

Collateral Sprouting as a Target for Improved Function after Spinal Cord Injury

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ABSTRACT

Functional recovery after spinal cord injury might be improved by enhancing the extent of innervation through stimulation of collateral sprouting, which is the growth of a new axon along the shaft of a non-injured axon. This review discusses (1) the spontaneous collateral sprouting of uninjured motor and sensory systems that has been shown after spinal cord injury in animal models, (2) experimental treatment strategies that are being developed to enhance collateral sprouting in motor systems and to reduce sensory sprouting which is associated with autonomic dysreflexia and pain, and (3) cell-surface and intracellular signaling mechanisms that are known to regulate axonal branching. The conclusion is that relatively little is known about collateral sprouting in adult mammals after spinal cord injury but that it may contribute to spontaneous functional motor recovery and causes sensory dysfunction. There is some promising data in rodents that collateral sprouting can be modulated for improved function, but the applicability to primates and relevance to human spinal cord injury remains to be determined.

Key words: cell-surface signaling; collateral sprouting; intracellular signaling; spinal cord injury

INTRODUCTION

AFTER MOST TYPES of spinal cord injury in humans, a proportion of the axonal projections remains intact (Kakulas, 1999, 2004). This suggests that it might be possible to reduce functional deficits by enhancing the extent of innervation from the spared fibers—by promoting collateral sprouting beyond the injury in the normal innervation territory. For example, after a high- or mid-cervical injury, increased innervation from spared fibers that reach the lower cervical levels might improve the voluntary control over muscles such as the triceps. This

would greatly improve the quality of life for people with spinal cord injury, as they would have much better control over a non-motorized wheelchair. Increased innervation by spared serotonergic projections might result in better bladder control (Burgard et al., 2003) and reduced pain (Hains et al., 2002). There is a “dark” side to collateral sprouting too. As will be discussed, uninjured sensory afferents can sprout and contribute to the development of pain and autonomic dysreflexia, two common, serious and debilitating consequences of spinal cord injury in humans (Karlsson, 1999; Weaver et al., 2002; Finnerup and Jensen, 2004).

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SPONTANEOUS COLLATERAL SPROUTING AFTER SPINAL CORD INJURY IN ADULTS

One of the first studies of collateral sprouting in the adult mammalian CNS was in 1958 and showed that sensory afferents undergo sprouting in the spinal cord after spinal cord injury in cats and monkeys (McCouch et al., 1958). Many subsequent studies in different neuronal systems documented collateral sprouting in the adult mammalian CNS as a compensatory and functional response to denervation. Collateral sprouting is now a well-accepted phenomenon.

Anatomical Evidence for Limited Collateral Sprouting of Corticospinal Projections in the Injured Adult Spinal Cord

The corticospinal motor system is the most important system for voluntary movement in humans and consequently has been investigated extensively for regeneration and collateral sprouting. Spontaneous collateral sprouting of spared corticospinal axons after spinal cord injury has been investigated in various species. An early retrograde tracing study provided some evidence for collateral sprouting of corticospinal axons distal to the injury, months after a unilateral pyramidotomy or a spinal cord hemisection in monkeys (Aoki et al., 1986). However, this study used few animals and was not accompanied by anterograde tracing. A small anterograde tracing study with radioactive amino acids also suggested that there is collateral sprouting in adult monkeys after a unilateral pyramidotomy (Kucera and Wiesendanger, 1985). Due to the autoradiographic technique used, it is unclear whether this was accompanied by an increase in axonal terminals. Therefore, it remains to be determined in more detail whether collateral sprouting of spared corticospinal axons occurs in primates, including humans. Unfortunately, there is no phenotypic marker for the corticospinal projections. One solution may be the post-mortem anterograde tracing with tracers such as DiI injected into the corticospinal tracts, although this technique too has its limitations. Considering the variability of human injuries, this will require careful quantification of the spared fibers.

In rodents, the corticospinal tract divides in the medulla into the main dorsal tract (90%), which crosses in the pyramidal decussation and is located in the ventromedial part of the dorsal columns of the spinal cord. In addition, there is a small crossed tract in the dorsolateral spinal cord and an uncrossed projection that courses through the ventral spinal cord (Brosamle and Schwab, 1997; Steward et al., 2004). In neonates and very young rats and hamsters with a hemisection or unilateral cortex removal,

the spared corticospinal axons sprout and grow from the unlesioned into the denervated side distal to the lesion (Hicks and D'Amato, 1970; Whishaw and Kolb, 1988; Barth and Stanfield, 1990; Kuang and Kalil, 1990; Asaika et al., 1999) (Fig. 1A). However, this crossed innervation by collateral sprouts does not occur in adults or is minimal (Hicks and D'Amato, 1970; Thallmair et al., 1998; Zhou et al., 2003). It is unclear why this does not occur in adults, as the uninjured ventral pathway sprouts into the medial motor column of lamina IX after bilateral transection of the dorsal corticospinal tracts in adult rats, (Weidner et al., 2001) (Fig. 1B). Moreover, a small amount of collateral sprouting occurs from spared corticospinal fibers on the denervated side 14 days after partial transection of both corticospinal tracts at the level of the medullar pyramids, known as a "pyramidotomy"

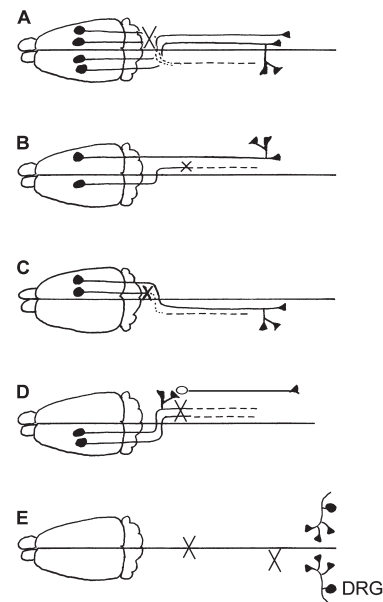


FIG. 1. Experimental models of collateral sprouting after spinal cord injury. (A) After a unilateral corticospinal tract lesion at the pyramids (pyramidotomy) in neonates or with treatments in adult rodents, axons from the non-injured side undergo collateral sprouting and grow into the denervated side of the spinal cord. (B) After a lesion of the dorsal corticospinal tract in adults, the ventral uncrossed corticospinal fibers sprout in the denervated spinal cord. (C) Alternatively, after a partial pyramidotomy, spared corticospinal fibers can sprout within the denervated spinal cord. (D) Injured corticospinal axons can sprout rostral to the injury to form new circuitry through propriospinal neurons in adult rats. B–D are illustrated as unilateral lesions but were bilateral in the described studies. (E) After a hemisection at T13 or a complete transection at T5 in adults, sensory afferents sprout into lamina II–V of the dorsal horn, and contribute to the development of allodynia and autonomic dysreflexia, respectively.

(Hagg et al., 2005) (Fig. 1C). One difference between the crossed and uncrossed sprouting models is the greater distance between the uninjured terminals and the denervated side in the crossed model. Thus, it is possible that diffusible factors from the denervated side do not reach sufficiently high concentrations on the spared side. Such an interpretation would also be consistent with the fact that the spared ventral and dorsal corticospinal fibers in the uncrossed models (Weidner et al., 2001; Hagg et al., 2005) are close to or amongst degenerating corticospinal projections. As will be discussed later, such diffusible agents might include neurotrophic factors. Injured adult corticospinal axons appear to respond differently. For example, after a thoracic lesion in adult rats corticospinal axons can sprout at the cervical level to form new circuitry onto propriospinal neurons (Fouad et al., 2001; Bareyre et al., 2004) (Fig. 1D). This may be related to a difference in the ability to initiate a collateral sprout from the axonal shaft. The important question is whether the modest amount of collateral sprouting in adult rodents has useful functional consequences.

Functional Consequences of Corticospinal Collateral Sprouting

It has become clear that plasticity in the cortex, subcortical nuclei and the spinal cord itself can contribute to functional recovery in animal models as well as in humans with spinal cord injury (Raineteau and Schwab, 2001; Edgerton and Roy, 2002; Edgerton et al., 2004). The collateral sprouting of corticospinal axons in the various adult models appears to improve functional recovery. For example, motor responses evoked by cortical microstimulation have revealed corticospinal plasticity in two monkeys with a unilateral pyramidotomy (Mitz and Humphrey, 1986). On the other hand, partial functional recovery is seen within 6 weeks in monkeys, but this was not accompanied by an increase in anterograde tracer in the spinal cord (Kucera and Wiesendanger, 1985). The caveat with those studies is the very small number of animals.

Therefore, together with the sparse anatomical data, there is little data to suggest that collateral sprouting occurs in primates and has functional consequences. Ultimately, it will be important to establish the extent of spontaneous corticospinal collateral sprouting in adult primates to help put the evidence for spontaneous and stimulated functional collateral sprouting in adult rats into perspective. However, this would be premature, as the understanding of adult corticospinal collateral sprouting in rodents needs to be improved first.

There is some evidence that spontaneous collateral sprouting has functional consequences in adult rats. Adult rats perform better on a pellet retrieval test (forelimb

reaching) 2–4 weeks after a transection of the dorsal corticospinal tract at C3 than rats with a complete corticospinal transection at the pyramids (Weidner et al., 2001). This was attributed to the sprouting response of the ventral corticospinal pathway, as a subsequent lesion of the ventral part of the cord abolished the improved pellet retrieval. There is a caveat, as the ventral part that was lesioned may have contained other systems important for motor functions (Loy et al., 2002). Also, after a unilateral pyramidotomy in adult rats, the uncrossed ventral pathway from the uninjured side does not contribute to recovery in a battery of tests, including reaching (Whishaw and Metz, 2002). On the other hand, it is possible that lesion models that also injure other systems, including the ascending sensory tract (Weidner et al., 2001), have differences in sprouting or functional recovery compared to the pure corticospinal lesions (pyramidotomy). This is an important issue to resolve as the mixed lesions most likely represent the human injuries, which rarely, if ever, involve only the corticospinal tract.

Injured corticospinal axons (thoracic lesion) can sprout and make a new relay circuitry onto propriospinal neurons in the cervical level (Fouad et al., 2001; Bareyre et al., 2004) (Fig. 1D). This sprouting response correlates with improved hindlimb placing response and EMG responses to cortical stimulation. The improvement disappears after completely removing the corticospinal projections by a pyramidotomy, evidence that the sprouting had contributed to the improved function (Bareyre et al., 2004). Although this is not purely collateral sprouting, such data add to the view that functional recovery of the corticospinal system might be possible in humans too. The question whether crossed collateral sprouting or the formation of new relay circuitry can function to ensure unilateral and selective voluntary movement only in the appropriate spinal cord levels below the injury in humans remains to be resolved.

Sensory Afferents Undergo Collateral Sprouting and Contribute to Dysfunction after Spinal Cord Injury

A number of studies in different species have documented collateral sprouting of sensory afferents after spinal cord injury in adults (Fig. 1E). This became clear in an early study with anatomical and electrophysiological evidence for collateral sprouting from L7 into the spinal cord distal to a chronic T12 hemisection in cats and after a focal spinal cord injury in monkeys (McCouch et al., 1958). The new fibers were seen in the corticospinal termination area and were thought to explain the development of spasticity during the chronic injury phase. Others have used tracing methods with radioactive proline to show increased innervation in lamina VI–VII of cats with

T12/L1 hemisections, which correlated with the enhanced intrinsic reflexes (Murray and Goldberger, 1974). The sprouting ipsilateral to the lesion has also been detected with a marker for dorsal root fibers and GAP43 in cats (Helgren and Goldberger, 1993). This sprouting probably contributes to the increased receptive field size in the dorsal horn of cats with a hemisection which spares the dorsal columns (Brenowitz and Pubols, 1981). In rats injured as neonates by a hemi-section, sensory afferents also undergo collateral sprouting above and below the lesion as seen by increased axon counts in the roots (Hulsebosch and Coggeshall, 1981). In adult rats, a hemisection at T13 appears to cause sprouting of unmyelinated (C) and thinly myelinated (A δ) afferents, as identified by an increase of CGRP and GAP43 in lamina II and IV at levels above and below the lesion, as far as C8 and L5, and even bilateral (Christensen and Hulsebosch, 1997; Ondarza et al., 2003). The anatomical changes observed in the T13 hemisection model correlate with the development of allodynia (Christensen et al., 1996). Whether actual aberrant sprouting or just changes in protein content occurred in this model remains to be confirmed. This is important as others have failed to detect intraspinal sprouting below a partial hemisection at various levels between T10 to L3 using HRP injections into the sciatic nerve (Rodin and Kruger, 1984). In summary, the evidence for a role of collateral sprouting in enhanced spinal sensitivity, including pain, after spinal cord injury may appear largely correlative. However, as discussed below, treatments that prevent the anatomical changes also reduce the pain symptoms, suggestive of a causative relationship. Moreover, plasticity in the sensory system clearly plays a role in pain after spinal cord injury in humans (Finnerup and Jensen, 2004).

Sensory collateral sprouting has also been linked to the development of autonomic dysreflexia in adult rats (Weaver et al., 2002). After a complete transection at T5, myelinated afferents anterogradely traced with CTB (which may only be taken up by myelinated fibers) sprout in laminae III-V of the lumbar cord after 2 weeks (Krenz and Weaver, 1998). CGRP immunoreactivity also increased, suggesting that unmyelinated fibers may also have sprouted or that the protein was upregulated in response to the spinal injury. Sprouting within the Clarke's nucleus was also detectable by the general tracer, DiI. Similar changes occur after a clip compression (Weaver et al., 2001) and in adult mice (Jacob et al., 2003). Spinal cord interneurons sprout and innervate the preganglionic sympathetic neurons of the intermediolateral cell column (Weaver et al., 1997), possibly relaying the information from the sprouted sensory afferents (Krenz and Weaver, 1998). A cautionary note is that changes in CGRP-positive afferents in this model is seen in 129Sv, but not

C57BL, mice, despite the fact that both strains develop autonomic dysreflexia (Jacob et al., 2003). This raises the possibility that the use of phenotypic markers such as CGRP alone is not adequate to document sprouting. A more radical interpretation would be that afferent sprouting is not needed for the development of autonomic dysreflexia in mice.

Plasticity and possibly sprouting of C-fibers also may underlie bladder voiding dysfunction that develops after spinal cord injury (Cheng and de Groat, 2004; Seki et al., 2004) and is common in injured humans. Therefore, collateral sprouting of sensory afferents potentially plays a role in dysfunction of multiple systems after spinal cord injury. This may prove to be an advantage for the development of spinal cord injury therapies as it would suggest that monotherapies that target the sensory system could improve a number of symptoms.

Evidence for local sprouting of sensory afferents also has been found in humans with spinal cord injury (Kakulas, 2004; Calancie et al., 2005) and may contribute to chronic pain that develops after spinal cord injury. Pain in spinal cord injury patients is a leading cause of disability and effective treatments for pain remain elusive (Finnerup and Jensen, 2004). Up to 60% of people with spinal cord injury have severe chronic and intractable pain. Up to half of the people with spinal cord injury have pain that can be evoked by non-noxious stimuli—i.e., allodynia (Finnerup et al., 2001). The sensory sprouting in humans may also contribute to the development of autonomic dyreflexia which is present in more than half of the people with spinal cord injury during the chronic phase (Karlsson, 1999; Weaver et al., 2002).

Finally, the sensory afferent sprouting in response to spinal cord injury is different from that caused by peripheral nerve injury and has been linked to neuropathic pain. This type of collateral sprouting may also play a role in humans with spinal cord injuries, as hemorrhage and injury has been found in the DRG in >30% of human cases with fatal blunt trauma, including some with spinal cord injury, in the first 7 days after accident (Taylor et al., 1998). Moreover, spinal cord injury often also includes injury to the roots. This raises the possibility that injury to the peripheral nerve compartment could also contribute to changes in the sensory afferent system after spinal cord injury in humans.

TREATMENT STRATEGIES TO AFFECT COLLATERAL SPROUTING AFTER SPINAL CORD INJURY

Increased collateral sprouting of corticospinal projections might improve voluntary movement in humans with

spinal cord injury. On the other hand, decreased sprouting of sensory afferents might reduce pain, autonomic dysreflexia and bladder dysfunction. Treatment strategies are being considered on the basis of the knowledge that axonal growth in the CNS is controlled in part by neurotrophic factors and inhibitory proteins.

Neurotrophin Treatment Can Enhance Corticospinal Collateral Sprouting after Spinal Cord Injury

There is ample evidence that neurotrophic factors are involved in collateral sprouting both *in vitro* and *in vivo* or can be used to enhance collateral sprouting in a number of different neuronal systems. *In vitro*, neurotrophins can induce branching of growing neurites (Patel and McNamara, 1995) and formation of new protrusions and growth cones on the sides of neurites (Gallo and Letourneau, 1998; Gibney and Zheng, 2003). Administration of NGF can promote and of NT-3 can inhibit branching of DRG axons in the spinal cord during early development (Zhang et al., 1994). Neurotrophins also play a role in collateral sprouting in the adult rat. For example, collateral sprouting of sensory and sympathetic axons in the skin in response to injury of other axons is caused by local increases in NGF in the skin (Diamond et al., 1987; Gloster and Diamond, 1992). Endogenous NGF and FGF-2 also play a role in collateral sprouting of cholinergic axons after partial denervation of the hippocampal formation (Van der Zee et al., 1992; Fagan et al., 1997). Collateral sprouting of adult mossy fibers in the hippocampal formation (Adams et al., 1997) and of serotonergic fibers in the cortex (Mamounas et al., 1995) can be enhanced by local treatment with neurotrophins. Therefore, it seems logical to assume that neurotrophins can also be used to increase the extent of collateral sprouting in the spinal cord.

The choice of neurotrophic factor to induce collateral sprouting of corticospinal motor axons was straightforward. Corticospinal neurons express TrkC, the receptor for NT-3 (Giehl and Tetzlaff, 1996). NT-3 can induce regenerative sprouting and regeneration of injured corticospinal fibers in the adult spinal cord (Schnell et al., 1994). Moreover, growing uninjured neonatal corticospinal axons sprout in response to NT-3 (Schnell et al., 1994), raising the possibility that collateral sprouting could also be induced in the adult after spinal cord injury. In fact, collateral sprouting from the non-injured to the denervated side (Fig. 1A) can be enhanced by overexpressing NT-3 in lower motor neurons of adult rats 2 weeks after a unilateral pyramidotomy in adult rats (Zhou et al., 2003). This NT-3 induced sprouting can be further enhanced by administration of BDNF or GDNF to the

cortex (Zhou and Shine, 2003), suggesting that changes in and around the cell body can play important roles in collateral sprouting. This is also suggested by the finding that NT-3 injection into the cortex induces collateral sprouting of the non-lesioned corticospinal fibers into the superficial dorsal horn in response to rubrospinal denervation in adult rats (Jeffery and Fitzgerald, 2001). An important finding of that study is the lack of a response after either NT-3 treatment or the rubrospinal lesion alone. This suggests that the corticospinal neurons do not respond with sprouting to cortical NT-3, unless there is an "injury" environment in the spinal cord. The fact that collateral sprouting does occur after partial corticospinal lesions (Weidner et al., 2001; Hagg et al., 2005) also suggests that only certain types of environments or short distances from the injury environment can induce spontaneous collateral sprouting.

In apparent contrast to the earlier studies, we have found that a 14-day NT-3 infusion into the cervical or lumbar cord reduces local collateral sprouting from spared fibers from within the denervated gray matter when started immediately after a bilateral partial pyramidotomy (Hagg et al., 2005). In our model, spared corticospinal fibers course through an injured environment. This raises the possibility that they respond opposite to NT-3 compared to fibers on a non-injured side (Zhou et al., 2003). One possibility is that NT-3 activated the p75 neurotrophin receptor which is transiently expressed by corticospinal neurons during the first week after an injury (Harrington et al., 2004). P75 is a co-receptor for the growth inhibitory Nogo receptor whose activation causes growth cone collapse (He and Koprivica, 2004). NT-3 also has a degeneration-inducing role through p75 in corticospinal neurons after an internal capsule lesion (Giehl et al., 2001). Only a few corticospinal fibers at the lesion site have p75 at 14 days after injury in our model (Hagg et al., unpublished). The absence of p75 at 14 days might thus explain why NT-3 delivered to the spinal cord can induce collateral sprouting when started 14 days post-injury (Zhou et al., 2003). In our study, NT-3 promoted sprouting of CGRP-positive fibers in the spinal cord region where the corticospinal sprouting was reduced, suggesting that the afferents may interfere with corticospinal sprouting. Consistent with such a speculation is the finding that the nerve encapsulation used to deliver the NT-3 that induced corticospinal collateral sprouting (Zhou et al., 2003), can reduce sprouting of sensory afferents (White and Kocsis, 2002). In summary, these various studies have uncovered the complexity of corticospinal collateral sprouting in response to treatments. There clearly are molecular, spatial and temporal conditions that need to be better understood in order to consider NT-3 as a therapeutic agent for improved function.

Moreover, it should be noted that none of the studies with NT-3 have analyzed the functional consequences of the treatments.

The idea that treatment around the corticospinal cell bodies can contribute to the induction of collateral sprouting is also supported by another study. Chronic infusion of the purine nucleoside inosine into the sensorimotor cortex induces corticospinal collateral sprouting from the non-injured to the denervated cervical cord after a unilateral pyramidotomy in adult rats (Benowitz et al., 1999). Inosine may act intracellularly by inducing expression of GAP-43, T alpha-1 tubulin, and the cell-adhesion molecule, L1 (Benowitz et al., 2002) and by regulating protein kinase N, which mediates NGF-induced neurite outgrowth of PC12 cells (Volonte et al., 1989). It is likely that some of the neurotrophic factors which can also affect expression of these genes, such as BDNF, would induce the same collateral sprouting response. This finding also suggests that the induction of regeneration-associated genes by targeting topographically defined cortical areas may be a way to induce sprouting of corticospinal axons that project to very select spinal cord levels.

Inhibition of the Myelin-Associated Inhibitor Nogo Induces Corticospinal Collateral Sprouting and Improves Function after Spinal Cord Injury

As in the regeneration field, neurotrophic factors and growth inhibitors most often play an opposing role. For example, inhibition of the myelin-associated inhibitor Nogo-A or its receptor promote corticospinal regenerative sprouting and regeneration in adult rats (Schnell and Schwab, 1993; Li et al., 2004). Collateral sprouting of corticospinal axons from the non-injured into the denervated side distal to a unilateral pyramidotomy (Fig. 1A) can also be enhanced in adult rats by inhibiting Nogo with antibodies (Thallmair et al., 1998). The non-injured corticospinal axons also crossed and grew into the red nucleus and sensory dorsal column nuclei. The Nogo antibody also causes crossed collateral sprouting of the corticorubral fibers after a unilateral pyramidotomy (Z'Graggen et al., 1998) and of rubrospinal projections into ventral horn after a bilateral pyramidotomy (Raineteau et al., 2002). Lastly, Nogo antibodies can also induce collateral sprouting of corticospinal axons into aberrant location in adult rats without corticospinal tract lesions (Bareyre et al., 2002). The effects of Nogo inhibition on multiple systems makes it difficult to decipher which system contributed to the improvements in motor and sensory functions as seen in two of those studies (Thallmair et al., 1998; Z'Graggen et al., 1998). One method that should become standard in such studies is to lesion the neurons or their axons that are thought to contribute to the functional recovery (Weidner et al., 2001;

Bareyre et al., 2004). The mechanisms of action of Nogo or Nogo receptor inhibition in vivo are not entirely understood (Hunt et al., 2002) but could include the induction of expression of growth-promoting genes in the spinal cord (Bareyre et al., 2002). Whether the blocking of Nogo or its receptor would cause functionally relevant collateral sprouting without causing pathological sprouting in humans, particularly with their more complex injuries is also unclear.

Inhibition of Endogenous NGF Reduces Sensory Collateral Sprouting and Dysfunction

Sensory neurons and axons are well-known for their responsiveness to neurotrophins. Therefore, the collateral sprouting that is associated with the development of pain, autonomic dysreflexia and bladder dyssynergia was thought to be caused in part by increased levels of neurotrophic factors that are seen in the spinal cord after various types of injury (Widenfalk et al., 2001; Brown et al., 2004). In fact, in the spinal cord hemisection model of pain, the increases in CGRP in spinal laminae I-IV are reduced by a 14-day intrathecal infusion of antibodies against NGF (Christensen and Hulsebosch, 1997). In the same model, hyperalgesia and sensory neuronal responsiveness are reduced by intraperitoneal administration of NGF antibodies (Gwak et al., 2003). It is unclear whether these systemically administered antibodies act in the spinal cord or in the periphery or whether the collateral sprouting was affected in that study. A similar systemic treatment with NGF antibodies can reduce collateral sprouting of nociceptive fibers in the skin and spinal cord in a skin denervation model (Pertens et al., 1999). The proposed role of NGF in sensory afferent sprouting and the development of autonomic dysreflexia has also been addressed by inhibiting NGF. For example, intrathecal infusion of NGF antibodies or an NGF-trapping TrkA-IgG fusion protein for 14 days blocks the development of autonomic dysreflexia in the spinal cord clip compression model (Krenz et al., 1999; Marsh et al., 2002). The NGF antibody treatment also reduced the expansion of CGRP immunoreactivity in the dorsal horn and the extent of innervation was correlated with the severity of the dysreflexia (Krenz et al., 1999). The bladder destrusor-sphincter dyssynergia that develops in adult rats after a complete T8 transection also can be reduced by intrathecal infusion of NGF antibody (Seki et al., 2004). The contribution of C-fibers to the development of the dyssynergia was shown by their removal with capsaicin. This is consistent with the idea that collateral sprouting of sensory afferents in response to elevated levels of NGF in the spinal cord contribute to bladder dysfunction. Despite the fact that these studies did not assess the effects of NGF neutralization on actual sprouting using antero-

grade tracing, they have identified a potential treatment strategy for very important aspects of spinal cord injury. Whether such a treatment would affect the necessary regeneration of injured sensory axons is unknown.

MOLECULAR MECHANISMS OF COLLATERAL SPROUTING

It is likely that a better understanding of the signaling pathways involved in collateral sprouting would lead to the discovery of more effective treatment strategies. This has been the case in the spinal cord regeneration field. Figure 2 summarizes several of the signaling pathways known to regulate neurite or axonal branching.

Potential Neurotrophin-Induced Signaling Mechanisms

As discussed above, neurotrophins can induce branching of growing neurites *in vitro* and collateral sprouting *in vivo*. However, not much is known about the mechanisms that regulate branching through Trk or other neurotrophic factor receptors. PI3K is a common downstream intracellular signaling molecule for such receptors and has been shown to be required for neurite outgrowth and branching of developing and adult sensory neurons *in vitro* (Gallo and Letourneau, 1998; Jones et al., 2003). Once a branch has formed along an axon *in vivo*, it is assumed that it will grow in response to a chemoattractive gradient of neurotrophic factors. The known pathways responsible for the TrkA-induced turning response include co-activation of PLC γ and PI3K (Ming et al., 1999). This

suggests that PI3K plays a critical role in a number of events required for collateral sprouting and that PI3K-selective activators might be useful for promoting collateral sprouting. However, it is unclear whether adult mammalian neurons have transmembrane neurotrophic factor receptors along their axon under normal or injury conditions *in vivo*, or can respond to neurotrophic stimulation from receptors along the axonal tract. *In vitro*, NGF presented along the sensory neurites causes collateral sprouting from that location (Gallo and Letourneau, 1998). Also, focal application of NT-3 along neurites of *Xenopus* neurons induces distant neurotransmitter release (Chang and Popov, 1999), suggesting that there are TrkC receptors along the neurites. Most convincingly, radiolabeled NGF injected into the cervical spinal cord level is transported to L4/5 DRG, suggesting that normal ascending sensory axons in the dorsal column have transmembrane TrkA along their entire length (Richardson and Riopelle, 1984).

Myelin-Associated Inhibitors May Act Predominantly at the Nodes of Ranvier

As discussed above, the inhibition of neurite outgrowth inhibitors can also lead to collateral sprouting. The main effect of blocking myelin-associated inhibitors with Nogo receptor antagonists or Nogo antibodies is an enhancement of sprouting rostral to the injury site. This suggests that the Nogo receptor is normally involved in preventing sprouting or branching of myelinated axons. Therefore, one expects that collateral sprouting occurs at the Nodes of Ranvier, where there is a gap in the myelin sheath. Various inhibitors are present at the nodes, in-

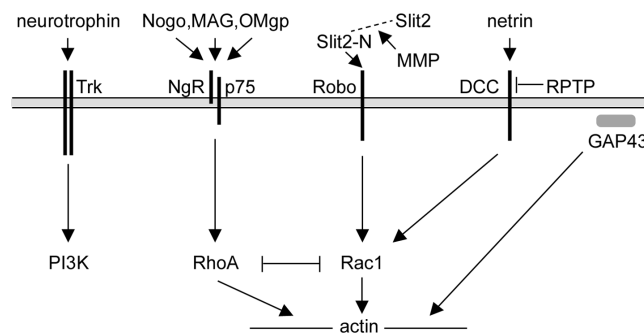


FIG. 2. Signaling pathways known to regulate neurite or axonal branching. Neurotrophins activate Trk receptors, which may mediate their branching effect through PI3K. Branching may reflect collateral sprouting after injury *in vivo*. The myelin-associated inhibitory proteins MAG, OMgp and NogoA activate the Nogo receptor/p75 complex which results in activation of RhoA, known to regulate actin dynamics and growth cone collapse. Slit2-N is cleaved from Slit2 by a metalloprotease and signals through the Robo receptor to induce neurite branching *in vitro* and during development. Intracellular signaling involves activation of Rac1, which can inhibit RhoA. Rac1 also regulates actin dynamics. GAP43 regulates F-actin dynamics at lipid rafts in the membrane. Netrins can signal through DCC and their effect on branching involves Rac1. DCC can be inhibited by a receptor protein tyrosine phosphatase (RPTP).

cluding the myelin-associated inhibitors MAG and OMgp, and probably NogoA (Scherer, 1996), and the chondroitin sulphate proteoglycan, versican (Oohashi et al., 2002) which in the CNS is produced by oligodendrocytes (Asher et al., 2002). Other inhibitors such as tenascin (Scherer, 1996) and Thy-1 (Liesi et al., 1990) are also found at the nodes. Despite this evidence it is unknown where along the axons or terminals collateral sprouting is initiated. Collateral sprouting in myelinated systems in response to neurotrophic factors or denervation may occur in the innervation territory only (Goodman and Horel, 1966). Systemic delivery of CNTF induces collateral sprouting of lower motor axons but only from the first few Nodes of Ranvier away from the neuromuscular junction (Gurney et al., 1992). It remains to be determined whether and how nodes in or close to the target are different with respect to inhibitory molecules.

The likelihood that collateral sprouting occurs only at the nodes of myelinated axons and the presence of myelin-associated molecules in myelin raises the question how collateral sprouting is normally prevented in unmyelinated systems. If this were known, a more rational design of treatments for unmyelinated systems could be devised. Presumably, these systems do not contain myelin-associated inhibitors, although they may be surrounded by inhibitory CSPGs. It should be recognized that approximately one third of the corticospinal tracts fibers in the spinal cord of adult rats is unmyelinated (Joosten and Gribnau, 1988). This suggests that current strategies which target myelin-associated inhibitors to induce collateral sprouting may have differential effects on these myelinated and unmyelinated corticospinal motor axons. However, in adult humans, the corticospinal tract consist predominantly of myelinated axons.

The myelin-associated inhibitors signal through the Nogo/p75 receptor complex which activates the small GTPase RhoA and inhibits the small GTPase Rac1, which in turn regulate actin cytoskeletal dynamics (Filbin, 2003; McGee and Strittmatter, 2003; He and Koprivica, 2004). The effect of inhibition of RhoA (Dergham et al., 2002) and of PKC, which is involved in both Nogo receptor and CSPG signaling (Sivasankaran et al., 2004) can promote regeneration after spinal cord injury. It will be important to test these strategies for collateral sprouting.

New Regulators of Adult CNS Collateral Sprouting May Come from the Developmental Biology Field

Many molecules regulate growth cone behavior and thus guidance of growing axons during embryonic development and *in vitro* (Huber et al., 2003; Guan and Rao, 2003). A few of these also regulate neurite branch-

ing, which may be similar to collateral sprouting *in vivo*. A better understanding of these regulators and their intracellular signaling may lead to novel approaches to induce collateral sprouting. Full-length Slit is well-known as a repellent and is expressed in the adult CNS (Marillat et al., 2002). However, the natural N-terminal Slit2 cleavage product induces branching of sensory DRG neurites *in vitro* (Wang et al., 1999; Nguyen-Ba-Charvet et al., 2001). This is not true for all neurons, as Slit2-N is a repellent for olfactory bulb neurites (Nguyen-Ba-Charvet et al., 2001). The context-dependent nature of Slit signaling is also clear from the fact that both Slit2 and Slit2-N act through the Robo receptor (Huber et al., 2003; Fan et al., 2003). Robo can activate Rac1, known to be involved in regulating actin dynamics. In fact, Rac1 is required for Slit2-induced axonal branching in developing *Drosophila* and may involve binding to Pak, one of the Robo-interacting proteins (Ng et al., 2002; Fan et al., 2003). Of interest is the finding that Rac1 can inhibit RhoA (Kozma et al., 1997), which is well-known for its role in neurite and axon repulsion, and is downstream of the inhibitory Nogo receptor (Filbin, 2003; McGee and Strittmatter, 2003; He and Koprivica, 2004). Finally, the transcription factor Islet-2 and its target, the transmembrane protein Plexin4A which colocalizes with Robo, are required for the branching effect of Slit2 on sensory neurons in developing zebrafish (Yeo et al., 2004; Miyashita et al., 2004).

How the two forms of Slit can differentially activate branching and repulsion is unclear, but may involve different Rac proteins (Ng et al., 2002) or complex interactions with the netrin-DCC signaling (Stein and Tessier-Lavigne, 2001; Fan et al., 2003). Of interest is the finding that the C-terminal but much less the N-terminal fragment of Slit2 interacts with glypican-1 (Ronca et al., 2001), which might be a regeneration associated protein (Bloechlinger et al., 2004). Glypican-1 is also co-expressed with Robo2. Thus, it is possible that glypican-1 neutralizes Slit2 during outgrowth, and the presence of glypican-1 determines the balance between repulsion and branching. Slit2 is a cleavage-dependent protein, which may be mediated by metalloproteases such as Kuzbanian/ADAM10 (Schimmelpfeng et al., 2001). Metalloproteases appear to play a role in promoting collateral sprouting in the adult rat dentate gyrus after an entorhinal cortex lesion (Reeves et al., 2003). Clearly, a great deal of information already exists on the role of Slits in neurite branching and it will be interesting to test the effects of Slit2-N on collateral sprouting of corticospinal projections after spinal cord injury. Conversely, the repellent effects of full-length Slit may be useful to reduce sensory sprouting.

Netrins and their transmembrane receptors may also play a complex, context-dependent role in axonal re-

pulsion and branching. The netrin receptor DCC plays a role in growth cone attraction and repulsion and the UNC-5 receptor and its mammalian homologs plays a role in repulsion (Guan and Rao, 2003). UNC-5 homologs are predominant in the normal adult rat spinal cord, perhaps inhibiting collateral sprouting (Manitt et al., 2004). Growth cone attraction through DCC involves Rac1 activation and the attraction, but not outgrowth, can be silenced by Robo (Stein and Tessier-Lavigne, 2001). Attractant netrin signaling through the DCC receptor is inhibited by a receptor protein tyrosine receptor (Chang et al., 2004), suggesting that selective tyrosine phosphatase inhibitors could enhance collateral sprouting. On the other hand, the transmembrane tyrosine phosphatase LAR facilitates the speed of collateral sprouting of cholinergic septohippocampal axons after entorhinal cortex lesions (Van der Zee et al. 2003). This reiterates the need for selective tyrosine phosphatase inhibitors if they are to be considered as therapeutic agents after spinal cord injury.

A few other proteins have been found to regulate neurite branching, although they have not been studied extensively. The neurexin-related protein, BAM-2, may function as a branch termination cue in *c. elegans* (Colavita et al., 2003). EphrinA3-EphA5 signaling appears to play a role in seizure induced collateral sprouting of hippocampal mossy fibers in adult rats (Xu et al., 2003). It will be interesting to investigate the presence of ephrins and Eph receptors in models of spinal cord collateral sprouting and to administer appropriate ephrins to potentially promote this sprouting.

Lastly, cytoskeletal proteins obviously play an important role in any axonal growth process, as has been shown extensively in studies of growth cone behavior (Huber et al., 2003; Guan and Rao, 2003). There are a few that have potential relevance to collateral sprouting. For example, MAP1B plays an inhibitory role in neurite branching as has been shown in adult DRG neurons from MAP1B^{-/-} mice (Bouquet et al., 2004). One of the first identified growth-associated proteins was GAP-43, discovered in 1981 (Bulsara et al., 2002). It is also associated with denervation-induced collateral sprouting of mossy fibers in the hippocampal formation of adult rats (Bendotti et al., 1997). Its role may be to regulate membrane mobility and F-actin polymerization associated with lipid rafts (Schmidt, 2004). It is unclear whether selective modulation of these cytoskeletal proteins would promote collateral sprouting. However, several of the treatments discussed in this review are known to induce GAP43, among other growth associated proteins. It would be useful to define the role of GAP43 in more detail, perhaps by genetic overexpression or inhibition in the corticospinal and sensory systems, respectively.

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