Acta Neurobiol. Exp. 2000, 60: 515-528

Brain injury: prolonged induction of transcription factors

Keith R. Pennypacker[1], Cheryl A. Kassed[1], Shabnam Eidizadeh[1] and **James P. O'Callaghan[2]**

[1]University of South Florida, College of Medicine, Department of Pharmacology and Therapeutics, 12901 Bruce B. Downs Boulevard MDC 9, Tampa, FL 33612, USA; [2]Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA

Abstract. A specific temporal order of events at the cellular and molecular level occurs in response to injury to the brain. Injury-compromised neurons degenerate while surviving neurons undergo neuritogenesis and synaptogenesis to establish neuronal connectivity destroyed in the injury. Several genes, such as those coding cytoskeletal proteins and growth factors, have been shown to be regulated by AP-1 and NF-κB transcription factors, two of the most studied DNA binding regulatory proteins. Our laboratory has discovered that Fos-related antigen-2 from AP-1 transcription factor family and NF-κB p65 and p50 subunits are induced long-term (days to months) in the brain after neurotoxic, excitotoxic or ischemic insult. Fos-related antigen-2 is induced in neurons in several models of injury and its elevated expression lasts days to months, corresponding to the severity. The time-course of FRA-2 induction is abbreviated with less severe insult (terminal damage) relative to the cell death, but the induction occurs during the period of regeneration and repair in both models. NF-κB p65 is basally expressed in hippocampal and cortical neurons, but is elevated in reactive astrocytes in hippocampus and entorhinal cortex starting at two days and lasting at least two weeks after kainate treatment. Neurons of the hippocampus surviving ischemic or neurotoxic injury increase expression of NF-κB p50 for at least a week after injury, suggesting a function for p50 in neuronal survival and/or repair. The extended expression of these transcription factors implies a role in the activation of genes related to repair and regeneration, such as growth factors and synaptic proteins, after injury to the CNS.

Correspondence should be addressed to K.R. Pennypacker, Email: kpennypa@hsc.usf.edu

Key words: AP-1, NF-κB, Fos-related antigen, neurotoxicity, ischemia, excitotoxicity

INTRODUCTION

After injury to the brain *via* chemical, mechanical or ischemic means, neurons in the area of damage either survive or die (Fig. 1). Death can occur by necrosis independent of gene expression or by apoptosis, requiring transcriptional activation of genes. Initiation of gene transcription relating to regeneration and repair forms new connections and synapses within neurons that survive injury to foster adaptation to an altered milieu. Glial cells, including both astrocytes and microglia, alter their morphology when reacting to injury. Within hours, microglial cells, the macrophages of the brain, become ameoboid in shape and migrate to the area of injury to remove cellular debris. Astrocytes become reactive in the first few days after injury by becoming hypertrophic and expressing elevated levels of glial acidic fibrillary protein. The function of reactive astrocytes is not clearly understood but they may play a role in supplying neurotrophic factors to the injured area. Transcription factors modulate the transcriptional activity of genes indicating that they are key molecular players whenever dramatic changes in gene expression occur, such as in the response to brain injury. Here, we report elevated levels of transcription factors from the AP-1 and NF-κB families, identified by our laboratory, that are expressed for extended periods by brain cells after injury.

The ultimate goal of our research is to identify and characterize the molecular processes that result from brain injury. Such knowledge is essential to the design and development of pharmacological agents that specifically target these processes and, consequently, promote neuronal regeneration and repair in an injured human brain. Transcription factors are ideal candidates for pharmacological intervention as their activities, which are typically subject to a variety of regulatory events, are easily manipulated at numerous levels. Although many transcription factors are activated in response to injury, an understanding of their contribution to neuronal survival remains obscure. Elucidation of the role of a particular transcription factor in the brain response to injury has been hampered by the complexity of this response, which encompasses a variety of cell types and occurs over an extended time period. Use of gene knock-out mice, however, now provides an effective means of establishing specific factor/function relationship in the context of the intact brain. As an alternative strategy, some investigators have resorted to use of tissue culture systems; such systems, however, do not recapitulate the *in vivo* situation and can lead to spurious results. For example, we have found that a number of transcription factors that are expressed by astrocytes in culture are not expressed by their i*n vivo* counterparts (Pennypacker et al. 1996). Moreover, the effects of the disruption of the blood brain barrier, of cell-cell and cell-substratum interactions and of late-occurring events are difficult if not impossible to assess in tissue culture models. The *in vivo* approach, while more technically demanding and less amenable to artificial manipulation, is the best way to investigate the process of brain injury. Our laboratory is examining several distinct models of brain injury in order to discern commonalities in the patterns of transcrip-

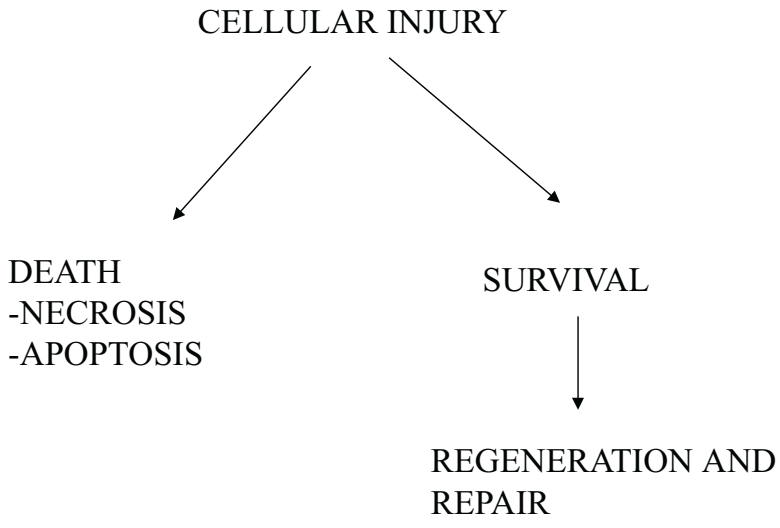

Fig. 1. Determination of cellular fate after injury.

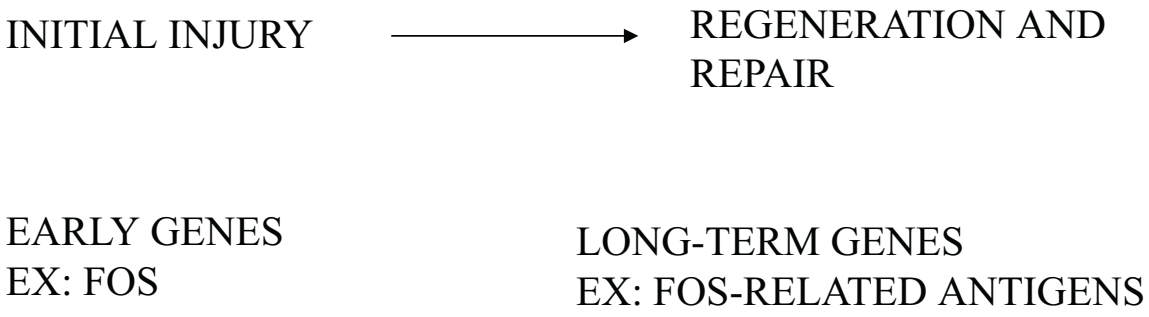

Fig. 2. Temporal order of expression of genes after injury.

tion factor expression during the period of regeneration and repair.

TEMPORAL ORDER OF EVENTS

There exists a specific temporal order of cellular events associated with brain injury. At the molecular level, there is a heirarchy of expression of transcription factors (Fig. 2), of which the temporal expression of AP-1 after injury to the brain is the best-characterized example (Dragunow and Preston 1995, Gass and Herdegen 1995). Fos, perhaps the most studied transcription factor in the CNS, is induced within minutes after a noxious stimuli and its expression remains elevated for about 24 h. Although its function remains unknown, it has been suggested that Fos is involved in the initiation of neuronal apoptosis. Jun expression is induced early (within hours), and its expression does not return to basal levels for several days. Jun is apparently bifunctional, with early expression being related to apoptosis while the later expression is in neurons that survive injury (Herdegen et al. 1997). There are several Fos-related antigens (FRA) that are induced within the first 24 h to several days after the CNS insult but whose expression remains heightened for days to months (Pennypacker et al. 1995). The extended expression of these factors suggests a role in regeneration and repair processes in the injured brain. The long-term expression of FRAs and NF-κB transcription factors in brain injury will be the focus of this paper.

Genes related to neuronal regeneration and repair are targets for NF-κB and AP-1 transcription factors (Fig. 3) and their expression is increased days to weeks after injury. Neurotrophic factors, such as nerve growth factor,

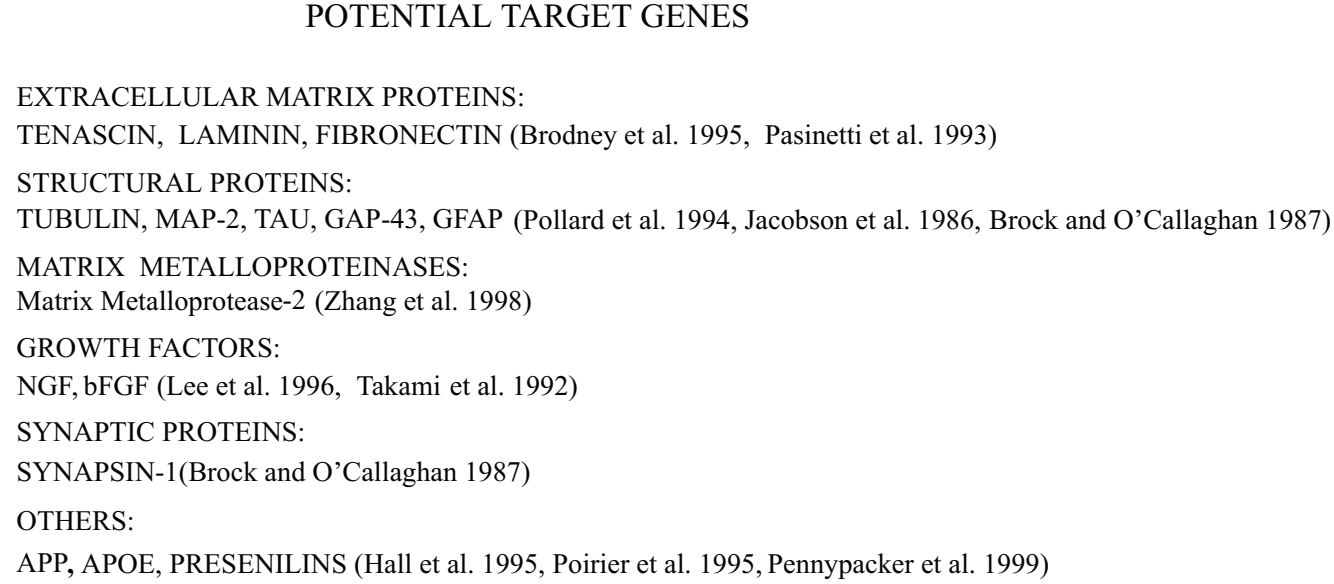

Fig. 3. Potential target of transcription factors expressed during the period of regeneration and repair.

are induced in neurons and reactive astrocytes and are secreted to salvage injured neurons and initiate neuritogenesis followed by synaptogenesis (Lee et al. 1996, Goss et al. 1998). For example, growth-associated protein-43 is an essential component of nascent axons while other proteins, such as microtubule-associated protein-2, aids in the formation of dendrites; both of these proteins are upregulated after brain injury (Jacobson et al. 1986, Pollard et al. 1994). Proteases, such as matrix metalloproteinase-2, are expressed after injury to degrade extracellular matrix proteins (Zhang et al. 1998) so nascent neurites can proceed through the extracellular environment for formation of new connections. Synaptic proteins, such as synapsin-1, are induced to construct new synaptic structures for completion of the neuronal circuitry (Brock and O'Callaghan 1987). Of interest, genes related to Alzheimer's disease, apolipoprotein E, presenilin-1, presenilin-2 and amyloid precursor protein show elevated protracted expression in brain injury suggesting a role for these proteins in the repair of the injured brain (Poirier 1994, Hall et al. 1995, Pennypacker et al. 1999).

Our laboratory has been interested in identifying inducible transcription factors that (1) are expressed for prolonged periods (days to months) after injury, and (2) are induced in many different brain injury models. The Fos-related antigen-2 (FRA-2) and NF-κB p65 and p50 subunits fit both criteria. Their extended period of expression in diverse types of brain injury suggests a role in governing the expression genes related to regeneration and repair in the CNS.

BRAIN INJURY MODELS

There are three major types of injury models (Fig. 4) for the study of brain injury. Percussive impact and puncture wounds to the head are two examples of traumatic injury. There are many methods to produce brain ischemia but middle cerebral artery occlusion is becoming the most accepted method for transient focal ischemia. After an embolus is introduced into the carotid artery to occlude blood flow to the middle cerebral artery for a specific period of time, an area of necrosis develops in striatum and frontal cortex. Chemically-induced injury does not necessitate surgery or injury to the head of the animal. Neurotoxicants, such as kainate and trimethyltin (TMT), are administered peripherally and cause damage to specific, well-characterized regions of the CNS. In contrast to traumatic and ischemic models, neurotoxicant administration does not compromise the blood brain barrier. Upon compromise of the blood brain barrier, lymphocytes and other immune cells gain access to the CNS, potentially confounding interpretation of results since lymphocytes release cytokines, chemokines and other biomolecules which are potent activators of transcription factors.

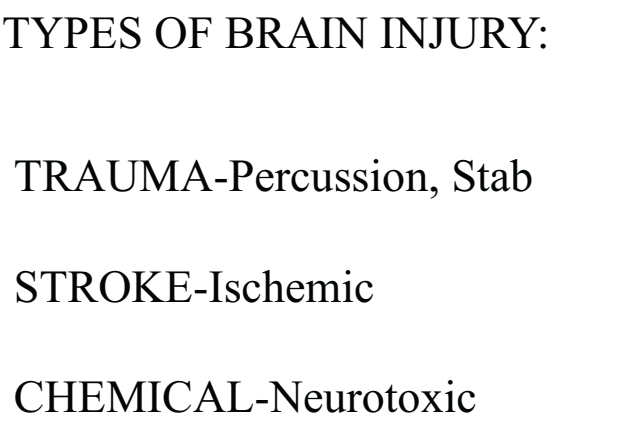

Fig. 4. Categories of brain injury models.

Brain injury by neurotoxicants can be further separated into categories involving cell body degeneration and terminal degeneration (Fig. 5). Kainate and TMT cause the former type of injury. Both agents cause neurodegeneration in the pyramidal cell layers of the hippocampus and other limbic regions of then brain. Reactive gliosis lasts for at least 35 days after TMT treatment and 200 days after kainate treatment indicating a prolonged response to injury (Brock and O'Callaghan 1987, Van-Den-Berg and Gramsbergen 1993). Agents, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), methamphetamine (METH) and methylene-dioxymethamphetamine (MDMA), do not cause death of neurons but do precipitate degeneration of dopaminergic terminals that project from the substantia nigra and synapse on medium spiny neurons in the striatum (O'Callaghan and Miller 1994). The injury caused by these drugs is subtle when compared to the damage caused by TMT or kainate. Reactive gliosis ended 14 to 21 days after treatment (O'Callaghan and Miller 1994). Our laboratory has been comparing the brain injury response between models that cause cell body degeneration (kainate, MCAO, and TMT) and those that cause terminal degeneration (MPTP, MDMA, METH).

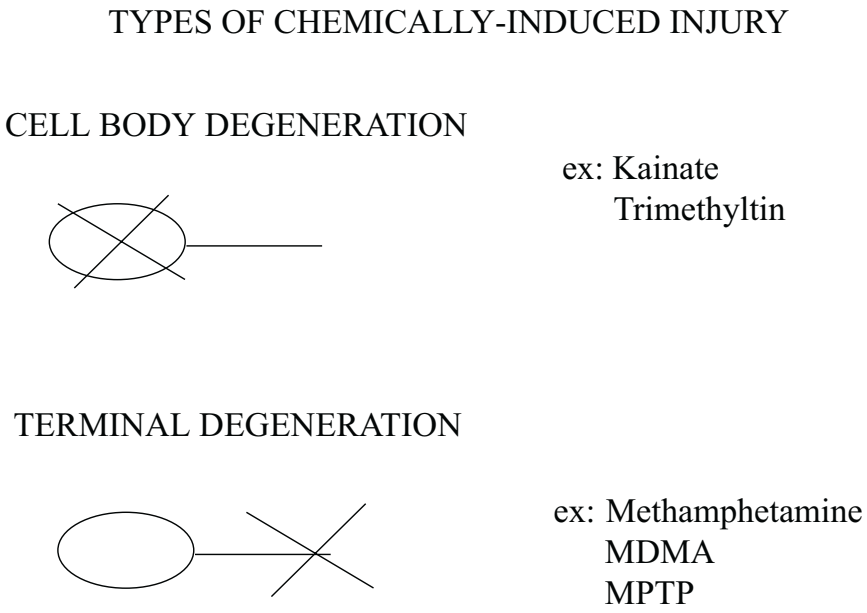

Fig. 5. Categories of chemically-induced brain injury.

AP-1 TRANSCRIPTION FACTORS

AP-1 transcription factors are among the best-characterized inducible DNA-binding proteins in the brain. This family includes c-Fos and Fos-related antigens (FRA) as well as the Jun-related factors. The AP-1 DNA-binding complex is composed of a Jun: FRA heterodimer, but Jun proteins can homodimerize or heterodimerize with other Jun-related factors (Hai and Curran 1991). The AP-1 DNA binding complex recognizes the consensus DNA sequence TGACTCA in the promoter regions of target genes; however, there are many variations on this theme. Therefore, different AP-1 dimer combinations may arise with varied affinities for DNA elements leading to differential effects on gene transcription.

The induction of these DNA binding proteins adjusts gene expression in response to a changing environment and integrates information from a host of stimuli. It has been proposed that AP-1 factors initiate genomic programs for functions such as cell death (Smeyne et al. 1993, Estus et al. 1994, Kamińska et al. 1994, Kasof et al. 1995) and learning and memory (Kaczmarek 1993). In brain, AP-1 transcription factors are induced by a variety of stimuli. Seizure activity dramatically increases AP-1 DNA binding activity in the hippocampus and other brain regions (Morgan et al. 1987, Sonnenberg et al. 1989, Pennypacker et al. 1993, Smeyne et al. 1993, Kamińska et al. 1994). In the striatum and nucleus accumbens, dopamine receptor agonists enhance the expression of these transcription factors, suggesting a role for these factors in the brain's reaction to drugs of abuse (Dragunow et al. 1990, Robertson et al. 1991, Pennypacker et al. 1992, Bronstein et al. 1994, Rosen et al. 1994).

As mentioned previously, the various members of the AP-1 transcription factor family are induced in a specific temporal pattern with certain proteins expressed short-term while others are present long-term. In the CNS, *c-fos* is induced in the hippocampus as a result of seizure activity (Morgan et al. 1987). Several FRA--immunoreactive proteins are also induced, yet the time-course of expression of these FRA proteins differs in a brain region-specific manner (Sonnenberg et al. 1989, Pennypacker et al. 1994, Kasof et al. 1995). The time course of expression of these FRA proteins is also dependent on which seizure-inducing drugs are used. Glutamate receptor agonists, such as kainate, produce prolonged FRA expression, while the chemoconvulsant pentylenetetrazol causes transient induction. Kainate delivered intraperitoneally induces seizure activity within one hour (lasting an additional 4-6 h), while pentylenetetrazol, a GABA receptor antagonist, causes a much shorter period of convulsive behavior. After kainate administration, expression of Fos protein declines within 1-4 h, however other FRA proteins persist.

FRA proteins of 46 kDa and 35 kDa in particular maintain an extended expression, and we have observed expression of the 35 kDa protein for several months after kainate treatment (Pennypacker et al. 1995). These observations led to the hypothesis that different FRA:Jun dimer combinations modulate genes in a distinct temporal sequence; acutely-induced AP-1 proteins modulate a distinct set of genes on a short-term basis while the persistently upregulated AP-1 factors alter expression of a different set of genes.

AP-1 regulatory sites have been located in a number of promoter regions including in genes associated with regeneration and repair. The growth associated protein-43 gene contains an AP-1 site that appears to function in transcriptional activity (Nedivi et al. 1992). Matrix metalloproteinase (MMP)-1 gene contains 2 AP-1 sites with one involved in basal transcription and the other in phorbol ester-induced transcription (White 1994). AP-1 regulation has also been reported for MMP-9 (Huhtala et al. 1991). Synapsin-1, a synaptic vesicle-associated protein present in all nerve terminals, declines initially after TMT treatment in the rat hippocampus, but expression rebounds 3 months later (Brock and O'Callaghan 1987) and its promoter has an AP-1-like site. Several genes related to regeneration and repair are transcriptionally upregulated or potentially upregulated by AP-1 transcription factors suggesting an AP-1 influence on neuroplastic gene expression. However, the AP-1 transcription factors that regulate long-term changes in gene expression related to neuroplasticity are not well-characterized.

35 kDa FRA IN KAINATE-INDUCED NEURODEGENERATION

Our laboratory became interested in transcription factors that exhibited prolonged expression with the discovery that a 35 kDa FRA is expressed in neurons of the dentate gyrus for extended periods of time after kainate treatment. There is a specific temporal expression of FRA transcription factors in the hippocampus after kainate administration. Initially, four FRA-immuno-reactive proteins are induced within four hours after treatment, but after three days only the 35 kDa band continues to be expressed in hippocampus and continues to be expressed at elevated levels for at least three months. The AP-1 DNA binding activity in the hippocampus is increased four hours after kainate administration and remains elevated for at least two weeks. There are very low levels of 35 kDa FRA protein in the untreated hippocampus but the majority of expression is in the dentate gyrus. Increased immunoreactivity is detected in the dentate gyrus one week after a single dose of kainate (Pennypacker et al. 1994). In saline-treated hippocampus, the neuronal layers of the dentate gyrus and pyramidal layers of the CA1, CA2 and CA3 subregions can be visualized with thionin staining; however, one week after kainate treatment, only dentate and CA2 neurons are intact.

In contrast to other brain regions examined in the adult rat, basal expression of the 35 kDa FRA is elevated in the adult olfactory bulb (OB) (Pennypacker et al. 1995) relative to the entorhinal cortex, midbrain, hypothalamus, hippocampus and cerebellum. The entorhinal cortex, striatum and hippocampus contained low levels of this 35 kDa protein; this protein was undetectable in the midbrain, cerebellum and hypothalamus. Immunohistochemical analysis revealed nuclear localization of the FRA protein in the olfactory bulb. Closer inspection revealed that the nuclear FRA immunoreactivity was arranged in rows or striations. The immunoreactive cells appeared to be granule cells, a neuronal population that divide throughout 90% of the lifetime of the rat (Brunjes 1994).

Both AP-1 DNA binding activity and FRA immunoreactivity are elevated in the olfactory bulb and hippocampus three months following one systemic injection of kainate (Pennypacker et al. 1995). Other brain regions showed little or no elevation in FRA immunoreactivity at three months. Like the hippocampus, the olfactory bulb is a site for kainate-induced neurotoxicity (Altar and Baudry 1990). To determine the components of the prolonged AP-1 DNA binding complex, antibodies specific to FRA, cAMP responsive element binding protein (CREB) and Jun-related factors were used to characterize the proteins in the AP-1 complex three months after kainate treatment. High basal levels of AP-1 DNA binding are observed during brain development (Pennypacker et al. 1995) and in the adrenal gland (Pennypacker et al. 1992). The AP-1 DNA binding complex in the olfactory bulb contained predominantly FRA and Jun immunoreactivity with some CREB protein also present. The components were similar for the hippocampal AP-1 DNA binding activity. In contrast, the cerebellar AP-1 complex was composed of both Jun and CREB proteins, but not FRA. Thus, FRA appears uniquely associated in areas recovering from neurodegeneration.

FRA-2

The FRA-2 protein was originally identified as a 46 kDa protein induced in growth-stimulated chicken embryo fibroblasts (Nishina et al. 1990). Unlike Fos, FRA-2 exhibits delayed and prolonged kinetics (Nishina et al. 1990) suggesting protracted regulation of target genes. The role of FRA-2 in gene expression in the brain is not well-studied, however, kainate treatment induces rapid and prolonged expression of FRA-2 mRNA in the rat hippocampus, providing a sound model for the study of this transcription factor in the rat hippocampus (Kasof et al. 1995). In rat retinal ganglion cells, axotomy decreases basal expression of FRA-2 but FRA-2 levels return within three-four weeks after regeneration (Robinson 1996). In contrast, FRA-2 expression is delayed and protracted after TMT-induced brain injury. Thus, FRA-2 expression is altered in diverse brain injury models during periods of neuronal regeneration suggesting a role of FRA-2 in neuroplasticity in the brain.

FRA-2 IN BRAIN INJURY

Recently, our laboratory has found that FRA-2 is induced after exposure to a variety of treatments that produce brain injury: (1) TMT, an organometallic compound that kills cells in the rat hippocampus, (2) MCAO, a model of stroke, (3) METH and MDMA, substituted amphetamines that damage dopaminergic nerve terminals in the mouse and (4) MPTP, a substituted pyridine used as a model for Parkinson's disease (Xiao et al. 1999, Pennypacker and O'Callaghan, unpublished). After TMT treatment, the elevation in the expression of FRA-2 in the rat hippocampus does not occur until at least one week after treatment and remains significantly elevated for 60 days. FRA-2 immunoreactivity is increased in neurons surviving TMT neurotoxicity throughout the hippocampus. The TMT-induced AP-1 DNA binding complex contains FRA-2 immunoreactivity suggesting the FRA-2-containing AP-1 complex is regulating AP-1 target genes in hippocampal neurons during and after TMT neurotoxicity (Xiao et al. 1999). One week after MCAO, levels of FRA-2 immunoreactivity are increased in the contralateral and ipsilateral hippocampi, and localized in hippocampal neurons that have survived MCAO-induced neurodegeneration. Elevated levels of FRA-2 are found in hippocampal neurons that survive due to ischemia or neurotoxicity (Pennypacker, unpublished).

FRA-2 expression is induced in neuronal injury models that do not involve cell death, in which reactive gliosis lasts two weeks (Miller and O'Callaghan 1994, O'Callaghan and Miller 1994). The expression of FRA-2 is increased in the MPTP and substituted amphetamine models of dopaminergic nerve terminal degeneration. The amphetamine congener, dexfenfluramine (DEX) which does not cause degeneration in dopaminergic terminals was used as a negative control and did not affect FRA-2 levels. MPTP, METH and MDMA caused 3-fold increases in striatal FRA-2 levels coincident with peak increases in GFAP and maximal decreases in dopamine; non-target regions were unaffected. Increments in FRA-2 and GFAP returned to control levels by 3-weeks post-dosing. Lowered ambient temperature blocks amphetamine but not MPTP-induced neurotoxicity, and a 7°C decrease in ambient temperature abolished the METH- and MDMA-induced FRA-2 levels but did not affect MPTP-induced FRA-2.

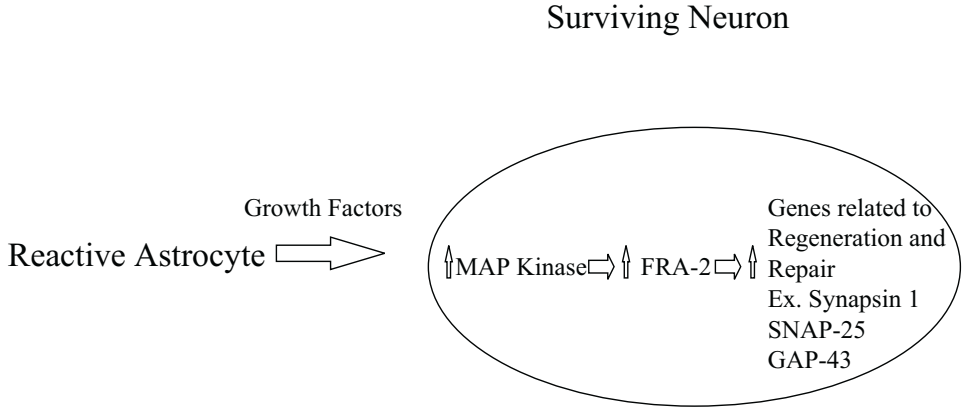

Fig. 6. Activation of FRA-2 in the brain injury response.

Together, these data indicate that enhanced expression of FRA-2 coincides with nerve terminal degeneration induced by dopaminergic neurotoxicants that do not cause neuronal cell death.

FRA-2 DNA binding activity is increased by phosphorylation of FRA-2 *via* MAP kinase (Gruda et al. 1994, Murakami et al. 1997). This kinase is activated by neurotrophic factors, which are secreted by reactive astrocytes. It is possible that growth factors are activating mitogen activated protein (MAP) kinases in neurons surviving injury to phosphorylate FRA-2 (Fig. 6). The FRA-2 DNA binding complex not only targets genes related to regeneration and repair, but has been shown to increase transcription levels of the FRA-2 gene.

IMPLICATIONS OF FRA-2 DATA

Induction of FRAs in the hippocampus is associated with neuronal survival in the case of MCAO and TMT. Clearly, however, neurotoxic effects in the absence of neurodegeneration can also be associated with induction of FRAs. Our finding that the substituted amphetamine, METH, induces FRA-2 shows that more subtle damage can result in activation of transcription factors. METH causes loss of dopaminergic nerve terminals (as evidenced by decreased dopamine, tyrosine hydroxylase and silver degeneration staining) and concomitant reactive gliosis (as evidenced by increases in GFAP) in the mouse neostriatum, with preservation of dopamine neuronal perikarya in the substantia nigra (O'Callaghan and Miller 1994). Thus, METH neurotoxicity offers a paradigm for studying the role of transcription factor activation in neuronal responses to injury that do not require neuronal cell death. Moreover, METH neurotoxicity (unlike TMT) can be manipulated by pharmacological agents, stress and the environment (Miller and O'Callaghan 1994). Finally, dopaminergic agonists are known to enhance the expression of FRAs in dopaminoceptive neurons of the neostriatum. Therefore, it possible to alter METH-induced neurotoxicity predictably, allowing for examination of the influence of terminal degeneration on expression of FRA-2. Thus, the FRA-2 transcription factor is induced after neuronal cell death and terminal degeneration indicating that activation of this signal transduction pathway is common to neurons undergoing regeneration. However the possibility exists that FRA-2 plays a different role in each of these brain injury models as with axotomy of retinal ganglion neurons (Robinson 1996).

The prolonged expression of FRA proteins has been connected to experimental models in which major and long-lasting changes in the biochemical milieu have occurred. FRA proteins dimerize with Jun proteins to form a complex that binds to AP-1 sequence in the promoter of genes to regulate transcription. Genes related to processes involved with neuronal plasticity may be targets for long-term FRA-containing AP-1 complexes during synaptogenesis and other neuroplastic events in the repair of the CNS. These results demonstrate that both degeneration of neuronal cell bodies (MCAO, TMT) or terminal degeneration (MPTP, METH, MDMA) induces this transcription factor in surviving neurons suggesting that FRA-2 may play an universal role in activating genomic programs relating to neuronal regeneration.

NF-κB

The NF-κB/Rel transcription factors regulate the expression of a number of genes after activation due to pathogen stimulation or injury (Baeuerle and Baltimore 1991). This family of transcription factors is ubiquitously expressed and includes seven known members and seven inhibitory proteins (O'Neill and Kaltschmidt 1997). The classic NF-κB transcription factor is composed of p50 and p65 subunits, and interacts with an inhibitory cytosolic protein (IκB) to maintain an inactive complex in the cytosolic compartment until the appropriate intracellular signal causes dissociation of the inhibitory protein (Baeuerle and Baltimore 1989). Phosphorylation of IκB by IκB kinases and its consequent degradation (Stancovski and Baltimore 1997) result in the migration of p65:p50 into the nucleus to modulate target gene expression (Shirakawa and Mizel 1989, Ghosh and Baltimore 1990, Link et al. 1992). The activated DNA binding complex recognizes promoter sequences of the consensus sequence, GGGRNNYYCC. Phosphorylation and release of IκB is insufficient for NF-κB activation, which requires IκB degradation to yield an activated complex (Henkel et al. 1993). Activation of NF-κB DNA binding activity can occur very rapidly within minutes upon cellular stimulation and appears to be an important mechanism for the relay of stress responses. A variety of stimuli including cytokines, bacterial and viral products, UV radiation and oxidative stress have been shown to initiate the activation of this transcription factor. Of particular interest, NF-κB is induced by oxidants (Meyer et al. 1993,

Schenk et al. 1993, Galter et al. 1994) demonstrating the importance of this factor in signal transduction related to cellular injury. The NF-κB complex is a molecular trigger poised for quick adaptation of the cell to extracellular environment, particularly noxious stimuli. No other transcription factor has as many target genes as NF-κB which suggests the importance of this factor in cellular regulation. Target genes include cytokines such as TNF, IL-2 and IL-6, inducible forms of nitric oxide synthase, cycloxygenase and magnesium superoxide dismutase (O'Neill and Kaltschmidt 1997), several of which play significant roles in response to brain injury. A recent report has shown that NF-κB activation *via* TNF1 receptor stimulation increases transcription of genes that suppress apoptosis (Wang et al. 1998).

NF-κB IN NEURONS AFTER INJURY

Neurons in the brain contain activated NF-κB in the nucleus, and neurons in the hippocampus contain the highest amount of this constitutive activity (Kaltschmidt et al. 1994). NF-κB activity in neurons is increased by exposure to glutamate (Kaltschmidt et al. 1995), and neurons exposed to glutamate undergo apoptosis, suggesting that NF-κB activation leads to neurodegeneration. The cytotoxic peptide, amyloid β-peptide, also increases NF-κB activity in neurons (Yan et al. 1995, 1996, Kaltschmidt et al. 1997) and this peptide is believed to be a major player in the pathology of Alzheimer's disease. However, the fact that a particular molecule activates NF-κB and induces apoptosis does not necessarily link NF-κB with apoptosis. For example, TNF-α induces NF-κB activity and also causes apoptosis but eliminating NF-κB activity renders cells more susceptible to TNF-α-mediated apoptosis (Beg and Baltimore 1996). Other investigators have found that activating NF-κB resulted in the enhanced survival of neurons exposed to amyloid β-peptide (Barger et al. 1995). The soluble forms of amyloid precursor protein are neuroprotective and these proteins activate NF-κB in neurons (Barger and Mattson 1996). A recent report has demonstrated that TNF-α-stimulated induction of the anti-apoptotic gene, IEX-1L, is mediated through NF-κB in immune cells (Wu et al. 1998). In further support of the role of NF-κB in cell survival, Bcl-2, another anti-apoptotic protein, activates NF-κB by degradation of IκB (de Moissac et al. 1998), and NF-κB activation increases the expression of anti-apoptotic genes to suppress expression of genes that cause apoptosis (Wang et al. 1998). Two groups have used mice lacking NF-κB p50 to determine the role of p50 in their model of brain injury. Instead of elucidating the role of p50, these reports have only added to the controversy surrounding the role of this subunit in neuronal injury. Schneider et al. (1999) have discovered increase survival of hippocampal neurons three days after ischemic insult in mice lacking p50. Yu et al. (1999) found decreased neuronal survival in mice lacking p50 expression eight hours after intrahippocampal kainate injections. Data from our laboratory indicate that p50 expression occurs in rat neurons that have survived injury. A similar controversy existed about the Jun transcription factor but it is now believed that Jun can perform dual functions as both a pro- and anti-apoptotic factor depending on its dimerization partner (Herdegen et al. 1997). It has been suggested that NF-κB is also multifunctional (Lipton 1997) and the temporal order of expression and activation may be the key to understanding this transcription factor in brain injury.

NF-κB IN GLIAL CELLS AFTER INJURY

Unlike in neurons, NF-κB is not constitutively activated in astrocytes. Astrocytes adjacent to areas of neurodegeneration are exposed to numerous agents, including TNF-α, IL-1β and free radicals, that are known to activate NF-κB. NF-κB immunoreactivity occurs in astrocytes reacting to ischemia and kainate-induced neurodegeneration (Perez-Otano et al. 1996, Terai et al. 1996). However, cultured astrocytes, which show similarities in gene expression to reactive astrocytes (McMillian et al. 1994), have been used as a model to study NF-κB activation by inflammatory cytokines and other factors that are known to be released by activated microglia after central nervous system injuries, which suggests that NF-κB may play a role in changing gene expression in reactive astrocytes. Studies *in vitro* have shown that NF-κB is activated in astrocytes in response to cytokines, such as IL-1β (Sparacio et al. 1992, Moynagh et al. 1993), where it regulates the expression of adhesion molecules, major histocompatibility complex Class II molecules, proenkephalin, growth factors, and other cytokines (i.e., IL-6) (for review see: O'Neill and Kaltschmidt 1997). While cultured astrocytes have proven to be ideal models to study expression of some genes, these cells artifactually express genes not expressed by their i*n vivo* counterparts (Pennypacker et al.

1996) so caution must be used when extrapolating culture data to the *in vivo* condition.

After injury to the brain, microglial cells are activated prior to neuronal apoptosis and reactive gliosis. These activated microglial cells constitute a source for many cytokines (IL-1β, TNF-α and TGFβ) (Giulian et al. 1993). It is conceivable that cytokines released by activated microglia, particularly IL-1β and TNF-α, which are well-characterized activators of NF-κB are responsible for NF-κB activation in reactive astrocytes and neurons. Free radical intermediates, known to be released by microglia and which may contribute to neurodegeneration, could also be involved in triggering NF-κB activation since oxidative stress is another well-known stimulator of this transcription factor. It is conceivable that cytokines and free radicals released by activated microglia, particularly IL-1β and TNFα, which are well-characterized activators of NF-κB, are responsible for NF-κB activation in reactive astrocytes and neurons.

GENE TARGETS OF NF-κB IN CNS INJURY

Putative NF-κB-response elements have been identified in a number of genes which encode proteins of physiological importance in modulating immune and inflammatory responses. These proteins include cytokines and adhesion molecules which are known to be upregulated in reactive astrocytes (Eddleston and Mucke 1993). TNF-α is expressed by astrocytes *in vivo* after brain ischemia and trauma (Kita et al. 1997, Uno et al. 1997). Interestingly, TNF-α is a potent activator of NF-κB which increases expression of itself *via* a NF-κB response element at -103 to -86. The function of this cytokine in CNS pathology has not yet been defined but there are some reports that it is beneficial (Bruce et al. 1996, Liu et al. 1998). Interleukin-6 that is expressed by astrocytes is another cytokine with putative neuroprotective properties (Sparacio et al. 1992, Lafortune et al. 1996, Schaninger et al. 1997). TNF and IL-1 treatment increases NF-κB binding to a site in the IL-6 promoter resulting in increased transcription of IL-6 (Zhang et al. 1990). Astrocytes express intercellular adhesion molecule-1 (ICAM-1) in injury and diseases of the CNS and the expression of this gene is regulated by IL-1β and TNF-α (Ballestas and Benveniste 1995). An NF-κB site (Voraberger et al. 1991) is involved in TNF-α activation of the ICAM-1 promoter activity (Wissnik et al. 1997).

Growth factors are another set of gene targets for NF-κB. Nerve growth factor (NGF), the best characterized neuronal growth factor, is induced by IL-1β in rat hippocampal astrocytes and its expression is mediated by NF-κB (Friedman et al. 1996). The NGF promoter contains an NF-κB regulatory site (Friedman et al. 1996). Reactive astrocytes are the major source of NGF in traumatic brain injury (Goss et al. 1998) and NGF expression increases for extended periods of time in reactive astrocytes in many models of brain injury including kainate, TMT and ischemia (Takamiet al. 1992, Lowenstein et al. 1993, Strauss et al. 1994, Lee et al. 1996, Oderfeld-Nowak and Zaremba 1998). Amyloid precursor protein (APP) is expressed in reactive astrocytes and its expression induced by exposure of astrocytes to IL-1β (Lahiri and Nall 1995). Its promoter contains several potential NF-κB sites (Quitsche and Goldgaber 1992). The secreted APP fragment acts as a growth factor which protects neurons in the hippocampal CA1 subregion against ischemic injury (Smith-Swintosky et al. 1994). Transgenic mice expressing various forms of human APP exhibited decreased damage to the hippocampus after excitotoxic insult (Masliah et al. 1997). Astroglial cells that support axonal outgrowth express APP in the CNS (Chauvet et al. 1997) and secreted APP has been reported to activate NF-κB (Barger and Mattson 1996) so it could be upregulating its own expression. These studies suggest that NF-κB activation in reactive astrocytes is regulating genes that are related to regeneration and repair of the brain after injury.

The only known kinase that responds to TNF-α and IL-1β is NF-κB-inducing kinase (NIK), which then stimulates IKKα and β to phosphorylate IκB. (Stancovski and Baltimore 1997). To our knowledge, the expression of these kinases has not been examined in brain cells either *in vitro* or *in vivo*. However, one study has shown that IκB-α expression is increased in astrocytes and other nonneuronal cells after peripheral lipopolysaccharide treatment (Quan et al. 1997). Expression and activity of the IKKs and IκB-α in the brain have not been investigated. Thus, studies are needed in this area to elucidate NF-κB signal transduction in astrocytes and neurons in response to injury since it is involved in the processes of apoptosis/anti-apoptosis and in regulating the expression of genes related to regeneration and repair.

One important point somewhat overlooked in brain injury studies is the role of the breakage of the blood

brain barrier. In traumatic injury and stroke models, the blood brain barrier is compromised allowing the infiltration of peripheral lymphocytes, which secrete cytokines, such as IL-1β and TNF-α, into the brain. These cytokines are potent activators of NF-κB and other transcription factors so the compromising of the blood brain barrier adds factors which may confound data interpretation. One strength of the TMT model is that the blood brain barrier is not compromised. Cytokines have not been detected in the hippocampus of adult rats after TMT treatment (Little and O'Callaghan 1999), but, NF-κB p50 expression is induced. Thus, factors other than cytokines are regulating this transcription factor.

NF-κB IN BRAIN INJURY RESPONSE

Our laboratory has found increased immunoreactivity to the NF-κB p65 subunit in reactive astrocytes of the rat hippocampus and entorhinal cortex after kainate-induced neurodegeneration (Perez-Otano et al. 1996). In control animals, sparse cytoplasmic NF-κB p65 immunoreactivity was visualized in neurons of the hippocampus, striatum, substantia nigra, and occasionally, in some cortical areas such as the entorhinal and pyriform cortex. Two days after systemic administration of kainate, but not at earlier time points (three h to one day), a pronounced increase in p65 protein was seen; induction was maximal four days after kainate administration and remained elevated for at least seven-ten days. Immunoreactivity against the p65 subunit of NF-κB was observed both in the nucleus and the cytoplasm, including the cell processes. Gel mobility-shift assays in hippocampal and entorhinal cortex extracts showed the induction of an NF-κB DNA binding complex after kainate lesioning. This NF-κB-binding complex is not present (or almost undetectable) in control animals, but is apparent 2-4 days after kainate administration. A complex composed of p65:p50 dimer persisted for at least 14 days after kainate treatment. Double immunocytochemistry experiments confirmed the localization of NF-κB immunoreactivity in the GFAP-positive astrocytes. The expression and activation of NF-κB in reactive astrocytes occurs when these cells are expressing genes known to be targets of this transcription factor, such as those encoding cytokines and growth factors. Therefore, these data suggest that p65 is transcriptionally regulating genes involved in regeneration and repair. Recently, our laboratory has been examining the expression of other NF-κB-related proteins, such as p50, IκB, and IκB kinase (IKK), after brain injury. Unlike p65, expression of p50 dramatically increases in neurons in the CA1, CA2, CA3, CA4 and dentate gyrus of the rat hippocampus one week after TMT and MCAO treatments. The level of p50 is increased at three days after TMT treatment and continues to increase at day seven. The pattern of immunocytochemical staining is very similar between the MCAO and TMT treatments. The cellular localization of p50 occurs in both the nuclear, cytosolic compartments and interestingly, in some dendrites. The contralateral hippocampus also contains increased p50 expression after MCAO. These data are in concordance with our finding that glial fibrillary acidic protein levels are increased on both sides of the brain after MCAO (Pennypacker et al. unpublished observation) demonstrating that the contralateral side of the brain is also reacting to the injury. Expression of p50 is not co-localized with the fluorescent dye, Fluoro-Jade, a marker of degenerating neurons as evidence that p50 is expressed in hippocampal neurons that survive TMT and MCAO neurodegeneration.

Both IKK, α and β, are modestly increased in the hippocampal neuronal layers after TMT treatment, but are absent in hippocampal neurons from saline-treated rats. Both isoforms of IKKs are increased in neurons throughout the hippocampus but the expression is more prominent in the CA2/3 region. In contrast to the other proteins, immunoreactivity to IκBα and β decreased in hippocampal neurons after TMT treatment. This observation is consistent with the view that NF-κB activation results in the degradation of IκB. With the loss of IκB expression, theoretically NF-κB activation should be elevated in hippocampal neurons. The phosphorylated form of IκBα on Western blots is increased at 24 h after MCAO and TMT treatments and levels remain elevated for at least a week after these treatments. Phosphorylation of IκB is an integral part of the activation of NF-κB signal transduction. These data show that NF-κB signal transduction remains activated for at least a week after injury. Phosphorylated IκBa declines seven days after TMT, which coincides with decreased levels of IκBα in immunostained sections. Again, the hippocampus contralateral to MCAO also has elevated levels of phosphorylated IκBα indicating that this side of the brain is also reacting to the injury.

Long-term expression of NF-κB in neurons surviving injury suggests a role in activating genes relating to regeneration and repair. Several genes related to regeneration and repair, such as nerve growth factor (NGF), have

been shown to be targets of NF-κB. Levels of NGF were elevated for days to weeks in many different brain injury models, including kainate, TMT and MCAO (Takami et al. 1992, Lowenstein et al. 1993, Strauss et al. 1994, Lee et al. 1996, Goss et al. 1998, Oderfeld-Nowak and Zaremba 1998). Recently, our laboratory has observed distribution of NGF expression in the rat hippocampus seven days after TMT and MCAO similar to that observed with p50 immunostaining. Cells throughout the layers of the CA1, CA3, CA4 and dentate gyrus show positive staining for NGF. While double-staining methodology is necessary to show NF-κB expression and activation in the NGF-positive cells, these data suggest that NGF will be co-localized with NF-κB. NF-κB may be one of the regulators of the long-term expression of NGF which is a well-characterized neurotrophic factor in the process of regeneration and repair.

IMPLICATIONS OF NF-κB DATA

Well-known activators of NF-κB, such as free radicals, TNF-α and IL-1β, have been shown to be released from microglia activated during brain injury. These secreted molecules initiate NF-κB signal transduction in neurons and astrocytes. In immune-derived cells, TNF-a and IL-1β receptor activation results in the phosphorylation of IκB by IKK, and the consequent translocation of NF-κB into the nucleus where it modulates the expression of target genes. This pathway could be operative in both neurons and astroglia (Fig. 7). In neurons, the role of NF-κB is controversial with studies suggesting that this transcription factor is regulating genes related to apoptosis while other reports show that antiapoptotic or neuroprotective genes are the potential targets. In astrocytes reacting to injury, NF-κB-driven

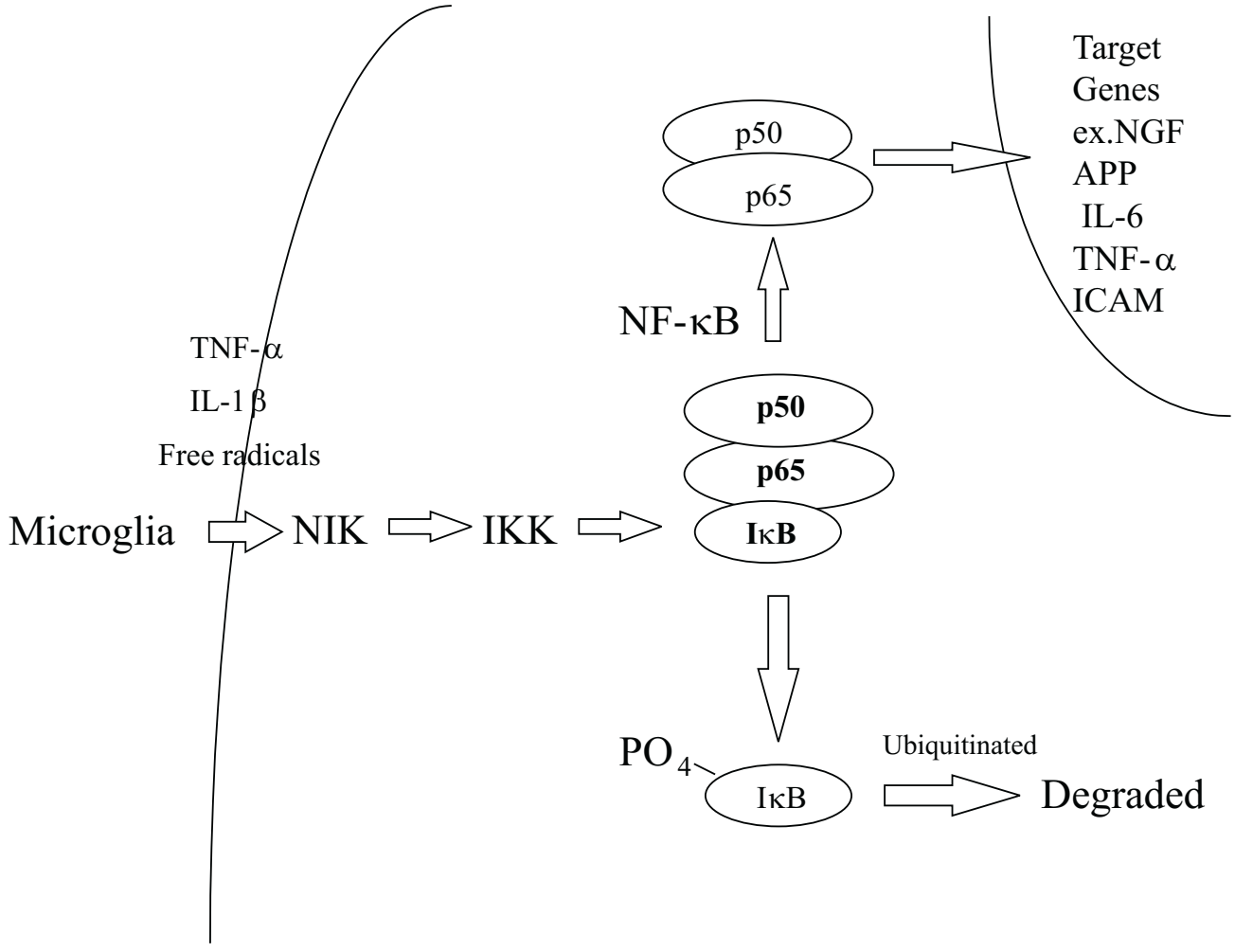

Fig. 7. Activation of NF-κB in the brain injury response.

transcription would target genes such as NGF, APP, TNF-α, ICAM and IL-6 which code for proteins that play a part in regeneration and repair. However, in TMT-induced neurodegeneration, cytokine levels are not increased, so there may be parallel pathways in different injury models leading to NF-κB activation or a yet to be discovered pathway common to all brain injury responses.

SUMMARY AND CONCLUSIONS

Neurons surviving brain injury express genes to adapt to changes in the post-injury state. Transcription factors are induced or modified to alter transcription of these genes. We have discovered transcription factors from the AP-1 and NF-κB families that are induced in neurons and astrocytes days to months after injury and are induced in different models of brain injury. These finding suggest that the induction of the above factors are common response mechanisms to the injury state. Thus, the understanding of their regulation and target genes will lead to discovery of new pharmacological targets to enhance recovery and repair processes common to brain injury.

ACKNOWLEDGEMENT

This work was supported by the American Heart Foundation Grant 9930072N to K.R.P.

REFERENCES

Altar C., Baudry M. (1990) Systemic injection of kainic acid: gliosis in olfactory and limbic brain regions quantified with [3H]PK 11195 binding autoradiography. Exp. Neurol. 109: 333-341.

Baeuerle P., Baltimore D. (1989) A specific inhibitor of the NFκB. Science 242: 540-546.

Baeuerle P., Baltimore D. (1991) The physiology of the NFκB transcription factor. In: Molecular aspects of cellular regulation (Eds. P. Cohen and J. Foulkes). Elsvier, Amsterdam, p. 409-432.

Barger S.W., Horster D., Furukawa K., Goodman Y., Krieglstein J., Mattson M.P. (1995) Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca^{2+} accumulation. Proc. Natl. Acad. Sci. USA 92: 9328-9332.

Barger SW., Mattson M.P. (1996) Induction of neuroprotective kappa B-dependent transcription by secreted forms of the Alzheimer's beta-amyloid precursor. Mol. Brain Res. 40: 116-126.

Barger S.W., Mattson M.P. (1996) Participation of gene expression in the protection against amyloid beta-peptide toxicity by the beta-amyloid precursor protein. Ann. NY Acad. Sci. 777: 303-309.

Beg F., Baltimore D. (1996) Essential role for NF-kB in preventing TNFalpha induced cell death. Science 274: 782-784.

Brock T., O'Callaghan J. (1987) Quanitative changes in the synaptic vesicle proteins synapsin 1 and the astrocyte-specific protein glial fibrillary acidic protein are assocaiated with chemical-induced injury to the rat central nervous system. J. Neurosci. 7: 931-942.

Bronstein D., Ye H., Pennypacker K., Hudson P., Hong J. (1994) Role of a 35 kDa fos-related antigen (FRA) in the long-term induction of striatal dynorphin expression in the 6-hydroxydopamine lesioned rat. Mol. Brain Res. 23: 191-203.

Bruce A., Boling W., Kindy M., Peschon J., Kraemer P., Carpenter M., Holtsberg F., Mattson M. (1996) Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nature Med. 2: 788-794.

Brunjes P. (1994) Unilateral naris closure and olfactory system development. Brain Res. Rev 19: 146-160.

Chauvet N., Apert C., Dumoulin A., Epelbaum J., Alonso G. (1997) Mab22C11 antibody to amyloid precursor protein recognizes a protein associated with specific astroglial cells of the rat central nervous system characterized by their capacity to support axonal outgrowth. J. Comp. Neurol. 377: 550-564.

de Moissac D., Mustapha S., Greenberg A., Kirshenbaum L. (1998) Bcl-2 activates the transcription factor NFκB through degradation of the cytoplasmic inhibitor IκBα. J. Biol. Chem. 273: 23946-23951.

Dragunow M., Preston K. (1995) The role of inducible transcription factors in apoptotic nerve cell death. Brain Res. Rev. 21: 1-28.

Dragunow M., Robertson G., Faull R., Robertson H., Jansen K. (1990) D2 dopamine receptor antagonists induced fos and related proteins in striatal neurons. Neuroscience 37: 287-294.

Eddleston M., Mucke L. (1993) Molecular profile of reactive astrocytes: Implications for their role in neurologic disease. Neuroscience 54: 15-36.

Estus S., Zaks W.J., Freeman R.S., Gruda M., Bravo R., Johnson E.M. (1994) Altered gene expression in neurons during programmed cell death: Identification of c-jun as necessary for neuronal apoptosis. J. Cell. Biol. 127: 1717-1727.

Friedman W., Thakur S., Seidman L., Rabson A. (1996) Regulation of nerve growth factor mRNA by interleukin-1 in rat hippocampal astrocytes is mediated by NFκB. J. Biol. Chem. 271: 31115-31120.

Galter D., Mihm S., Droge W. (1994) Distinct effects of glutathione disulfide on the nuclear transcription factors

kappaB and the activator protein-1. Eur. J. Biochem. 221: 639-648.

Gass P., Herdegen T. (1995) Neuronal expression of AP-1 proteins in excitoxic-neurodegenerative disorders and following nerve fiber lesions. Prog. Neurobiol. 47: 257-290.

Ghosh S., Baltimore D. (1990) Activation in vitro of NFκB by phosphorylation of its inhibitor Iκβ. Nature 344: 678-682.

Giulian D., Vaca K., Corpuz M. (1993) Brain glia release factors with opposing actions on neuronal survival. J. Neurosci. 13: 29-37.

Goss J., O'Malley M., Zou L., Styren S., Kochanek P., DeKoskey S. (1998) Astrocytes are the major source of nerve growth factor upregulation following traumatic brain injury in the rat. Exp. Neurol. 149: 301-309.

Gruda M., Kovary K., Metz R., Bravo R. (1994) Regulation of Fra-1 and Fra-2 phosphorylation differs during the cell cycle of fibroblasts and phosphorylation in vitro by MAP kinase affects DNA binding activity. Oncogene 9: 2537-2547.

Hai T., Curran T. (1991) Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. USA 88: 3720-3724.

Hall E., Oostveen J., Dunn E., Carter D. (1995) Increased amyloid protein precursor and apolipoprotein E immunoreactivity in the selectively vulnerable hippocampus following transient forebrain ischemia in gerbils. Exp. Neurol. 135: 17-27.

Henkel T., Machleidt T., Alkalay I., Kronke M., Ben-Neriah Y., Baeuerle P. (1993) Rapid proteolysis of Iκβ is necessary for activation of transcription factor NFκB. Nature 365: 182-185.

Herdegen T., Skene P., Bahr M. (1997) The c-Jun transcription factor-bipotential mediator of neuronal death, survival and regeneration. TINS 20: 227-231.

Huhtala P., Tuuttila A., Chow L., Lohi J., Keski-Oja J., Tryggvason K. (1991) Complete structure of the human gene for 92-kDa type IV collagenase. Divergent regulation of expression for the 92- and 72-kilodalton enzyme genes in HT-108 cells. J. Biol. Chem. 266: 16485-16490.

Jacobson R., Virag I., Skene J. (1986) A protein associated with axon growth, GAP-43, is widely distributed and developmentally regulated in rat CNS. J. Neurosci. 6: 1843-1855.

Kaczmarek L. (1993) Molecular biology of learning: is c-fos a new beginning? J. Neurosci. Res. 34: 377-381.

Kaltschmidt B., Uherek M., Volk B., Baeuerle P.A., Kaltschmidt C. (1997) Transcription factor NF-kappaB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. Proc. Natl. Acad. Sci. USA 94: 2642-7.

Kaltschmidt C., Kaltschmidt B., Baeuerle P.A. (1995) Stimulation of ionotropic glutamate receptors activates transcription factor NF-kappa B in primary neurons. Proc. Natl. Acad. Sci. USA 92: 9618-22.

Kaltschmidt C., Kaltschmidt B., Neumann H., Wekerle H., Baeuerle P.A. (1994) Constitutive NF-kappa B activity in neurons. Mol. Cell. Biol. 14: 3981-92.

Kamińska B., Filipkowski R., Żurkowska G., Lason W., Przewłocki R., Kaczmarek L. (1994) Dynamic changes in the composition of the AP-1 transcription factor DNA-binding activity in rat brain following kainate-induced seizures and cell death. Eur. J. Neurosci. 6: 1558-1566.

Kasof G., Mandelzys A., Maika S., Hammer R., Curran T.,.Morgan J. (1995) Kainic acid-induced neuronal death is associated with DNA damage and a unique immediate-early gene response in c-fos-lacZ transgenic rats. J. Neurosci. 15: 4238-4249.

Kita T., Liu L., Tanaka N., Kinoshita Y. (1997) The expression of tumor necrosis factor-alpha in the rat brain after fluid percussive head injury. Internat. J. Legal. Med. 110: 305-311.

Lafortune L., Nalbantoglu J., Antel J.P. (1996) Expression of tumor necrosis factor alpha (TNF alpha) and interleukin 6 (IL-6) mRNA in adult human astrocytes: comparison with adult microglia and fetal astrocytes. J. Neuropathol. Exp. Neurol. 55: 515-21.

Lahiri D., Nall C. (1995) Promoter activity of the gene encoding the beta-amyloid precursor protein is up-regulated by growth factors, phorbol ester, retinoic acid and interleukin-1. Mol. Brain Res. 32: 233-240.

Lee T., Kato H., Kogure K., Itoyama Y. (1996) Temporal profile of nerve growth factor-like immunoreactivity after transient focal ischemia in rats. Brain Res. 713: 199-210.

Link E., Kerr L., Shreck R., Zabel U., Verma I., Baeuerle P. (1992) Purified I kappa-beta is inactivated upon dephosphorylation. J. Biol. Chem. 267: 239-246.

Lipton S. (1997) Janus faces of NF-kB: neurodestruction versus neuroprotection. Nature Med. 3: 20-22.

Little A., O'Callaghan J. (1999) TNF-alpha, IL-1alpha, and IL-1beta gene expression is not altered in response to trimethyltin-induced neuronal damage in the adult rat hippocampus. Soc. Neurosci. Abstr. 25: 1535.

Liu J., Marino M., Wong G., Grail D., Dunn A., Bettadapura J., Slavin A., Old L., Bernard C. (1998) TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. Nature Med. 4: 78-83.

Lowenstein D., Seren M., Longo F. (1993) Prolonged increases in neurotropic activity associated with kainate-induced hippocampal synaptic reorganization. Neuroscience 56: 597-604.

Masliah E., Westland C., Rockenstein E., Abraham C., Mallory M., Veinberg I., Sheldon E., Mucke L. (1997) Amyloid precursor proteins protect neurons of transgenic mice against acute and chronic excitotoxic injuries in vivo. Neuroscience 78: 135-146.

Meyer M., Schreck R., Baeurle P. (1993) H2O2 and antioxidants have opposite effects on activation of NF-κB and

AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. EMBO J. 12: 2005-2015.

Miller., O'Callaghan J. (1994) Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. J. Pharm. Exp. Ther. 270: 752-760.

Morgan J., Cohen D., Hempstead J., Curran T. (1987) Mapping patterns of c-fos expression in the central nervous system after seizure. Science 237: 192-197.

Murakami M., Sonobe M., Ui M., Kabuyama Y., Watanabe H., Wada T., Handa H., Iba H. (1997) Phosphorylation and high level expression of Fra-2 in v-src transformed cells: a pathway of activation of endogenous AP-1. Oncogene 14: 2435-2444.

Nedivi E., Basi G., Akey I., Skene J. (1992) A neural-specific GAP-43 core promoter located between unusual DNA elements that interact to regulate its activity. J. Neurosci. 12: 691-704.

Nishina H., Sato H., Suzuki T., Sato M., Iba H. (1990) Isolation and characterization of fra-2, an additional member of the fos gene family. Proc. Natl. Acad. Sci. USA 87: 3619-3623.

O'Callaghan J., Miller D. (1994) Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. J. Pharm. Exp. Ther. 270: 741-751.

O'Neill L., Kaltschmidt C (1997) NFκB: a crucial transcription factor for glial and neuronal cell function. TINS 20: 252-258.

Oderfeld-Nowak B., Zaremba M.. (1998) GM1 ganglioside potentiates trimethyltin-induced expression of interleukin--1beta and nerve growth factor in reactive astrocytes in the rat hippocampus: an immunocytochemical study. Neurochem. Res. 23: 443-453.

Pennypacker K., Hernandez H., Benkovic S., Morgan D., Willing A., Sanberg P. (1999) Induction of presenilins in the rat brain after middle cerebral arterial occlusion. Brain Res. Bull. 48: 539-543.

Pennypacker K., Hong J., Douglass J.,McMillian M. (1992) Constitutive expression of AP-1 transcription factors in the rat adrenal. J. Biol. Chem. 267: 20148-20152.

Pennypacker K., Hong J., McMillian M. (1995) Implications of prolonged expression of Fos-related antigens. TIPS 16: 317-321.

Pennypacker K., Hong J., Mullis S., Hudson P., McMillian M. (1996) Transcription factors in primary glial cultures: changes with neuronal interactions. Mol. Brain Res. 37: 224-230.

Pennypacker K., Hudson P., Hong J., McMillian M. (1995) DNA binding activity of CREB transcription factor during ontogeny of the central nervous system. Dev. Brain Res. 86: 242-249.

Pennypacker K., Lennard D., Hudson P., Hong J., McMillian M. (1995) Basal expression of 35 kDa fos-related antigen in olfactory bulb. Mol. Brain Res. 34: 161-165.

Pennypacker K., Thai L., Hong J., McMillian M. (1994) Prolonged expression of AP-1 transcription factors in the rat hippocampus after systemic kainate treatment. J. Neurosci. 14: 3998-4006.

Pennypacker K., Walczak D., Thai L., Fannin R., Mason E., Douglass J., Hong J. (1993) Kainate-induced changes in opioid peptide genes and AP-1 protein expression in rat hippocampus. J. Neurochem. 60: 204-211.

Pennypacker K., Zhang W., Ye H., Hong J. (1992) Apomorphine induction of AP-1 DNA binding in the rat striatum after dopamine depletion. Mol. Brain Res. 15: 151-155.

Perez-Otano I., McMillian M., Chen J, Bing G., Hong J., Pennypacker K. (1996) Induction of NFκB-like transcription factors in brain areas susceptible to kainate toxicity. Glia 16: 306-315.

Poirier J. (1994) Apolipoprotein E in animal model of CNS injury and in Alzheimer's disease. TINS 17: 525-530.

Pollard H., Khrestchatisky M., Moreau J., Ben-Ari Y., Represa A. (1994) Correlation between reactive sprouting and microtubule protein expression in epileptic hippocampus. Neuroscience 61: 773-787.

Quan N., Whiteside M., Kim L., Herkenham M. (1997) Induction of inhibitory factor kB alpha mRNA in the central nervous system after periphral lipopolysaccharide administration: an in situ hybridization histochemistry study in the rat. Proc. Natl Acad. Sci. USA 94: 10985-10990.

Quitsche W., Goldgaber D. (1992) The amyloid beta-protein precursor promoter. J. Biol. Chem. 267: 17362-17368.

Robertson G., Herrera D., Dragunow M., Robertson H. (1991) L-DOPA activates c-fos in the striatum ipsilateral to a 6-hydroxydopamine lesion of the substantia nigra. Eur. J. Pharmacol. 150: 99-100.

Robinson G. (1996) Changes in expression of transcription factors ATF-2 and Fra-2 after axotomy and during regeneration in rat retinal ganglion cells. Mol. Brain Res. 41: 57-64.

Rosen J., Chuang E., Iadarola M. (1994) Differential induction of Fos protein and a Fos-related antigen following acute and repeated cocaine administration. Mol. Brain Res. 25: 168-172.

Schaninger M., Neher M., Viegas E., Schneider A., Spranger M. (1997) Stimulation of interleukin-6 secretion and gene transcription in primary astrocytes by adenosine. J. Neurochem. 69: 1145-1150.

Schenk H., Klein M., Erdbrugger W., Droge W., Schulze--Osthoff K. (1993) Distinct effects of thioredoxin and antioxidants on the activation of NF-kappaB and AP-1. Proc. Natl. Acad. Sci. USA 91: 1672-1676.

Schneider A., Martin-Villaba A., Weih F., Vogel J., Wirth T., Schwaninger M. (1999) NF-kappaB is activated and promotes cell death in focal cerebral ischemia. Nature Med. 5: 554-559.

Shirakawa F., Mizel S. (1989) In vitro activation and nuclear translocation of NFκB catalyzed by cAMP-dependent pro-

tein kinase and protein kinase C. Mol. Cell. Biol. 9: 2424-2430.

Smeyne R.J., Vendrell M., Hayward M, Baker S., Miao G., Schilling K., Robertson L., Curran T., Morgan J. (1993) Continuous c-fos expression precedes programmed cell death in vivo. Nature 363: 166-169.

Smith-Swintosky V., Pettigrew L., Craddock S., Culwell A. Rydel R., Mattson M. (1994) Secreted forms of beta-amyloid precursor protein protect against ischemic brain injury. J. Neurochem. 63: 781-784.

Sonnenberg J., Macgregor-Leon P., Curran T., Morgan J. (1989) Dynamic alterations occur in the levels and composition of transcription factor AP-1 complexes after seizure. Neuron 3: 359-365.

Sparacio S., Zhang Y., Vilcek J., Benveniste E. (1992) Cytokine regulation of interleukin-6 gene expression in astrocytes involves activation of an NFκB-like nuclear protein. J. Neuroimmunol 39: 231-242.

Stancovski I., Baltimore D. (1997) NF-κB activation: The IκB kinase revealed. Cell 91: 299-302.

Strauss S., Otten U., Joggerst B., Pluss K., Volk B. (1994) Increased levels of nerve growth factor (NGF) protein and mRNA and reactive gliosis following kainic acid injection in the rat striatum. Neurosci. Lett. 168: 193-196.

Takami K., Iwane M., Kiyota Y., Miyamoto M., Tsukuda R., Shiosaka S. (1992) Increase in basic fibroblast growth factor immunoreactivity and its mRNA level in rat brain following transient forebrain ischemia. Exp. Brain Res. 90: 1-10.

Uno H., Matsuyama T., Akita H., Nishimura H., Sugita M. (1997) Induction of tumor necrosis factor-alpha. J. Cereb. Blood Flow Metab. 17: 491-499.

Van-Den-Berg K., Gramsbergen J. (1993) Long-term changes in glial fibrillary acidic protein and calcium levels in rat hippocampus after a single systemic dose of kainic acid. Ann. NY Acad. Sci. 679: 394-401.

Voraberger G., Schafer R., Stratowa C. (1991) Cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5'-regulatory region. J. Immunol. 147: 2777-2786.

Wang C-Y., Mayo M., Korneluk R., Goeddel D., Baldwin A.J. (1998) NF-kB Antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680-1683.

White L. (1994) Two activator protein-1 elements in the matrix metalloproteinase-1 promoter have differential effects on transcription and bind JunD, c-Fos and Fra-2. Matrix Biol. 14: 715-725.

Wissnik S., Stolpe A., Caldenhoven E., Koenderman L., Van Der Saag P. (1997) NF kappaB/Rel family members regulating the ICAM-1 promoter in monocytic THP-1 cells. Immunobiology 198: 50-64.

Wu M., Ao Z., Prasad K., Wu R ., Schlossman S. (1998) IEX-1L, an apoptosis inhibitor involved in NF-κB-mediated cell survival. Science 281: 998-1000.

Xiao Y., Harry G., Pennypacker K. (1999) Expression of AP-1 transcription factors in rat hippocampus and cerebellum after trimethyltin neurotoxicity. Neurotoxol. 20: 761-766.

Yan S.D., Chen X., Fu J., Chen M., Zhu H., Roher A., Slattery T., Zhao L., Nagashima M., Morser J., Migheli A., Nawroth P., Stern D., Schmidt AM (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature 382: 685-91.

Yan S.D., Yan S.F., Chen X., Fu J., Chen M., Kuppusamy P., Smith M.A., Perry G., Godman G.C., Nawroth P. (1995) Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid beta-peptide. Nature Med. 1: 693-9.

Zhang Y., Lin., Vilcek J. (1990) Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a κB sequence. Mol. Cell. Biol. 10: 3818-3823.

Zhang, J.W., Deb S., Gottschall, P.E. (1998) Regional and differential expression of gelatinases in rat brain after systemic kainic or biculine administration. Eur. J. Biol.10: 3358-3368.