

Volume transmission in activity-dependent regulation of myelinating glia

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Abstract

The importance of neural impulse activity in regulating neuronal plasticity is widely appreciated; increasingly, it is becoming apparent that activity-dependent communication between neurons and glia is critical in regulating many aspects of nervous system development and plasticity. This communication takes place not only at the synapse, but also between premyelinating axons and glia, which form myelin in the PNS and CNS. Recent work indicates that neural impulse activity releases ATP and adenosine from non-synaptic regions of neurons, which activates purinergic receptors on myelinating glia. Acting through this receptor system, neural impulse activity can regulate gene expression, mitosis, differentiation, and myelination of Schwann cells (SCs) and oligodendrocytes, helping coordinate nervous system development with functional activity in the perinatal period. ATP and adenosine have opposite effects on differentiation of Schwann cells and oligodendrocytes, providing a possible explanation for the opposite effects of impulse activity reported on myelination in the CNS and PNS. © 2003 Elsevier Ltd. All rights reserved.

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1. Introduction

Volume transmission would seem particularly relevant in activity-dependent interactions between neurons and glial cells ensheathing axons. This is a region far removed from access to neurotransmitter from synapses, and there is evidence that impulse activity can influence development of myelinating glia. Although myelination is highly correlated with the physiological properties of axons, the molecular mechanisms inducing myelination are unknown. Moreover, the means of communicating axonal firing to myelinating glia are not well understood, particularly during nervous system development, when impulse activity in premyelinated axons could have an instructive influence on myelinating glia. Curiously, studies of the effect of electrical activity on myelination, report opposite effects in the PNS and CNS, but the mechanism for these observations is unknown (for review see [Zalc and Fields, 2000](#)).

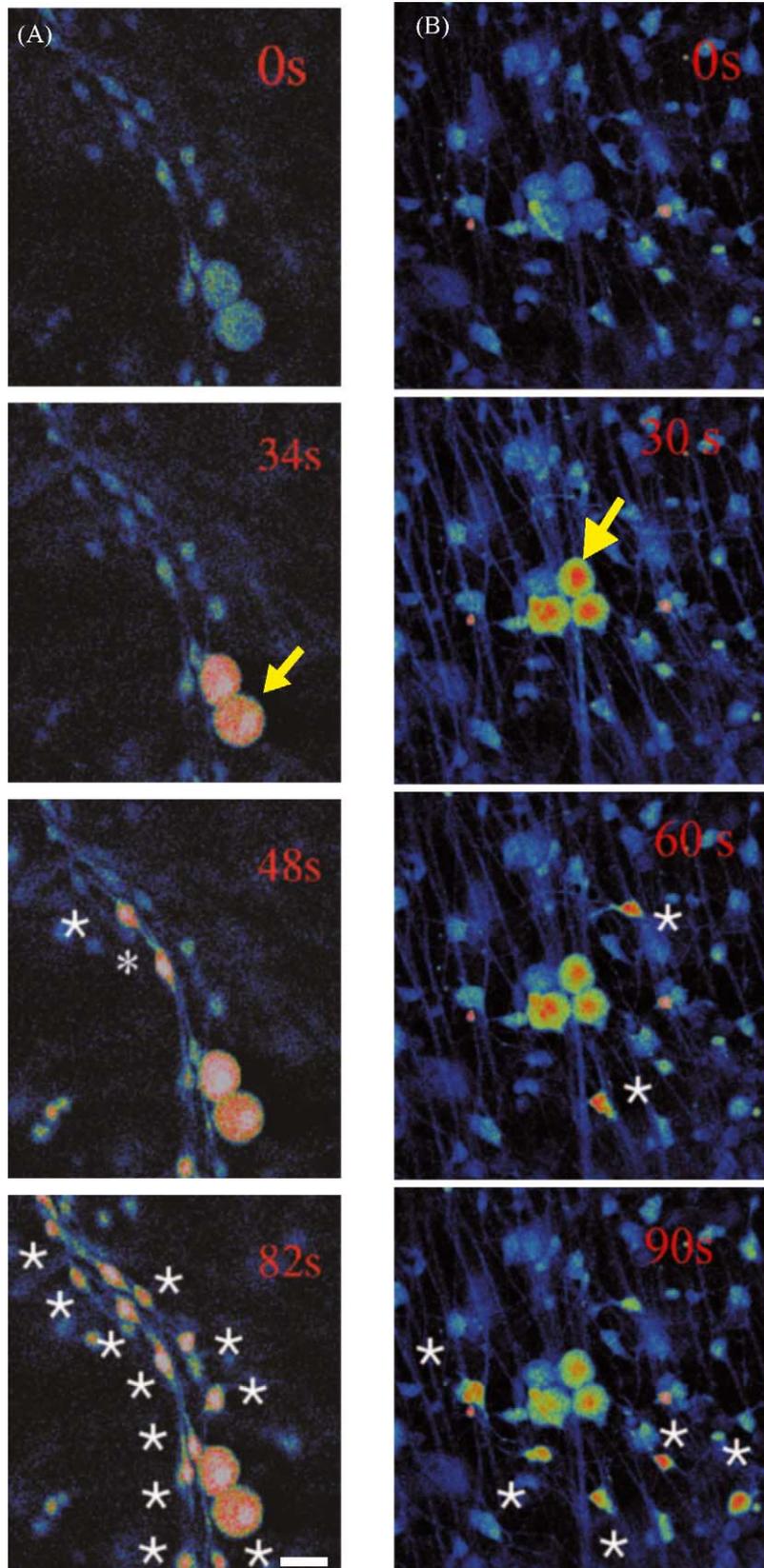
2. Activity-dependent communication between axons and myelinating glia

Intracellular calcium imaging has provided evidence for neuron–glia communication at synapses ([Jahromi et al., 1992](#); [Reist and Smith, 1992](#)), and nodes of Ranvier (for review see [Fields and Stevens–Graham, 2002](#); [Fields and Stevens, 2000](#)). Intracellular calcium transients in paranodal Schwann cells (SCs) have been detected following intense stimulation of axons, which is thought to be mediated by extracellular K⁺ accumulation ([Lev-Ram and Ellisman, 1995](#)). This may occur during pathophysiological conditions, but these responses have not been seen in other studies using more physiologically relevant stimulation ([Wachtler et al., 1998](#)). Much less is known about activity-dependent signaling between neurons and myelinating glia during embryonic development. Yet it is during this period that myelinating glia are highly plastic and are particularly sensitive to instructive signals from axons ([Mirsky and Jessen, 1996](#)).

3. Volume conduction between axons and myelinating glia mediated by extracellular ATP

Our laboratory has been investigating whether volume conduction could allow activity-dependent communication

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between premyelinating axons and glia, and if so what molecular mechanisms are involved. Secondly, we have used an *in vitro* preparation to determine whether functional effects of this activity-dependent signaling on glial development and myelination can be identified, and to compare the differences in myelinating glia of the CNS with the PNS. These studies are facilitated by using DRG neurons, which have bipolar axons that are myelinated in the CNS by oligodendrocytes and by Schwann cells in the PNS.

Mouse DRG neurons were grown in multicompartiment cell culture chambers (Campanot chambers), equipped with platinum electrodes for stimulation (Fields et al., 1992). After 3 weeks in culture, Schwann cells or oligodendrocyte progenitor cells (OPCs) were added and allowed to associate with axons and eventually undergo myelination. Calcium imaging was used to determine whether action potentials in premyelinated axons could be detected by Schwann cells or oligodendrocyte progenitor cells, via increases in calcium acting as an intracellular signaling molecule. Electrical stimulation of DRG axons in co-culture with either OPCs (Stevens et al., 2002), or Schwann cells (Stevens and Fields, 2000) resulted in calcium signals in the glia with a latency of 15 s or more from the start of stimulation (Fig. 1). High frequency stimulation was not required: responses were seen after 3 or 1 Hz stimulation, but fewer glia responded, and the amplitude of the calcium response was smaller than with 10 Hz stimulation (Stevens and Fields, 2000).

Liberation of ATP from DRG axons can be shown by measuring the increase in concentration of ATP in the culture medium following electrical stimulation of axons (Stevens and Fields, 2000). Measurements in multicompartiment chambers indicate that ATP is released from both the cell body and axonal compartments (Fields and Stevens, unpublished observations), and that this is blocked by stimulation in the presence of TTX, which blocks sodium-dependent action potentials. The calcium responses in Schwann cells induced by action potentials in DRG axons were blocked completely by stimulation in the presence of apyrase, an enzyme that rapidly degrades extracellular ATP. This and other evidence indicates that although electrical activity might release many different signaling molecules from axons, the activity-dependent communication with Schwann cells in these experiments was mediated entirely by the increase in extracellular ATP (Stevens and Fields, 2000).

4. Impulse activity in axons stimulates different purinergic receptors in PNS and CNS myelinating glia

The activity-dependent communication between DRG axons and OPCs was also significantly inhibited by stimulation in the presence of apyrase, but the calcium responses in OPCs were not entirely blocked by apyrase (Stevens et al., 2002). This suggests that other signaling molecules generated by electrically active axons are involved in communication with OPCs.

Application of ATP results in calcium responses in SCs and OPCs in monoculture, indicating that these cells have P2 purinergic receptors. However, adenosine can be generated from extracellular ATP by the action of ectoenzymes that dephosphorylate ATP, ADP, and AMP (Zimmermann et al., 1998; Burnstock, 1997). Adenosine application caused a large calcium response in OPCs in monoculture, but no response was detected in SCs (Stevens et al., 2002). This suggests that in contrast to Schwann cells, OPCs have adenosine receptors acting through intracellular calcium. RT-PCR analysis of cultured and freshly dissociated OPCs indicate the presence of A1 and A3 adenosine receptors, which operate via release of calcium from intracellular stores, and A2a and A2b receptors, which act primarily through cAMP (Stevens et al., 2002).

The absence of calcium responses in SC to direct application of adenosine, suggest that central and peripheral axons of DRG neurons could communicate with OPC and SC selectively in the CNS and PNS via differential signaling molecules; i.e., ATP release from axons could be detected by either central or peripheral myelinating glia, but only the central glia could detect the adenosine generated by breakdown of ATP, via activation of g-protein coupled adenosine receptors mobilizing intracellular calcium in OPCs.

5. Controversy on the role of impulse activity on myelination

Early studies were interpreted as suggesting that increased impulse activity might promote the formation of myelin in the optic nerve. Mice reared in the dark developed fewer myelinated axons in the optic nerve compared with normally reared mice (Gyllenstein and Malmfors, 1963). Additionally,

Fig. 1. Activity-dependent volume conduction between DRG axons and central and peripheral myelinating glia is revealed by intracellular calcium imaging: (A) Schwann cells (SC) and DRG neurons were co-cultured in multi-compartment chambers equipped with platinum electrodes for eliciting action potentials in axons, and confocal microscopy was used to monitor changes in fluorescence of an intracellular calcium indicator (fluo-3). Increasing calcium levels are represented by warmer colors, and a sudden increase was detected in DRG neurons (yellow arrow) upon electrical stimulation (10 Hz). This is caused by influx of calcium through voltage-sensitive calcium channels. This response in DRG neurons was followed within seconds by calcium increases in several SC situated along DRG axons (*). (B) Similar experiments performed on co-cultures of DRG neurons (yellow arrow) and OPCs (*) resulted in similar axon-glial signaling responses. This is an example of activity-dependent volume conduction, as there are no synapses in these DRG cultures, revealed by electrophysiology and immunocytochemistry. Panel (A) is derived from Stevens and Fields (2000). Panel (B) is derived from Stevens et al. (2002).

myelination is decreased in the optic nerve of the naturally blind cape mole rat (Omlin, 1997). Conversely, premature eye opening increased the level of myelin protein expression in the optic nerve of rabbit (Tauber et al., 1980).

In contrast, other studies have reported that intraocular injections of the sodium channel blocker tetrodotoxin (TTX), which blocks action potential activity, had no effect on the number of myelinated fibers or the time of myelination onset in optic nerves of rat (Colello et al., 1995; Crespo et al., 1995; Colello and Pott, 1997). In experiments on goldfish, action potential blockade by intraocular injection of TTX during optic nerve regeneration had no effect on myelination of regenerated fibers compared with controls (Hayes and Meyer, 1989). Also, dark-rearing of kittens (Moor et al., 1976) and rats (Fukui et al., 1991) has been reported as having no effect on the initiation of myelination of the visual pathway during early postnatal development, and mouse spinal cord explants undergo normal oligodendrocyte development and myelination in the presence of TTX (Shrager and Novakovic, 1995).

More recent studies in cell culture reveal a pronounced effect of electrical activity in stimulating myelination of CNS axons (Demerens et al., 1996). Neurons and oligodendrocyte progenitors dissociated from embryonic mouse brain cerebral hemispheres were maintained in co-culture, and when cultures at 8 DIV were treated with TTX for 2 or 4 days, the number of myelinated fibers at 18–21 DIV was decreased by approximately 80%.

Conversely, when cultures at 8 DIV were treated for 2 days with alpha saxatxin (α -ScTX), which increases excitability of neurons, the number of myelinated segments observed 10 days later increased by a factor of 2.4. The molecular mechanism for the effect of impulse activity in stimulating myelination is completely unknown. Since glial cells have many of the same ion channels expressed by neurons, it is important to consider that pharmacological treatments in many of these studies could have direct effects on glia apart from the intended effects on axonal firing.

Overall, the literature indicates either no effect or a stimulatory effect of impulse on myelination in the CNS, but this is not the case in the PNS. In contrast to the many studies of myelination by oligodendrocytes in the CNS, there is little information on effects of action potentials on myelination by Schwann cells in the PNS, but our work has shown two ways in which impulse activity inhibits myelination by Schwann cells: by changes in axonal membrane properties (Stevens et al., 1998), and by generating a soluble activity-dependent signal (ATP) (Stevens and Fields, 2000).

The first mechanism acts later in development and only on axons firing at low frequency. One day after co-culturing Schwann cells with the DRG neurons in the multi-compartment chambers, axons were stimulated at a rate of either one action potential every 10 s (0.1 Hz) or one action potential every 1 s (1 Hz) for 5 days. These two frequencies resemble the different firing rates that are seen during early, premyelinating periods and late myelinating periods of development. The results showed that although

the ultrastructure of myelin was normal in stimulated and unstimulated cultures, only one-third as many myelin profiles were present on axons stimulated at 0.1 Hz, compared with either unstimulated controls or axons stimulated at 1 Hz (Stevens et al., 1989). This result reveals the importance of action potential firing frequency, which may not be mimicked in experiments using pharmacological agents to inhibit axonal firing.

The molecular mechanism for the inhibitory effects of impulse activity on myelination by Schwann cells was traced to activity-dependent regulation of cell adhesion molecules on the DRG axon. Levels of the cell adhesion molecule L1 in DRG neurons are reduced after 5 days stimulation in axons firing at 0.1 Hz, but not by stimulation at 1 Hz (Itoh et al., 1995, 1997). L1, has been shown to have a critical role in initiation of myelination (Seilheimer et al., 1989; Wood et al., 1990). When homophilic binding between L1 molecules on DRG axons and Schwann cells is blocked with L1 antibodies, early ensheathment and the initiation of myelination are blocked. The second mechanism inhibiting myelination by SCs resulted from the activity-dependent liberation of ATP from axons. This intercellular communication was found to regulate development and subsequently myelination of Schwann cells and OPCs.

6. Differential effects of impulse activity on myelination of peripheral and central DRG axons

When electrical activity was applied earlier in development (i.e., before the myelinating glia had matured to a promyelinating stage), several effects of impulse activity on SCs and OPCs were detected. Tritiated thymidine incorporation assay and BrdU staining revealed that electrical activity in DRG axons inhibited cell proliferation of SCs (Stevens and Fields, 2000) and OPCs (Stevens et al., 2002). Pharmacological studies showed that this effect could be mediated by stimulation of ATP receptors on SCs (P2 receptors), but specific ATP receptor agonists had little effect on OPC proliferation. However, stimulation of adenosine (P1) receptors strongly inhibited OPC proliferation. The functional significance of the inhibitory effect of impulse activity on proliferation of SCs and OPCs may relate to developmental progression from an early phase characterized by rapid cell proliferation and migration, to a phase of cell differentiation and maturation as the nervous system matures and becomes electrically active. The effects of electrical activity, acting through release of ATP and adenosine, on differentiation of SCs and OPCs were therefore investigated, and this revealed unexpected differences between myelinating glia in the CNS and PNS.

After the developmental phase characterized by rapid cell proliferation, SCs differentiate either into myelinating or non-myelinating phenotypes (Mirsky and Jessen, 1996). These two terminally differentiated cells have very different functions and cell morphology. The non-myelinating

phenotype surrounds multiple small diameter axons, but myelinating SCs form a one-to-one relationship with large diameter axons and form the multilayered myelin sheath around axons, which is essential for rapid impulse conduction. When the effects of ATP receptor stimulation on SC differentiation was investigated, it was found that rather than promoting differentiation into either the myelinating or non-myelinating phenotype, ATP arrested development of SC at a highly plastic stage immediately prior to differentiation into either myelinating or non-myelinating lineage (Stevens and Fields, 2000). Even after prolonged periods in co-culture with DRG neurons in differentiating medium, which resulted in myelination in control cultures, treatment with ATP arrested SC at the immature O4 negative stage, and no myelination was detected (Fig. 2).

In contrast, OPCs treated with ATP differentiated normally; however, treating monocultures of OPCs with

adenosine greatly stimulated differentiation of OPCs to a premyelinating stage. These cells are characterized by multibranched processes and they expressed the O1 antigen and myelin basic protein (MBP), which are indicative of more highly differentiated oligodendrocytes. The acceleration of differentiation (and inhibition of proliferation) could be induced by electrical stimulation of co-cultures of DRG neurons and OPCs, and blocked by adenosine receptor antagonists (Stevens et al., 2002). As would be predicted from the accelerated differentiation, in co-cultures treated with adenosine, the number of axons undergoing early myelination was greatly increased compared with cultures without added adenosine. This effect was due to adenosine acting directly on the OPCs, because even a transient treatment of OPCs with adenosine prior to co-culturing them with DRG axons resulted in more highly differentiated cells and an increase in myelination (Fig. 2).

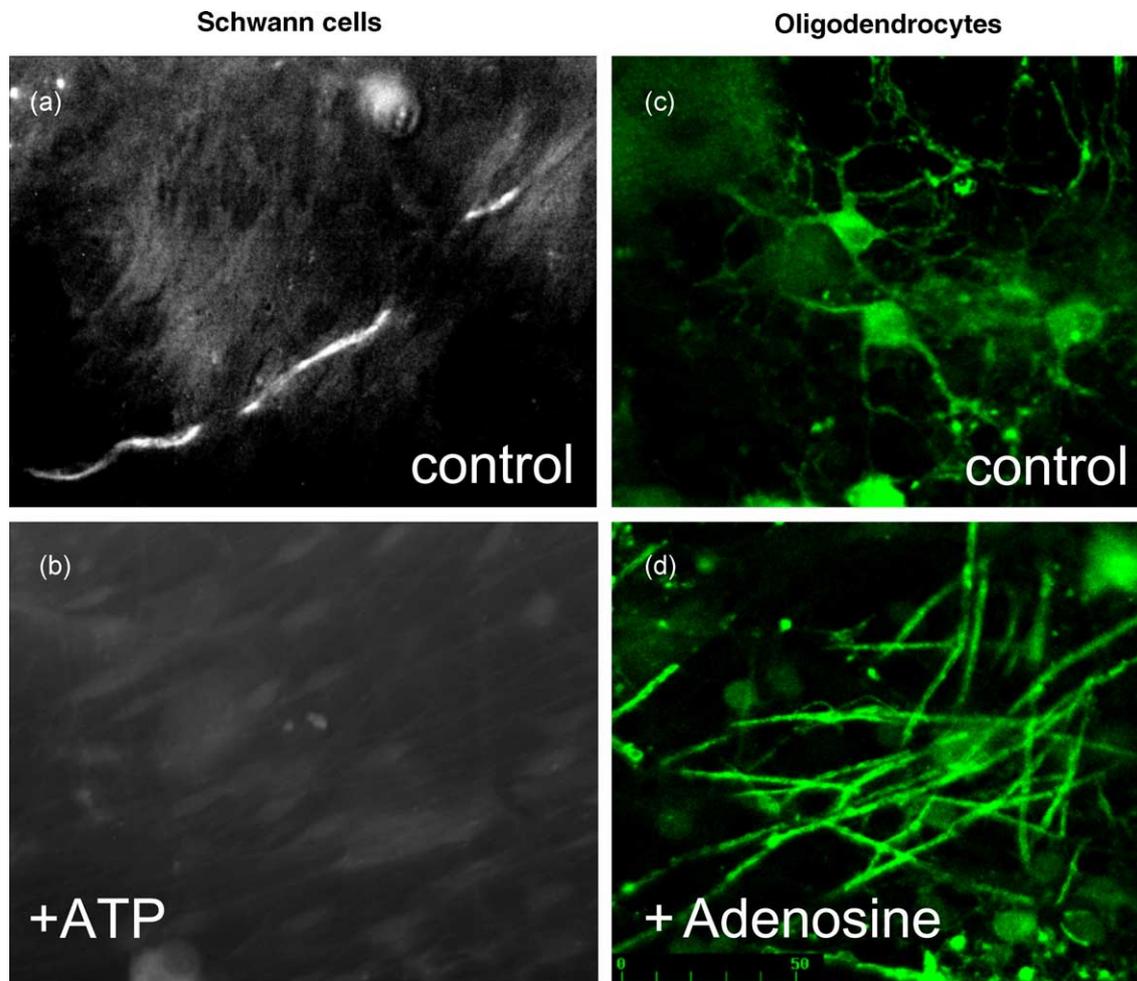


Fig. 2. Electrical activity has opposite effects on differentiation and myelination of DRG axons by Schwann cells and oligodendrocytes, mediated by different axon-glia signaling molecules. In the PNS, ATP released by electrically active axons inhibits differentiation and myelination, as can be seen by comparing the myelin basic protein (MBP) labeled co-cultures of DRG neurons and SCs in panels (a) and (b). In the CNS, a breakdown product of ATP, adenosine, stimulates differentiation and promotes myelination, as seen by comparing MBP labeled co-cultures of DRG neurons and OPCs in panels (c) and (d). Panels (a) and (b) are derived from Stevens and Fields (2000); and panels (c) and (d) are derived from Stevens et al. (2002).

7. Summary

Electrical activity, acting through ATP and adenosine in the PNS and CNS, respectively, has opposite effects on differentiation and myelination. In the PNS, ATP released by electrically active axons inhibits differentiation and therefore myelination, but in the CNS, adenosine (presumably generated by the breakdown of ATP released from the central axons) stimulates OPC proliferation and promotes myelination. This may be consistent with differences in development of myelination in the PNS and CNS, with respect to the onset of electrical activity during development. Central axons do not begin to grow into the spinal cord until after peripheral DRG axons have already reached the periphery and become electrically active (Fitzerald, 1987). Although SCs are associated with peripheral axons, the onset of myelination is delayed until the early post-natal period. This would be consistent with the inhibitory effects of electrical activity on SC proliferation and differentiation in culture. In contrast, OPCs are associating with axons and differentiating at a later stage of development when the nervous system is functionally active. Therefore, activity-dependent stimulation of differentiation and myelination by OPCs is consistent with the situation in vivo.

A large number of factors promote nervous system development, but inhibitory factors are also important in coordinating nervous system development. This inhibitory effect of electrical activity, acting through extracellular ATP, may be an example of the importance of inhibitory factors in regulating nervous system development. The signals that induce myelination are unknown, but they are axon specific, since only the appropriate axons become myelinated. Many signals, such as those associated with the increased diameter of the axons, do not develop until 1–2 weeks after birth. It would seem disadvantageous if SCs, in the absence of these myelin-inducing signals, were to differentiate into terminally differentiated non-myelinating SCs on the small diameter axons, because these cells would no longer be available for myelination. The action of electrical activity in arresting SC differentiation at the stage prior to differentiating into either the myelinating or non-myelinating lineage, may assure an ample population of cells at a stage available to differentiate into a premyelinating phenotype when the axon reaches the appropriate stage. Because astrocytes perform a function analogous to the non-myelinating SC in the CNS, a similar requirement to inhibit development of OPCs might be unnecessary. To be consistent with the developmental time course, impulse activity in the CNS should either have no effect or promote differentiation and myelination by OPCs.

The presence of different purinergic receptors on central and peripheral myelinating glia, provides a way for impulse activity in DRG neurons to have differential and selective effects on myelinating glia along its central and peripheral axons. ATP liberated by electrical activity in peripheral axons inhibits differentiation of SC, and on the central axons, ATP had no effect on OPC differentiation. Instead, the

breakdown product of ATP, adenosine, stimulates differentiation and myelination centrally, while having no stimulatory effect on SC differentiation on the peripheral axon.

8. Future directions

Yet to be determined are the mechanisms for the non-synaptic release of ATP from DRG axons and the regulation of enzymes controlling the breakdown of ATP to adenosine. Understanding how these mechanisms are regulated during development and in different regions of the nervous system will be important. There are a large number of distinct ATP and adenosine receptors, which have selective biological effects. It will be important to determine if there are changes in expression of these receptors in central and peripheral myelinating glia to understand how different developmental processes may be regulated selectively by impulse activity acting through different purinergic receptors at appropriate stages of development. Each of these may be subject to regulation in association with disease or injury, and understanding this form of axon glial signaling may be helpful in therapeutic approaches to treating demyelinating conditions.

9. Note added in proof

Recent research has detected the presence of adenosine receptors in Schwann cells of a subtype that activates cAMP, but does not increase intracellular calcium (Stevens et al., 2004). Activation of these receptors by adenosine also inhibits Schwann cell proliferation through a different mechanism from ATP, but it does not inhibit Schwann cell differentiation or myelination.

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