

## Chapter 7

# The Role of JNK Pathway in the Process of Excitotoxicity Induced by Epilepsy and Neurodegeneration

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**Abstract** The c-Jun N-terminal kinases (JNKs) are members of the MAPK family and can be activated in neurons by different neurotoxins such as kainic acid (an experimental model of epilepsy), beta amyloid, and nitropropionic acid. Although JNKs have different physiological functions they have been linked mainly to the apoptotic process in neurons and other cell types. Therefore, the JNK signaling pathway constitutes an important target to prevent the apoptotic cell death in epilepsy and neurodegeneration. In the present chapter, the role of JNKs, specifically the JNK3 isoform, as a potential target for epilepsy and neurodegenerative diseases will be discussed. In addition, the pharmacological compounds that inhibit the JNKs signaling pathway constitutes a potential therapeutic intervention to prevent neuronal death.

**Keywords** C-Jun N-terminal kinase • JNK3 • Epilepsy • Alzheimer's disease • Neurodegeneration • Apoptosis

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## 7.1 Introduction

One of the biggest health problems that we have today is the development of effective drugs for the treatment of neurodegenerative diseases and epilepsy. The cause is clear, in the case of the neurodegenerative diseases the population over 65 is growing, at least in developed countries and favors the emergence of neurological diseases (Smith et al. 1999). In the treatment of epilepsy, the pharmacoresistance is probably one of the main problems in the treatment of this neurological disease. Thus, in next coming years, the aims will be develop effective drugs for the prevention of neuronal death process that occurs in neurological diseases.

Although it is well known the participation of more than one pathway in the process of neuronal loss, for example the cell cycle activation, GSK3 $\beta$ , cdk5, oxidative stress among them, c-Jun N-terminal kinase (c-JNKs) constitutes one of the main pathway related with cell death (McCubrey et al. 2006; Levy et al. 2009). Thus c-JNKs are an interesting target for the development of drugs for the treatment of neurodegenerative disorders (Borsello and Forloni 2007; Braithwaite et al. 2010).

It is well know that c-JNKs is a member of the family of serine and threonine mitogen-activated protein kinases (MAPKs) which participates in numerous physiological processes such as tissue differentiation, cancer, diabetes, cell survival and apoptosis, and other pathogenic processes (Bevilaqua et al. 2003; Borsello and Bonny 2004; Eshraghi et al. 2007; Borsello et al. 2003). Thus, MAPKs allows the cell to respond to exogenous and endogenous stimuli integrating signals into complex cytoplasmic and nuclear processes. Currently, it has been characterized four mammalian MAPK cascades: (a) extracellular signal-regulated kinase 1 and 2 (ERK1/2), (b) c-Jun N-terminal kinases (c-JNKs), (c) p38 consisting of four isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ), and (d) ERK5 (Bozyczko-Coyne et al. 2002; Brecht et al. 2005; Borsello and Forloni 2007).

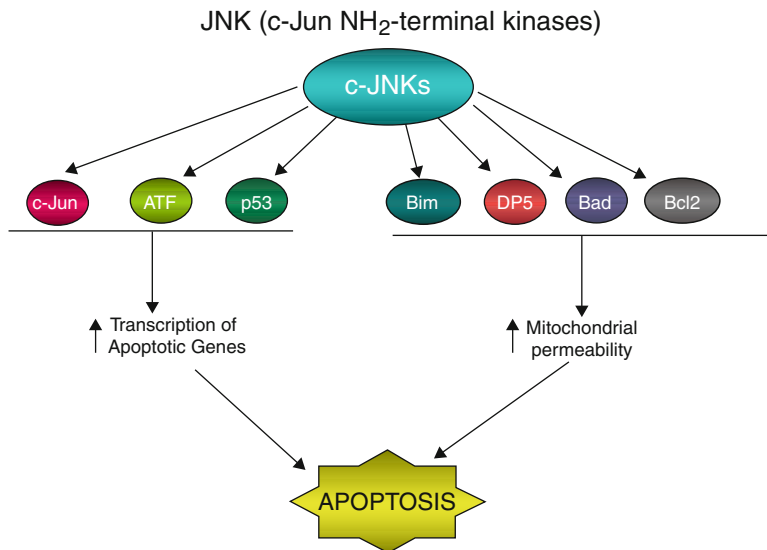
Three genes encoding for c-JNKs have been characterized in humans (*Jnk1*, *Jnk2*, and *Jnk3*). Some differences exist among the three isoforms codified by these genes, since whereas JNK1 and JNK2 are widely distribute in all organism tissues, JNK3 mainly shows a neuronal localization and thus constitutes a target for neuronal death prevention (Brecht et al. 2005; de Lemos et al. 2010). MAPK pathways are activated either as a result of a series of interactions between the kinase components or through the formation of a signaling complex that contains multiple kinases, driven by a scaffold protein (Behrens et al. 1999; Morrison and Davis 2003; Björkblom et al. 2008; Borsello et al. 2003). C-JNKs are activated by phosphorylation of Thr and Tyr residues in the activation loop by mitogen-activated protein kinase kinase 4 (MKK4) and kinase kinase 7 (MKK7) (Weston and Davis, 2007). Interactions between proteins and scaffold proteins are crucial for MAPK function and regulation in the cell. For instance, kinase suppressor of Ras-1 (KSR) and MEK partner 1 (MP1) act as scaffold proteins for the ERK signaling pathway, whereas JNK-interacting proteins (JIPs) serve as scaffold proteins for the c-JNK pathway. Likewise,  $\beta$ -arrestin 2 acts as a scaffold protein for both the ERK and the JNK signaling pathways. Following activation, c-JNKs can phosphorylate and modulate the

activities of hundreds of substrates (Borsello and Forloni 2007). Among the substrates identified that are phosphorylated in the nucleus include some hormone receptors, as well as transcription factors such as the activator protein-1 (AP-1), the family of Jun factors (c-Jun, JunB, JunD), Elk-1, p53, the anti-activation transcription factor-2 (ATF-2), JDP2, c-Myc, the NAFT family, the STAT family, and the PAX family (Bogoyevitch and Kobe). For many of these factors, phosphorylation increases its activity and induces transcriptional gene expression. Currently, the most studied substrate for c-JNKs is c-Jun; however, it is not known which isoform is responsible for its phosphorylation. Interestingly, the high expression of both *c-Jun* gene and the great protein levels precedes or coincides with periods of cell death, such as that occurring during embryonic development (Herdegen et al. 1997; Coffey et al. 2000), after trauma (Bozyczko-Coyne et al. 2002; Suckfuell et al. 2007), cerebral ischemia (Tian et al. 2005; Hu et al. 2008), and seizures (Gass et al. 1993; de Lemos et al. 2010). This link of c-JNKs to neuronal death have attracted enormous interest of this pathway in several neurodegenerative disorders such as Alzheimer diseases (AD) and Parkinson diseases (PD) (Resnick and Fennell 2004). In this chapter, we reviewed the progress in understanding the role of c-JNKs in the pathophysiology of neurodegenerative diseases and the potential role of JNK inhibitors to treat neurodegenerative disorders.

## 7.2 Role of c-Jun N-Terminal Kinase Activation in Apoptosis

Some mechanisms have been proposed to explain how the c-JNKs pathway governs the whole process of neuronal death by apoptosis. For example, c-JNKs directly phosphorylate and regulate the pro- and anti-apoptotic activity of members of the B-cell lymphoma 2 (Bcl-2) families (Sun et al. 2005; Vogel et al. 2009; Donovan et al. 2002; Björkblom et al. 2008). The Bcl-2 family proteins can be divided into three major subgroups: (1) Anti-apoptotic proteins, such as Bcl-2, Bcl-XL, and Mcl-1, which typically share four conserved motifs termed Bcl-2 homology (BH) domains and can form heterodimers with Bax, inhibiting mitochondrial cytochrome *c* release and protecting against cell death; (2) The pro-apoptotic proteins, such as Bax, Bak, and Bok, which typically have three BH domains but promote cytochrome *c* release and apoptosis; (3) The BH3-only proteins, including Dp5/HRK (death protein 5/harakiri), Bim (Bcl2-interacting mediator of cell death), Bid, Bad, Puma, and Noxa, which share the BH3 domain. Thus BH3-only proteins are critical initiators of apoptosis and during this process are stringently regulated at the transcriptional and posttranslational levels depending on the cell type and apoptotic stimulus (Morishima et al. 2001; Puthalakath and Strasser 2002) (Fig. 7.1).

Dp5 is one of the BH3-only proteins of particular interest to studies of apoptosis in the nervous system. Dp5 was the first found to be induced by NGF deprivation in sympathetic neurons. The induction of Dp5 is also observed in cerebellar granule neurons (CGNs) deprived of potassium, cortical neurons exposed to toxic concentrations of  $\beta$ -amyloid protein, retinal ganglion cells of axotomized rat retinas, and



**Fig. 7.1** Proposed mechanisms by which JNKs could regulate the apoptotic process. JNKs can induce the expression of nuclear genes that promote neuronal apoptosis. Furthermore, pro-apoptotic proteins can be phosphorylated by JNKs and are translocated to the mitochondria and induce neuronal apoptosis

postnatal mouse motoneurons (Ma et al. 2007). Over expression of Dp5 in sympathetic neurons or CGNs induces apoptosis in a Bax-dependent manner, and this effect can be attenuated by co-expression of anti-apoptotic Bcl-2. Deletion of Dp5 delays sympathetic neuron apoptosis triggered by NGF withdrawal and rescues motoneurons from axotomy-induced apoptosis (Coults et al. 2007). These studies suggest that Dp5 plays a critical role in neuronal apoptosis.

Studies from several laboratories have demonstrated that JNK is involved in *dp5* upregulation during neuronal injury or apoptosis. Although JNK has been shown to be involved in *dp5* upregulation, the mechanism of how JNK regulates *dp5* expression is not clarified.

c-JNKs phosphorylate and decrease the anti-apoptotic activity of both Bcl-2 and Bcl-XL. In addition, these kinases phosphorylate the pro-apoptotic protein BAD at Ser-128, thereby potentiating its pro-apoptotic effect (Donovan et al. 2002; Bogoyevitch and Kobe 2006). Similarly, c-JNKs phosphorylate the pro-apoptotic proteins Bim and Bcl-2-modifying factor (Bmf), causing their release and translocation to the mitochondria, where they promote the release of mitochondrial proteins such as cytochrome c, apoptosis-inducing factor (AIF), and other mitochondrial pro-apoptotic death mediators. Therefore, the release of cytochrome c and other pro-apoptotic proteins from the mitochondria is regulated by the Bcl-2 protein family. Finally, all these processes favor the process of cell death by activation of a caspase-dependent or independent pathway.

### 7.3 Experimental Data with JNK Inhibitors

Since the activation of the c-JNKs pathway may be a common step in neurodegeneration the pharmacological inhibition of c-JNKs is a potential strategy to protect against neuronal loss. In this sense, we highlight three chemical inhibitors: CEP-1347, an inhibitor of the MLK family of the c-JNKs pathway, and SP600125 and AS601245, both selective inhibitors of c-JNKs activity (Chen et al. 2010; Chambers et al. 2011).

The main limitation of the CEP-1347 inhibitor is its poor selectivity because it acts upstream of JNK activators, namely the MLKs (Saporito et al. 2002). This compound has shown neuroprotective effects both *in vitro* and *in vivo* against  $\beta$ -amyloid toxicity, trophic withdrawal in PC12 cells, MPP+ exposure and apoptosis in cerebellar granule cells following serum and potassium deprivation (Maroney et al. 1999). Moreover, in addition to inhibiting the pro-apoptotic JNK pathway, this drug activates neurotrophic pathways, including the neurotrophin BDNF in a mouse model of Huntington disease (HD). Specifically, CEP-1347 increases BDNF mRNA levels in the brain compared to vehicle, which correlates with a reduction of disease progression in R6/2 mice, an experimental model of HD (Apostol et al. 2008).

However, although the PRECEPT clinical trial showed that CEP-1347 was safe and well-tolerated in a randomized placebo-controlled study in PD subjects, it was concluded that this drug was not effective to treat PD. Nevertheless, further studies are needed to assess why this drug was not effective (The Parkinson Study Group PRECEPT Investigators 2007).

SP600125 is a reversible ATP-competitive inhibitor that can inhibit JNK, including JNK-1, -2, and -3 isoforms, with high selectivity. The neuroprotective effect of SP600125 has been seen in ischemic processes (Guan et al. 2005). Other studies have further demonstrated that SP600125 can inhibit the phosphorylation of c-Jun and prevent the expression of IL-2, IFN- $\gamma$ , TNF- $\alpha$ , and COX-2, while inactivating Bcl-2 and blocking IL-1-induced accumulation of p-c-Jun and inducing c-Jun transcription (Guan et al. 2005; Choi et al. 2010; Chen et al. 2010; Chambers et al. 2011).

SP600125 blocked the induction of BH3-only protein Bim after seizures in mice, suggesting that the JNK pathway may be critical in epilepsy/neurodegeneration. Interestingly, Chen and colleagues (2010) reported that SP600125 was effective in the treatment of experimental temporal lobe epilepsy (TLE) in rats (Murphy et al. 2010). Moreover, SP600125 exerts neuroprotective effects against MPTP-induced neurotoxicity in mice, inhibiting JNK signaling and also reducing COX-2 expression (Wang et al. 2004). Likewise, SP600125 displays neuroprotective functions in  $\beta$ -amyloid-injected rats, as it has potent memory-enhancing effects and blocks learning deficits induced by  $\beta$ -amyloid (Ramin et al. 2011). In addition to the neuroprotective properties of SP600125, this compound also improves neuroplasticity. Effectively, this JNK inhibitor increases synaptic transmission in the hippocampus after treatment with  $\beta$ -amyloid in the CA1 area, suggesting a role of JNK in regulating short-term memory formation. More studies are required to evaluate the effects of SP600125 in  $\beta$ -amyloid production in AD models; however, its low water solubility limits its usefulness in human treatment.

AS601245 is also a reversible ATP-competitive inhibitor that inhibits the three JNK isoforms, with a higher affinity for JNK3. This compound exhibits neuroprotective effects in models of ischemia (Carboni et al. 2004). It also decreases microglial activation and exerts beneficial effects on memory deficits.

As mentioned above, JNK activity can be regulated by JNK-interacting proteins, such as JIP-1, a protein that integrates the positive and negative regulators of JNK, facilitating the activity of the JNK signaling pathway. In mice, JIP-1 contains a JNK-binding domain (JBD) that mediates the sequestration of JNK in the cytoplasm, thus inhibiting the expression of genes that are activated via the JNK signaling pathway and acts as a functional inhibitor of JNK. Therefore, in addition to chemical inhibitors, JIP-derived peptides have been developed to inhibit JNK activity based on the properties of the protein JIP-1. The peptide is XG-102, also called D-JNK-permeable peptide 1 (D-JNKI), exerts neuroprotective effects against different models of excitotoxicity in vitro and plays a neuroprotective role in experimental models of ischemia, preventing cell death by apoptosis (Pan et al. 2010; Liu et al. 2010). Thus, rather than inhibiting the enzymatic activity of JNKs as classical chemical inhibitors do, XG-102 selectively blocks the access of JNK to different substrates, preventing protein–protein interactions without interfering with its activation. Moreover, XG-102 has been observed to show beneficial effects on both hair cell death and the permanent loss of hearing induced by sound trauma (Wang et al. 2007). A phase I/II clinical study with XG-102 are currently underway to evaluate the efficacy of this compound in patients with acute acoustic trauma; the study will be completed in 2012. Therefore, in the coming years, more clinical data will shed light on the neuroprotective potential of these compounds.

## 7.4 Role of the JNK3 Pathway in Epilepsy

Knockouts for the mammalian *Jnk* genes *Jnk1*, *Jnk2*, and *Jnk3* are viable and have enabled the study of the physiological and pathological roles of the JNK pathway (de Lemos et al. 2010). The first evidence of the role of *Jnk3* in neurotoxicity was provided by Yang and colleagues (1997), who demonstrated that in comparison to wild-type mice, *Jnk3* (–/–) mice were less sensitive to seizures induced by kainic acid and to neuronal death in the hippocampal CA1 and CA3 areas. Furthermore, *Jnk3* (–/–) mice remain viable during development and show normal brain structure. These studies were the first which demonstrated that *Jnk3* deletion is a suitable target to prevent both neuronal cell death and seizures elicited (Yang et al. 1997). Subsequent studies demonstrated that *Jnk3* (–/–) mice had increased p110-beta protein levels and PI3K activity because of an upregulation of the *pik3cb*. This gene selectively increases neuroprotective cell pathways; however, it is unknown how the PI3K/Akt signaling pathway is activated in the absence of JNK3 (Junyent et al. 2011). On the other hand, *Jnk1* (–/–) null mice did not show any changes in AKT activity in the hippocampus and probably could explain why loss of *Jnk1* or *Jnk2* did not show any effects against KA treatment (Brecht et al. 2005).

All these experimental data suggest that JNK3 is required to induce neuronal stress and apoptosis in adult hippocampus. The disruption of *Jnk1* or *Jnk2* does not affect the nervous system, but double knockout *Jnk1* ( $-/-$ ) *Jnk2* ( $-/-$ ) mice die during embryonic development with major alterations in the neural phenotype (Kuan et al. 1999). This is because the neural tube fails to close due to a deficiency in apoptosis. However, the opposite effect has been observed in the developing cortex brain, where there is an increase in apoptosis (Kuan et al. 1999). This indicates that *Jnk1* and *Jnk2* are necessary for the development of cell death in the neural tube and, in turn, for promoting cell survival during cerebral cortex development. Thus, although c-JNKs and c-Jun proteins are pro-apoptotic in different cell types, they may have other functions, as already mentioned. Furthermore, induction of axonal regeneration in axotomised peripheral neurons in an adult organism appears to be associated with increased expression of c-Jun, suggesting that this transcription factor regulates the expression of genes related to regeneration (Herdegen et al. 1997).

As we have commented above, the first evidence of the involvement of c-JNKs in experimental epilepsy models was derived from the reduction of seizures activity and prevention of apoptosis in JNK3-deficient mice treated with kainic acid (Yang et al. 1997). In addition, mice with an inactive form of the c-jun gene (Jun AA: alanine instead of serine at positions 63 and 73) showed resistance to excitotoxic neuronal death. These data opened the study of a specific target to protect against epilepsy seizures and also neurodegenerative disorders. Thus, blocking the access of c-JNKs to their substrate c-Jun may offer a suitable target in neuroprotection (Behrens et al. 2001).

Interestingly Murphy and colleagues demonstrate that Bim has a causal role in the status epilepticus-induced cell death process because neurodegeneration was reduced in *bim*( $-/-$ ) mice (Murphy et al. 2010). Bim is a downstream BH3-only protein target regulated by JNKs. These data indicate that loss of a specific BH3-only can reduce seizure damage in vivo.

## 7.5 Role of JNK3 in Alzheimer's Disease

Alzheimer's disease (AD) is currently the leading global cause of dementia in the elderly. At the initial stages, AD is characterized by a mild loss of memory and then progresses to a severe loss of cognitive performance in the advanced stages (Xu et al. 2009; Zhu et al. 2001; Mondragón-Rodríguez et al. 2010). AD is characterized by a series of histological markers that include neurofibrillary tangles, senile plaques, and a large loss of neurons (Castellani et al. 2009). Apart from these markers, the loss of neurons is associated with apoptosis, which is probably mediated by several inducers such as reactive oxygen species,  $\beta$ -amyloid, mitochondrial alteration, and an inflammatory process that induces microglial activation in the AD brain (Su et al. 2008). In this process of neuronal demise in AD, different signaling pathways are activated and among them, the c-JNKs pathway plays a prominent role. This is based on several lines of evidence: (a) c-JNKs can phosphorylate tau and

(b)  $\beta$ -amyloid activates the c-JNKs pathway that then promotes neuronal loss and this activation mediates  $\beta$ -amyloid toxicity (Ramin et al. 2011; Morishima et al. 2001; Colombo et al. 2009; Mazzitelli et al. 2011). Furthermore, it has been previously reported that expression of c-Jun increases in the AD brain and neurons from c-Jun-null mice are resistant to  $\beta$ -amyloid toxicity (Mazzitelli et al. 2011).

Morishima and colleagues were the first to demonstrate that neuronal hippocampal and cortical cultures of JNK3 knockout mice were partially protected from neuronal apoptosis mediated by  $\beta$ -amyloid (Morishima et al. 2001). However, c-Jun phosphorylation was not completely inhibited, indicating that JNK1 or JNK2 may be involved in this phosphorylation (Morishima et al. 2001). On the other hand, a very important point in AD is the formation of  $\beta$ -amyloid fragments that are derived from amyloid precursor protein (APP) after cleavage by beta/gamma secretase. The C-terminal intracellular region (AICD) of APP plays an important functional role in regulating APP metabolism (Slomnicki et al. 2008). AICD contains eight potential phosphorylation sites, but one of them, specifically T668, is phosphorylated by several kinases including GSK3 $\beta$ , JNK3, Cdc2, and Cdk5. Likewise, these kinases are associated with neurotoxicity and have been implicated in neurodegenerative diseases. Interestingly, JNK3 is specifically involved in the physiological regulation of AICD during neuronal differentiation, suggesting a role of JNK3 in synaptogenesis (Kimberly et al. 2005).

Moreover, Colombo and colleagues used the JNK inhibitor peptide (D-JNKI1) to demonstrate that JNK plays a prominent role in APP production and that the extracellular  $\beta$ -amyloid fragments are also reduced (Colombo et al. 2009). It has been observed that  $\beta$ -secretase (BACE1) is regulated by BACE1 gene transcription through the JNK/c-Jun signaling pathway (Sclip et al. 2011). This is important because it has been hypothesized that  $\beta$ -amyloid fragments are mainly responsible for the neurodegeneration in AD.

Studies performed in neuronal cell cultures have shown that JNK3 is involved in the apoptotic process mediated by  $\beta$ -amyloid. This process involves MLK3–MKK7–JNK3 activation, as well as downstream events including p-JNK nuclear localization, c-Jun phosphorylation and Bad translocation to the mitochondria, with the mitochondria then releasing pro-apoptotic proteins (Sclip et al. 2011).

## 7.6 Role of JNK3 in Experimental Models of Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease. The mechanisms involved in its pathogenesis include oxidative stress production, mitochondrial dysfunction, and protein aggregation, which promote the loss of dopaminergic neurons in the substantia nigra pars compacta (Levy et al. 2009). Currently, the main problem of PD and all neurodegenerative diseases is that therapy is focused on symptomatic relief. It is necessary to develop



neuroprotective therapies that will slow disease progression. The investigation of cell death mechanisms common to several models of experimental PD may identify new drug targets for treatment.

It has been reported that in mice exposed to MPTP, a PD neurotoxin that inhibits mitochondrial complex I, dopaminergic neurons degenerate in the substantia nigra (Peng and Andersen 2003; Saporito et al. 2000; Hunot et al. 2004; Pan et al. 2009; Choi et al. 2010). Inhibiting c-JNKs or their upstream signals may reduce dopamine-mediated neuronal death induced by MPTP, suggesting a possible therapeutic application for c-JNK inhibitors in PD (Pan et al. 2010). Additionally, dopaminergic neuronal death induced by MPTP, rotenone, paraquat, and 6-hydroxydopamine all require JNK3 activation (Hunot et al. 2004; Pan et al. 2007; Pan et al. 2009; Pan et al. 2010). Therefore, JNK3 is a critical and common mediator of dopaminergic neuronal death in PD experimental models.

Hunot and colleagues demonstrated that mice treated with MPTP showed increased COX-2 expression that was mediated by JNK (Hunot, et al. 2004). Interestingly, COX-2 expression is upregulated in PD brains and is generally induced by stress stimuli. Moreover, COX-2 localizes in neurons and its expression is upregulated in numerous pathological conditions, including Alzheimer's disease. Therefore, COX-2 induction might represent an important step in the cascade of molecular events leading to neuronal loss in PD.

Disappointingly, a clinical trial using CEP1347 to treat PD was terminated because it failed to produce significant improvements. The main problem of this compound is its poor selectivity against JNK3, the main target involved in apoptosis. As we previously discussed, JNK1 and 2 are ubiquitously expressed in adult tissues and have important physiological functions; hence, the side effects associated with inhibiting these enzymes limit the tolerable doses of JNK inhibitors. Accordingly, general inhibition of all JNK isoforms, such as that achieved by CEP1347, may be of limited benefit to treat neurodegenerative diseases. On the other hand, JNK3 is neural-specific and does not exhibit high basal activity in the brain. Therefore, selective or specific inhibition of the JNK3 isoform may be more specific to slow down PD progression.

## 7.7 JNK and Huntington's Disease

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an autosomal dominant mutation in either of the two copies of the huntingtin gene. Specifically, this disorder is caused by an abnormal expansion of a CAG codon in exon-1 of the gene (Liu 1998; Garcia et al. 2002).

Systemic administration of mitochondrial toxin 3-nitropropionic acid (3-NPA) to experimental animals, such as nonhuman primates and rodents, produces symptoms similar to those of human HD. The toxin irreversibly inhibits the succinate dehydrogenase (SDH) enzyme, the main constituent of the mitochondrial respiratory chain complex (MCC) II (Garcia et al. 2002). Treatment of rats and in vitro primary

striatal cultures with 3-NPA activates the JNK pathway and contributes to neuronal death (Perrin et al. 2009). This neuronal loss depends on c-Jun because expression of dominant negative c-Jun protects striatal neurons from cell death mediated by this complex II inhibitor. Likewise, JNK activation appears to be a major factor in the apoptotic death of HN33 cells induced by polyglutamine-expanded huntingtin (Liu 1998). Mutated huntingtin with 48 or 89 polyglutamine repeats enhances JNK activation and may trigger apoptosis, while normal huntingtin with 16 repeats fails to activate the JNK pathway.

However, a recent study demonstrated that the intraperitoneal administration of 3-NPA to *Jnk3*( $-/-$ ) mice was not neuroprotective in contrast to the neurotoxin KA (Junyent et al. 2012). This suggests that although the JNK pathway may be activated in this model, JNK3 is probably not mainly responsible for neuronal death and other pathways may be involved in neuronal loss.

## 7.8 Role of JNK3 and Ischemia

Pirianov and colleagues were the first to describe the neuroprotective role of the specific isoform of JNK3 in a model of hypoxic–ischemic injury (Pirianov et al. 2007). They demonstrated that the deletion of JNK3 had a neuroprotective role by reducing ATF-2 phosphorylation, which was associated with the size of the infarct. Therefore, it was proposed that the JNK3/c-Jun/ATF-2 pathway was likely to be the main route in neural cell death induced by hypoxic–ischemic injury. Moreover, the authors demonstrated that JNK3 activation phosphorylated c-Jun, which has been shown to trigger the transcription of a large number of death genes including the pro-apoptotic Bcl-2 family member, Bim, and the death receptors TNFR (p55) and CD95/Fas (Qi et al. 2010). Furthermore, JNK3 signaling is implicated in the mitochondrial release of cytochrome c, leading to caspase-3 activation either via a Bim-dependent mechanism or through direct targeting of the mitochondria (Morishima et al. 2001; Murphy et al. 2010). *Jnk3* knockout in perinatal brain injury has been linked to a decrease in caspase-3 activity, as well as a reduction in the levels of the pro-apoptotic proteins PUMA and Bim (Tian et al. 2005; Pirianov et al. 2007; Qi et al. 2010).

The postsynaptic density protein 95 (PSD-95) is a scaffold protein characterized by the presence of several protein-binding domains, including three N-terminal PDZ domains, a signal Src homology region 3 domain, and a C-terminal guanylate kinase-like domain (Han et al. 2008; Hu et al. 2008). Moreover, the PDZ domains bind to the C-terminus of the NMDA receptor NR2 and KA receptor GluR6 subunit, which is crucial for the grouping of NMDA receptors and KA receptors in the post-synaptic membrane. It has been reported that brain ischemia alters the GluR6-PSD-95-MLK3 complex in the hippocampus, which affects JNK3 phosphorylation and activation. Likewise, inhibition of the JNK3 signaling pathway is involved in the neuroprotective role of GABA against ischemic injury.

## 7.9 Future Perspectives of Inhibiting the c-JNKs Pathway in the Treatment of Neurological Disorders

The development of neuroprotective drugs is undoubtedly an area of increasing relevance due to the high incidence and prevalence of neurological disorders and the lack of effective treatments. However, since the exact mechanism of neuronal cell death in neurological disorders is not known, this limits the success in searching for effective drugs. Given that the process of neuronal death is complex, to at least find a drug that effectively blocks a pathway involved in cell death or delays the progress of AD, PD, or HD is considered a success. Apart from c-JNKs activation in neurodegenerative diseases, other biochemical parameters such as oxidative stress, mitochondrial alteration, cell cycle reentry, cytoskeletal alteration, GSK-3 activation, and inhibition of pro-survival pathways (such as the AKT pathway) might also contribute to the neurodegenerative process. Therefore, targeting the c-JNKs pathway with effective inhibitors at least provides a powerful way to experimentally achieve neuroprotection, as well as preserving cognitive function, inhibiting apoptosis, and having a trophic function. Unfortunately, clinical studies with CEP-1347 in PD have failed, but the loss of drug efficacy could have been due to multiple causes, such as whether the clinical trial (selected patients) for the specific compound was well designed or not. Other possible causes could have been a failure of the dose, administering the drug when the neurons were already dead, or the drug rescuing nonfunctioning neurons that could not perform their physiological roles. Therefore, it is necessary to conduct more clinical studies with inhibitors of the c-JNK pathway. It would probably be interesting to consider clinical trials with two drugs, such as an antioxidant, a GSK3 $\beta$  inhibitor, or other c-JNKs antagonists, since more than one pathway may be involved in neuronal death and this might be more effective in treating neurodegenerative diseases.

However, the inhibition of the c-JNK pathway has limitations due to the biological functions involved. For example, it has been reported that c-JNK inhibitors can rescue axotomised neurons, but prevent its regeneration. A possible alternative would be to develop direct targets against specific molecules of the c-JNK pathway; however, this requires more information about the individual actions of the different c-JNK isoforms. In this sense, the work of Zhao and colleagues demonstrated that inhibiting the mitochondrial complex MKK: JNK3 attenuated apoptosis without affecting cellular functions (Zhao et al. 2012). Likewise, the study of Björkblom and colleagues suggests that the nuclear localization of c-JNKs is the main factor responsible for cell death, while the cytoplasmic localization is responsible for its physiological functions (Björkblom 2005). This is an important factor to consider when designing drugs to treat neurological disorders.

**Acknowledgements** This study was funded by grant 2009/SGR00853 from the *Generalitat de Catalunya* (autonomous government of Catalonia), grants BFU2010-19119/BFI, SAF2011-23631, and SAF2009-13093 from the Spanish *Ministerio de Ciencia e Innovación*, grant PI080400 and PS09/01789 from the Instituto de Salud Carlos III, and grant 610RT0405 from Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED).

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