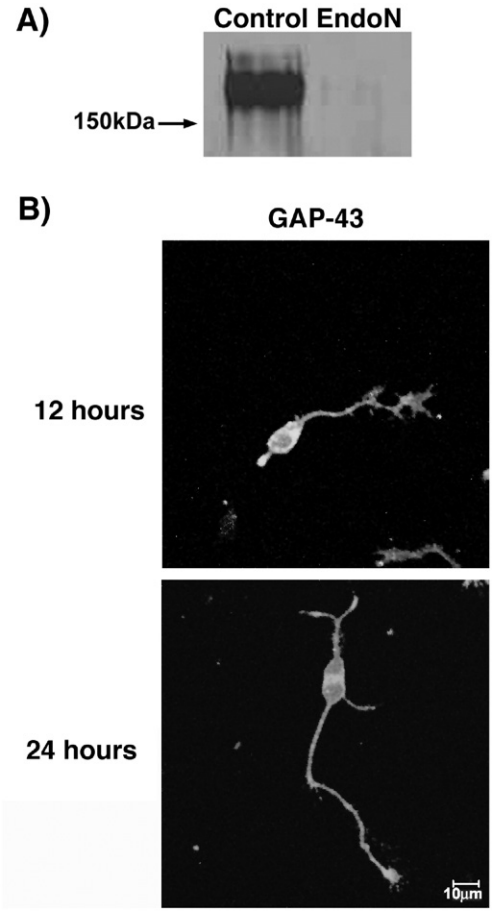

Fig. 1 – (A) Endoneuraminidase N (EndoN) completely removes PSA from the neuronal culture as confirmed by a lack of PSA immunoreactivity in western blotting analysis. (B) Septal neurons expressing growth associated protein-43 (GAP-43) at 12 and 24 h in culture. GAP-43 is used to visualize neurites and measure their length.