IB4-SAP Prevents Axotomy-Induced Sprouting of Aß Fibers

(continued from page 1)

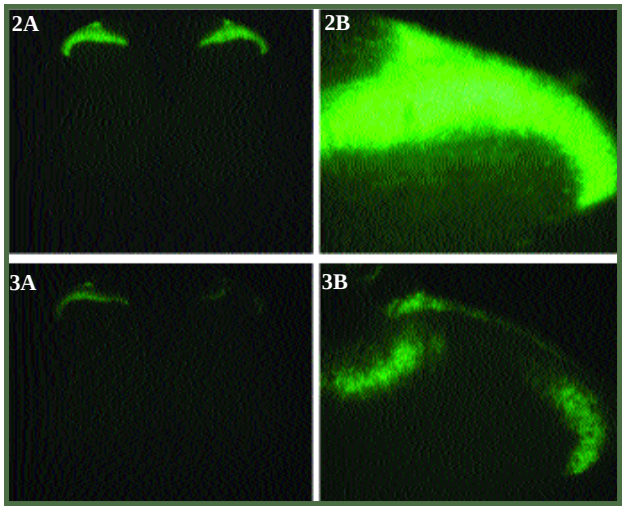
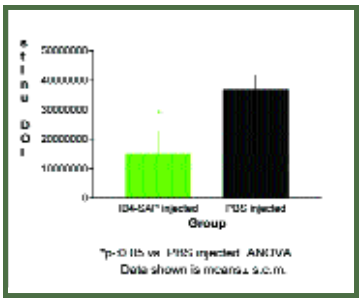


Fig. 2 Isolectin B4 labels the central terminals of small diameter nociceptors in lamina II of the spinal cord.

Fig 3. Intraneural IB4-SAP reduces Isolectin B4 staining in lamina II.

formalin and the relevant tissue was dissected and post-fixed overnight in the same fixative. The spinal cords and dorsal root ganglia were sectioned using a microtome at 60 and 40 µm thickness, respectively. Sections were stained using either IB4-FITC or goat anti-CTB-FITC.



Results: IB4-SAP treatment resulted in a reduction of IB4 staining in lamina II of the spinal cord as compared to PBS-treated animals (Fig. 2 compared to Fig. 3). This result confirms previous work demonstrating that, when injected into the sciatic nerve, IB4-SAP is retrogradely transported to the dorsal root ganglia where it is subsequently toxic to a subpopulation of small diameter nociceptors.[2] Image analysis demonstrated that the decrease in IB4 staining was both large and statistically significant as compared to PBS-injected controls ($p \leq 0.05$; Fig. 4).

As previously described[1] sections from rats that received a sciatic axotomy followed by intraneural CTB displayed an obvious presence of CTB in lamina II in addition to the motor neurons, laminae I and II-IV (Fig. 5). In these sections the borders of lamina II were not discernable. In all cases, lamina II was devoid of stain on the side contralateral to the injection.

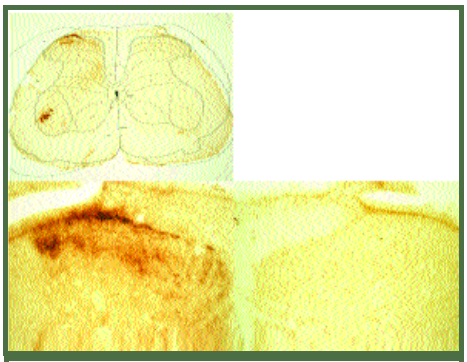


Fig. 5 Evidence of Aβ sprouting in lamina II following peripheral axotomy.

Spinal cord sections from rats that had received sham surgery followed by intraneural injection of CTB displayed prominent CTB immunostaining in the motor neurons, lamina I and lamina III-VI on the side ipsilateral to the injection (Fig. 6). Lamina II was devoid of positive immunostaining for CTB and boundaries between lamina I, II and III (arrows) were easily discernable.

Spinal cord sections from rats that received an intraneural IB4-SAP injection and sciatic axotomy followed by an intraneural injection of CTB (Fig. 7) displayed prominent CTB immunostaining in motor neurons, lamina I and lamina III-VI on the side ipsilateral to the injections. Lamina II was devoid of positive immunostaining for CTB.

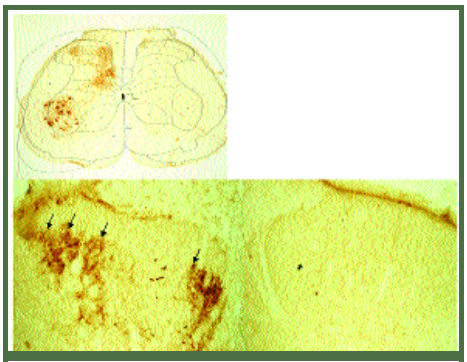


Fig. 6 No evidence of Aβ sprouting in lamina II following sham axotomy.

These data confirm the previous report that intraneural IB4-SAP ablates a population of small diameter nociceptors in the dorsal root ganglion and reduces the quantity of IB4-positive terminals in the spinal cord. Following ablation of this cell population, the extent of axotomy-induced CTB staining in lamina II is reduced as compared to control animals. This study strongly suggests that C-fibers contribute significantly to CTB staining that is observed in the spinal cord following nerve injury.

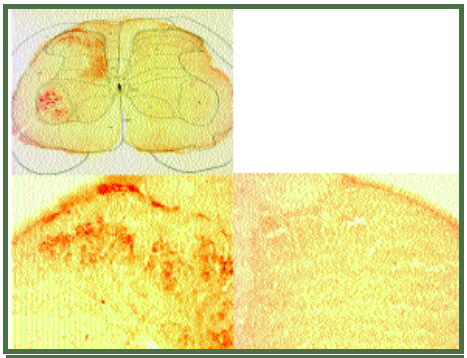


Fig. 7 No evidence of Aβ sprouting in lamina II following IB4-SAPinjection and peripheral axotomy.

References:

1. Woolf, CJ *et al.* (1992) *Nature*, 335:75-78.

2. Honda, CN *et al.* (2001) *Neuroscience*, 108:143-155.