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Neuronal Regeneration Enhances the Expression of the Immunophilin FKBP-12

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Immunophilins are a group of proteins that serve as receptors for the immunosuppressant drugs cyclosporin A and FK506. The immunophilin designated FK-506 binding protein-12 (FKBP-12) is concentrated more than 10 times higher in the brain than in immune tissues. The complex of FK506 and FKBP-12 inhibits the calcium activated phosphatase, calcineurin, increasing phosphorylated levels of calcineurin substrates with growth associated protein-43 (GAP-43), being most prominent in the brain. We now demonstrate an association of FKBP-12 with neuronal regeneration and GAP-43 disposition. Facial nerve crush markedly augments expression of FKBP-12 mRNA in the facial nucleus with a time course paralleling changes in GAP-43 mRNA. Following sciatic nerve lesions, similar increases in FKBP-12 mRNA occur in lumbar motor neurons and dorsal root ganglia neuronal cells. Increased FKBP-12 expression appears linked to regeneration rather than degeneration as facial nerve lesions elicited by ricin injection, which produce neuronal death without regeneration, fail to augment FKBP-12 expression in the facial nucleus. The time course for accumulation of FKBP-12 in sciatic nerve segments following nerve crush indicates rapid axonal transport at a rate similar to GAP-43.

[Key words: FK506, GAP-43, growth-associated proteins, immunosuppressants, calcineurin, axonal transport]

Immunophilin is a term referring to a group of proteins that bind the immunosuppressant drugs cyclosporin A and FK506 and mediate their pharmacologic actions (McKeon, 1991; Schreiber, 1991; Schreiber and Crabtree, 1992). The major immunophilins, cyclophilin and FK506 binding protein (FKBP), respectively, bind to cyclosporin A and FK506. Cyclophilin and FKBP occur in several isoforms, most of which are small soluble proteins (Harding et al., 1986; Siekierka et al., 1989). The immunosuppressant actions of these drugs arise when the drug–immunophilin complex associates with the calcium/calmodulin dependent protein phosphatase, calcineurin, to inhibit its phosphatase activity (Liu et al., 1991, 1992; Fruman et al., 1992; Swanson et al., 1992). Characterization of the immunophilins has focused almost exclusively upon lymphocytes and other cells of the immune system. Recently, we reported that levels of FKBP in the brain are more than 10 times greater than those in peripheral immune tissues (Steiner et al., 1992). Moreover, FKBP-12 mRNA and protein display striking regional variations in density which closely parallel those of calcineurin, suggesting coordinated functional activities.

Treatment of brain cytosol with FK506 enhances levels of several phosphorylated proteins, indicating that FKBP-12 and calcineurin are functionally linked in the brain. The physiological relevance of this enhanced phosphorylation has been demonstrated for nitric oxide synthase (NOS), which is a calcineurin substrate whose phosphorylation is augmented by FK506 (Dawson et al., 1993a). Phosphorylation of NOS decreases its catalytic activity (Bredt and Snyder, 1994). NOS inhibition prevents the neurotoxicity elicited by glutamate acting at NMDA receptors in cerebral cortical cultures (Dawson et al., 1991a, 1993b). Treatment of these cultures with FK506 also blocks NMDA neurotoxicity associated with inhibition of NOS catalytic actions (Dawson et al., 1993a).

GAP-43, a major neuronal phosphoprotein implicated in neuronal regeneration (Benowitz and Routtenberg, 1987; Snipes et al., 1987; Skene, 1989), is the most prominent phosphorylated protein whose levels are augmented by FK506 treatment (Steiner et al., 1992). Since phosphorylation of GAP-43 may facilitate its ability to target neuronal processes (Meiri et al., 1991), we sought a functional relationship between FKBP-12 and GAP-43. We now report enhanced expression of FKBP-12 coincident with enhanced expression of GAP-43 following peripheral nerve lesions.

Materials and Methods

In situ *hybridization and ^{3}H FK506 autoradiography.* Rats were perfused transcardially with 150–200 ml of ice cold phosphate-buffered saline (PBS) (0.1 M, pH 7.4). Tissues were removed and immediately frozen in isopentane (−80°C). Cryostat sections (18 μm thick) were cut and thaw mounted on gelatin coated slides.

In situ hybridization was performed as previously described (Steiner et al., 1992; Dawson et al., 1994), using antisense oligonucleotide probes end labeled with ^{35}S dATP. For FKBP-12, three separate oligonucleotides complementary to the following regions of the cloned cDNA (Maki et al., 1990; Standaert et al., 1990) were used: 70–114, 214–258, 441–485. For GAP-43, three separate antisense oligonucleotides complementary to nucleotides 961–1008, 1081–1128, 1201–1248 of the cloned cDNA (Rosenthal et al., 1987) were used. For calcineurin Aα antisense oligonucleotides complementary to the nucleotides 1363–1410 and 1711–1758, (Ito et al., 1989) and for calcineurin Aβ 1339–

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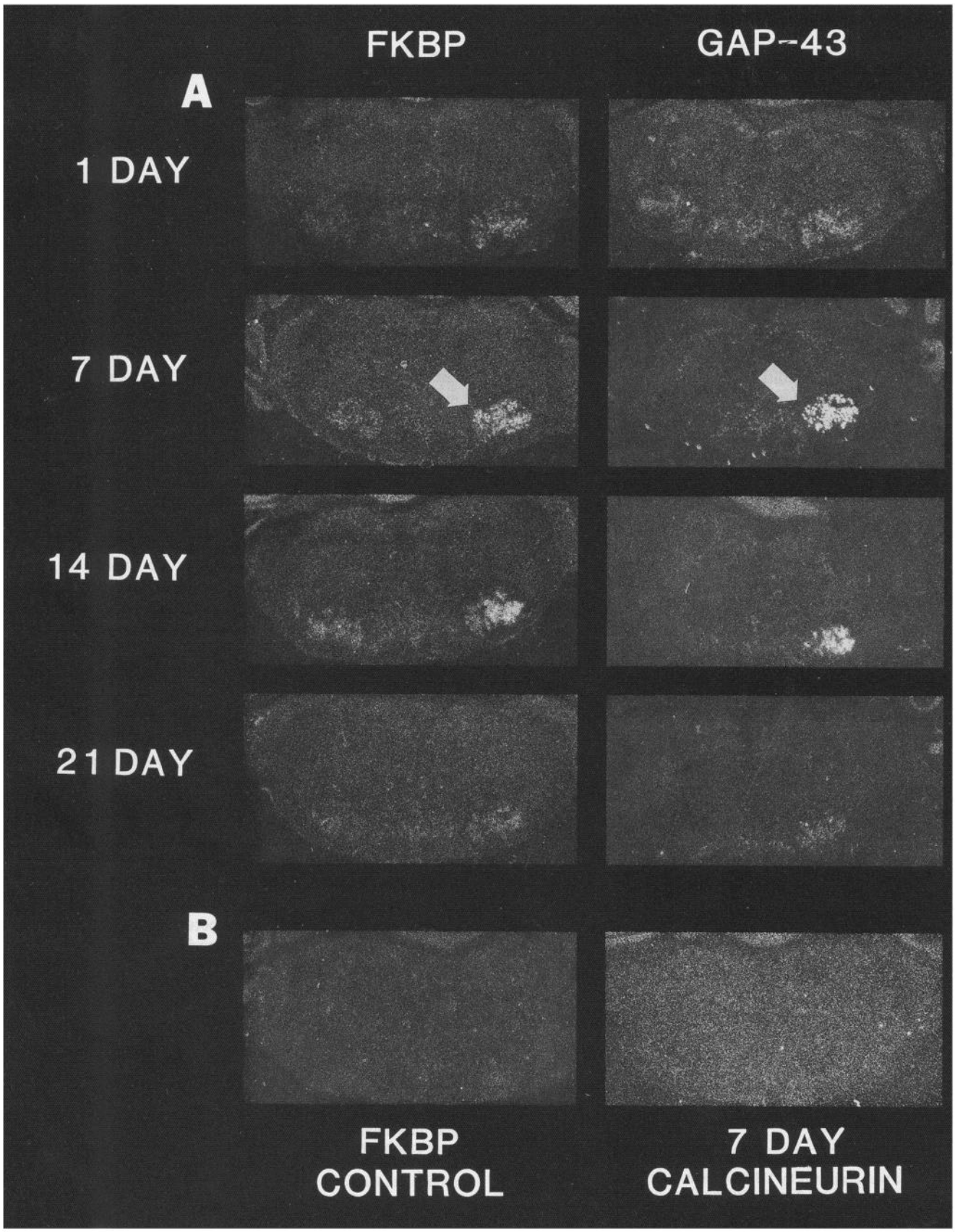

Figure 1. FKBP-12 and GAP-43 expression in the facial nucleus after nerve crush. *A, In situ* hybridization comparing the time course of expression of mRNA in the facial nucleus for FKBP-12 (*left*) and GAP-43 (*right*). The right facial nucleus is ipsilateral to the crush, and the left side is an unoperated control. *B, In situ* hybridization for FKBP-12 on an untreated control (*left*) and for calcineurin Aα, β 7 d following facial nerve crush (*right*). Experiments were replicated at least three times with similar results.

Table 1. ^{3}H-FK506 binding to sciatic nerve and growth cones

Tissue	B_{max} (fmol/5 mm segment)	B_{max} (pmol/mg protein)
A. ^{3}H-FK506 binding in sciatic nerve		
Adult rat		
Sciatic Nerve		22.1
Cerebral Cortex		48.0
Thymus		9.5
Spleen		8.0
Neonatal rat		
Forebrain		25.5
Growth cones		10.2
B. ^{3}H-FK506 binding after sciatic nerve crush		
Unoperated	31.8 ± 2.1	21.2 ± 1.4
7 d crush	136.5 ± 15.7*	40.1 ± 2.0*

^{3}H-FK506 binding was assayed as described in Materials and Methods. In A experiments were replicated three times with less than 10% variation. In B values are presented as the mean ± SEM (n = 3).

* $P \leq 0.05$, Students' t test for independent means.

1386 and 1569–1616 (Kuno et al., 1989) were used. Sections were thawed and allowed to dry, and then fixed for 5 min in 4% freshly depolymerized paraformaldehyde in PBS. Following two rinses in PBS, sections were acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine 0.9% NaCl (pH 8.0), and then dehydrated in graded alcohols, defatted in chloroform for 5 min, rehydrated to 95% ethanol and allowed to air dry. Hybridization was performed overnight at 37°C in buffer containing 50% deionized formamide, 10% dextran sulfate, 4 × SSC, 1 × Denhardt's solution, 20 mM phosphate buffer, 0.1 mg/ml salmon sperm DNA, 0.1 mg/ml yeast transfer RNA, 10 mM dithiothreitol, 2.0% betamercaptoethanol (BME), 1.0 mM EDTA and labeled probe (2,000,000 dpm/section). Following hybridization, sections were rinsed in 1 × SSC, 1.0% BME for 15 min at room temperature, and then twice for 10 min at 55°C, air dried and placed on film or dipped in Kodak NTB-2 emulsion. Sense probes corresponding to the antisense oligos were also used in hybridization experiments and gave no signal. Pretreatment with RNase also completely eliminated the hybridization.

^{3}H-FK506 autoradiography was carried out as described (Steiner et al., 1992; Dawson et al., 1994) on unfixed sections which were thawed and air dried before preincubation for 1 hr in buffer consisting of 50 mM HEPES, 2 mg/ml bovine serum albumin, 5% ethanol, and 0.02% Tween 20 pH 7.4. Sections were then exposed to 1 nM ^{3}H-FK506 (86.5 Ci/mMol; Du Pont-NEN, Boston, MA) for 1 hr at room temperature in preincubation buffer. Nonspecific binding was defined by addition of 1 μM FK506. Following incubation, the slides were washed 4 × 5 min in ice cold preincubation buffer and air dried. The radiolabeled sections were then juxtaposed to tritium-sensitive film or coverslips coated with Kodak NTB-2 emulsion.

Peripheral nerve crush axotomies. Sprague–Dawley rats (175–200 gm) were anesthetized with a mixture of Rompun (12 mg/kg) and Ketamine (30 mg/kg). Using aseptic techniques, the facial nerve was crushed with jewelers forceps 2 × 30 sec 2 mm distal to its exit from the stylomastoid foramen. Identical procedures were used to crush the sciatic nerve at the level of the mid-thigh. For ricin experiments, 0.5 μg of ricin RCA 60 (Sigma, St. Louis, MS) was injected at the crush site of the facial nerve according to the method of Streit and Kreutzberg (Streit and Kreutzburg, 1988) in 0.5 μl of PBS, 0.1% fast green.

Axonal transport studies. For axon transport experiments, classic ligature techniques were used following the methods of Tetzlaff et al. (1989). One week following sciatic nerve crush two collection ligatures (5/0 sutures) were placed on the nerve approximately 10 mm apart with the distal most ligature positioned 10 mm proximal to the initial crush sight. Six hours later, 5–3 mm segments of the nerve were collected from regions proximal to, distal to, and between the collection ligatures as illustrated in Figure 6. The nerve segments were prepared for ^{3}H-FK506 binding assays.

^{3}H-FK506 binding. Tissues were prepared for ^{3}H-FK506 binding by homogenizing in 10 volumes of 50 mM Tris-HCl, pH 7.4. Homogenates were centrifuged at 15,000 × g for 20 min at 4°C, and supernatants were collected and assayed for total protein concentration using the Coomassie blue dye binding assay (Pearce). ^{3}H-FK506 binding was carried out as described (Steiner et al., 1992) on aliquots containing 2 μg of total soluble protein in a final volume of 0.4 ml assay buffer consisting of 50 mM Tris-HCl, pH 7.4, 2 mg/ml bovine serum albumin, 250 pM ^{3}H-FK506. By varying concentrations of unlabeled FK506, estimates of the affinity and Bmax were obtained. Following incubation at 25°C for 60 min, 0.35 ml was layered over a 0.8 ml column of LH-20 Sephadex (Pharmacia LKB) and washed with 0.4 ml of assay buffer. The eluates were collected and counted in a scintillation counter. All binding studies were replicated at least three times with triplicate samples.

Growth cones. Growth cones were purified from 5 d old rat forebrain essentially as described by Gordon-Weeks and Lockerbie (1984) (Gordon-Weeks and Lockerbie, 1984).

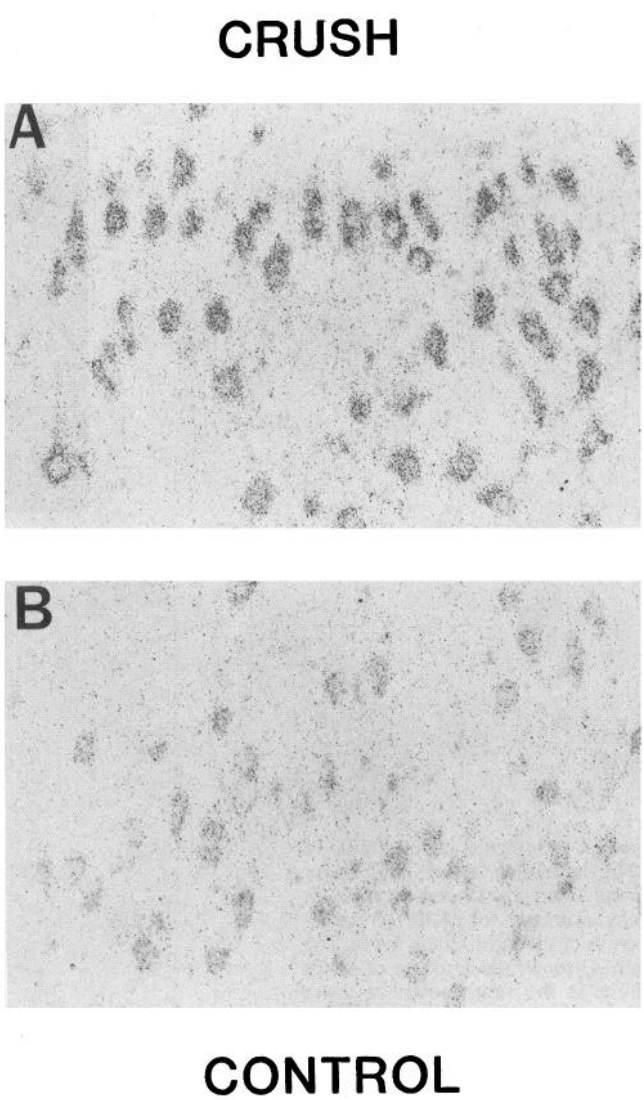

Figure 2. Localization of FKBP-12 to facial motor neurons following nerve crush. Bright-field photomicrographs of *in situ* hybridization for FKBP-12 in motor neurons of the facial nucleus 7 d after crush (*A*), and in motor neurons of control facial nucleus (*B*).

Results

High levels of FKBP in normal peripheral nerve and increases following nerve crush

If FKBP were physiologically associated with neuronal process extension in the actions of GAP-43, then one might anticipate substantial levels of FKBP in growth cones paralleling the enrichment of GAP-43 in this structure and low levels of FKBP

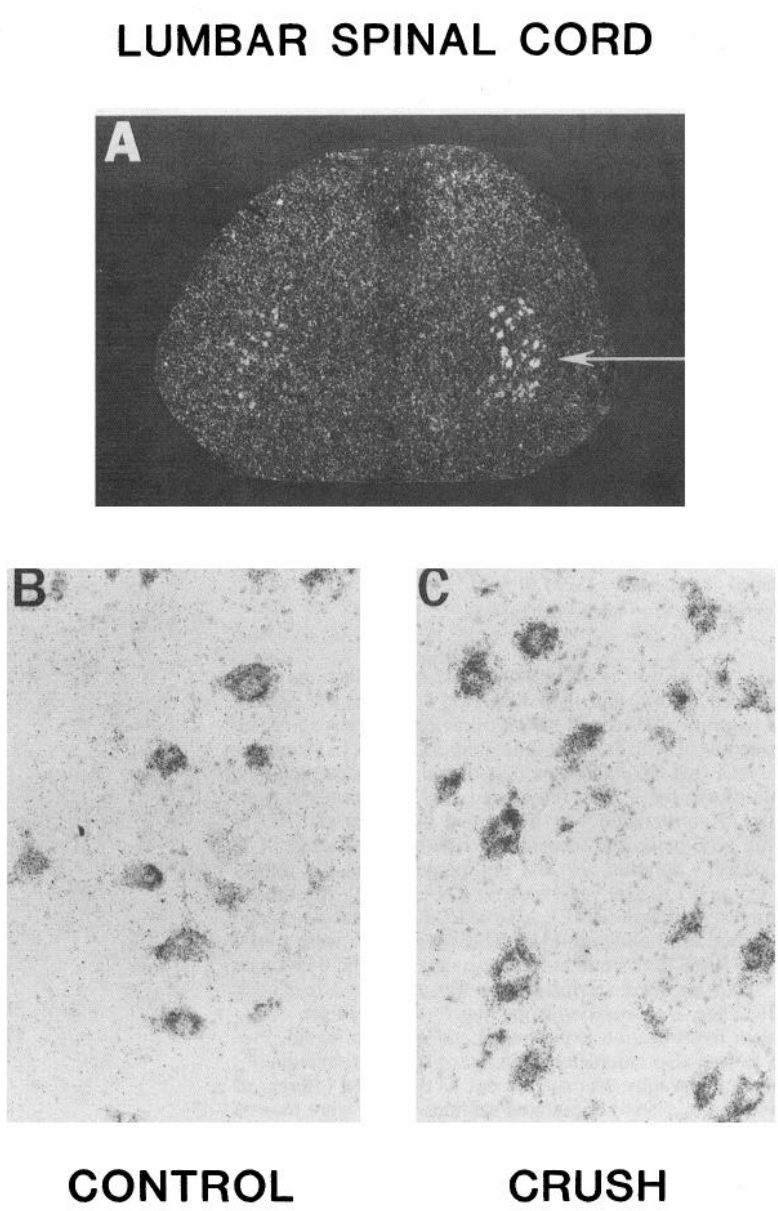

Figure 3. Upregulation of FKBP-12 mRNA in lumbar spinal cord motor neurons after sciatic nerve crush. *In situ* hybridization for FKBP-12 7 d after crush of the right sciatic nerve. Top panel (*A*) shows the response of motor neurons in the ventral horn of lower lumbar spinal cord (indicated by the *arrow*). Bright-field photomicrographs of corresponding motor neuron pools are shown in the bottom panels: *B*, left side contralateral to nerve crush; *C*, right side ipsilateral to the nerve crush. This experiment was repeated three times with similar results.

in peripheral nerve paralleling the low level of GAP-43 in peripheral nerve. Accordingly, we measured ^{3}H-FK506 binding in rat sciatic nerve, as well as in growth cones isolated from 5 d old rat pups, and compared values with those of the cerebral cortex and several peripheral tissues (Table 1A). Sciatic nerve binding levels are somewhat lower than those of the cerebral cortex and about two to three times levels in the thymus and spleen, which contain FKBP associated with lymphocytes. ^{3}H-FK506 binding levels in growth cones are 40% of values in neonatal rat forebrain (Table 1A).

Evidence for a role of FKBP in nerve regeneration comes from experiments in which we crushed the sciatic nerve of adult rats and 7 d later measured ^{3}H-FK506 binding in a 5 mm segment immediately proximal to the nerve crush, the region where GAP-43 accumulates following nerve crush (Tetzlaff et al., 1989) (Table 1B). Total binding in the segment proximal to the crush is quadrupled compared to control values. Since total protein is also substantially augmented in the proximal segment, ^{3}H-FK506 binding per milligram of protein is only doubled in the proximal segment.

Facial nerve lesions augment the coincident expression of FKBP and GAP-43

Following crush of the facial nerve, mRNA levels of GAP-43 increase in the facial nerve nucleus (Tetzlaff et al., 1989, 1991). Utilizing *in situ* hybridization, we examined mRNA levels of FKBP-12, GAP-43 and calcineurin following facial nerve crush (Fig. 1*A*). Striking enhancement of FKBP-12 and GAP-43 expression is observed. As early as 24 hr following facial nerve crush FKBP-12 expression is increased with peak levels evident

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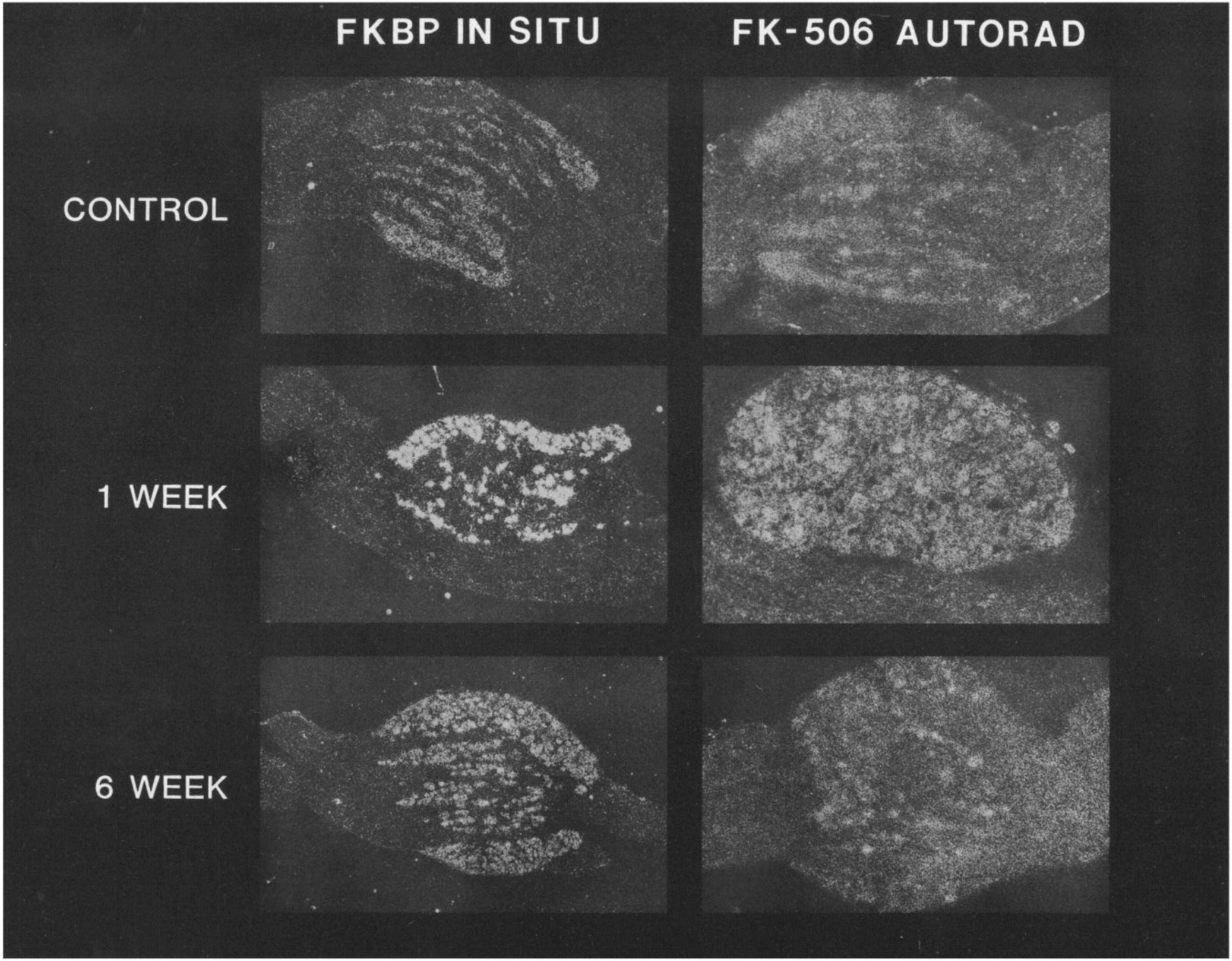
Figure 4. Induction of FKBP and FKBP-12 mRNA in the dorsal root ganglion 1 and 6 weeks after sciatic nerve crush. Dark-field photomicrographs of sections through the L4 dorsal root ganglion ipsilateral to sciatic nerve crush processed for FKBP *in situ* hybridization are shown in the *left panels* and for ^{3}H-FK506 autoradiography in the *right panels*. These results were replicated three times for each time point.


FKBP IN SITU
FK-506 AUTORAD
CONTROL
1 WEEK
6 WEEK

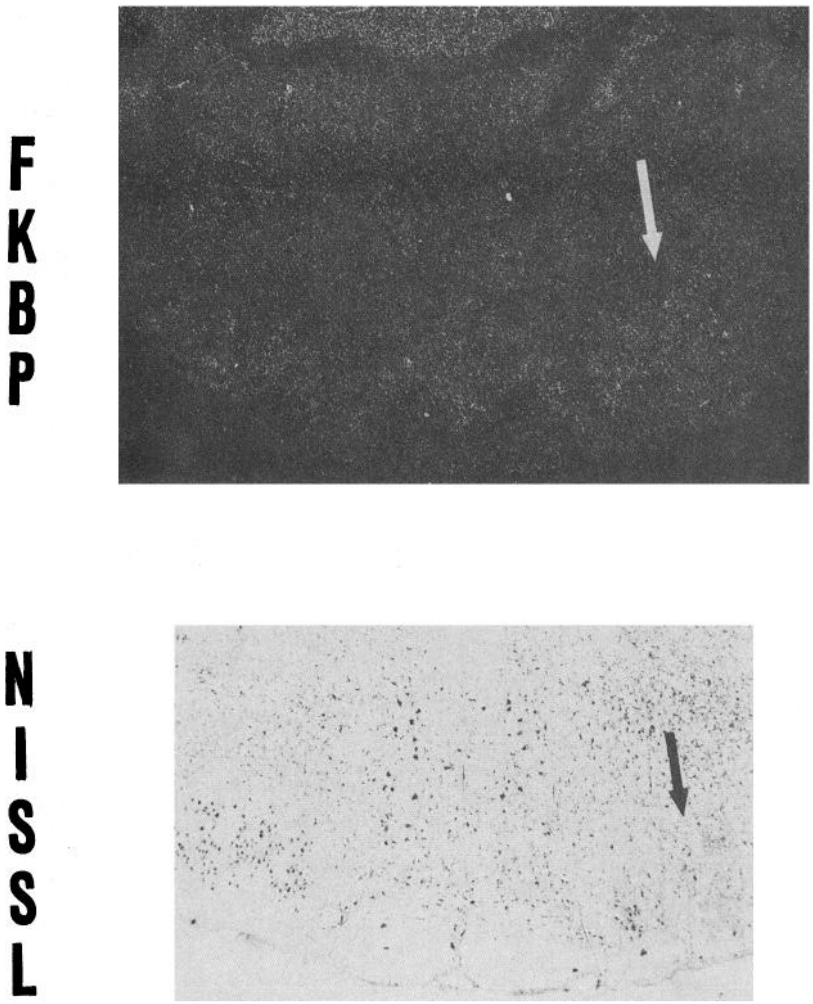


Figure 5. Ricin lesion of the right facial nerve. Nissl stain (*bottom panel*) reveals extensive degeneration of motor neurons in the right facial nucleus with an accompanying glial proliferation 7 d following injection of ricin into the facial nerve. *In situ* hybridization for FKBP mRNA 7 d after ricin lesion of the facial nerve/nucleus is shown in the *top panel.* This experiment was replicated three times with similar results.

at 1–2 weeks, while mRNA concentrations diminish substantially at 3 weeks. Transient increases in FKBP-13 and cyclophilin mRNA also occur following facial nerve crush (data not shown). GAP-43 mRNA levels follow a time course closely similar to those of FKBP-12. By contrast, no changes in calcineurin expression are detected at any of the time points examined (Fig. 1*B*). On the unlesioned side a modest increase in silver grains compared to control sections are observed. This is consistent with findings that contralateral neurons also respond to axotomy (Lieberman, 1971). Examination under higher magnification reveals that the increased levels of silver grains for FKBP-12 mRNA are confined to neuronal cell bodies (Fig. 2). No changes in ^{3}H-FK506 binding are observed in the facial nucleus following nerve crush (data not shown).

Following facial nerve crush, rats develop a facial nerve palsy, which is evident by the lack of whisker movement with functional recovery at 3 weeks coincident with the completion of nerve regeneration (Saika et al., 1991). In our rats we also observe a loss of whisker movement following nerve crush with a return of function at 3 weeks. Thus, the time course of increased expression of GAP-43 and FKBP-12 mRNA corresponds to the process of nerve regeneration.

Alterations in FKBP and GAP-43 associated with sciatic nerve regeneration

Following sciatic nerve lesions GAP-43 mRNA levels are enhanced in both spinal cord motor neurons and in dorsal root ganglia neuronal cells (Basi et al., 1987; Bisby, 1988; Hoffman, 1989; Van der Zee et al., 1989; Verge et al., 1990; Woolf et al., 1990; Schreyer and Skene, 1991; Sommervaille et al., 1991; Wiese and Emson, 1991; Chong et al., 1992; Wiese et al., 1992). In rats subjected to sciatic nerve crush, we observe a striking enhancement in mRNA levels for FKBP-12 in motor neurons at L-4, 5 (Fig. 3) and in dorsal root ganglia neuronal cells (Fig. 4) coincident with the reported enhancement of GAP-43 expression. At high magnification the FKBP-12 mRNA silver grains are localized to neuronal cell bodies (Fig. 3). We monitored FKBP protein levels by autoradiography of ^{3}H-FK506 binding under conditions in which it binds selectively to FKBP (Steiner et al., 1992) (Fig. 4). Increased FKBP is detected in the primary sensory neurons in the dorsal root ganglia, though no increases in protein levels are evident in motor neuronal cells following sciatic nerve crush (data not shown), consistent with the lack of elevated FKBP levels in the facial nucleus following crush of the facial nerve. This is consistent with findings that GAP-43 protein elevations are only detectable in primary sensory neurons in the dorsal root ganglia and not in motor nuclei following similar lesions (Van der Zee et al., 1989; Woolf et al., 1990; Schreyer and Skene, 1991).

The selective neuronal association of augmented FKBP-12 expression with regeneration is further supported by experiments with ricin. When injected into peripheral nerves ricin is transported back into the cell body which is destroyed without as-

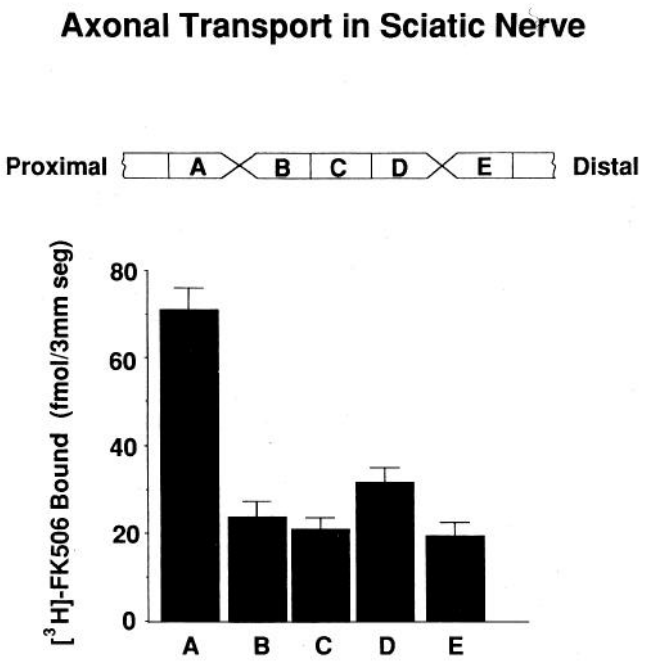


Figure 6. ^{3}H-FK-506 binding in segments of sciatic nerve 7 d following crush. The diagram illustrates the 3 mm segments of nerve taken: constrictions indicate positions of ligatures applied at day 7 for the 6 hr collection time as described in Materials and Methods. The distal ligature site is 10 mm proximal to the original crush site. Anterograde transport of FKBP is 124 mm/d. Data are the means ± SEM (n = 3).

sociated nerve regeneration (Wiley et al., 1982). We injected ricin into the facial nerve at the same site where crushes had been performed in other experiments and conducted *in situ* hybridization localization studies for FKBP-12 mRNA at 2, 4, and 7 d following ricin treatment (Fig. 5, data not shown). No increase in FKBP-12 mRNA is observed following ricin treatment. ^{3}H-FK506 binding is also unchanged after ricin injections (data not shown). Gliosis occurs both following ricin treatment and nerve crush. The failure of FKBP-12 mRNA to increase following ricin treatment fits with the selective neuronal localization of FKBP-12 mRNA in the facial nucleus.

FKBP is rapidly transported in the sciatic nerve

The failure of FKBP protein to increase in motor neurons following sciatic or facial nerve crush despite the increase in FKBP-12 mRNA suggests that the protein is rapidly transported out of the cell body into nerve processes. This fits with our earlier observations that FKBP mRNA is concentrated in granule cells of the cerebellum which contain low levels of FKBP protein, while FKBP protein levels are highly concentrated in the molecular layer in the cerebellum associated with the parallel fibers arising from granule cells (Steiner et al., 1992; Dawson et al., 1994). To examine for possible transport of FKBP, we crushed the sciatic nerve and 7 d later applied ligatures 10 and 20 mm proximal to the crush. Six hours following ligature, we monitored ^{3}H-FK506 binding in 3 mm segments spanning the area of the ligatures (Fig. 6). ^{3}H-FK506 binding levels are highest in the segment just proximal to the ligature 20 mm from the

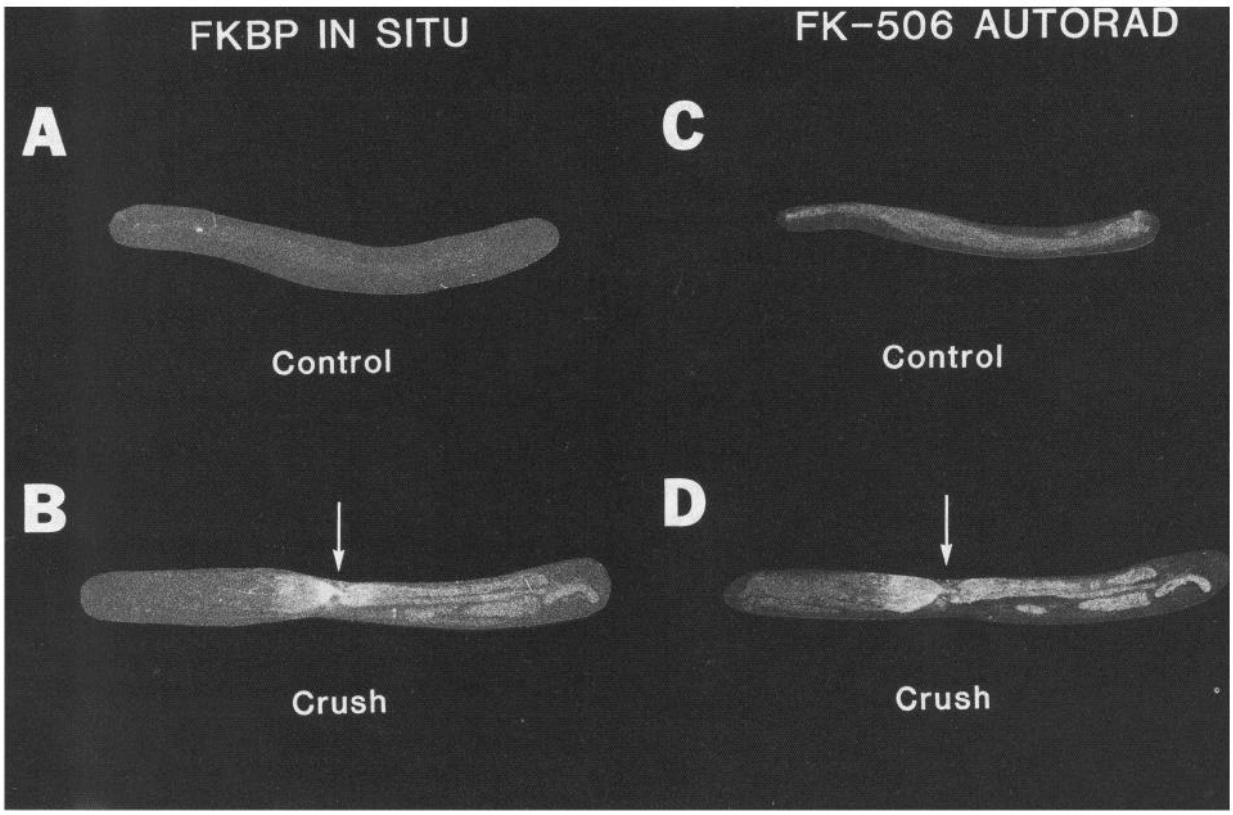


Figure 7. Transport of FKBP in the sciatic nerve. Dark-field photomicrographs of sections through a control (untreated) sciatic nerve and a 7 d sciatic nerve crush site processed for FKBP-12 *in situ* hybridization (*A*, *B*) and for ^{3}H-FK-506 autoradiography (*C*, *D*). *Arrows* indicate the sight of the nerve crush. This experiment was repeated three times with similar results.

crush, three to four times levels in the other segments. Based on the levels of ^{3}H-FK506 binding in segments A–D, we calculated the rate of anterograde transport for FKBP to be 124 mm per day, essentially the same as transport rate for GAP-43 (Skene and Willard, 1981a,b; Tetzlaff et al., 1989), among the most rapid for neuronal proteins (Grafstein and Forman, 1980).

To visualize the accumulation of FKBP following nerve crush, we applied a loose ligature to mark the site of crush of the sciatic nerve and conducted *in situ* hybridization for FKBP-12 mRNA as well autoradiography for ^{3}H-FK506 binding (Fig. 7). Most FKBP-12 mRNA and ^{3}H-FK506 binding accumulates immediately proximal to the crush to levels considerably higher than in control uncrushed sciatic nerve. Examination of the *in situ* hybridization and autoradiography preparations at high magnification reveals silver grains associated with neuronal fibers. Some silver grains occur in cells whose identity we could not determine definitively, so that they may be Schwann cells, macrophages, or fibroblasts (data not shown). Striking elevations in FKBP and its mRNA are also observed along the distal stump of the crushed sciatic nerve.

Discussion

Several findings suggest that FKBP-12 is a growth associated protein similar to GAP-43. Both occur at high levels in growth cones. Levels of both increase in neuronal cell bodies following peripheral nerve injury with a similar time course. Both are transported at the same rate. Moreover, FK506 through binding to FKBP-12, inhibits the protein phosphatase, calcineurin, and FK506 enhances phosphorylation of GAP-43 more than that of any other proteins evaluated (Steiner et al., 1992).

What function of FKBP-12 might relate to GAP-43? Phosphorylation of GAP-43 is thought to influence its activities. Mieri et al. (1991) reported increases in phosphorylated but not unphosphorylated GAP-43 protein as axons approach their targets, suggesting that phosphorylation of GAP-43 may be critical for this targeting process. Alternatively, GAP-43 may get targeted to growth cones by other means and then be more amenable to being phosphorylated. For instance, studies from Zuber et al. (1989) indicate that the targeting of GAP-43 to the growth cone can be attributed in large part to sequences in the N-terminus. The localization of FKBP-12 near GAP-43 at neuronal processes may reflect a role for FKBP-12 interacting with calcineurin to act as a physiological regulator of GAP-43 phosphorylation. In addition, FKBP-12/calcineurin may also regulate the phosphorylation of other growth associated phosphoproteins such as α- tubulin-1 (Tetzlaff et al., 1991). FKBP-12 only inhibits calcineurin when bound to a ligand such as FK506. Thus, in order for FKBP-12 to influence the phosphorylation of growth associated proteins, an endogenous ligand for FKBP-12 may exist that modulates calcineurin activity and the phosphorylation of its substrate proteins. FKBP-12 may be associated with other proteins and cellular compartments in addition to GAP-43. For instance, FKBP is highly enriched in peripheral nerves, whereas GAP-43 is barely detectable in peripheral nerves. The levels of GAP-43 expression in the normal, mature state are very low, and the small amounts that are transported down the nerve rapidly end up in nerve terminals. GAP-43 is highly enriched in growth cones (Meiri et al., 1986; Skene et al., 1986), whereas FKBP-12 shows considerably lower levels in growth cones than in the brain as a whole. Furthermore, GAP-43 is highly expressed in developing rat brain and FKBP-12 is not (Dawson et al., 1994). In addition, FKBP-12 may act at other sites besides calcineurin. FKBP-12 binds to the ryanodine receptor (Jayaraman et al., 1992; Timerman et al., 1993) and the inositol-triphosphate (IP_3) receptor (A. M. Cameron, J. P. Steiner, D. M. Sabatini, A. I. Kaplan, L. Walensky, and S. H. Snyder, unpublished observations). In skeletal muscle sarcoplasmic reticulum, FK506 dissociates FKBP-12 from the ryanodine receptor to facilitate Ca^{2+} induced Ca^{2+} release mechanism (Brillantes et al., 1993) and FK506 dissociates FKBP-12 from the IP_3 receptor to facilitate IP_3 mediated calcium release (A. Cameron, J. S. Steiner, D. M. Sabatini, A. I. Kaplin, L. Walensky, and S. H. Snyder, unpublished observations). Type I receptors of the TGF-β family also interact with FKBP-12 and excess FK506 competes for binding to TGF-β Type I receptors (Wang et al., 1994). Thus, FKBP-12 may regulate other targets that may have important functions in neuronal regeneration, although the role of these additional targets in neuronal regeneration has not been clarified. A prediction of whether FKBP-12 plays a role in neuronal regeneration is that FK506 should influence neurite outgrowth. In PC-12 cells, we have shown that low nanomolar concentrations of FK506 markedly enhance differentiation and process extension elicited by NGF (Lyons et al., 1994). Also, in isolated neonatal rat dorsal root ganglia as little as 1 pM FK506 increases the neurite extension.

Following both sciatic and facial nerve crushes calcineurin mRNA does not change despite marked increases in FKBP-12 and GAP-43. This suggests that rate-limiting steps in phosphorylation of neuronal proteins associated with process extension more likely involve the dynamics of FKBP-12 than calcineurin. This finding emphasizes the potential importance of FKBP-12 as a physiological regulator of neuronal protein phosphorylation. Alternatively, FKBP-12 may influence neuronal regeneration through its other targets that are potentially not related to protein phosphorylation.

The cellular localization of FKBP might be an important determinant of its functional activities. In the brain we have only detected FKBP prominently in neurons (Steiner et al., 1992; Dawson et al., 1994). However, we observed limited amounts of FKBP in white matter, which could reflect non-neural elements of nerves. In the sciatic nerve distal to the crush, FKBP-12 protein and mRNA levels increase and, at high magnification, appear associated with cells. Since the sciatic nerve contains no neuronal cell bodies, FKBP-12 presumably occurs in non-neural cells. Unfortunately, despite repeated efforts we have not been able to obtain micrographs of sufficient resolution to discriminate between Schwann cells, fibroblasts, and macrophages. It is unclear whether FKBP-12 made in these cells influences GAP-43, other calcineurin substrates or its additional targets in nerves or whether this pool of FKBP-12 serves a nonneural function. Interestingly, Tetzlaff et al. (1989) observed substantial levels of GAP-43 protein in Schwann cells surrounding the regenerating sciatic nerve and Woolf et al. (1992) showed an enormous upregulation in GAP-43 in Schwann cells that have lost their axonal contacts, particularly at the muscle endplate. Conceivably, during particular states of differentiation FKBP-12 and GAP-43 may interact in the Schwann cells as well as in neurons.

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