Title
p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway.

Permalink
https://escholarship.org/uc/item/4xm667p6

Journal
The Journal of cell biology, 177(6)

ISSN
0021-9525

Authors
Sachs, Benjamin D
Baillie, George S
McCall, Julianne R
et al.

Publication Date
2007-06-01

DOI
10.1083/jcb.200701040

Peer reviewed
Introduction

Tissue scarring, characterized by cell activation, excessive deposition of ECM, and extravascular fibrin deposition, is considered a limiting factor for tissue repair. Fibrin, the major substrate of the serine protease plasmin, is a provisional matrix deposited after vascular injury (Bugge et al., 1996). The two plasminogen activators (PAs), namely tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), and their inhibitors, such as plasminogen activator inhibitor-1 (PAI-1), are key modulators of scar resolution by spatially and temporally regulating the conversion of plasminogen to plasmin resulting in fibrin degradation and ECM remodeling (Lijnen, 2001). In the peripheral nervous system, previous work by us and others showed that inhibition of fibrinolysis in mice deficient in plasminogen or tPA exacerbated axonal damage (Akassoglou et al., 2000) and impaired functional recovery after nerve injury (Siconolfi and Seeds, 2001). In accordance, mice deficient for fibrinogen showed increased regenerative capacity (Akassoglou et al., 2002). Studies of fibrin deposition in human diseases, in combination with experiments from mice deficient in plasminogen and PAs, have provided information about a wide range of physiological and pathological conditions, such as wound healing, metastasis, atherosclerosis, lung ischemia, rheumatoid arthritis, muscle regeneration, and multiple sclerosis (MS) (Degen et al., 2001; Adams et al., 2004).

We have discovered a new mechanism in which phosphodiesterase PDE4A4/5 interacts with p75NTR to enhance cAMP degradation. The p75NTR-dependent down-regulation of cAMP results in a decrease in extracellular proteolytic activity. This mechanism is supported in vivo in p75NTR-deficient mice, which show increased proteolysis after sciatic nerve injury and lung fibrosis. Our results reveal a novel pathogenic mechanism by which p75NTR regulates degradation of cAMP and perpetuates scar formation after injury.
However, the molecular mechanisms that regulate proteolytic activity remain unclear.

In our current work, we focus on the mechanisms that regulate fibrinolysis after injury. Our previous studies demonstrated a correlation between fibrin deposition and expression of p75 neurotrophin receptor (p75NTR) after nerve injury (Akassoglou et al., 2002). Up-regulation of p75NTR is observed in MS (Dowling et al., 1999), stroke (Park et al., 2000), and spinal cord (Beattie et al., 2002) and sciatic nerve injury (Taniuchi et al., 1986), all of which are associated with fibrin deposition. p75NTR is also expressed in non-neuronal tissues (Lomen-Hoerth and Shooter, 1995) and is up-regulated in non-neuronal system diseases associated with defects in fibrin degradation, such as atherosclerosis (Wang et al., 2000), melanoma formation (Herrmann et al., 1993), lung inflammation (Renz et al., 2004), and liver disease (Passino et al., 2007). p75NTR has been primarily characterized as a modulator of cell death (Wang et al., 2000) and differentiation (Passino et al., 2007) in non-neuronal tissues. The expression of p75NTR by cell types such as smooth muscle cells and hepatic stellate cells, which actively participate in tissue repair by migration, and secretion of ECM and extracellular proteases, raises the possibility for a functional role of p75NTR in disease pathogenesis that extends beyond apoptosis and differentiation.

We find that p75NTR is involved in the regulation of proteolytic activity and fibrin degradation. Mice deficient for p75NTR (Lee et al., 1992) show increased proteolytic activity and decreased fibrin deposition in two disease models: sciatic nerve injury and lung fibrosis. p75NTR regulates proteolytic activity by simultaneously down-regulating tPA and up-regulating PAI-1 via a novel cAMP/PKA pathway. p75NTR decreases cAMP via interaction with the cAMP-specific phosphodiesterase (PDE) isoform PDE4A4/5. This is of particular note, as selective PDE4 inhibitors have an anti-inflammatory action and have potential therapeutic utility in inflammatory lung disease, as well as in a wide range of neurologic diseases such as depression, spinal cord injury, MS, and stroke (Gretarsdottir et al., 2003; Nikulina et al., 2004; Houslay et al., 2005). Overall, the regulation of plasminogen activation by p75NTR identifies a novel pathogenic mechanism whereby p75NTR interacts with PDE4A4/5 to degrade cAMP and thus perpetuates scar formation that could possibly render the environment hostile for tissue repair.

Results

Fibrin deposition is reduced in p75NTR−/− mice

To examine whether p75NTR regulates fibrin deposition in the sciatic nerve we compared fibrin levels in wild-type (wt) and p75NTR−/− mice after injury. In wt mice, there is a dramatic increase of fibrin deposition (Fig. 1 c) and p75NTR expression (Fig. 1 d) after injury, when compared with uninjured nerves (Fig. 1, a and b). In contrast, p75NTR−/− mice show reduced fibrin deposition after injury (Fig. 1 e). Quantification of immunoblots reveals that p75NTR−/− mice have decreased fibrin by threefold 3 d and fourfold 8 d after injury (Fig. 1 g). Quantification of fibrin immunostaining also reveals that p75NTR−/− mice have significantly decreased fibrin (Fig. 1 h, P < 0.003). These results suggest that loss of p75NTR decreases the levels of fibrin in the sciatic nerve after injury.

p75NTR regulates expression of tPA in the sciatic nerve after crush injury

Analysis of total fibrinogen levels were similar in the plasma of wt and p75NTR−/− mice (unpublished data), suggesting the decrease in fibrin deposition is not the result of hypofibrinogenemia. Because fibrin removal depends on proteolytic activity (Bugge et al., 1996), we hypothesized that the decreased fibrin in the p75NTR−/− mice reflects an up-regulation of the proteolytic activity. p75NTR−/− mice have increased proteolytic activity (Fig. 2 b)
when compared with wt mice (Fig. 2 a) that is statistically significant (Fig. 3 i, P < 0.05). Uninjured nerves exhibit minimal proteolytic activity (Fig. 2 i), as expected (Akassoglou et al., 2000). Injured p75NTR−/− sciatic nerves do not show lysis of fibrin in the absence of plasminogen (Fig. 2 c), suggesting that the proteolytic activity is plasminogen dependent.

The tPA/plasmin system regulates fibrin clearance after nerve injury (Akassoglou et al., 2000). A specific tPA inhibitor, tPASTOP, blocks proteolytic activity in p75NTR−/− mice (Fig. 2 d). p75NTR is strongly activated by withdrawal of axons (Lemke and Chao, 1988) and its expression correlates with proliferating, non-myelin producing Schwann cells (SCs) (Zorick and Lemke, 1996). After sciatic nerve injury both p75NTR (Fig. 2 e, red) and tPA (Fig. 2 e, green) increase when compared with uninjured controls (Fig. 2 j), but show little colocalization (Fig. 2, e and h), suggesting that p75NTR-reexpressing SCs do not express tPA. Expression of tPA (Fig. 2 k, red) and p75NTR (Fig. 2 l, red) in SCs is confirmed using double immunofluorescence with the SC marker S100 (Fig. 2, k and l; green).

**Genetic loss of tPA rescues the effects of p75NTR deficiency**

To examine genetically whether the increased proteolytic activity in the p75NTR−/− mice was due to tPA, we crossed p75NTR−/− mice with tPA−/− mice and generated p75NTR−/−tPA−/− double-knockout mice. p75NTR−/− mice show a decrease in fibrin deposition (Fig. 3 b) and an increase in proteolytic activity (Fig. 3 f), compared with wt control mice (Fig. 3, a and e, respectively). In contrast, p75NTR−/−tPA−/− mice show increased fibrin deposition (Fig. 3 c) when compared with p75NTR−/− mice (Fig. 3 b) and no evidence of proteolytic activity (Fig. 3 g). As a control, tPA−/− mice also show no evidence of proteolytic activity after sciatic nerve crush injury (Fig. 3 h), as described previously (Akassoglou et al., 2000). Quantification of proteolytic activity is shown in Fig. 3 i. The evidence derived from the genetic depletion of tPA in the p75NTR−/−tPA−/− mice (p75NTR−/−tPA−/− mice, Fig. 3 g) are in accordance with the pharmacologic inhibition of tPA activity in the p75NTR−/− sciatic nerve using tPASTOP (Fig. 2 d). Overall, these results suggest that up-regulation of proteolytic activity in the sciatic nerve of p75NTR−/− mice is due to up-regulation of tPA.

**p75NTR−/− SCs show increased expression of tPA and increased fibrinolysis**

Because SCs are a major source for tPA after injury, we isolated primary SCs from wt and p75NTR−/− mice and cultured them on a three-dimensional (3D) fibrin gel. Wt SCs, which express high levels of p75NTR, form a monolayer on the fibrin gel (Fig. 4 a). In contrast, p75NTR−/− SCs degrade the fibrin gel (Fig. 4 b) and show a 2.7-fold increase of fibrin degradation (Fig. 4 c). p75NTR−/− SCs show a sixfold increase in tPA levels, when compared with wt SCs (Fig. 4 d; P < 0.01). These results suggest that p75NTR down-regulates tPA activity and blocks fibrin degradation in SCs in vitro.

**Expression of p75NTR inhibits tPA and fibrinolysis**

After finding a biological function for p75NTR in the regulation of tPA using SCs and sciatic nerves from p75NTR−/− mice, we used stable and transient transfections of p75NTR as well as

---

**Figure 2.** p75NTR regulates expression of tPA in the sciatic nerve after crush injury. In situ zymography in the presence of plasminogen on wt (a) and p75NTR−/− (b) mice and in the absence of plasminogen (c) or in the presence of plasminogen and tPASTOP (d) in p75NTR−/− mice. Arrows indicate the lytic zone. Double immunofluorescence for tPA (green) or p75NTR (red) on wt (e and h), p75NTR−/− (f) and p75NTR−/−tPA−/− mice (g). Uninjured wt sciatic nerve exhibits minimal proteolytic activity (i) and minimal tPA and p75NTR immunoreactivity (l). Zymographies have been performed on n = 10 wt and n = 10 p75NTR−/− mice. Representative images are shown. tPA (k) and p75NTR (l) expression in SCs was verified by double immunofluorescence with an S100 (SC marker) antibody. Arrows indicate double-positive cells (k and l, yellow). The experiment was repeated at two different time points (4 and 8 d after crush injury) in n = 4 mice per genotype per time point and representative images are shown. Bar: 400 μm (a–d, i), 150 μm (e–g, j), 20 μm (h, k, and l).
siRNA against p75NTR to test the properties of p75NTR in heterologous systems. To examine whether p75NTR could inhibit fibrin degradation, we first used NIH3T3 fibroblasts stably transfected with p75NTR that exhibit high levels of p75 NTR (10^5 receptors/cell) (Hsu and Chao, 1993). NIH3T3 cells on a 3D fibrin gel degrade fibrin (Fig. 5a), whereas NIH3T3p75 NTR cells do not (Fig. 5b). Expression of p75NTR inhibits fibrin degradation by 12-fold (Fig. 5c; P < 0.001). NIH3T3 cells form lytic areas (Fig. 5d), whereas NIH3T3p75NTR cells grow uniformly on fibrin (Fig. 5e). NIH3T3 cells fully degrade the plasmin substrate casein (Fig. 5f) but NIH3T3p75NTR cells do not degrade casein (Fig. 5g), suggesting impaired proteolysis in NIH3T3p75NTR cells. Aprotinin, a general inhibitor of serine proteases, completely inhibits fibrin degradation by NIH3T3 cells (not depicted).

In fibroblasts both tPA and uPA are involved in activation of plasminogen and fibrin degradation. tPA activity is significantly decreased in the NIH3T3p75NTR cells (Fig. 5h). In contrast, expression of p75NTR has no effect on uPA activity (Fig. 5i).

tPA is a transcriptionally regulated immediate-early gene (Qian et al., 1993). Indeed, expression of p75NTR down-regulates tPA transcripts (Fig. 5j). In addition, mRNA of PAI-1 is also up-regulated in NIH3T3p75NTR cells (Fig. 5j). Real-time quantitative PCR shows a 10.1-fold decrease in tPA mRNA, a fourfold increase in PAI-1 mRNA, and a twofold decrease in uPA mRNA in NIH3T3p75NTR cells. Upon expression of p75NTR, the decrease of uPA RNA does not affect uPA activity (Fig. 5i). In contrast, the decrease of tPA RNA in NIH3T3p75NTR cells results in a corresponding decrease in tPA activity (Fig. 5h; P < 0.01).

Figure 3. Loss of tPA rescues the effects of p75NTR deficiency in plasminogen activation and fibrin deposition in the sciatic nerve. Increased fibrin deposition in the crushed sciatic nerve of p75NTR−/− tPA−/− mice (c), when compared with crushed p75NTR−/−/tPA−/− sciatic nerve (b). Wt (a) and tPA−/− (d) nerves are used for control. In situ zymography shows lack of proteolytic activity in the crushed p75NTR−/−/tPA−/− sciatic nerves (n = 5) (g), when compared with crushed p75NTR−/− sciatic nerves (n = 20) (f). Crushed wt (e) and tPA−/− (h) nerves are used for control. Fibrin immunostainings and zymographies were performed on n = 5 p75NTR−/−/tPA−/−, n = 20 p75NTR−/−, n = 20 wt, n = 5 tPA−/− mice. Representative images are shown. (i) Quantification of proteolytic activity 4 d after crush injury shows statistically significant increase for proteolytic activity in p75NTR−/− mice. Quantification results are based on n = 5 p75NTR−/−, n = 5 p75NTR−/−/tPA−/−, n = 5 tPA−/− and n = 4 wt mice. Bar graph represents means ± SEM (*, P < 0.05; by ANOVA). Bar: 50 μm (a–d), 300 μm (e–h).

Figure 4. p75NTR-mediated regulation of tPA and fibrinolysis in SCs. Primary SC cultures from wt (a) or p75NTR−/− mice (b) on a 3D fibrin gel. Arrowheads indicate the border of fibrin degradation. Quantification of fibrin degradation (c) and tPA activity (d) from wt and p75NTR−/− SCs. Experiments were performed three times in duplicates. Representative images are shown. Bar graph represents means ± SEM (P < 0.01; by t test). Bar, 130 μm.
After injury, sciatic nerves of \(p75^{NTR}^{-/-}\) mice show a fourfold increase of \(tPA\) RNA when compared with wt (Fig. 5 k). Moreover, \(p75^{NTR}^{-/-}\) mice show an increase in \(tPA\) RNA in primary cerebellar granule neurons (CGNs) (Fig. S1 c, available at http://www.jcb.org/cgi/content/full/jcb.200701040/DC1), and increased proteolytic activity in the cerebellum (Fig. S1, a and b). Overall, these data suggest that expression of \(p75^{NTR}\) inhibits the \(tPA/plasmin\) system both in vivo in the cerebellum and after sciatic nerve injury, as well as in vitro in primary neurons, SCs, as well as fibroblasts.

\(p75^{NTR}\) regulates \(tPA\) and PAI-1 via a PDE4/cAMP/PKA pathway

Transcriptional regulation of \(tPA\) depends on the cAMP/PKA pathway (Medcalf et al., 1990). Indeed, elevation of cAMP, using dibutyryl-cAMP (db-cAMP), overcomes the inhibitory effect of \(p75^{NTR}\) (Fig. 6 a). Moreover, cAMP elevation, elicited using the general PDE inhibitor IBMX, elevates \(tPA\) activity in NIH3T3p75\(^{NTR}\) to the levels seen in NIH3T3 cells (Fig. 6 b). IBMX does not affect basal levels of \(tPA\) in NIH3T3 cells (Fig. 6 b). These data suggest that PDE activity is required for the \(p75^{NTR}\)-induced \(tPA\) decrease.

PKA activity is decreased in NIH3T3p75\(^{NTR}\) cells (Fig. 6 c, lanes 3 and 4) compared with NIH3T3 cells (Fig. 6 c, lanes 1 and 2), suggesting that \(p75^{NTR}\) expression reduces PKA activity. KT5720, a specific PKA inhibitor, decreases \(tPA\) activity in NIH3T3 cells (Fig. 6 b). Because the cAMP/PKA pathway enhances \(tPA\) transcription and suppresses PAI-1 secretion (Santell and Levin, 1988), we tested whether the cAMP/PKA pathway influences the \(p75^{NTR}\) regulation of \(tPA\) and PAI-1. Forskolin-induced cAMP elevation increases, whereas KT5720-induced PKA inhibition decreases \(tPA\) RNA in NIH3T3 cells (Fig. 6 d). Forskolin treatment of NIH3T3p75\(^{NTR}\) cells also increases both \(tPA\) RNA (Fig. 6 d) and activity (not depicted), whereas forskolin decreases PAI-1 RNA in both NIH3T3 and NIH3T3p75\(^{NTR}\) cells (Fig. 6 e).

Similar to NIH3T3 cells, elevation of cAMP increases the activity of \(tPA\) in both wt and \(p75^{NTR}^{-/-}\) SCs (Fig. 6 f). Brain-derived neurotrophic factor (BDNF)/TrkB signaling has been shown to regulate \(tPA\) in primary cortical neurons (Fiumelli et al., 1999). In contrast to cortical neurons, SCs are known to express minute levels of TrkB but high levels of \(p75^{NTR}\) (Cosgaya et al., 2002). We show here that treatment of SCs with either BDNF or nerve growth factor (NGF) has no effect on \(tPA\) (Fig. 6 f). Similar results are obtained after treatment of SCs with pro-NGF, the high-affinity ligand of \(p75^{NTR}\) (Lee et al., 2001) (unpublished data). In addition, in NIH3T3 and NIH3T3p75\(^{NTR}\) cells, which do not express Trk receptors, the \(p75^{NTR}\)-mediated suppression of \(tPA\) activity occurs independent of neurotrophins or serum (unpublished data). In accordance, in NIH3T3 cells transient expression of the intracellular domain (ICD) of \(p75^{NTR}\) decreases \(tPA\) similar to the full-length (FL) \(p75^{NTR}\) (Fig. 6 g).
These data suggest that neurotrophin/p75NTR signaling is not involved in the regulation of tPA in SCs and fibroblasts and that regulation of tPA by p75NTR is independent of neurotrophins.

**p75NTR** decreases cAMP via PDE4

Because the effects of p75NTR were overcome by elevating cAMP, we examined whether p75NTR reduced cAMP levels. Indeed, cAMP is decreased 7.8-fold in NIH3T3p75NTR cells (Fig. 7 a; P < 0.0001). Transient expression of p75NTR in NIH3T3 cells decreases levels of cAMP (Fig. 7 b; P < 0.0005). Furthermore, siRNA knockdown against p75NTR leads to increased cAMP levels in both NIH3T3 and NIH3T3p75NTR cells. Inhibition of PKA by KT5720 decreases cAMP levels (Fig. 7 c). Overall, these data suggest a neurotrophin-independent PDE4/cAMP pathway downstream of p75NTR, which consequently leads to decreases in extracellular proteolysis.

Down-regulation of cAMP can be mediated either by inhibition of cAMP synthesis via the action of G_{i}, a G protein that inhibits adenylyl cyclase or via the action of PDEs. Treatment of cells with pertussis toxin (PTX) that blocks interactions between the G_{i} and G protein coupled receptors, does not rescue the p75NTR-mediated down-regulation of cAMP (Fig. 7 a; P > 0.5). In contrast, the PDE inhibitor IBMX resulted in significant increase of cAMP in NIH3T3 cells (Fig. 7 a; P < 0.000001). Use of specific chemical inhibitors for PDE isoforms shows that only rolipram, a specific inhibitor of PDE4, significantly increases cAMP levels in NIH3T3p75NTR cells (Fig. 7 a; P = 0.051), suggesting that the p75NTR-induced cAMP decrease is mediated via PDE4.

**Figure 6.** p75NTR regulates tPA and PAI-1 via a PDE4/cAMP/PKA pathway. (a) db-cAMP induces fibrinolysis in NIH3T3p75NTR cells. (b) IBMX increases tPA activity of NIH3T3 cells to the levels of NIH3T3p75NTR cells (P < 0.0001). (c) PAI-1 activity assay shows decrease of tPA activity in NIH3T3 and NIH3T3p75NTR cells (P < 0.0001). (d) Forskolin increases cAMP levels in NIH3T3 and NIH3T3p75NTR cells. Inhibition of PKA by KT5720 decreases PKA expression. (e) Quantification of PAI-1 mRNA shows a fourfold increase of PAI-1 mRNA in NIH3T3p75NTR cells compared with NIH3T3 cells. (f) Forskolin increases tPA activity in both wt (P < 0.001) and p75NTR-/- (P < 0.00001) SCs. NGF and BDNF do not affect activity of tPA (P < 0.8 and P > 0.3, respectively). (g) Transient overexpression of FL p75NTR or p75 ICD leads to decreased levels of tPA in NIH3T3 cells. Experiments were performed at least 5 times in duplicates. *, P < 0.0001; **, P < 0.05; ***, P < 0.01. NS: non-significant. Bar graphs represent means ± SEM (statistics by ANOVA).
p75NTR interacts with PDE4A4/5 and targets cAMP degradation to the membrane

Recruitment of PDE4 to subcellular structures such as the plasma membrane concentrates the activity of PDEs and reduces PKA activity by enhancing degradation of cAMP (Brunton, 2003; Houlsay and Adams, 2003). We therefore examined whether p75NTR regulates cAMP via recruitment of PDE4. In NIH3T3p75NTR cells, p75NTR coimmunoprecipitates (co-IPs) with endogenous PDE4A (Fig. 8 a). No association is observed with the other three PDE4 sub-families, namely PDE4B, PDE4C, or PDE4D (unpublished data), suggesting that the effect was PDE4A specific. Based on the molecular weight of PDE4A at 109 kD, we determined that p75NTR co-IPs with the PDE4A5 isoform. Endogenous co-IP in CGNs (Fig. S3 a, available at http://www.jcb.org/cgi/content/full/jcb.200701040/DC1) and in injured sciatic nerve (Fig S3 b) shows that p75NTR and PDE4A5 interact at endogenous expression levels. Analysis of lysates shows that the levels of PDE4A are similar in NIH3T3 and NIH3T3p75NTR cells (Fig. S3 c). These results show that p75NTR forms a complex with PDE4A5.

A functional consequence of the p75NTR–PDE4A5 interaction would be recruitment of PDE4A5 to the membrane resulting in decreased membrane-associated cAMP/PKA signaling.

To investigate whether p75NTR reduces membrane-associated PKA activity, we modified the genetically encoded A-kinase activity reporter, AKAR2 (Zhang et al., 2005) and generated pm-AKAR2.2, a membrane-targeted fluorescent reporter of PKA activity that generates a change in fluorescence resonance energy transfer (FRET) when it is phosphorylated by PKA in living cells (Fig. S4 a). As expected, NIH3T3 cells show a dramatic emission ratio change for the pm-AKAR2.2 in response to forskolin (Fig. 8 b). In contrast, NIH3T3p75NTR cells show an attenuated response, revealing reduced PKA activity at the plasma membrane (Fig. 8 b). Transient transfection of p75NTR confirmed the results observed in the stable NIH3T3p75NTR cells using the latest generation of plasma membrane–specific PKA biosensor AKAR3 (Allen and Zhang, 2006) (Fig. S4 b, available at http://www.jcb.org/cgi/content/full/jcb.200701040/DC1). As expected, increased cAMP degradation at the plasma membrane results in decreased intracellular cAMP (Fig. S4 c; Fig. 7, a and b). Overall, our results showing reduced membrane-associated PKA activity upon expression of p75NTR suggest that p75NTR targets cAMP degradation to the membrane via its interaction with PDE4A5.

To verify the specificity of p75NTR–PDE4A5 association, a series of mapping studies were conducted using deletion mutants. PDE4A5 interacts with FL p75NTR, as well as deletions Δ3, Δ62,
∆83, but not a deletion missing the distal 151 amino acids, ∆151 (Fig. 8 c), suggesting that the interaction between p75NTR and PDE4A5 occurs in the juxtamembrane region of p75NTR, requiring sequences between residues 275 and 343. To explain the specificity of the interaction of p75NTR with a single PDE4 isoform, we reasoned that p75NTR would interact with a unique region of PDE4A5 that is not present in other PDE4s. Although the PDE4 isoforms are highly homologous, PDE4A5 contains a unique C-terminal region with a yet unknown biological function (Houslay and Adams, 2003). Co-IP experiments in HEK293 cells using the PDE4A4 δCT mutant that is missing the C-terminal region (aa 721–886) abolishes the interaction with p75NTR (Fig. 8 d).

To examine whether p75NTR could interact with PDE4A5 in a direct manner, we performed in vitro pull-down assays using recombinant proteins. A GST fusion protein of p75NTR encoding the entire ICD interacts with both recombinant PDE4A5 and its human homologue PDE4A4 (Fig. 8 e). In contrast, p75NTR ICDD does not interact with recombinant PDE4D3 (Fig. 8 e). These results are in accordance with both the endogenous co-IPs in cells (Fig. 8, a and c; Fig. S3) and the PDE4A4 mutagenesis data (Fig. 8 d) because similar to PDE4A4 δCT, PDE4D3 does not contain the unique C-terminal domain of PDE4A4/5. We have used peptide array technology to define sites of direct interaction in other PDE4s (Bolger et al., 2006). Screening a peptide array library of overlapping 25-mer peptides that scanned the sequence of PDE4A4 with GST-ICD p75NTR identified interactions with the LR1 domain, whose sequence is unique to the PDE4A subfamily (peptides 40 and 41), the catalytic domain (peptides 135 and 136) and the unique C-terminus (peptides 172 and 173). Alanine scanning mutagenesis shows that substitution of C862 abolishes the interaction of p75NTR with the 173 peptide that is unique to PDE4A.
p75NTR regulates plasminogen activation and fibrin deposition in a model of lipopolysaccharide-induced pulmonary fibrosis

Because expression of p75NTR inhibits fibrinolysis in fibroblasts, we hypothesized that the role of p75NTR as a modulator of fibrinolysis extends to tissues outside of the nervous system that express p75NTR after injury or disease. Because p75NTR is expressed in the lung (Ricci et al., 2004), we compared the levels of fibrin in the lung of wt and p75NTR−/− mice in a model of lipopolysaccharide (LPS)-induced lung fibrosis (Chen et al., 2004). LPS-treated wt mice showed widespread extravascular fibrin deposition (Fig. 9 b) and decreased proteolytic activity after LPS treatment (Fig. 9 e), when compared with saline-treated wt mice (Fig. 9 a and d). In contrast, p75NTR−/− mice show a 2.58-fold decrease of fibrin immunoreactivity (Fig. 9 c and j) and increased proteolytic activity (Fig. 9 f).

Decreased proteolytic activity in the lung after injury depends on the up-regulation of PAI-1 (Idell, 2003). Loss of PAI-1 protects from pulmonary fibrosis in LPS-induced airway disease, hyperoxia, and bleomycin-induced fibrosis (Savov et al., 2003). Because p75NTR increases PAI-1 (Fig. 5 j and Fig. 6 e), we examined whether p75NTR regulates expression of PAI-1 in vivo. PAI-1 is up-regulated in LPS-treated wt mice (Fig. 9 h) when compared with saline-treated wt mice (Fig. 9 g). In contrast, LPS-treated p75NTR−/− mice show similar immunoreactivity for PAI-1 (Fig. 9 i) as saline-treated wt mice (Fig. 9 g), suggesting that p75NTR induces up-regulation of PAI-1 after injury in the lung.

Western blots show a decrease in PAI-1 in the lungs of p75NTR−/− mice (Fig. 9 k). Similar to the p75NTR−/− mice, rolipram reduces fibrin deposition in the lung (Fig. S5, a and b; available at http://www.jcb.org/cgi/content/full/jcb.200701040/DC1) and sciatic nerve (Fig. S5, d–f), and decreases PAI-1 in the lung (Fig. S5 c), suggesting the involvement of PDE4 in p75NTR-mediated inhibition of fibrinolysis in vivo. Collectively, our data show that p75NTR increases fibrin deposition via a PDE4-mediated inhibition of plasminogen activation in both LPS-induced lung fibrosis and sciatic nerve crush injury. These data suggest a role for p75NTR/PDE4 signaling as a general regulator of plasminogen activation and fibrinolysis at sites of injury.

Discussion

Our study shows a novel direct interaction of p75NTR with PDE4A4/5, a specific PDE4 isoform, which results in the regulation of cAMP, a major intracellular signaling pathway, and mediates a major biological function of extracellular proteolysis and fibrinolysis (Fig. 10). p75NTR is expressed in a wide range of tissue injury models, where repair depends upon both cell differentiation and ECM remodeling. For example, we recently showed that in the absence of plasminogen the effects of p75NTR in tissue repair are protective due to its beneficial effects in cell differentiation (Passino et al., 2007). Similarly, in the flow-restricted carotid artery model of vascular injury that depends on uPA and not on tPA-mediated fibrinolysis (Kawasaki et al., 2001), p75NTR is protective due to the induction of smooth
muscle cell apoptosis (Kraemer, 2002). In the sciatic nerve p75NTR appears to have a dual role by sustaining fibrin deposition (our study), and also promoting myelination (Cosgaya et al., 2002; Song et al., 2006). Examination of functional recovery in p75NTR−/− mice after peripheral nerve injury would reveal the contribution of p75NTR-mediated ECM remodeling and remyelination to the regeneration process. Overall, the biological role of p75NTR after tissue injury would probably depend on the relative contributions of its role as a regulator of cell death and differentiation and its role as an inhibitor of fibrinolysis.

We identify regulation of cAMP as a novel signaling mechanism downstream of p75NTR. Previous studies showed that β2-adrenergic receptors target degradation of cAMP to the membrane via recruitment of multiple PDE4 isoforms, such as PDE4B1, PDE4B2, and PDE4Ds (Perry et al., 2002). Our finding of interaction between p75NTR and PDE4A4/5 represents the first example of recruitment of a single PDE4 isoform to a transmembrane receptor. While interaction of β2-adrenergic receptors with PDE4s is mediated via β-arrestin, our study suggests that the interaction of p75NTR with PDE4A4/5 could be potentially mediated by direct binding to PDE4A domains, such as the C-terminal domain that is unique to this sub-family. In addition, in co-IP experiments we do not detect an interaction between p75NTR and β-arrestin (unpublished data). It is possible that the unique C-terminal domain of PDE4A could regulate isoform-specific PDE4 recruitment to subcellular locations. Biological roles have been described for PDE4D in ischemic stroke (Gretarsdottir et al., 2003) and heart failure (Lehnart et al., 2005) and for PDE4B in schizophrenia (Millar et al., 2005). Our study identifies a biological function for PDE4A4/5 as a molecular mediator of p75NTR/cAMP/PKA signaling involved in the regulation of tPA and fibrinolysis.

In spinal cord injury in rodents, elevation of cAMP via the PDE4 inhibitor, rolipram, promotes axonal regeneration and functional recovery (Nikulina et al., 2004). In the sciatic nerve, reduction of cAMP after injury is attributed primarily to up-regulation of PDE4 by SCs (Walikonis and Poduslo, 1998). Based on our findings, it is possible that reexpression of p75NTR after injury could contribute to the activation of PDE4 and down-regulation of cAMP. BDNF, but not NGF, increases cAMP in neurons via TrkB (Gao et al., 2003). Moreover, BDNF/TrkB signaling overcomes the inhibition of nerve regeneration by myelin proteins via inhibition of PDE4 (Gao et al., 2003).

We provide the first evidence for p75NTR in the regulation of cAMP by using genetic depletion, siRNA knockdown or up-regulation of the p75NTR. Our results suggest that p75NTR might exert the opposite function as Trk receptors by recruiting PDE4A4/5 and decreasing cAMP. Interestingly, PDE4A has been detected as the predominant PDE4 isoform at the corticospinal tract (Cherry and Davis, 1999). Because p75NTR can act as a coreceptor for NogoR, a mediator of the inhibition of nerve regeneration, PDE4A interaction with p75NTR could play an inhibitory role in nerve regeneration by competing with neurotrophin signaling via Trk receptors.

It is possible that the increased expression of p75NTR by neurons, glia, and brain endothelial cells could regulate the temporal and spatial pattern of tPA expression during brain injury or inflammation. p75NTR might also be upstream of other non-fibrinolytic functions associated with tPA, such as neurodegeneration, synaptic plasticity, and long-term potentiation (Samson and Medcalf, 2006). Given the dependence of p75NTR functions on the availability of ligands and coreceptors (Teng and Hempstead, 2004; Reichardt, 2006), further analysis will determine the role of p75NTR in extracellular proteolysis and ECM remodeling in different cellular systems. We show that expression of p75NTR can inhibit tPA in the absence of neurotrophin ligands. Constitutive expression of p75NTR may signal in a neurotrophin-independent manner to induce neuronal apoptosis (Rabizadeh et al., 1993), activation of Akt (Roux et al., 2001), and RhoGTPase (Yamashita et al., 1999). The regulation of cAMP identified here is an effect of expression of p75NTR that does not appear to depend on neurotrophin signaling. The cellular distribution of PDE4A4/5 would determine the involvement of p75NTR in the regulation of cAMP. It is possible that non-neurotrophin ligands that bind directly to p75NTR, such as β-amyloid (Teng and Hempstead, 2004), as well as the myelin/NogoR p75NTR-dependent inhibitors of nerve regeneration (Filbin, 2003), are able to regulate both cAMP and plasminogen activation by p75NTR. Because cAMP analogues decrease expression of p75NTR (Baron et al., 1997), it is possible that p75NTR by decreasing cAMP contributes to the positive regulation of its expression. Because PKA phosphorylates p75NTR and regulates its translocation to lipid rafts (Higuchi et al., 2003), p75NTR via regulation of PKA might regulate its own subcellular localization. Given the multiple genes regulated by cAMP and PKA, other cellular functions may be regulated by p75NTR/cAMP signaling.
NGF/p75NTR signaling has been suggested to enhance local neurogenic inflammation to exacerbate pulmonary disease (Renz et al., 2004). Our study suggests an additional pathway for p75NTR as a regulator of expression of PAI-1 and a mediator of fibrosis. p75NTR in the lung is detected mainly in basal epithelial cells of bronchioles (unpublished data). Similar to p75NTR, PAI-1 is expressed by bronchial epithelial cells (Savov et al., 2003) and its expression results in an antifibrinolytic environment within the airway wall. Fibrin regulates both inflammation and airway remodeling (Idell, 2003; Savov et al., 2003). It is therefore possible that p75NTR-mediated regulation of PAI-1 via PDE4 could influence inflammatory and tissue repair processes in pulmonary disease. Although in chronic obstructive pulmonary disease the PDE4A4 isoform is specifically up-regulated (Barber et al., 2004) and considered a pharmacologic target nary disease the PDE4A4 isoform is specifically up-regulated in pulmonary disease. Although in chronic obstructive pulmonary disease the PDE4A4 isoform is specifically up-regulated (Barber et al., 2004) and considered a pharmacologic target.

Collectively, we have identified a novel cAMP-dependent signaling pathway initiated by p75NTR that specifically regulates plasminogen activity and scar formation after sciatic nerve and lung injury. Though p75NTR is responsible for a variety of cell survival and death decisions (Chao, 2003), our data has revealed an unrecognized property of this receptor to regulate the degradation of cAMP. This property provides a potential mechanism to account for how p75NTR acts at sites of injury to promote ECM remodeling. The impact of high levels of p75NTR expression upon inhibition of extracellular proteolysis indicates that the detrimental effects of p75NTR extend beyond cell growth and axon inhibition. Finally, the dramatic inhibitory effect of p75NTR signaling on plasminogen activation suggests that the p75NTR/PDE4A4 interaction represents a novel target for therapeutic intervention in both neuronal and non-neuronal tissues.

Materials and methods

Animals, sciatic nerve crush, and induction of lung fibrosis

p75NTR−/− mice (Lee et al., 1992) and iPA−/− mice (Carmeliet et al., 1994) were in C57Bl/6 background and purchased from The Jackson Laboratory. Double p75NTR−/−/iPA−/− mice were generated by crossing p75NTR−/− mice with iPA−/− mice. C57/Bl6 mice were used as controls. Sciatic nerve crush was performed as described previously (Akassoglou et al., 2000). Lung fibrosis was induced as described previously (Chen et al., 2004).

Immunohistochemistry

Immunohistochemistry was performed as described in Akassoglou et al. (2002). Primary antibodies were sheep anti-human fibrinogen (1:200; US Biologicals), rabbit anti-human iPA (1:300; Molecular Innovations), rabbit anti-p75NTR clone 9651 (1:1,000), goat anti-p75NTR (1:200; Santa Cruz Biotechnology, Inc.), rabbit anti-mouse PAI-1 (1:500; a gift from David Loskutoff, Scripps Research Institute, La Jolla, CA), and mouse anti-S100 (1:200; Neomarkers). For immunofluorescence, secondary antibodies were anti-rabbit FITC and anti-goat Cy3 (1:200; Jackson Immunochromicals). Images were acquired with an Axioplan II epifluorescence microscope (Carl Zeiss Microimaging, Inc.) using dry Plan-Neofluar lenses using 10× 0.3 NA, 20× 0.5 NA, or 40× 0.75 NA objectives equipped with Axiocam HRc digital camera and the Axiovision image analysis system.

Immunoblot

Immunoblot was performed as described previously (Akassoglou et al., 2002). Antibodies used were rabbit anti-p75NTR clone 9992 and 9651 (1:5,000), mouse anti-fibrin (1:500; Accurate Chemical & Scientific Corp.), rabbit anti-myosin (1:1,000; Sigma-Aldrich), rabbit anti-GAPDH (1:1,500; Abcam), and rabbit anti-PAI-1 (1:5,000; a gift of David Loskutoff). Quantification was performed on the Scion NIH Imaging Software. Fibrin precipitation and quantification from lung tissues was performed exactly as described previously (Lung et al., 2004).

Co-IP

Co-IP was performed as described previously (Khursigara et al., 1999). Cells lysates were prepared in 1% NP-40, 200 mM NaCl, 1 mM EDTA, and 20 mM Tris-HCl, pH 8.0. IP was performed with an anti-p75NTR antibody (9992) and immunoblot with anti-PDE4A4, PDE4B, PDE4C, and PDE4D (Fabgenetics). The co-IP buffer using NP-40 has been previously used to examine interactions of p75NTR with other intracellular proteins, such as TRAF-6 (Khursigara et al., 1999) and PKA (Higuchi et al., 2003). For mapping experiments, PDE4A5 cDNA was cotransfected with HA-tagged p75NTR deletion constructs into HEK293 cells. IP was performed with an anti-HA antibody (Cell Signaling). Cells lysates were probed with an anti-PDE4A4 or an anti-p75NTR antibody (9651). For Co-IP experiments using recombinant proteins, equimolar amounts (2 μM) of purified recombinant MBP-PDE4A5, MBP-PDE4A4 (McPhee et al., 1999), MBP-PDE4D (Yarwood et al., 1999), and GST-p75NTR-ICD (Khursigara et al., 2001) were mixed in binding buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2 mM MgCl2, 1 mM DTT, 0.5% Triton X-100, and 0.5% BSA) and incubated for 1 h at 4°C. Washed glutathione-Sepharose beads were added according to the manufacturer’s instructions for an additional hour. Beads were sedimented by centrifugation (10,000 g for 1 min) and washed three times. Proteins associated with the beads were eluted by boiling in loading buffer and separated by SDS-PAGE.

RT-PCR and real-time PCR

RT-PCR was performed as described previously (Akassoglou et al., 2002). Primers for iPA, uPA, and PAI-1 genes were used as described previously (Yamamoto and Loskutoff, 1998). Real-time PCR was performed using the Optical DNA Engine 2 (MJ Research) and the Quantitect SYBR Green PCR kit (QIAGEN). Results were analyzed with Opticon 2 software using the comparative Ct method as described previously (Livak and Schmittgen, 2001). Data were expressed as ΔΔCt for the iPA gene normalized against GAPDH.

Quantification of iPA and uPA activity

Quantification of iPA and uPA activity in SC and fibroblast in lysates and supernatants was performed according to the directions of the activity-assay kits from American Diagnostica and Chemicon, respectively. To elevate cAMP cells were treated with 2 mM db-cAMP (Sigma-Aldrich) or with 10 μM forskolin (Sigma-Aldrich) for 16 h. To block PKA activity, cells were treated with 200 nM KT5720 (Calbiochem). Induction with neurotrophins was performed using 100 ng/ml NGF and 50 ng/ml BDNF for 16 h before iPA assay.

Fibrin degradation assay

Coating with fibrin was prepared as described previously (Lansink et al., 2004). To quantify fibrin degradation, the supernatant was collected (10,000 g for 1 min) and washed three times. Proteins associated with the beads were eluted by boiling in loading buffer and separated by SDS-PAGE.

Cell culture and transfections

Murine SCs were isolated as described previously (Syroid et al., 2000). NIH3T3 or HEK293 cells were cotransfected either with p75NTR FL, ICD deletion constructs into HEK293 cells. Cells were harvested and processed for immunostaining.
cAMP/PKA assays

10⁶ fibroblasts or 500,000 SCs were lysed in 0.1 N HCl solution and cAMP was measured using a competitive binding cAMP ELISA (Assay Designs). Cells were treated with 100 ng/ml PTX for 16 h. For inhibition of PDE activity, cells were treated for 16 h with 500 µM isobutyl methylxanthine (IBMX; Calbiochem), 18.7 µM 8-methoxy methyl-3-isobutyl-1-methylxanthine (PDE1 inhibitor; Calbiochem), 90 µM erythro-9-(2-Hydroxy-3-nonyl)adenine (PDE2 inhibitor; Calbiochem), 100 nM trequinsin (PDE3 inhibitor; Calbiochem), and 10 µM rolipram (PDE4 inhibitor; Calbiochem). Cells were treated with forskolin in the presence of the inhibitors for 1 h. Because these inhibitors specifically inhibit a PDE isoform and have no effect on the other PDE isoforms (Beavo and Reifsnyder, 1990), they are extensively used for the identification of the specific PDE isoform that is involved in different cellular functions. Induction with neurotrophins was performed using 100 ng/ml NGF or 50 ng/ml BDNF, 750 ng/ml of FcTrkβ, or 1.35 µg/ml of Fcp75^NTR for 1 h before cAMP assay. For the qualitative and quantitative PKA assay (Promega), cells were treated with 10 µM forskolin for 30 min, lysed in 1% NP-40 buffer with 150 mM NaCl, 50 mM Tris, and 1 mM EGTA, and proteolytic PKA sensor, AKAR2 (Zhang et al., 2005). pm-AKAR2.2 consists of NIH3T3 cells and NIH3T3p75 NTR cells were transiently transfected with the FLAG epitopes is located immediately after Lys-747. This strategy generates a C-terminal truncate of PDE4A4 from 1–721.

Peptide array mapping

Peptide libraries were synthesized by automatic SPOT synthesis (Frank, 2002). Synthetic overlapping peptides (25 amino acids in length) were spotted on Whatman 50 cellulose membranes according to standard protocols by using Fmo-chemistry with the AutoSpot Robot ASS 222 (Intavis Bioanalytical Instruments AG). Membranes were overlaid with 10 µg/ml recombinant GST-p75^NTR-CD. Bound recombinant GST-p75^NTR-CD (Khrusi et al., 2001) was detected using rabbit anti-GST (1:2,000; GE Healthcare) followed by secondary anti-rabbit horseradish peroxidase antibody (1:2,500; Dianova). Alanine scanning was performed as described previously (Bolger et al., 2006).

Online supplemental material

Fig. S1 shows that genetic loss of p75^NTR increases tPA mRNA levels and proteolytic activity in the cerebellum. Fig. S2 demonstrates that treatment with neurotrophins has no effect on cAMP levels in NIH3T3 cells. Fig. S3 shows endogenous communoprecipitations of PDE4A5 and p75^NTR from injured sciatic nerve and from primary CGNs. Fig. S4 shows results from transient transfections of NIH3T3 cells with p75^NTR and the PKA activity reporters, AKAR3 and pm-AKAR3. Fig. S5 shows that inhibition of PDE4s with rolipram decreases fibrin deposition both in LPS-induced lung fibrosis and sciatic nerve crush injury. The online version of this article is available at http://www.jcbin.org/cgi/content/full/jcb.200701040/DC1.

We thank David Luszkoff for the anti-PAI-1 antibody, Joan Heller Brown, Lawrence Brunton, Roger Y. Tsien, Barbara Hermstead, Juan Carlos Arevals, Hiroko Yano, and David Arthur for discussions, Paul Insel, Palmer Taylor, and Dan Litman for equipment access; Susan Taylor for assistance with the cAMP/PKA assays; Lisa Gallegos and Alexandra Newton for assistance with FRET experiments; and Birnai Zhang for advice on CGN culture. We thank Zusana Pearson and Niccolò Zampieri for help with experiments and Xiaolin Tan, Andrew Maleson, and Priscila Kim for expert technical assistance.

Supported in part by the Pharmacology National Institutes of Health (NIH) training grant ST32GM07752 to B.D. Sachs and M.A. Passino, the DFG postdoctoral fellowship to C. Schachtrup, and the ASPET fellowship to J.R. McCall. The Alliance for Cell Signaling and NIH grant NS27177 to Roger Y. Tsien supported J. Zhang. This work was supported by NIH grants NS521072 and HD52315 to M.V. Chao; and NIH grants NS51470 and NS52189 to K. Akassoglou.

This work is dedicated to the memory of our parents Beatrice Du Chao, Evangelia Akassoglou, and Douglas Houslay, who passed away during the preparation of this manuscript.

Submitted: 8 January 2007
Accepted: 21 May 2007

References

Adams, R.A., M. Passino, B.D. Sachs, T. Nuriel, and K. Akassoglou. 2004. Fibrin mechanisms and functions in nervous system pathophysiology. Mol. Interv. 4:163–176.

Akassoglou, K., W.K. Kombinr, J.L., Degen, and S. Strickland. 2000. Tissue plasminogen activator-mediated fibrinolysis protects against axonal degeneration and denervation after sciatic nerve injury. J. Cell Biol. 149:1157–1166.

Akassoglou, K., W.-M. Yu, P. Apkinar, and S. Strickland. 2002. Fibrin inhibits peripheral nerve regeneration by arresting Schwann cell differentiation. Neuron. 33:861–875.

Allen, M.D., and J. Zhang. 2006. Subcellular dynamics of protein kinase A activity visualized by FRET-based reporters. Biochem. Biophys. Res. Commun. 348:716–721.

Barber, R., G.S. Baillie, R. Bergmann, M.C. Shepherd, R. Sepper, M.D. Houslay, and R. Sepper. 2000. human plasminogen. Am. J. Physiol. Lung Cell. Mol. Physiol. 287:L332–L343.

Bolger, P., E. Scarpini, S. Pizzul, F. Zotti, G. Conti, D. Pleasure, and G. Scarlato. 1997. Immunocytochemical expression of human muscle cell p75 neurotrophin receptor is down-regulated by cyclic adenosine 3′,5′-monophosphate. Neurosci. Lett. 234:79–82.
Beattie, M.S., A.W. Harrington, R. Lee, J.Y. Kim, S.L. Boyce, F.M. Longo, I.C. Bresnahan, B.L. Hempstead, and S.O. Yoon. 2002. ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. Neuron. 36:375–386.

Beavo, J.A., and D.H. Reifsnyder. 1990. Primary sequence of cyclic nucleotide phosphodiesterases and the design of selective inhibitors. Trends Pharmacol. Sci. 11:150–155.

Bolger, G.B., G.S. Baillie, X. Li, M.J. Lynch, P. Herzyk, A. Mohamed, L.H. Mitchell, A. McCauli, C. Hundsrucker, E. Kussmann, et al. 2006. Scanning peptide array analyses identify overlapping binding sites for the signalling scaffolding proteins, beta-arrestin and RACK1, in cAMP-specific phosphodiesterase PDE4D5. Biochem. J. 389:23–36.

Brunton, L.L. 2003. PDE4: arrested at the border. Sci. STKE. 2003:PE44.

Bugge, T.H., K.W. Kombrinck, M.J. Flick, C.C. Daugherty, M.J. Danton, and J.L. Degen. 1996. Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. Cell. 87:709–719.

Carmeliet, P., L. Schoonjans, L. Kieckens, B. Ream, J. Degen, R. Bronson, R. De Vos, J.J. van den Oord, D. Collen, and R.C. Mulligan. 1994. Physiological consequences of loss of plasminogen activator gene function in mice. Nature. 368:419–424.

Chao, M.V. 2003. Neurotrophins and their receptors: a convergence point for development and disease. Trends Neurosci. 26:419–427.

Chen, D., K. Giannopoulos, P.G. Shiels, Z. Webster, J.H. McVey, G. Kemball, K. Balk, T. Tuukkanen, M. Moore, R. Lechler, and A. Doring. 2005. Inhibition of intravascular thrombosis in murine endotoxemia by targeted expression of hirudin and tissue factor pathway inhibitor analogs to activate endothelium. Blood. 104:1344–1349.

Cherry, J.A., and R.L. Davis. 1999. Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. J. Comp. Neurol. 407:287–301.

Cosgaya, J.M., J.R. Chan, and E.M. Shooter. 2002. The neurotrophin receptor p75NTR as a positive modulator of myelination. Science. 298:1245–1248.

Degen, J.L., A.F. Drew, J.S. Palumbo, K.W. Kombrinck, J.A. Bezerra, M.J. Danton, K. Holmgren, and T.T. Sul. 2001. Genetic manipulation of fibrinogen and fibrinolysis in mice. Ann. N. Y. Acad. Sci. 936:276–290.

Dowling, P., X. Ming, S. Raval, W. Husar, P. Casacchia-Bonnefil, M. Chao, S. Cook, and B. Blumberg. 1999. Up-regulated p75NTR neurotrophin receptor on glial cells in MS plaques. J. Neuroimmunol. 96:193–202.

Fiumelli, H., D. Jabaudon, P.J. Magistretti, and J.-L. Martin. 1999. BDNF stimulates expression, activity and release of tissue-type plasminogen activator in mouse cortical neurons. Eur. J. Neurosci. 11:1639–1646.

Frank, R. 2002. The SPOT-synthesis technique. Synthetic peptide arrays on chip. Cell Mol. Biol. 48:1205–1216.

Gao, Y., E. Nikulina, W. Mellado, and M.T. Fiblin. 2003. Neurotrophins elevate cAMP to reach a threshold required to overcome inhibition by MAG through extracellular signal-regulated kinase-dependent inhibition of phosphodiesterase. J. Neurosci. 23:11770–11777.

Greterdottir, S., G. Thorleifsson, S.T. Reynisdottir, S.M. Bjarnadottir, O.B. Einarsson, T.J. Jonsdottir, T. Gudmundsdottir, J.J. van den Oord, D. Collen, and R.C. Mulligan. 2006. Physiological effects of plasminogen deficiency. Cell. 69:737–749.

Lee, R., P. Kermani, K.K. Teng, and B.L. Hempstead. 2001. Regulation of cell survival by secreted pro-neurotrophins. Science. 294:1945–1948.

Lemke, G., and M. Chao. 1998. Axons regulate Schwann cell expression of the major myelin and NGF receptor genes. Development. 102:499–504.

Lijnen, H.R. 2001. Elements of the fibrinolytic system. Ann. N. Y. Acad. Sci. 936:226–236.

Ling, Q., A.T. Jacobova, A. Deora, M. Febbraio, R. Simantov, R.L. Silverstein, B. Hempstead, W.H. Mark, and K.A. Hajjar. 2004. Annexin II regulates fibrin homeostasis and neuroangiogenesis in vivo. J. Clin. Invest. 113:38–48.

Livak, K.J., and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 25:402–408.

Lomen-Hoerth, C., and E.M. Shooter. 1995. Widespread neurotrophin receptor expression in the immune system and other nonneuronal rat tissues. J. Neurochem. 64:1780–1789.

McPhee, S.J., J.Y. Yawood, G. Scotland, E. Huston, M.B. Beard, A.H. Ross, E.S. Houslay, and M.D. Houslay. 1999. Association with the SRC family tyrosine kinase LYN triggers a conformational change in the catalytic region of human cAMP-specific phosphodiesterase HSPDE4A4B. Consequences for rolipram inhibition. J. Biol. Chem. 274:11796–11810.

Medcalf, R.L., M. Riegger, and W.D. Schleuning. 1990. A DNA motif related to the cAMP-responsive element and an exon-located activator protein-2 binding site in the human tissue-type plasminogen activator gene promoter cooperates in basal expression and convey activation by phorbol esters and cAMP. Cell. 265:14618–14626.

Millar, J.K., B.S. Pickard, S. Mackie, R. James, S. Christie, S.R. Buchanan, M.P. Malloy, J.E. Chubb, E. Huston, G.S. Baillie, et al. 2005. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science. 310:1187–1191.

Miotla, J.M., M.M. Teixeira, and P.G. Hellewell. 1998. Suppression of acute lung injury in mice by an inhibitor of phosphodiesterase type 4. Am. J. Respir. Cell Mol. Biol. 18:411–420.

Nikulina, E., J.L. Tidwell, H.N. Dai, B.S. Bregman, and M.T. Fiblin. 2004. The phosphodiesterase inhibitor rolipram delivered after a spinal cord lesion promotes axonal regeneration and functional recovery. Proc. Natl. Acad. Sci. U.S.A. 101:8786–8790.

O’Connell, J.C., J.F. McCallum, M.J. McPhee, J. Wakefield, E.S. Houslay, W. Waskar, G. Bolger, M. Frame, and M.D. Houslay. 1996. The SH3 domain of Src tyrosyl protein kinase interaction with the N-terminal splice region of the PDE4A4 cAMP-specific phosphodiesterase RPDE-6 (RNPDE4A5). Biochem. J. 318(Pt 1):255–261.

Park, J.A., J.Y. Lee, T.A. Sato, and J.Y. Koh. 2000. Co-induction of p75NTR and p75NTR-associated death executor in neurons after zinc exposure in cortical culture or transient ischemia in the rat. J. Neurosci. 20:9096–9103.

Passino, M.A., R.A. Adams, S.L. Sikorski, and K. Akassoglou. 2007. Regulation of hepatic stellate cell differentiation by the neurotrophin receptor p75NTR. Science. 315:1856–1859.

Perry, S.M., G.S. Baillie, T.A. Kohout, I. McPhee, M.M. Magiera, K.L. Ang, W.E. Miller, A.J. McLean, M. Conti, M.D. Houslay, and R.J. Lefkowitz. 2002. Targeting of cyclic AMP degradation to beta 2-adrenergic receptors by beta-arrestins. Science. 298:834–836.

Qian, Z., M.E. Gilbert, M.A. Colcos, E.R. Kandel, and D. Kuhl. 1993. Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature. 361:453–457.
Rabizadeh, S., J. Oh, L.T. Zhong, J. Yang, C.M. Bitler, L.L. Butcher, and D.E. Bredesen. 1993. Induction of apoptosis by the low-affinity NGF receptor. Science. 261:345–348.

Reichardt, L.F. 2006. Neurotrophin-regulated signalling pathways. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361:1545–1564.

Ren, H., S. Kerzel, and W.A. Nockher. 2004. The role of neurotrophins in bronchial asthma: contribution of the pan-neurotrophin receptor p75. Prog. Brain Res. 146:325–333.

Ricci, A., L. Felici, S. Mariotta, F. Mannino, G. Schmid, C. Terzano, G. Cardillo, F. Amenta, and E. Bronzetti. 2004. Neurotrophin and neurotrophin receptor protein expression in the human lung. Am. J. Respir. Cell Mol. Biol. 30:12–19.

Roux, P.P., A.L. Bhakar, T.E. Kennedy, and P.A. Barker. 2001. The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. J. Biol. Chem. 276:23097–23104.

Samson, A.L., and R.L. Medcalf. 2006. Tissue-type plasminogen activator: a multifaceted modulator of neurotransmission and synaptic plasticity. Neuron. 50:673–678.

Santelli, L., and E. Levin. 1988. Cyclic AMP potentiates phorbol ester stimulation of tissue plasminogen activator release and inhibits secretion of plasminogen activator inhibitor-1 from human endothelial cells. J. Biol. Chem. 263:16802–16808.

Savov, J.D., D.M. Brass, K.G. Berman, E. McElvania, and D.A. Schwartz. 2003. Fibrinolysis in LPS-induced chronic airway disease. Am. J. Physiol. Lung Cell. Mol. Physiol. 285:L940–L948.

Siconolfi, L.B., and N.W. Seeds. 2001. Mice lacking tPA, uPA, or plasminogen genes showed delayed functional recovery after sciatic nerve crush. J. Neurosci. 21:4348–4355.

Song, X.Y., F.H. Zhou, J.H. Zhong, L.L. Wu, and X.F. Zhou. 2006. Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. J. Neurochem. 96:833–842.

Syroid, D.E., P.J. Maycox, M. Soili-Hanninen, S. Petratos, T. Bucci, P. Burrola, S. Murray, S. Cheema, K.F. Lee, G. Lemke, and T.J. Kilpatrick. 2000. Induction of postnatal Schwann cell death by the low-affinity neurotrophin receptor in vitro and after axotomy. J. Neurosci. 20:5741–5747.

Tanuchi, M., H.B. Clark, and E.M. Johnson Jr. 1986. Induction of nerve growth factor receptor in Schwann cells after axotomy. Proc. Natl. Acad. Sci. USA. 83:4094–4098.

Teng, K.K., and B.L. Hempstead. 2004. Neurotrophins and their receptors: signaling trios in complex biological systems. Cell. Mol. Life Sci. 61:35–48.

Walikonis, R.S., and J.F. Poduslo. 1998. Activity of cyclic AMP phosphodiesterases and adenyl cyclase in peripheral nerve after crush and permanent transection injuries. J. Biol. Chem. 273:9070–9077.

Wang, S., P. Bray, T. McCaffrey, K. March, B.L. Hempstead, and R. Kraemer. 2000. p75(NTR) mediates neurotrophin-induced apoptosis of vascular smooth muscle cells. Am. J. Pathol. 157:1247–1258.

Yamamoto, K., and D.J. Loskutoff. 1996. Fibrin deposition in tissues from endotoxin-treated mice correlates with decreases in the expression of urokinase-type but not tissue-type plasminogen activator. J. Clin. Invest. 97:2440–2451.

Yamashita, T., and M. Tohyama. 2003. The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. Nat. Neurosci. 6:461–467.

Yamashita, T., K.L. Tucker, and Y.A. Barde. 1999. Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. Neuron. 24:585–593.

Yarwood, S.J., M.R. Steele, G. Scotland, M.D. Houslay, and G.B. Bolger. 1999. The RACK1 signaling scaffold protein selectively interacts with the CAMP-specific phosphodiesterase PDE4D5 isoform. J. Biol. Chem. 274:14909–14917.

Zhang, J., C.J. Hopfeld, S.S. Taylor, J.M. Olefsky, and R.Y. Tsien. 2005. Insulin disrupts beta-adrenergic signalling to protein kinase A in adipocytes. Nature. 437:569–573.

Zorick, T.S., and G. Lemke. 1996. Schwann cell differentiation. Curr. Opin. Cell Biol. 8:870–876.