

Regulation of the Growth Associated Protein, GAP-43, in Cultured Neurons

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Abstract

Following physical injury to the mammalian central nervous system (CNS), long axon growth is generally unsuccessful. This may be due to low expression of growth associated proteins (GAPs) required for long axon growth, to the physical constraints of the environment, and/or to the presence of growth inhibitory molecules. Dorsal root ganglion (DRG) neurons of the peripheral nervous system (PNS) are able to regenerate their peripheral axons following injury provided that a favorable pathway is kept intact. This regeneration is associated with increased expression of GAPs, which subsequently become down-regulated if target is re-innervated. Central axotomy of DRG neurons has little effect on GAP expression and regenerative growth is poor. These and other observations lead to the hypothesis that in the intact, mature state a peripheral target derived factor represses expression of the GAPs and long axon growth by DRG neurons. In culture these neurons spontaneously extend multiple neurites and up-regulate expression of the growth associated protein GAP-43. Experiments were designed to search for influences which might prevent the up-regulation of GAP-43 and inhibit neurite outgrowth by DRG neurons in culture and may therefore represent target derived repression. Regulation of GAP-43 expression and neurite outgrowth was also investigated using the neuron-like cell line, RN46A, as a CNS neuron model.

Primary myotube cultures and the myogenic cell line L8, were used as target models. Contact with these cells, or exposure to factors derived from them, were demonstrated to repress GAP-43 protein and mRNA expression in DRG cultures. However, an L8-derived factor did not repress GAP-43 expression in RN46A cells. Exposure to myelin fragments, several growth factors (LIF, CNTF, IGF-1, GDNF) or neurotrophins (NGF, BDNF, NT-3, NT-4), were shown not to repress GAP-43 up-regulation. Chronic application of the membrane permeable cAMP analogus dBcAMP or 8-BrcAMP, or induction of endogenous cAMP by cholera toxin or forskolin, repressed GAP-43 protein levels in both DRG and RN46A cultures. No GAP-43 repressive activity was observed by the application of either pertussis toxin or dBcGMP. The adenylate cyclase inhibitor SQ22,536 was

able to reverse the effects of cholera toxin but not the L8 factor on GAP-43 repression, suggesting that the L8 factor operates independently of cAMP. In all situations, changes in GAP-43 levels were not due to variations in neuronal survival.

An L8-derived factor repressed neurite growth by DRG neurons. Unexpectedly, elevated cAMP increased neurite growth by both the DRG neurons and RN46A cells. Neurite morphology analysis indicated that cAMP induced growth entailed an increase in velocity of neurite advancement independently of growth cone initiation or neurite branching. Cyclic-AMP induction of neurite growth was demonstrated to occur very rapidly, and in the presence of the transcription inhibitors actinomycin-D or 5,6-dichlorobenzimidazole riboside. Cyclic-AMP-induced neurite growth is therefore independent of new protein synthesis, including up-regulation of GAP-43.

These results suggest two mechanisms of GAP-43 regulation. A muscle derived factor operating on DRG neurons represses GAP-43 expression at both the protein and mRNA levels, while simultaneously repressing neurite growth. This may be related to repression of Gap-43 and repression long axon growth in DRG neurons in vivo following target innervation, consistent with my initial hypothesis. A second GAP-43 repressing mechanism is mediated by cAMP. Cyclic-AMP also apparently operates locally at the growth cone to accelerate neurite advancement independent of new transcription. Thus, at least one form of axon growth enhancement is not coupled to expression of GAP-43.