BIOCHEMICAL PHARMACOLOGY 76 (2008) 1476–1484

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c-Jun N-terminal kinases mediate Fas-induced neurite regeneration in PC12 cells

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ARTICLE INFO

Article history:
Received 13 June 2008
Accepted 11 July 2008

Keywords:
Sprouting
Nerve regeneration
Nerve growth factor
c-Jun N-terminal kinases
Fas

ABSTRACT

In response to injury, peripheral neuronal cells initiate complex signalling cascades to promote survival and regeneration. In the present study, we used a model of experimental injury in the rat pheochromocytoma cell line PC12 to investigate receptor signals that lead to neurite outgrowth. Nerve growth factor (NGF) dose-dependently induced sprouting and the expression of the NGF receptors Trk tyrosine kinase receptor (TrkA) and p75 neurotrophin receptor ($p75^{NTR}$) as well as Fas and Fas ligand. Neurite regeneration was decreased by chemical inhibition of TrkA, but not $p75^{NTR}$, and by the Fas inhibitor protein Fas-Fc. The mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinases (JNKs) were activated in response to NGF and both significantly contributed to neurite re-growth. Interestingly, otherwise apoptotic Fas ligation supported neuronal recovery exclusively via JNKs and promoted sprouting parallel to NGF. These findings suggest a novel signal integration from the NGF and Fas pathways in the JNK axis of MAPK signalling, where JNKs function as "physiological" mediators of normally apoptotic signals.

1. Introduction

Neuronal regeneration is orchestrated by a complex interplay between diffusible cues and their corresponding cell surface receptors. Well characterized neuronal differentiation factors are the neurotrophins, which use two types of receptors: the Trk tyrosine kinase receptor (TrkA) and the p75 neurotrophin receptor ($p75^{NTR}$). Nerve growth factor (NGF) is the best characterized member of the neurotrophin family and binds to TrkA and $p75^{NTR}$ to initiate signalling [1]. Its secretion is important not only for brain development, but also for neuronal repair after injury [2]. Similarly, death receptors and their ligands are expressed in the nervous system. Apart from developmental processes, especially the Fas–Fas-ligand system is involved not only in a variety of neurological disorders [3], but also in neurite outgrowth and regeneration [4,5]. To accomplish their manifold functions, cell surface receptors need complex intracellular pathways with multifunctional signalling molecules.

MAPK pathways are central mediators in cellular differentiation in general and sprouting in particular [1,2,6]. As a paradigm of the highly conserved MAPK cascade hierarchy, extracellular signal-regulated kinase 1/2 (ERK1/2) is activated by MAPK/ERK kinase 1 (MEK1), which is in turn activated by MEK kinase 1 (MEKK1). ERK1/2 have been implicated in growth factor responses underlying proliferation and differentiation [7,8] and their activation has been linked to the development of peripheral neurons [9] and to regeneration via neurotrophin induction [10]. In contrast, the importance of the p38 kinases, the second MAPK family, for neuronal differentiation and repair has been discussed controversely [11,12]. The third major MAPK group, the c-Jun N-terminal kinases (JNKs), has many different functions in the nervous system. It consists of the ubiquitously expressed JNK1 and JNK2, and JNK3, which

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.07.014

are primarily expressed in the heart, brain, and testis, respectively [13]. JNKs have mostly been studied in the context of cellular stress and apoptosis [14,15]. However, their functions also include important physiological actions, such as the regulation of neurite outgrowth and the response to growth factors [11,16–18].

In the present study, we have studied the expression and function of neurotrophin and death receptors as well as MAPK signalling after experimental injury in the rat pheochromocytoma cell line PC12 [19,20]. NGF induced sprouting and the expression of TrkA, $p75^{NTR}$, Fas and Fas ligand. Receptor inhibition showed that only TrkA significantly contributed to neurite re-formation. The mitogen-activated protein kinases (MAPKs) ERK1/2 and JNKs are activated in response to NGF and crucially affect neurite re-growth. Moreover, Fas ligation was found to support neuronal recovery and to exert its effects via JNK, but not ERK1/2, signalling.

2. Materials and methods

2.1. Cell culture and neurite outgrowth

If not indicated otherwise, chemicals were purchased from Sigma–Aldrich (Taufkirchen, Germany). Rat pheochromocytoma PC12 cells (ATCC/LGC Promochem; Wesel, Germany) were cultured on collagen-coated plates in RPMI 1640 (Invitrogen; Karlsruhe, Germany) supplemented with 5% fetal bovine serum (FBS; Cambrex/Lonza; Basel, Switzerland) and 10% horse serum (Invitrogen) at 37 °C and 5% CO_2. For differentiation, PC12 cells were kept in growth medium for 24 h, and afterwards in medium supplemented with 0.5% FBS for 72 h to synchronize cell cycle activity. Finally, recombinant mouse 2.5 S NGF (10 or 50 ng/ml; Alomone Labs/ICS; Munich, Germany) was added for 7 days until the neurites were removed.

2.2. Injury of differentiated PC12 cells

The following injury technique for differentiated PC12 cells was adapted from Galbiati et al. [20]. PC12 cells were differentiated with NGF (50 ng/ml) in RPMI 1640 with 0.5% FBS. After 7 days, cells were rinsed 3 times with phosphate-buffered saline (PBS), dislodged by PBS with 0.5 mM EDTA and centrifuged (600 × *g*, 10 min). To detach all neurites, cells were triturated with a Pasteur pipette after resuspension in low serum medium (0.5% FBS). PC12 cells were plated at low density (8×10^5 cells/100 mm collagen-coated dish; collagen was obtained from Biochrom; Berlin, Germany) in low serum medium containing NGF, Fas-Fc, Superfas ligand (SFL) or inhibitors alone or in combinations. The following pre-incubation times were used with the inhibitors before adding 10 or 50 ng/ml NGF: K252a (100 and 200 nM; Calbiochem/Merck; Darmstadt, Germany) for 30 min, Pep5 (100 nM and 1 μM; Calbiochem) for 30 min, SP600125 (2 μM; kindly provided by Celgene; Munich, Germany) for 30 min and U0126 (5 μM; Calbiochem) for 30 min. Fas-Fc (4 μg/ml; R&D Systems; Wiesbaden, Germany) and SFL (100 ng/ml; Alexis/Axxora; Lörrach, Germany) were added simultaneously with NGF. Injury treatment killed only few cells, which were removed before experiments by a medium change 24 h after injury. Generally, cell survival was determined by trypan blue counts. The percentage of cells with neurites longer than 1.5 diameters of the cell body were counted on days 3 and 5 after injury using the software LeicaQwin (Leica; Solms, Germany).

2.3. Whole cell extracts

Proteins were extracted on days 3 and 5 after injury. Before harvesting, cells were washed with PBS. Cells were resuspended in lysis buffer [20 mM Tris (pH 7.4), 1% sodium dodecyl sulfate (SDS), 1% phosphatase inhibitor and protease inhibitor], incubated at 95 °C for 5 min, briefly sonicated and centrifuged to remove insoluble material (15,000 × *g*, 15 min, 4 °C). Protein extracts were stored at −80 °C.

2.4. Western blots

Twenty microgram of total protein were separated on 10 or 12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore; Schwalbach, Germany). Membranes were blocked with 4% non-fat dry milk and incubated with the primary antibody according to the manufacturer's instructions. After 3 washing steps with Tris-buffered saline with Tween-20 (TBST), membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 30 min. Western blots were developed with the ECL chemiluminescence system and Hyperfilm ECL (GE Healthcare; Freiburg, Germany). Between stainings with phosphospecific antibodies and total kinase or transcription factor antibodies, blots were stripped in 2% SDS, 62.5 mM Tris, and 100 mM mercaptoethanol (50 °C, 30 min), washed with TBST, and blocked again. Measurements of dual-phosphorylated kinases were normalized by hybridization with antibodies against total kinase protein. To confirm equal loading, membranes were finally stained with Ponceau S. Only films with subsaturating levels of intensity were selected for analysis. Antibodies against the following targets were purchased from the indicated sources: mouse IgG (GE Healthcare); ERK1/2, JNK, phospho-ERK1/2 and rabbit IgG (Cell Signaling Technologies/New England Biolabs; Frankfurt, Germany); phospho-JNK (Promega; Mannheim, Germany); Fas and Fas ligand (Santa Cruz; Heidelberg, Germany).

2.5. Statistics and replication rate

Statistical significance of the normally distributed data was determined using the *t*-test for independent samples, and the respective results are displayed as means ± standard deviation. All experiments and measurements were replicated at least three times.

3. Results

3.1. NGF-induced neurite re-growth and NGF receptor expression after injury in PC12 cells

The first aim of the present study was to measure neurite re-growth of injured PC12 cells and to examine whether the

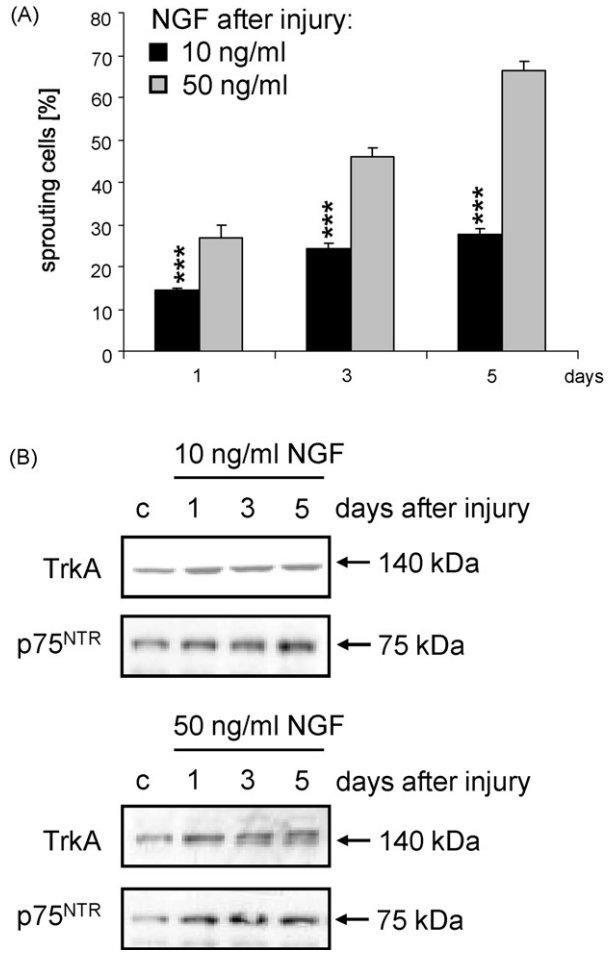

Fig. 1 – NGF induces sprouting after injury of PC12 cells. (A) 50 ng/ml NGF significantly increased neurite re-growth as compared to 10 ng/ml NGF. (B) PC12 cells constitutively expressed the NGF receptors TrkA and p75^{NTR}, as determined by Western blot analysis. Stimulation with 50 ng/ml NGF clearly elevated receptor expression. c, undifferentiated control; ***$p < 0.001$ compared to 50 ng/ml NGF.

expression of NGF receptors was related to sprouting. In response to 10 ng/ml NGF, the regeneration of neurites was slow: after 5 days, only 28% of the cells treated with 10 ng/ml NGF had re-formed neurites compared to 66% of the cells stimulated with 50 ng/ml NGF (Fig. 1A).

Expression analysis of the NGF receptors TrkA and p75^{NTR} showed that both receptors were permanently detectable regardless of the differentiation status. NGF stimulation dose-dependently increased the expression of TrkA and p75^{NTR}. Addition of 10 ng/ml NGF led to a slight upregulation of both receptors compared to control cells (Fig. 1B). When injured PC12 cells were incubated with 50 ng/ml NGF, the expression of TrkA and p75^{NTR} distinctly increased (Fig. 1B). To investigate whether both NGF receptors were necessary for neurite regeneration, we used receptor-specific inhibitors. K252a, an inhibitor of TrkA, significantly and dose-dependently decreased the number of neurite-bearing cells, while p75^{NTR} inhibition by Pep5 did not significantly affect neurite re-growth (Fig. 2).

3.2. *Expression and effects of Fas and Fas ligand*

Although NGF signals are exclusively mediated by its receptors, it is known that death receptors may additionally contribute to neuronal differentiation and regeneration [4]. Therefore, we examined whether Fas and Fas ligand, which can both be induced in differentiated PC12 cells after cell stress [21], were involved in the re-formation of neurites after injury. Both 10 and 50 ng/ml NGF increased the expression of Fas and Fas ligand (Fig. 3A). The induction of Fas and Fas ligand was slightly delayed after stimulation with 10 ng/ml NGF, showing a clear increase after 3 days, whereas 50 ng/ml NGF enhanced the expression of Fas and Fas ligand already after 1 day (Fig. 3A).

The next step was to investigate whether the Fas system had any effect on sprouting. When cells were co-stimulated with 50 ng/ml NGF and Fas-Fc, which inhibits Fas signals, the re-formation of neurites was significantly attenuated, whereas co-stimulation with NGF and the Fas agonist Superfas ligand had the same sprouting effect as NGF alone (Fig. 3B).

As SFL alone has been reported to induce sprouting in dorsal root ganglia to a similar extent as NGF [4], we examined whether Fas ligation could compensate for NGF stimulation in our system. Interestingly, SFL alone induced substantial neurite re-growth (39 and 55%, respectively), although significantly less than NGF alone (46 and 66%, respectively). When Fas ligation was blocked by Fas-Fc, sprouting was significantly reduced (23 and 38%, respectively), indicating that Fas ligation also supports sprouting (Fig. 3C).

The impaired neurite formation after treatment with Fas-Fc and Superfas ligand raised the question whether the lower sprouting rate was due to a reduced cell viability. Trypan blue assays revealed that significantly fewer PC12 cells survived when they were cultivated without NGF (Fig. 3D). Nevertheless, more than 80% of the cells survived treatment with Fas-Fc and SFL, suggesting that additional NGF stimulation had a positive effect on cell survival, but incubation without NGF does not necessarily lead to apoptosis.

3.3. *MAPK activation after PC12 cell injury*

Since MAPKs play a central role in neurite regeneration [4,22], we examined expression and activation of ERK1/2 and JNKs after PC12 cell injury. ERK1/2 was activated by both 10 and 50 ng/ml NGF (Fig. 4A). JNKs were only slightly phosphorylated (activated) after 3 and 5 days in response to 10 ng/ml NGF, whereas 50 ng/ml NGF induced a strong JNK activation (Fig. 4B). No changes in kinase expression were observed (Fig. 4A and B). Inhibition of ERK1/2 by U0126 (5 μM) or JNK inhibition by SP600125 (2 μM) significantly reduced the sprouting effect of NGF (Fig. 4C).

3.4. *ERK1/2 and JNK activity after receptor inhibition*

Finally, the question remained how ERK1/2 and JNKs were involved in TrkA or Fas signalling after PC12 cell injury. First of all, activation of both kinases was examined after TrkA

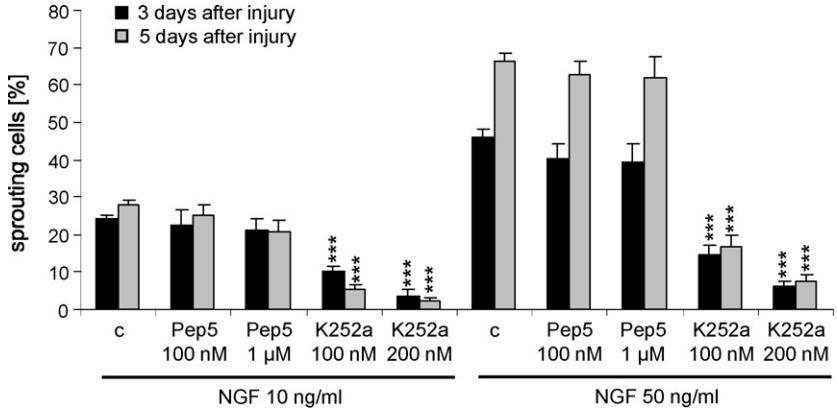

Fig. 2 – Selective inhibitory effect of the TrkA inhibitor K252a on sprouting of PC12 cells. Whereas pre-incubation with the $p75^{NTR}$ inhibitor Pep5 did not affect sprouting, the addition of the TrkA inhibitor K252a significantly reduced neurite formation after both 10 and 50 ng/ml NGF. c, NGF-treated control; $^{***}p < 0.001$ compared to 10 or 50 ng/ml NGF.

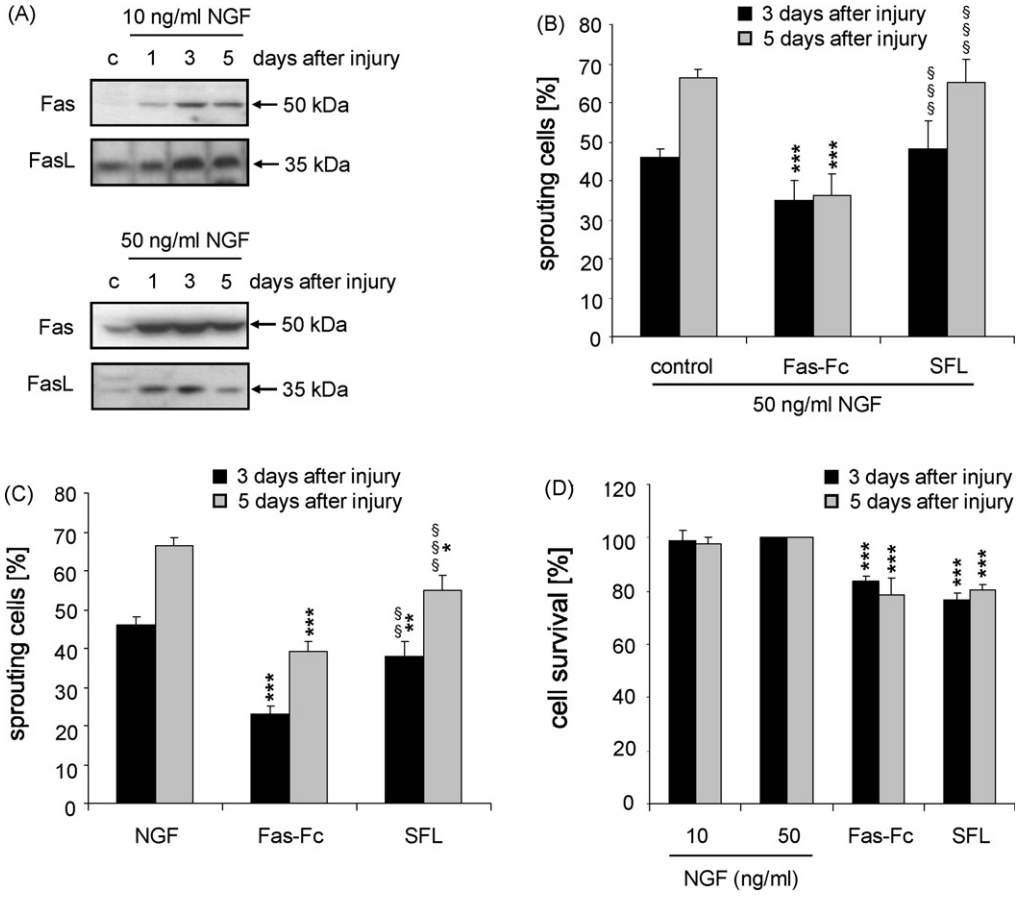

Fig. 3 – Fas and Fas ligand are involved in sprouting of PC12 cells. (A) Both 10 and 50 ng/ml NGF induced the expression of Fas and Fas ligand. (B) Only co-stimulation with Superfas ligand (SFL, 100 ng/ml) and 50 ng/ml NGF had the same sprouting effect as NGF alone. Addition of the Fas inhibitor Fas-Fc (4 μg/ml) to cells stimulated with 50 ng/ml NGF diminished sprouting. (C) Stimulation with Fas-Fc and Superfas ligand alone did not have the same neurite-inducing effect as 50 ng/ml NGF. (D) Cell viability was reduced when cells were incubated with Fas-Fc and Superfas ligand alone, whereas all cells cultured with NGF after injury did not display any significant loss of cell viability. c, control, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to 50 ng/ml NGF; $^{§§}p < 0.01$, $^{§§§}p < 0.001$ compared to 4 μg/ml Fas-Fc.

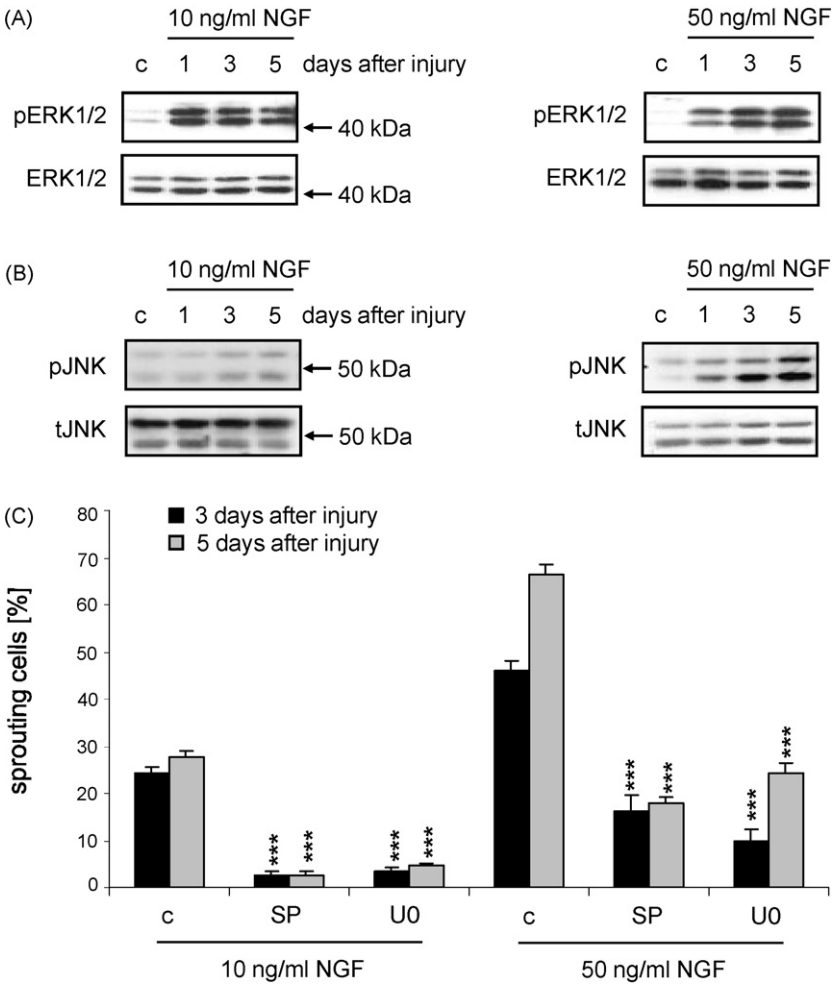

Fig. 4 – ERK1/2 and JNKs are activated in response to NGF after injury of PC12 cells. While ERK1/2 was strongly activated by both NGF concentrations (A), JNKs were only clearly phosphorylated after stimulation with 50 ng/ml NGF (B). Inhibition of both kinases significantly reduced sprouting (C). c, control; SP, SP600125; U0, U0126; ***$p < 0.001$ compared to NGF-treated controls.

inhibition by K252a. Interestingly, the activation of ERK1/2 was not affected by K252a, whereas JNK phosphorylation was dose-dependently decreased (Fig. 5A).

Co-stimulation with NGF (50 ng/ml) and Superfas ligand (100 ng/ml) activated ERK1/2 and JNKs to a similar extent as NGF alone (Fig. 5B). However, co-stimulation with Fas-Fc (4 μg/ml) and 50 ng/ml NGF led to a reduced phosphorylation of ERK1/2 and JNKs compared to NGF alone (Fig. 5B). Since the addition of Superfas ligand to NGF did not change the activity of ERK1/2 and JNKs, we investigated whether the formation of neurites was still induced by both pathways to a similar extent. Interestingly, pre-incubation with SP600125 (before addition of NGF and SFL) decreased sprouting more efficiently than pre-incubation with U0126 (Fig. 5C).

To summarize, ERK1/2 and JNKs were both activated in response to NGF. In contrast to ERK1/2, JNK phosphorylation was attenuated by TrkA inhibition. Similarly, co-stimulation with SFL and NGF caused ERK1/2 and JNK activation, but neurite formation was more strongly reduced after JNK inhibition than after ERK1/2 inhibition, which suggests that JNKs are more important for neurite re-growth in this model system and turn apoptotic Fas ligation into sprouting signals.

4. Discussion

In the present study, we investigated the importance of the NGF receptors and the Fas system for neurite re-growth and their effect on the activation of MAPKs after experimental injury of differentiated PC12 cells. We found that NGF induced sprouting and the expression of its own receptors as well as of Fas and Fas ligand. While only TrkA significantly contributed to neurite re-formation after stimulation with NGF alone, signal transduction supporting neuronal recovery seems to be altered after Fas ligation, which suggests a parallel sprouting induction via NGF and Fas signalling. The MAPKs ERK1/2 and JNKs are activated in response to NGF and crucially affect neurite re-growth, whereas only JNKs mediate Fas signalling in this context.

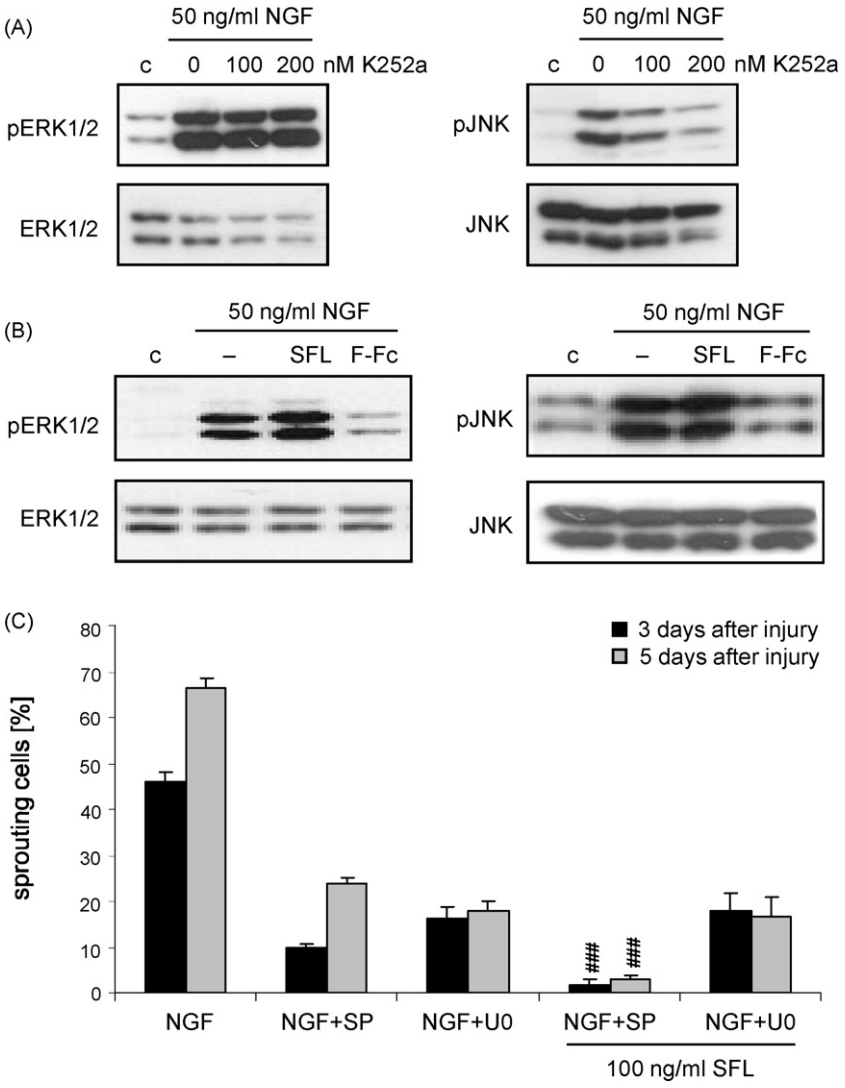

Fig. 5 – Interactions between Fas and NGF signalling. (A) The activation of ERK1/2 was not affected by the TrkA inhibitor K252a, whereas JNK phosphorylation was dose-dependently decreased. (B) 50 ng/ml NGF alone or in combination with 100 ng/ml Superfas ligand (SFL) caused a similar activation of ERK1/2 and JNKs, whereas co-stimulation with 50 ng/ml NGF and 4 μg/ml Fas-Fc (F-Fc) decreased their activity. (C) Pre-incubation with SP600125 (SP, 2 μM) significantly reduced sprouting compared to U0126 ($^{###}$ $p < 0.001$) when 100 ng/ml Superfas ligand was added to 50 ng/ml NGF.

4.1. *Neurite re-growth and receptor regulation*

During experimental injury, the neurites of differentiated PC12 cells were detached, and the injured cells were subsequently stimulated with NGF to induce neurite re-growth. Whereas cell survival after injury was independent of the NGF concentrations used, rapid sprouting was only achieved with 50 ng/ml, but not with 10 ng/ml NGF, indicating that a certain threshold concentration of NGF is necessary to enhance neurite formation. This dose-dependent sprouting of injured PC12 cells is very comparable to NGF-mediated differentiation of PC12 cells [11], suggesting a common mechanism for neurite outgrowth during differentiation and after injury.

Similarly, treatment with 50 ng/ml NGF enhanced the expression of its receptors during neurite re-growth to a much larger extent than the 10 ng/ml dose. This type of clear-cut receptor regulation after injury is rare. In animal models, an increase of neuronal p75 after injury is either involved in anti-inflammatory responses [23] or it is only present in intact neurons which are not directly affected by the lesion [24]. After spinal cord injury and axotomy, TrkA expression decreases at the lesion site and cannot be influenced by adding NGF [25–27]. Only after optic nerve lesions, TrkA increases during regeneration [28]. Nevertheless, TrkA is important for neuronal recovery and survival [29]. First, its expression is slowly restored after injury [27]. Secondly, the enhanced p75 expression in intact neurons facilitates the signalling of residual TrkA receptors [24]. This synergistic receptor interaction is also described for PC12 cells [30], suggesting that enhanced NGF signalling is achieved by an increased receptor activity rather than by a stronger receptor expression.

With regard to receptor function, TrkA was the crucial mediator of NGF signals, which has also been shown in dorsal root ganglia (DRGs) [29]. Generally, the presence of p75

enhances NGF binding of TrkA [2] and facilitates TrkA signalling [34,35], whereas p75 alone is normally associated with growth cone collapse [31] or apoptosis [32]. In both cases, TrkA is either needed to induce p75 shedding to disinhibit neuronal growth [31,33] or to block apoptotic signals [2]. In PC12 cells, p75 only induces apoptosis when it is over-expressed [32]. Interestingly, the supporting and stabilizing function of p75 is obviously not necessary for neurite re-formation after PC12 cell injury.

4.2. *Fas signalling after injury of PC12 cells*

Fas and Fas ligand are widely expressed in the nervous system and their expression is increased in various neurological disorders and after injury, e.g. of the spinal cord [3,36]. Even oxidative stress alone, which also occurs under neuropathological conditions, can induce Fas and Fas ligand in PC12 cells [37] and might even lead to apoptosis [38]. Therefore, blocking Fas signalling has been an option to increase cell survival, e.g. after spinal cord injury [39]. Differentiated PC12 cells, however, are protected against Fas-induced apoptosis. Although NGF does not suppress the production of Fas ligand [37], it induces the expression of the protective Fas apoptosis inhibitory molecule (FAIM) [40]. After mechanical injury, PC12 cells treated with Fas-Fc or Superfas ligand alone showed decreased survival. As Fas inhibition and Fas stimulation both had the same effect, the reduced cell viability seems to result from NGF withdrawal, which usually leads to a potentiated activity of the Fas system and subsequently to apoptosis [21].

Co-stimulation with Superfas ligand and NGF induced sprouting to the same extent as NGF alone, whereas the addition of Fas-Fc reduced neurite re-growth. In other neuronal systems, the activation of Fas and Fas ligand can increase branching [5] or contribute to differentiation and regeneration after sciatic nerve injury [4]. Especially the strong neurite-forming effect of Superfas ligand in DRGs [4] was not observed in PC12 cells, as their survival is NGF-dependent, which makes NGF a prerequisite for sprouting. However, Fas signalling seems to act in parallel to NGF, as its inhibition by Fas-Fc significantly reduces sprouting compared to NGF alone. In this case, one could expect an additional effect of a co-stimulation with NGF and Superfas ligand. However, this effect has not been observed, indicating that the recovery of injured PC12 cells already proceeds at maximum rate in this injury model.

4.3. *The involvement of MAPKs in neurite re-growth of injured PC12 cells*

ERK1/2 and JNKs are activated during neurite re-formation, and their inhibition significantly reduces sprouting. Several studies have described the importance of ERK1/2 for neuronal recovery in experimental cell models [22] or axon regeneration of developmental peripheral neurons [9].

JNKs are considered important mediators of the regenerative cell body response [41–43] and have been mostly implicated in the induction of subsequent apoptosis (reviewed by [6]). However, in experimental cell models or DRGs, JNK activity is strongly connected with neurite outgrowth [22,44]. Even after sciatic nerve transection, axonal JNKs are locally activated [45], and this activity can lead to cell death or nerve recovery. During axonal regeneration, JNK activation is sustained [45,46] and returns to basal levels after the regenerative process has been completed.

Concerning the mechanism of MAPK activation, TrkA inhibition did not reduce ERK1/2, but only JNK phosphorylation. These findings are surprising, since TrkA signalling is known to involve ERK1/2 activity [1,2], whereas JNK is usually associated with $p75^{NTR}$ activation and the induction of cell death [32,47,48]. Only after cellular stress, the TrkA inhibitor K252a has been described to function as a JNK inhibitor and to promote survival by activating ERK1/2 and Akt [49], similar to its derivative and MLK3 inhibitor CEP-1347. For neurite regeneration, however, the link between JNKs and TrkA has not been shown before.

After co-stimulation with Fas-Fc and NGF, the activity of ERK1/2 and JNK was reduced, whereas co-stimulation with SFL and NGF did not change the phosphorylation patterns of both kinases compared to stimulation with NGF alone. The role of ERK1/2 in Fas signalling has been reported to be either antagonistic by blocking Fas-mediated apoptosis [50,51] or agonistic by promoting phenotypic maturation of dendritic cells or cytokine release in human glioma cells in response to Fas ligation [52,53]. Also after sciatic nerve injury, ERK1/2 mediate Fas-ligand-induced neurite re-growth [4]. In injured PC12 cells, however, ERK1/2 do not seem to participate in Fas signalling. After co-stimulation with Fas-Fc and NGF, ERK1/2 activity was reduced compared to NGF alone, but remained above basal levels, suggesting that ERK1/2 still mediated NGF signals. In contrast, ERK1/2 activity was similar after stimulation with either NGF alone or NGF plus SFL. Moreover, the inhibition of sprouting by pre-incubation with the MEK1/ERK1/2 inhibitor U0126 was independent of Fas activation by SFL, suggesting that ERK1/2 are involved in NGF signalling irrespective of an additional Fas stimulation.

In the literature, JNKs have mostly been implicated in Fas-mediated apoptosis by executing apoptotic signals after Fas ligation as well as inducing the expression of Fas and Fas ligand [21,46,54]. After experimental injury of differentiated PC12 cells, however, their role is different. Stimulation with SFL seems to initiate a pathway parallel to NGF to support neurite formation and recruiting one part of the JNK pool, which might otherwise be involved in NGF signalling. When the Fas inhibitor Fas-Fc is added, JNK activity and sprouting are reduced. Moreover, pre-treatment with SP600125 especially attenuates neurite formation after the co-stimulation with NGF and SFL, which further underlines that sprouting signals are altered after Fas ligation. All of these findings support the hypothesis that Fas signalling contributes to neurite regeneration after injury via JNKs. In contrast to the normal apoptotic features of Fas ligation and subsequent JNK activation, the simultaneous activity of the NGF cascade might lead to the activation of a hypothetical ''physiological JNK pool'', which is also accessed by the Fas pathway after injury and therefore initiates sprouting rather than degeneration.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Annika Dorst and Elke Schroeder for excellent technical assistance. This project was supported by the Deutsche Forschungsgemeinschaft (SFB 415).

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