Invited Review

Transcription factors in brain injury

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Summary. After brain injury, neuronal genes are regulated to adjust to an altered environment; however, if neurons are damaged then genes related apoptosis are activated. Glial cells, astrocytes and microglia, respond to neuronal death by transcribing genes to enhance the survival of remaining neurons and for regeneration and repair. AP-1 transcription factors are induced in the neuronal response to injury. Depending on the AP-1 dimer combination, neuronal genes related to either apoptosis or survival are transcribed. A 35 kDa Fos-related antigen:JunD dimer is present in neurons that survive injury. Jun and JunD exists in neurons prior to undergoing apoptosis. Neuronal death activates gene expression in astrocytes and microglia. NFκß transcription factors are induced in astrocytes reacting to neuronal injury. In the microglial response, STATs appear to be activated to regulate gene transcription. These transcription factors that modulate the genes involved in the cellular processes of brain injury are examined in this review.

Key words: Neuronal apoptosis, AP-1 transcription factors, NFκß transcription factors, Reactive astrocytes, Microglia

Introduction to mechanisms of cell death

The homeostatic challenge of all cells is to constantly adapt to a changing environment. External stimuli cause the release of signaling molecules, such as hormones and growth factors, to communicate environmental changes to other cells. Binding of these extracellular molecules to their respective cellular receptors activate signal transduction pathways composed of protein kinase and phosphatase cascades to adjust the intracellular biochemistry for both gene expression-dependent and -independent mechanisms. For gene expression-dependent events, transcription factors are the final target for signal transduction systems to modulate mRNA levels. Gene transcription is altered in response to these extracellular signals to enhance or repress the

quantity of intracellular proteins to maintain homeostasis.

Injury to cells activates intracellular transduction pathways to produce molecules for cellular survival and repair. However, if cells become too compromised to recover then death occurs. Cell death is divided into 2 basic categories; necrosis in which the cell dies rapidly and apoptosis in which cell death is a slow, orderly process (Buja and Eigenbrodt, 1993).

Necrosis occurs when there is damage to the plasma membrane due to irreversible injury (Buja and Eigenbrodt, 1993). Damage to the plasma membrane leads to disruption of the ionic gradients and the cell begins to accumulate organic phosphate and hydrogen ions resulting in lowered pH (Farber, 1982). A decrease in the cellular levels of ATP results in increased intracellular sodium levels causing cell swelling. At this time, the membrane becomes non-specifically permeable and calcium homeostasis is lost activating proteases and lipases with consequent dismantling of the cellular structure. Necrosis differs from apoptosis by causing a random DNA fragmentation and cytokine-mediated inflammatory response which magnifies the tissue damage (Searle et al., 1982).

In contrast to necrosis, apoptosis proceeds in an orderly manner with some forms requiring de novo protein synthesis as demonstrated by blockade of protein synthesis inhibitors (Buja and Eigenbrodt, 1993). In these cases, cell death is delayed due to dependence on the activation of gene expression. The apoptotic program causes a time delay to allow some cells to migrate and proliferate followed by a death execution program. Genes encoding proteases and endonucleases are activated to degrade proteins and internucleosomal DNA breaks (Oberhammer, 1993). Like necrosis, calcium is a major player in this process. Since protein synthesis is required for most types of apoptosis, transcription factors are induced or activated to execute this genomic program leading to cell death.

Injury to CNS

Injury to the brain involves a complex interplay between neurons, astroglia and microglia. Neurons are the most vulnerable cell type to damage while glial cells attempt to repair the injured area. Neurons maintain a

highly regulated electrochemical gradient so mechanical or chemical injury to the cell can cause a loss of this gradient that leads to neuronal death, necrosis or apoptosis depending on the extent of the injury. During neuronal death, microglia, the CNS macrophages, become activated and begin to secrete substances that kill compromised neurons and perhaps stimulate astroglia to become reactive. The microglia scavenge the damaged area engulfing the cellular debris (Moore and Thanos, 1996). Reactive astrocytes increase their size, their number of cytoplasmic processes, and express cytokines, growth factors and structural protein markers, such as glial fibrillary acidic protein (Eddleston and Mucke, 1993). The function of reactive astrocytes is not well-stood but they appear to fill in the space around the area of the lesion to provide a substrate for axonal regrowth and neurotrophic support (Reier et al., 1989).

Transcription factors are the final target of signal transduction pathways that starts from an extracellular signal and ends at altered gene transcription. As mentioned above, gene expression in all cell types is dramatically altered after brain injury. Neurons alter gene expression in an attempt to survive and in response to glial signals, and if they do not, then apoptotic genes are activated. Astrocytes undergo their morphology and upregulate the transcription of usually inactive or low-basally expressed genes. Expression of genes, such as nitric oxide synthase, are enhanced in microglia to produce toxic molecules to degrade the cellular debris and over-reaction to the insult can kill surviving neurons.

One of the primary mechanisms of neuronal damage is through the neurotransmitter, glutamate, an excitatory neurotransmitter. There are several glutamate membrane receptors but the NMDA receptor is the one associated with initiating neuronal death in many pathological situations. Binding to NMDA membrane receptors causes an influx of calcium, which under normal conditions relays transduction signals for memory and other neuroplastic functions. Overstimulation of this receptor due to pathological conditions, initiates the process of neuronal death through increased calcium influx activating proteases and endonucleases. Thus, understanding the signal transduction pathways may permit insight into pharmacological agents to regulate expression of genes involved in both survival (neuroprotective) and death/apoptosis.

Transcription factors in brain injury

Examination of signal transduction pathways and specific intracellular messengers which are activated following brain injury has been a major area of neuroscience research. The induction of transcription factors has been a major focus of this research. The rapidly-induced AP-1 factors, Fos and Jun, have been examined extensively in models of brain injury; however, other inducible factors such as zif-268, have been examined (Dragunow and Preston, 1995; Gass and Herdegen, 1995). While most factors have been linked to

neurons, NFκß transcription factors, whose DNA binding activity is activated by calcium pathways, are induced in reactive astroglia (Perez-Otano et al., 1996). The transcription factors that are activated though phosphorylation on tyrosine residues, signal transduction activators of transcription, STATs, (Briscoe et al., 1996) are expressed in microglia (Jonakait et al., 1994).

AP-1 transcription factors are among the best-characterized DNA-binding proteins in the brain (Morgan and Curran, 1991; Pennypacker et al., 1994a). This family which is part of the basic leucine zipper superfamily (bZIP), includes Fos, FosB and fos-related antigens (Fra), Fra-1 and Fra-2, as well as the Jun-related factors, Jun, JunB and JunD. The AP-1 DNA-binding complex is a dimer of one member of the Jun-related proteins plus a member of Fos-associated factors, but Jun proteins can homodimerize or heterodimerize with other Jun-related factors (Hai and Curran, 1991). Jun proteins can also form dimers with non-inducible cAMP responsive element protein (CREB) whose phosphorylation state is altered during brain injury (Herdegen et al., 1992). 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 classic cAMP signal transduction pathway modulates gene transcription via CRE-binding factors. CREB is the best characterized of CRE-binding proteins which includes the activating transcription factors (ATF) and the inducible cAMP responsive element modulators (Lalli and Sassone-Corsi, 1994). CREB, usually a constitutively expressed protein, activates transcription via phosphorylation at serine 133 by protein kinase A to change the conformation of the DNA-bound CREB. CREB expression is regulated during brain development (Pennypacker et al., 1995b) and after chronic morphine treatment in the nucleus accumbens (Widnell et al., 1996). Again, as with the AP-1 proteins, CREB forms homo- or heterodimers with other CRE-binding and AP-1 proteins through the leucine zipper generating diverse array of combinations.

The NFκß transcription factors were initially discovered as important regulator of immunoglobin genes in B and T cells (Grimm and Bauerle, 1993). The DNA binding complex composed of a p65:p50 dimer resides inactive in the cytoplasm bound to inhibitory factor, Ikß. After an external stimulus, the Ikß protein is phosphorylated by protein kinase C, protein kinase A or Raf-1 followed by translocation of the p65:p50 complex to the nucleus. This transcription factor recognizes the consensus sequence GGGACTTTCCC in the promoter regions of genes. As with AP-1 transcription factors, most promoters contain a NFκß-like motif. This protein family includes a number of factors so that many dimer combinations are possible.

Several members of the zinc finger family of

transcription factors have also been studied in brain injury paradigms. A zinc ion binding to these proteins enables the formation of finger-like folding to bind to the DNA (Pabo and Sauer, 1992) which is a mechanism also used for by steroid receptors (Schwabe and Rhodes, 1991). Zif-268 (also called NGF1A, EGR-1 or KROX-24) is another highly inducible protein that has been studied relating to visual stimulation, seizure activity and drug abuse and its regulation has been well-studied in brain injury. Expression of KROX-20, which resembles Zif-268 only in the zinc finger motif, has also been described in neuronal damage. Both of these proteins contains 3 zinc finger motifs to bind to GC-rich regulatory sequence.

STAT are a recently discovered family of transcription factors (Briscoe et al., 1996). The STATs are cytosolic proteins which are phosphorylated on tyrosine residues by Janus kinases and then enter the nucleus to modulate gene expression. Cytokines and growth factors activate this transduction pathway. While the bulk of the work on these proteins has been related to immune function, recent studies have been examining their function in brain cells (Jonakait et al., 1994).

In this review, the models of brain injury, ischemia, mechanical and excitotoxicity, will be examined and commmonalities emphasized, since all share the element of glutamate toxicity. In these models, there is much confusion over the mechanism of degeneration whether necrosis or apoptosis. However, it appears this controversy arises from differing degrees of brain injury provoked by each experimental paradigm. These ambiguities are further enhanced by the methodology used to examine the transcription factor's expression. Observing expression of transcription factor mRNA is a common method of study. However, a limitation of this approach is that, at least in some paradigms, mRNA is expressed without translation to protein (Kiessling et al., 1993). Thus, no modulation of gene transcription will follow and the increased levels of transcription factor mRNA appears to be a non-functional event.

Ischemia

Ischemia involves the lack of oxygen to the brain due to restriction of the blood flow. Both focal and global ischemic models have been used to study the induction of inducible transcription factors (ITFs). Neuronal death occurs in a matter of hours after tissue damage from prolonged ischemia. The current understanding of mechanism of pathogenesis is that there is the activation of glutamate receptors followed by the influx of calcium causing neurodegeneration (Choi and Rothman, 1990). In global ischemia, the pyramidal neurons of the CA1 are particularly susceptible to neurodegeneration.

The ITFs, Fos, FosB, Jun, JunB, JunD and Krox-24, have been examined in this model with contradictory results. Some of these discrepancies can be explained by the severity of the ischemic event; moderate ischemia induces apoptosis while severe ischemia causes necrosis

(Beilharz et al., 1995). Prolonged expression of Jun has been implicated in the delayed death of CA1 neurons (Dragunow et al., 1993, 1994). However, other investigators have not observed Jun protein expression in these dying neurons (Kiessling et al., 1993), which may be explained by failure of protein synthesis in these irreversibly compromised neurons (Thilmann et al., 1986). Using an ischemic tolerance model in which CA1 neurons survive (Kitagawa et al., 1990), Jun protein expression showed a protracted expression related to the survival of these cells (Gass and Herdegen, 1995). However, these discrepancies may be caused by differences in the experimental paradigms. Furthermore, the Jun protein could be serving dual functions. The specificity of the DNA binding is altered with different dimer partners, so Jun dimerizing with one bZIP protein would modulate genes related to apoptosis, but with another dimer combination would upregulate of genes related to neuronal survival.

Seizures and neuronal death

Glutamate is an excitatory neurotransmitter; overstimulation of the glutamate receptor increases intracellular calcium levels which kills neurons. Many glutamate receptors agonists have been used to study excitotoxicity. Direct and systemic administration of this agents has been used to observe neuronal death, although local application can confound the study by breaking the blood:brain barrier with further mechanical damage. Systemic administration of glutamate receptor agonists induces seizure activity and thus has been used as a model for epilepsy.

Various glutamate receptor agonists, as well as GABA receptor antagonists, have been used to induce seizure activity. These agents cause seizure activity, but only a few lead to neuronal death. Kainate has been used extensively in studying convulsions and later neuronal death. Kainate administration causes short-term, seizure activity and in the long-term, selective neuro-degeneration. Systemically applied kainate will induce seizure behavior within 90 min with prolonged, robust convulsions (4-6 hours) followed by death of hilar and pyramidal neurons of the CA1 and CA3 within 3 days, while the neurons of the dentate gyrus appear unaffected. The dying neurons have been shown to be undergoing apoptosis as determined by DNA fragmentation (Dragunow and Preston, 1995; Kasof et al., 1995).

The duration and intensity of seizure activity appears to play a role in kainate-induced neuronal death since other agents induce convulsions without damaging neuronal cells. Metrozol, a GABA receptor antagonist, induces seizure activity and transcription factors in the hippocampus without neuronal damage. However, the duration of the seizures is considerably shorter than those induced by kainate. The initial pattern of AP-1 transcription factor expressed after metrozol treatment is similar to that after kainate, but again, expression is more prolonged in the kainate-treated animal (Kasof et

al., 1995). In fact, a 35 kDa Fra remains elevated for months and maybe permanently upregulated in neurons of the dentate gyrus and olfactory bulb (Pennypacker et al., 1995a,c).

Kainate treatment induces an extended expression of ITFs expressed in a specific spacial-temporal order (Popovici et al., 1990, Pennypacker et al., 1994a-b; Kasof et al., 1995). The ITFs, Jun, JunB, JunD, Zif-268, Fos, FosB, Fra-1, Fra-2 and some uncharacterized Fra-immunoreactive proteins at 46 and 35 kDa, are induced within the first hour of kainate administration and their expression is prolonged which may be due to long periods of seizure activity. Fos first appears in the dentate neurons followed by expression in the pyramidal neurons (Popovici et al., 1990). Jun B expression is only transiently expressed (1-2 days), Jun expression is intermediate (1-2 weeks) and JunD, while moderately up-regulated by this treatment, is constitutively expressed. Fos is only transiently expressed, while the other Fras are expressed from a few days to a week. However, the 35 kDa Fra is elevated in the neurons of the dentate gyrus for several months after kainate treatment (Pennypacker et al., 1994b, 1995c). The AP-1 DNA binding complex that predominates early after kainate in the rat hippocampus is composed of a Fos:JunB dimer, but within a week JunD:35 kDa Fra complex is the dominant complex (Kaminska et al., 1994; Pennypacker, et al., 1994b). Thus, different AP-1 DNA binding complexes are formed during kainate-induced seizure activity and later after neuro-degeneration, consequently changing specificity of AP-1 driven gene transcription.

Neuronal death in the CA1 and CA3 occurs within 2-4 days after kainate administration, so investigators have been examining the role of ITFs in apoptosis. Protein synthesis is necessary for kainate-induced apoptosis (Schreiber et al., 1993). Fos has been implicated in initiating the apoptotic program in pyramidal neurons in fos-lacZ mice and rats (Smeyne et al., 1993; Kasof et al., 1995); however other studies have not seen Fos expression (Popovici et al., 1990; Gass et al., 1995). Several studies are now downplaying Fos' importance in apoptosis (Gajate et al., 1996; Roffler-Tarlov et al., 1996). Jun has also been observed in pyramidal neurons undergoing apoptosis (Dragunow et al., 1993; Dragunow and Preston, 1995); however, other investigators propose Jun is involved in a neuronal rescue program (Gass and Herdegen, 1995). The specificity of Jun's transcriptional modulation depends the dimer combination so Jun could theoretically be involved in both processes. However, further studies in which Jun expression is directly blocked are needed to elucidate the role of Jun expression during the period of pyramidal neuron death.

The neurons of the dentate gyrus remain unaffected from kainate-induced neurotoxicity. These cells adapt to loss of the CA3 target, loss of innervation from entorhinal cortex and loss of the inhibitory input from hilar neurons. The levels of 35 kDa Fra is induced in these neurons as well as in olfactory bulb and remain elevated for at least 3 months (Pennypacker, et al., 1995c). A Fra:JunD complex could drive the transcription of genes related to neuroplasticity that contain AP-1 sites, such as GAP-43 (Nedivi et al., 1992). Granule neurons of the olfactory bulb express high levels of the 35 kDa Fra (Pennypacker et al., 1995c); these cells grow throughout the lifetime of the rat supporting the idea that 35 kDa Fra-containing DNA binding complex is activating neuroplasticity-related genes (Pennypacker et al., 1995a). Fra immunoreactivity is regulated by sensory stimulus in the olfactory bulb (Klintsova et al., 1995). Some reports have indicated that this Fra may be a truncated form of FosB (Nakabeppu and Nathans, 1991), but so far the evidence is not conclusive.

Axotomy and related injury models

Many different mechanical injury paradigms have been used to study ITFs, ranging from puncture wound to axotomy. The AP-1 transcription factors are expressed during mechanical injury and glutamate receptor stimulation is involved. As expected many diverse results emanate from the various models used and from the experimental variance within each model. Mechanical injury to the rat cortex induces a rapid and transient increase in Jun and Fra proteins (Dragunow and Robertson, 1988; Herdegen et al., 1995). A long-term induction in Fra immunoreactivity which is mediated by glutamate has been reported (Herrera and Robertson, 1990; Sharp et al., 1990; Herrera and Cuello, 1992). Many studies have used axotomy since this is a more specific treatment to determine the ITFs involved in neuronal regeneration and/or death.

As with the other studies, the Jun, Fras and Zif-268 have been the primary ITFs examined after axotomy. Jun and JunD are consistently induced with extended expression in peripheral and cranial nerves (Jenkins and Hunt, 1991; Herdegen et al., 1992a,b; Jenkins et al., 1993a,b; Koistinaho et al., 1993) as well as in neurons of the central nervous system (Dragunow 1992; Jenkins et al., 1993a,b; Leah et al., 1993). Since the neurons survive in peripheral nervous system models, Jun and JunD appear to modulate regenerative-related gene transcription, but in the CNS there is an argument whether these factors modulate genes related to neuronal survival or death. Medial septal neurons survive axotomy and exhibit long-lasting induction of Jun suggesting the regeneration argument (Haas et al., 1996). In contrast, Jun has been shown to be essential for apoptosis in cultured sympathetic neurons after nerve growth factor deprivation (Estus et al., 1994). Again, it must be emphasized that the dimerization partner plays a key role in dictating the specificity of gene modulation. Thus different dimer complexes could be involved in modulating gene expression for diametrical opposed processes, survival and death.

In summary, neuronal cell response to brain injury

has been extensively studied using models that mimic epilepsy and seizures, ischemia and stroke, as well as mechanical damage. Much contention exists over the function of ITFs in brain injury, ie., whether they modulate genes that lead to regeneration or degeneration of neurons. Evidence exists that Jun, JunD and 35 kDa Fra are important factors in the neuronal response to injury. Depending on the composition of the DNA binding complex, genes associated with apoptosis or regeneration will be modulated .

Non-neuronal cells during brain injury

Neurons have been the cell type emphasized in most brain injury studies; however, astrocytes and microglia play important roles in neurodegeneration that have been overlooked until recent years. The signal transduction pathways in glia have not been as well-characterized as their neuronal counterparts. In contrast to neurons, these cells are not excitable cells and usually do not have as rapid an induction of ITFs.

Microglia

These cells are considered the macrophages of the brain (Moore and Thanos, 1996). Whenever toxic, pathologic or mechanical damage occurs in the CNS, microglia scavenge the cellular debris and may thereby create an environment conducive for regeneration and reorganization in the damaged area. During development, they remove the neuronal debris resulting from programmed cell death, a fundamental process in the formation of the brain. Although still not completely understood, microglia are thought to be of hematopoeitic origin. Microglia are observed in 2 forms in the brain, ramified and ameboid. Ramified microglia have been proposed to be a quiescent state transforming into the ameboid form for phagocytic purposes, but ramified cells have been observed engulfing cellular debris (Thanos, 1992).

Following injury, microglia usually form phagocytic clusters that surround the dying neurons (Streit et al., 1988). In response to cellular degeneration, many cell surface markers are upregulated, such as CR3, MHC I, and MHC II (Suzumura et al., 1987; Graeber et al., 1988). Cultured microglia have been shown to produce many substances which may also occur in response to toxic or pathologic conditions. Growth factors such as FGF and NGF are produced to aid compromised neurons (Mallat et al., 1989) while cytokines are secreted to promote proliferation and phagocytic transformation of the microglia (Griffin et al., 1989; Dickson et al., 1993). Microglia release a number of cytotoxic substances such as glutamate, hydrogen peroxide and nitric oxide which have given them a bad reputation in neurodegeneration (Giulian and Baker, 1986; Piani et al., 1991; Kolb and Kolb-Bachofen, 1992). Other genes that are transcriptionally activated include urokinase plasminogen activator which degrades extracellular matrix proteins to

perhaps aid in their migration (Nakajima et al., 1992).

Many genes are activated in microglia responding to neurodegeneration. The signal transduction pathways that regulate the modulation of the microglial genes have yet to be completely elucidated. Since microglia respond to interferons and interleukins, one would assume that transduction would be similar to those found in other immune cells. Receptors for the interferons, growth factors and interleukins are coupled to a phosphotyrosine JAK-STAT transduction pathway. Receptor occupation activates the Janus tyrosine kinases which phosphorylate the cytosolic STAT proteins on tyrosine residues which then translocate into the nucleus and bind to recognition sites in gene promoters (Briscoe et al., 1996). At least one report exists that shows the activation of this pathway in cultured microglia with gamma interferon treatment (Jonakait et al., 1994). Further indication of the importance of phosphotyrosine signal transduction is the high level of phosphotyrosine staining in microglia using antibodies that are specific for phosphotyrosine residues (Tilotson and Wood, 1989). In fact, these antibodies can be used to as a marker for microglia and seem to preferentially stain ramified cells. Nitric oxide synthase gene expression is dependent on STAT factors in macrophages (Kolb and Kolb-Bachofen, 1992) while inhibitors of tyrosine kinases are able to block the formation of NO in cultured microglia (Kong et al., 1996). These results suggest the possible involvement of JAK-STAT signal transduction in microglial production of growth factors and cytokines.

Dying neurons are communicating their compromised situation to microglia through an uncharacterized mechanism, probably through changes in cell surface molecules and/or secretable substances (Giulian et al., 1993) to activate phosphotyrosine second messenger systems and thereby modulate microglial gene transcription. Release of growth factors would aid in the survival of partially damaged neurons while fully injured ones would be eliminated by substances produced from cytotoxic genes.

Astroglia

Astrocytes respond to an injurious events in the brain by becoming "reactive" 2-3 days after a neurotoxic, neuropathological and other events that cause neuronal damage (Norenberg, 1994). After neurological insult, astrocytes increase their size, the number of cytoplasmic processes and content of glial marker proteins such as glial fibrillary acidic protein (GFAP) and S100. Genes for growth factors, nerve growth factor, basic fibroblast growth factor and brain-derived growth factor, and cytokines, interleukins 1 and 6, interferons and tumor necrosis factor are upregulated, which may aid neuroregeneration and repair (Eddleston and Mucke, 1993). The purpose of reactive gliosis is still unclear but may stabilize the tissue around the lesion, occupy the space of the lesion and provide a substrate for axonal elongation (Reier et al., 1989).

In most studies the neuronal response to injury has been the primary focus with the non-neuronal cells' response being incidental so up until recently the signal transduction in reactive gliosis has not received as much attention. Many studies have focused on acute treatments of under 24 hours while astroglia usually became reactive 2-3 days after injury. Many different genes are modulated in reactive astrocytes; however, ITFs such as Fos and Jun are not as readily induced as in neurons. For example, focal brain injury induced expression of seven factors (Fos, FosB, Jun, Krox-24, Krox-20, JunB, JunD) in neurons while non-neuronal cells expressed only Fos, Jun and Krox-24 and weakly stained for JunB and JunD (Dragunow and Hughes, 1993). Injury to the superior cervical ganglion induced Jun in the neurons while several factors are induced in glia at least 6 days after injury (Koistinaho et al., 1993). After kainate-induced lesions in the hippocampus, a small population of astroglia in the hilar region expressed Fra-immuno-reactive proteins (Pennypacker, et al., 1994b). However, none of these studies have convincingly determined the ITFs that activate gene transcription in reactive gliosis.

While basal levels of NFκβ expression and DNA binding have been reported in neurons (Bakalkin et al., 1993; Cauley and Verma, 1994; Kalstchmidt et al., 1993, 1994), reactive astrocytes express elevated levels of these factors in kainate-induced neuronal degeneration in the hippocampus and entorhinal cortex (Perez-Otano et al., 1996). Other treatments that caused reactive gliosis, such as 6-hydroxydopamine, also induced NFκß in reactive astrocytes (unpublished observation). Primary cultures of astrocytes express NFκß when treated with cytokines (Sparacio et al., 1992; Friedman et al., 1993; Massa et al., 1993). Activated microglia are a major source of cytokines proposed to mediate astrocytic reaction to injury (Bakalkin et al., 1993; Giulian et al., 1993, 1988); these cytokines induce expression and DNA binding of NFκß transcription factors. NFκß transcription factor binding is also induced by free radicals which are released by degenerating cells and microglia (Giulian and Baker, 1986). Cytokines, particularly TNF-α and IL-1ß, have been shown to regulate nerve growth factor in astrocytes (Yoshida and Gage, 1992), a potential target gene for NFκß DNA binding. Microglia responding to neuronal degeneration release cytokines and/or free radicals which may induce NFκß in astrocytes to activate gene expression associated with reactive gliosis.

Thus, during neurodegeneration, astrocytes undergo the process of reactive gliosis from an unknown signal which may be a cytokine or oxygen radical generated from microglia. The NFκß transcription factors are induced in reactive astrocytes and appear to be involved in modulating the expression of genes related to gliosis, such as GFAP.

The cell to cell interaction during CNS injury is poorly understood. However, cultured astrocytes express high levels of proteins, such as GFAP, that are induced during reactive gliosis (Eddleston and Mucke, 1993). Levels of AP-1 transcription factors, Jun, JunD and Fras, are increased in cultured astrocytes but are decreased with potential target genes, such as GFAP, with the addition of neurons to the glial culture (Pennypacker et al., 1996). Some of the phenotypic changes in astroglial cells due to neuronal damage may be induced through the removal neuronal-glia interactions

Glial cells in PNS

Unlike the central nervous system, the peripheral nervous system has the ability to regenerate after injury. This regenerative capability is at least partially due to Schwann cells (SC), which change phenotype in response to injury. The SC are the oligodendrocytes of the PNS in that they wrap around axons and secrete myelin to form a laminar covering around the axon. Removal of the SC:axon interaction induces phenotypic changes by suppressing the expression of myelination-associated genes and by activating transcription genes associated with axonal regeneration.

Jun protein is expressed in SC after loss of axonal contact; Jun expression is lost by activating cAMP signal transduction and the expression of SCIP, a transcription factor expressed in myelinating SC, is induced (Monuki et al., 1989). Jun protein is elevated early in SC development, when SC are non-myelinating (Shy et al., 1996). Jun protein appears to be a marker for the ability of SC to aid in neuronal regeneration (Vaudano et al., 1996) since Jun-expressing SC have the ability to regenerate CNS axons. Thus, Jun may play a role in activating the non-myelinating, regenerative genomic program while SCIP directs transcription of the myelination-associated genes.
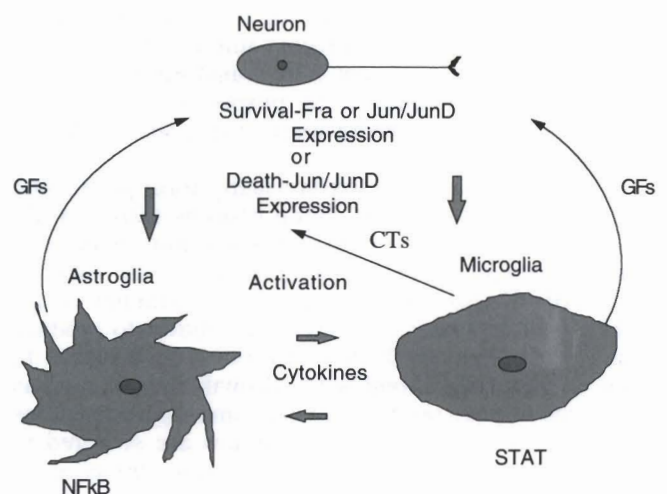
Fig. 1. Summary of the communication between and within brain cells after brain injury. Depending on the AP-1 transcription factors induced within a neuron, the neuron undergoes degeneration or survives injury. The degenerative process activates NFκß and STAT signal transduction in astroglia and microglia, respectively. Cytokine (CT) and growth factor (GF) genes are transcribed in these glial cells and their gene products play key roles in the brain injury response.

Conclusion

After an insult to the brain due to neurotoxins, excitotoxins, mechanical damage, ischemia, etc., neurons react by activating genes which initiate adaptation to this noxious stimulus (Fig. 1). However, if neurons can not maintain homeostasis then apoptotic-associated genes are activated. AP-1 transcription factor are involved in the modulation of both genomic programs; a 35 kDa Fra, JunD and Jun regulate genes related to survival and regeneration, while Jun and JunD have also been implicated in apoptosis. Neuronal death activates microglia and astrocytes through an un-identified signal(s). Both microglia and astrocytes produce growth factors (GFs) to aid surviving neurons while cytotoxins (CTs) released by microglia kill the dying neurons. Cytokines released by glial cells function in intercellular communication. NFκβ transcription factors are induced in reactive astrocytes to modulate gene transcription. Since oxidative stress activates NFκβ DNA binding activity, free radicals produced from dying neurons and/or generated by microglia could be an additional signal for reactive gliosis. The STAT transcription factors, activated by cytokines, may play the pivotal role in modulating microglial gene trans-cription, cytotoxins, growth factors and cytokines.

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