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Intraganglionic interactions between satellite cells and adult sensory neurons

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ABSTRACT

Perineuronal satellite cells have an intimate anatomical relationship with sensory neurons that suggests close functional collaboration and mutual support. We examined several facets of this relationship in adult sensory dorsal root ganglia (DRG). Collaboration included the support of process outgrowth by clustering of satellite cells, induction of distal branching behavior by soma signaling, the capacity of satellite cells to respond to distal axon injury of its neighboring neurons, and evidence of direct neuron-satellite cell exchange. In vitro, closely adherent coharvested satellite cells routinely clustered around new outgrowing processes and groups of satellite cells attracted neurite processes. Similar clustering was encountered in the pseudounipolar processes of intact sensory neurons within intact DRG in vivo. While short term exposure of distal growth cones of unselected adult sensory neurons to transient gradients of a PTEN inhibitor had negligible impacts on their behavior, exposure of the soma induced early and substantial growth of their distant neurites and branches, an example of local soma signaling. In turn, satellite cells sensed when distal neuronal axons were injured by enlarging and proliferating. We also observed that satellite cells were capable of internalizing and expressing a neuron fluorochrome label, diamidino yellow, applied remotely to distal injured axons of the neuron and retrogradely transported to dorsal root ganglia sensory neurons. The findings illustrate a robust interaction between intranganglionic neurons and glial cells that involve two way signals, features that may be critical for both regenerative responses and ongoing maintenance.

1. Introduction

The response of sensory neurons to injury involves local changes at the level of the injured axon but also a series of retrograde alterations in parent neuronal perikarya. It has long been held that local satellite cells surrounding neurons within parent ganglia, contribute to the survival and plasticity of the sensory neuron, especially after injury. Perineuronal satellite cells surround, support and possibly protect neurons in sensory ganglia. Features include an intimate association with neurons, scant cytoplasm, a high surface-volume laminar structure and a basement membrane (Pannese, 1981; Pannese et al., 1999). They are thought to secrete growth factors, and may scavenge free radicals (Calcutt et al., 1992; Pannese, 1981; Powell et al., 1991; Zhou et al., 1999). Satellite cells also express p75, the low affinity NGF receptor (Zhou et al., 1996). There is literature to suggest that perineuronal satellite cells have dynamic properties and alter their behavior in response to neuronal changes after injury (Fenzi et al., 2001; Hanani et al., 2002). Satellite cells somehow sense neuron injuries involving axon processes far remote from the ganglia. Thus, a two way interchange between this unique cell population with neurons must exist, but its mechanism and importance are not well understood. No evidence that molecules can be interchanged and internalized between perineuronal satellite cells and primary sensory neurons has been reported.

While the analysis of the behavior of adult sensory neurons in culture is crucial in understanding their responses to injury, most investigations have supposed that harvested neurons are studied in isolation. While many laboratories add steps to remove glial cells from harvested sensory neurons, few routinely stain or analyze what complement of cells remain. Their presence offers an opportunity to examine peripheral neuron-glial interaction.

In this work, we show that adult sensory neurons in vitro cultures capture a population of adherent satellite cells. We identify relationships between clusters of these retained cells and neurite outgrowth. In vivo we show that perineuronal satellite cells sense injury to distal axons and respond by enlarging and by proliferating. Finally we show that sensory neurons can transfer small molecules to their surrounding glial partners. Overall these investigations provide evidence that there is ongoing and bilateral interaction between sensory neurons and perineuronal satellite cells.

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2. Methods

2.1. Animals, surgery

Adult male Sprague–Dawley rats weighing 200–300 g were used for this protocol. To address cell turnover in adult rats after injury we studied ipsilateral L4 and L5 DRGs following sciatic nerve transection (left midsciatic transection and resection of the distal nerve and its branches to prevent regeneration) or sciatic nerve crush (compression of the nerve for 30 s between the jaws of a plastic coated forceps). All interventions were carried out with pentobarbital anesthesia (65 mg/kg ip). Rats were sacrificed at endpoint using an overdose of pentobarbital. The protocol was reviewed and approved by the University of Calgary Animal Care Committee in conjunction with the guidelines of the Canadian Council of Animal Care (CCAC).

2.2. In vitro analysis of adult sensory neurons

Prior to tissue harvesting, rats were anesthetized using isoflurane (Halocarbon Laboratories, River Edge, NJ) then sacrificed. DRG neurons were dissociated and maintained in vitro using a modification from the method of Lindsay (Andersen et al., 2000; Lindsay, 1988). Briefly, L4 and L5 DRGs were dissected from rats and placed into L15 (Invitrogen, Burlington, Ontario) medium where the axon roots and dural tissue were manually removed. The DRGs were rinsed three times in L15 medium and then transferred to a tube containing 2 ml 0.1% collagenase (Invitrogen,Burlington, Ontario)/L15. Following a 90 min incubation at 37 °C, the DRGs were placed into single-cell suspension by triturating 10–15 times every 5 min through three 1 ml pipette tips and then three 200 μl pipette tips. The single-cell suspension was spun for 5 min at 800 rpm at 4–8 °C and the cell pellet was washed three times in 2 ml L15. After the final 5 min 800 rpm spin, the cells were resuspended in L15 and passed through a 70 μm mesh (VWR International Co., Mississauga, Ontario). In an additional procedure to partially remove glial cells in some of the experiments, the cell suspension was loaded onto 15% BSA (Sigma, St. Louis, MO) in L15 and spun at 900 rpm for 10 min and the pellet collected (passaged). The cells were then washed once more with L15 and spun again then placed into a culture medium of Dulbecco's Modified Eagle Medium/F12 (DMEM/F12; Invitrogen, Burlington, Ontario) + 1:100 dilution N2 (Invitrogen, Burlington, Ontario), 0.5–0.8% BSA (Sigma Aldrich, Oakville, Ontario) and 0.2 ng/ml NGF (Cedarlane Laboratories Ltd, Horby, Ontario) plus 50U Penicillin (1 ml), 50 μg Streptomycin (1 ml) (Invitrogen, Burlington, Ontario) and plated onto poly-L-lysine (Sigma Aldrich, Oakville, Ontario) and 10 μg/ml mouse laminin (Invitrogen, Burlington, Ontario) coated plates. Half of the cell medium was changed every 2 days. The cultures involved 6 rats with axotomy and 6 sham injured rats (2 each per culture day) for nonpassaged analysis and similar animal and culture numbers for passaged neurons.

2.3. Soma signaling experiments

The procedures were modifications of a growth cone turning assay modified for adult mammalian DRG neurons from previous embryonic neuron cultures in Xenopus laevis (Lohof et al., 1992; Nishiyama et al., 2003; Zheng et al., 1994) as reported in our laboratory and others (Murray et al., 2012; Webber et al., 2008a) (Guo et al., 2014). Two DRG samples were harvested from 1 rat to generate 6 plates. Briefly, gradients were created and maintained using a Picospritzer II (Parker Hannafin, Fairfield, NJ) ejecting at a pressure of 3 psi, frequency of 2Hz, for 20 milliseconds using a pulse generator (SD9; Grass Instrument Co., Quincy, MA) from a micropipette with a 0.5- to 1 μm opening. The micropipette was positioned 100 μm from soma of the neuron or the growth cone center at an angle of 45° with respect to the last 10 μm segment of the axon shaft. The turning assay was performed on preconditionally lesioned DRG cultures at 1 to 3 DIV. For analysis, the trajectories of the outgrowing neurites were traced onto a graph and we calculated the percentage of total growth cones examined that exhibited a branch (% of growth cones with branches), and the ratio of primary or secondary branch numbers to total growth cones examined (ratio of branches to growth cones [as a %]). Primary branch were classified as having emerged from the main advancing growth cone process and secondary branches as arising from a primary branch. All analyses were carried out 60 min following onset of the continuous picospritzer application.

2.4. Immunocytochemistry and immunohistochemistry

For in vitro studies, cell cultures were fixed in 2% paraformaldehyde for 10 min, washed with PBS, blocked for 30 min in PBS with 5% fetal bovine serum and 0.1% BSA then incubated with primary and secondary antibodies (Table 1).

Tissue samples were fixed in modified Zamboni's fixative (2% paraformaldehyde, 0.5% picric acid and 0.1% phosphate buffer) overnight at 4 °C. Tissues were then washed in PBS 5 times, cryoprotected in 20% sucrose/PBS and left at 4 °C overnight. After embedding in optimum cutting temperature (OCT) compound (Miles), 20 μm thick sections were placed onto poly-L-lysine coated slides. For indirect immunofluorescence, slides were incubated for 48 h at 4 °C with primary antibodies. Slides were then washed with PBS and incubated with secondary antibodies for 1 h at room temperature. After further PBS washing, cover slips were mounted with bicarbonate-buffered glycerol (PH 8.6) or Vectashield Mounting Medium with DAPI (Vector Laboratories,Burlington, Canada) and slides were viewed with a fluorescent microscope (Zeiss, Axioskope, Zeiss Canada, Toronto, Canada). Negative controls included omission of primary antibodies or secondary antibodies on parallel sections.

To examine retrograde neuron labeling, we administered DY (diamidino yellow, Sigma), a fluorochrome retrograde tracer, by transecting the sural nerve distal to a crush zone through a separate incision and immersing the stump into a capsule filled with 1.5 μl of 2.5% DY for 30 min, then gently rinsing with saline. Seven days after crush the L5 DRGs were fixed in Zamboni's fixative and sections made at 14 μm for additional immunohistochemistry. In separate experiments, we administered DY to the site of a sciatic transection, then

Table 1
Antibodies and Primers.

(i) Primary antibodies used
anti-β-Tubulin-III, (1:100), mouse monoclonal, Sigma, St. Louis, Mo
anti-NF200 (neurofilament 200, 1:800), mouse monoclonal, Sigma, St. Louis, Mo
anti-S100 (1:200), mouse monoclonal S100 beta chain, Santa Cruz, Santa Cruz, Ca
anti-BrdU (1:50), monoclonal, Cedarlane, Hornby, Ont
anti-BrdU (1: 200), monoclonal, Sigma, St. Louis, Mo,
anti-NeuN (1: 100), monoclonal, Chemicon International, Temecula, Ca
anti-GFAP (1:250), polyclonal rabbit DakoCytomation, Carpinteria, CA
(ii) Secondary antibodies used
anti-mouse IgG CY3 conjugate (1:100) sheep, Sigma, St. Louis, Mo
anti-goat (1:200) Alexa Fluor 488 donkey, Invitrogen Canada, Burlington, Ont
anti-rabbit IgG (H + L) conjugate (1:400) Alexa Fluor 488 goat Cedarlane, Burlington, Ont
anti-mouse (1:400) Cy3 sheep, Alexis Biochemicals, San Diego, Ca
(iii) Primers used
CNTF R F 5′- TTCTGCCTTTGCCTACCAGCT-3′
CNTF R R 5′-AGACCACCATCTCCAACTGTGG-3′
HGF RM F 5′-AACACAGCTTTTTGCCTTCGAG-3′
HGF RM R 5′-CTGGATTGCTTGTGAAACACCA-3′
Protrudin R F 5′- TGATGAGGCGGAAGTACCACA-3′
Protrudin R R 5′- GGCTTCCAGTCGGATCAAGAA-3′
ErbB2 R F 5′- ACATCTCAGCATGGCCAGACA-3′
ErbB2 R R 5′- TGTCAATGAGTACGCGCCATC-3′
Connexin 43 (GJA1 R) F 5′- AGCACGGCAAGGTGAAAATG-3′
Connexin 43 (GJA1 R) R 5′- TACCACTGGATGAGCAGGAAGG-3′
Connexin 32 (GJB1 R) F 5′-TTTTTCCCCATCTCCCATGTG-3′
Connexin 32 (GJB1 R) R 5′-ATGTGTTGTTGGTGAGCCACG-3′
RPLP0 F 5′- TACCTGCTCAGAACACCGGTCT-3′
RPLP0 R 5′- GCACATCGCTCAGGATTTCAA-3′