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AMPA-receptor trafficking and injury-induced cell death

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Abstract

AMPA receptors (AMPARs) are critical for synaptic plasticity, and are subject to alterations based on subunit composition and receptor trafficking to and from the plasma membrane. One of the most potent regulators of AMPAR trafficking is the pro-inflammatory cytokine tumor necrosis factor (TNF)α, which is involved in physiological regulation of synaptic strength (Beattie *et al*., (2002) *Science*, **295**, 2282–2285; Stellwagen and Malenka, (2006) *Nature*, **440**, 1054–1059) and is also present at high concentrations after CNS injury. Here, we review evidence that TNF can rapidly alter the surface expression of AMPARs so that the proportion of Ca^{++}-permeable receptors is increased and that this increase, in combination with increased levels of extracellular glutamate after injury, plays an important role in enhancing excitotoxic cell death after CNS injury. Thus, the pathophysiological hijacking of a critical regulator of synaptic plasticity and homeostasis by the secondary injury cascade may represent a new therapeutic target for neuroprotection.

Keywords

cell death; cell imaging; CNS injury; excitability & synapse; glia-neuron interaction

Introduction

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) is thought to be the most prevalent neurotransmitter receptor in the central nervous system (CNS). AMPARs are ionotropic glutamate receptors consisting of four transmembrane subunits (GluR1-4) that form multiple heteromers with different ion channel properties and conductances (see Derkach *et al*., 2007). Changes in AMPARs are known to be involved in synaptic plasticity, and these changes are mediated in part by tight regulation of the trafficking of AMPARs to and from the extrasynaptic and synaptic plasma membranes of neurons (Malinow & Malenka, 2002). In this article, we will review the substantial evidence that AMPAR trafficking also contributes to changes in excitability and ionic conductances after injury to the CNS, and that these changes can be critical in determining the survival of neurons and neural tissue after various kinds of CNS injury. We will also review the evidence that pro-inflammatory cytokines, especially tumor necrosis factor alpha (TNF), are involved in the modulation of AMPAR trafficking, in both normal and pathologic states.

CNS injury and excitotoxic cell death

Damage to the CNS can occur through trauma, infection, ischemia and a variety of neurodegenerative process. These can all produce release of extracellular glutamate, through a variety of mechanisms including release from damaged neurons and axons and enhanced

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glutamate concentrations produced by injury-induced or chronic inflammatory signaling, and reduction of astrocyte-mediated glutamate uptake. Acute injury to the brain or spinal cord can produce dramatic increases in extracellular glutamate within minutes (e.g. Panter & Faden, 1992). In addition, release of ATP from damaged cells can activate purinergic receptors on microglia and macrophages, promoting additional release of glutamate (see Wang *et al.*, 2004), as well as the release of pro-inflammatory cytokines including interleukin (IL)-1*β* and TNF, and the release of inflammatory mediators such as NO and reactive oxygen species. Astrocytes normally clear glutamate from the extracellular space via glutamate transporters (Rothstein *et al.*, 1996), and these can fail under pathological conditions. Further, TNF can increase the astrocytic release of glutamate (Vesce *et al.*, 2007). Thus injury or chronic inflammation can, by themselves, increase the probability of glutamate-related toxicity mediated by both AMPARs and *N*-methyl-D-aspartate (NMDA)-type receptors (Matute *et al.*, 2001; Beattie, 2004), and inflammatory reactions to injury can combine with excitotoxicity to produce an ongoing cascade of secondary injury (e.g. Beattie, 2004). Increased glutamate thus activates AMPARs which allow influx of Na^{+} and depolarization which, in turn, allows glutamate to act on NMDA receptors. Both NMDARs and some AMPARs allow Ca^{+} influx, contributing to Ca^{++} load in Ca^{++}-mediated signaling pathways, and this can lead to cell damage and death. There are numerous protective mechanisms including the desensitization and inactivation of AMPARs, although these can be swamped by excess glutamate. These ideas have come from numerous *in vitro* studies and have been supported by *in vivo* studies of cell death after spinal cord and brain trauma and ischemia. Drugs that block AMPARs and NMDARs have shown efficacy in models of CNS injury, but many of these are toxic or not usable clinically (Walters *et al.*, 2005; Chen & Lipton, 2006), suggesting a need to develop alternative therapeutic approaches for modulating excitotoxicity.

Chronic inflammation occurs in neurodegenerative diseases, and this can also lead to increased extracellular glutamate as well as increased expression and secretion of inflammatory cytokines (Agrawal & Fehlings, 1997; Pitt *et al.*, 2000; Blanchard *et al.*, 2004; Lai *et al.*, 2006; Liu *et al.*, 2006; Tobinick & Gross, 2008). Further, glutamate-mediated ‘excitotoxic’ cell death is not limited to neurons. Oligodendrocytes also contain AMPARs (and NMDARs), and can be quite sensitive to glutamate-mediated cell death after injury (e.g. McDonald *et al.*, 1998; Park *et al.*, 2004).

In most of these situations AMPARs seem to be particularly involved, and the theme of this paper will be to provide evidence that AMPAR trafficking, modulated by inflammatory cytokines, especially TNF, may play a critical role in exacerbating excitotoxic cell death.

AMPAR subunit composition regulates conductance properties and trafficking

AMPARs are constructed of subunits GluR1–4 and are heteromers or homomers with different conductance properties (Hollmann *et al.*, 1991); AMPARs containing GluR2 are not permeable to Ca^{++} and are resistant to conductance increases induced by CAMKII-mediated phosphorylation of GluR1 ser 831 (reviewed in Derkach *et al.*, 2007). This would seem to make GluR2-lacking AMPARs more likely participants in Ca^{++}-mediated changes associated with excitotoxicity.

Trafficking of AMPARs with different subunits is also differentially regulated, with constitutive continuous replacement of GluR2/3-containing receptors, and stimulus-dependent exo- and endocytosis of GluR1-containing receptors in hippocampal neurons (Shi *et al.*, 2001). There is a large and compelling literature that suggests that such trafficking is critical for the modulation of synaptic strength during learning and memory [e.g. long-term

potentiation (LTP) and long-term depression (LTD)] in both the cerebellum and hippocampus, and that AMPAR trafficking is also crucial to the long-term adjustments needed to maintain the information imparted in short-term changes in synaptic strength. Thus, AMPAR trafficking has been implicated in both homeostatic synaptic scaling (Stellwagen *et al.*, 2005; Stellwagen & Malenka, 2006; Turrigiano, 2006, 2007, 2008; Kaneko *et al.*, 2008), and other forms of long-term synaptic strength adjustments (metaplasticity). And AMPARs with GluR2 subunits may be especially important (Gainey *et al.*, 2009). Interestingly, as developed below, TNF serves an important and perhaps even necessary role in the AMPAR changes associated with this long-term neural plasticity, and is also implicated in the regulation of synaptic strengths associated with sleep (Krueger *et al.*, 2008). Thus, AMPAR trafficking, and its regulation by TNF (and other stimuli) seem to be critical components of the normal homeostatic regulatory system controlling synaptic plasticity.

Glutamate and pro-inflammatory cytokine co-operativity

Evidence for synergistic effects of cytokines and glutamate in excitotoxic cell death comes from studies in experimental allergic encephalomyelitis (EAE) models of cell death (see Matute *et al.*, 2001), and spinal cord injury (SCI; Beattie, 2004). In a completely different context, Rogers and co-workers had shown that TNF applications to neurons in the nucleus of the solitary tract (NTS) produced enhancement of these neurons' responses to afferent activity elicited by stomach distension as part of the gastrogastric vagovagal reflex (Emch *et al.*, 2000). As circulating TNF can reach the vagal complex circuitry via the permeable capillaries in the area postrema, this result suggested a possible mechanism for reduction of gastric motility seen during peripheral inflammation caused by injury or cancer-related cachexia. In addition, as the vagal afferents to the NTS are glutamatergic, this result suggested that TNF was somehow enhancing the responsiveness of glutamate receptors on the postsynaptic neurons in the NTS. As both TNF and glutamate are elevated after SCI (Panter & Faden, 1992; Wang *et al.*, 1996), we wondered whether TNF might be enhancing the excitability of spinal cord neurons to excess glutamate after injury and thus exacerbating cell death. We tested this in collaboration with the Rogers' lab by making nanoinjections of TNF along with low doses of the glutamate agonist kainic acid (KA) into the spinal cord gray matter (Hermann *et al.*, 2001). Injections of femtomoles of TNF that by themselves produced no cell death were effective in producing large areas of cellular loss 90 min after injection when combined with small doses of KA that also by themselves produced little cell loss. The cell loss was indistinguishable from that produced by contusion lesions of the cord at 90 min, except that no extravasated blood was present. Further, this cell loss due to combined injections was completely blocked by co-injections of the AMPA/KA antagonist CNQX, suggesting that AMPA and or KA receptors were mediating the effects. Thus, small doses of TNF appeared to be altering glutamate receptor excitability, as had been shown in the NTS, but in this case the result was excitotoxic cell death.

As it was known that synaptic strength and excitability could be altered by AMPAR trafficking (Beattie *et al.*, 2002) it was logical to ask whether TNF affected AMPAR trafficking, and this was confirmed in a series of studies of hippocampal neurons *in vitro* (Beattie *et al.*, 2002). Immunocytochemical and electrophysiological studies showed that TNF applied to hippocampal cultures increased the surface expression of GluR1-containing subunits by > 60% within 15 min, and that these changes in surface expression were accompanied by dramatic changes in AMPAR-mediated excitatory postsynaptic currents. Moreover, blockade of endogenous TNF using a soluble receptor protein or blocking antibodies, reduced the excitability of glutamatergic synapses. These data strongly suggested a role for TNF in the physiological modulation of synaptic strength in the hippocampus (Beattie *et al.*, 2002), and this was confirmed in an elegant series of studies by Stellwagen,

Malenka, E. Beattie and colleagues (Stellwagen *et al.*, 2005; Stellwagen & Malenka, 2006) who showed that the TNF action was mediated via the TNFR1 receptor through a PI3K–akt pathway (Stellwagen *et al.*, 2005). Further, while TNF- or TNFR-knockout mice were not deficient in LTP or LTD, they were unable to exhibit homeostatic synaptic scaling (Stellwagen & Malenka, 2006). Thus, studies aimed at evaluating the role of TNF–AMPAR interaction in CNS injury yielded new insights into the normal regulation of synaptic plasticity. However, given the many possible roles of TNF in cell death, it was still not determined whether the TNF effect on AMPAR trafficking was actually involved in the enhancement of cell death seen after spinal cord injury (Hermann *et al.*, 2001) or in models of EAE (Matute *et al.*, 2001).

To answer this question, Leonoudakis *et al.* (2008) used a model of hippocampal cell excitotoxic death to determine whether TNF could enhance cell death by altering AMPAR trafficking (Leonoudakis *et al.*, 2008). They replicated the results of Beattie *et al.* (2002), showing that by 15 min after application of TNF (100 ng/mL), GluR1-containing AMPARs are increased on the membrane surface. Interestingly, this effect began to reverse over the course of an hour and, by the end of the 1-h exposure, there was an increase in surface expression of GluR2 (evident in the membrane preparation Western blots, but not in the surface expression immunocytochemical assays). In addition, both microscopic fluorescence and membrane fractionation approaches showed that GluR1-containing AMPARs appeared to be inserted preferentially into the extrasynaptic membrane, although they were also found to increase to a lesser extent in the synaptic fraction. This is consistent with a number of other studies suggesting that initial insertion into the extrasynaptic membrane is followed by regulated lateral diffusion into the postsynaptic density (Adesnik *et al.*, 2005; Derkach *et al.*, 2007). However, synaptic localization may not be necessary for activation of AMPARs by extracellular glutamate. This study used the lactate dehydrogenase assay to measure cell death induced by 18 h exposure to 20 μM KA. Pretreatment with 100 ng/mL TNF increased KA-induced cell death by ~20%. This, and additional basal cell death, was blocked by treatments that reduced TNF-induced GluR1 increases (the PI3K inhibitor LY294002), or by the AMPA/KA antagonist CNQX and, importantly for the GluR2-lacking hypothesis, the joro spider toxin analog NASPM, which selectively blocks permeability of Ca^{++}-permeable AMPARs. These data strongly support the hypothesis that increased GluR2-lacking AMPARs lead to higher susceptibility to glutamate-mediated excitotoxicity, and lend further support to the important role of TNF.

However, it is clear that the role of TNF in AMPAR-mediated cell death is complicated, and may vary over time after injury and according to concentration. Bernardino *et al.* (2005) examined the effects of TNF and its partner in inflammation interleukin-1β on AMPA-induced excitotoxicity in mouse hippocampal slice cultures (Bernardino *et al.*, 2005). They found that pretreatment of cultures with 10 ng/mL TNF potentiated AMPA-induced neuronal death, similar to that shown by Leonoudakis *et al.* (2008), but that 1 ng/ml pretreatment actually had a neuroprotective effect. The authors suggest that the ‘high-dose’ effect is mediated by TNFR1 while the low-dose effect is mediated by TNFR2, and they demonstrated that mice lacking each of those receptors showed different profiles of protection and death. However, in this model, the AMPA-induced cell death appeared to be regulated by microglial activation; AMPAR trafficking was not measured in neurons in this work. The importance of TNFR1 in the AMPA-mediated excitotoxicity in this study is consistent with the results of Stellwagen *et al.* (2005) in that TNFR1 was required for AMPAR increases in GluR1-containing receptors. However, microglial activation is clearly important in the elaboration of the secondary injury cascade after SCI or other CNS damage (see (Beattie, 2004). Thus the pleiotropic effects of glutamate and cytokines on the multiple cell types in the CNS parenchyma complicates predictions of the role of AMPARs in secondary injury. Indeed, although there is general agreement that pro-inflammatory

cytokines play a role in cell death, especially early after injury, there is compelling evidence from studies of ischemic injury that TNFR1 and TNFR2 activation may be important at some point in the cascade for neuroprotection or repair (Bruce *et al.*, 1996). Therefore, even with the rather clear evidence from *in vitro* studies that AMPAR trafficking in response to TNF could be behind the TNF–KA synergistic effects on cell death seen in the nanoinjection study by Hermann *et al.* (2001), the role for this process in promoting damage after spinal cord injury was not proven.

Therefore, Ferguson *et al.* (2008a,b) undertook a series of studies aimed at determining whether AMPAR trafficking is involved in post-SCI cell death (Ferguson *et al.*, 2008b). The strategy was to evaluate whether TNF effects of AMPAR trafficking could be detected in the spinal cord after nanoinjections or injury and, if so, whether reduction of injury-induced TNF could reverse the AMPAR increases and also provide protection from contusion injuries.

Measuring surface expression of AMPARs in tissue culture can be accomplished by using antibodies that recognize extracellular epitopes of AMPAR subunits (Beattie *et al.*, 2002), or by using streptavidin (Leonoudakis *et al.*, 2008). Localizing AMPAR subunits to the plasma membrane in the spinal cord is problematic, as tissue sections allow antibody penetration into the intracellular spaces. Membrane fractionation techniques can be used both *in vitro* and *in vivo* but *in vivo* the membrane preparations are derived from a multitude of cells types. In order to address this, we used a combination of membrane fractionation and enrichment techniques in combination with optical membrane localization techniques using confocal microscopy and 3-D blind, iterative image deconvolution, using antibodies generated against AMPAR subunits and markers of synaptic membranes (e.g. presynaptic synaptophysin).

First, we asked whether we could detect changes in AMPAR surface expression in the early period following a spinal cord contusion injury. A mild unilateral weight drop injury with dura intact was given to rats after a laminectomy (see Gensel *et al.*, 2006). Ninety minutes after injury, rats were perfused and the tissue taken for evaluation of membrane localization of GluR1, using confocal microcopy to estimate the proportion of postsynaptic membrane in the neuropil that was occupied by GluR1-containing AMPARs. Presynaptic elements were identified using an antibody to synaptophysin, which was also used to define the membrane contour of large neurons in the parenchyma in later experiments (see Fig. 1). To estimate the region of spreading secondary injury, we looked for activated neurons expressing c-fos (Hermann *et al.*, 2001). At 90 min, c-fos-labeled cells defined a penumbra region in which the colocalization of GluR1 and synaptophysin, indicating close apposition and thus synaptic membrane localization of GluR1, was substantially increased compared to the contralateral uninjured gray matter (Fig. 1). Thus, this initial estimate of increased synaptic localization of GluR1 supported the hypothesis that injury, and resultant TNF, increases surface expression of AMPARs. We then asked whether the nano-injections of TNF that had potentiated excitotoxic cell death in the Hermann *et al.* (2001) studies would also increase surface AMPARs. We used both an ‘optical membrane fractionation’ technique, in which the cellular surface was defined by the pattern of synaptophysin labeling outlining the cell, and biochemical membrane fractionation techniques to provide converging evidence for membrane localization. Both methods showed that 90 min after nano-injections of TNF into the spinal cord gray matter, GluR1 protein was significantly increased in the plasma membrane. In addition, comparison of GluR1 and GluR2 showed that GluR1 was increased while GluR2 labeling was decreased. Thus, as shown by Leonoudakis *et al.* (2008) using *in vitro* hippocampal neurons, spinal cord neurons *in vivo* also responded to TNF by increasing the proportion of GluR1-containing AMPARs and decreasing the proportion of GluR2-containing receptors. The optical fractionation method allowed us to estimate synaptic and

extrasynaptic membrane fractions. Both exhibited dose-dependent increases in GluR1 staining and decreases in GluR2 staining. These results, in combination with the *in vitro* work (Stellwagen *et al.*, 2005; Leonoudakis *et al.*, 2008), strongly support the notion that TNF can rapidly increase the proportion of Ca^{++}-permeable, GluR2-lacking AMPARs, leading to enhanced excitotoxicity. However, the techniques used to estimate membrane localization are somewhat indirect, and could be contaminated by proteins localized very close to but not really within the plasma membrane. In support of those methods, a recent analysis of changes in GluR1/GluR2 ratios in membrane postsynaptic to nociceptive afferents sensitized with capsaicin reached remarkably similar conclusions using electron microscopy of postembedding immunogold labeling, which allowed for resolution on the nanometer scale (Larsson and Broman, 2008). Other studies from the pain literature have implicated TNF in nociceptive sensitization, providing additional convergence of TNF signaling and AMPAR trafficking mechanisms in the spinal cord (Schafers *et al.*, 2003a,b, 2008). Nevertheless, a fuller test of the hypothesis requires the demonstration that concurrent reduction in TNF activity and AMPAR trafficking lead to reductions in cell death.

This was demonstrated by experiments in which the TNF-induced changes in GluR1/GluR2 ratios were blocked by application of soluble TNF-receptor 1 protein (sTNFR1), and an experiment showing that sTNFR1 could reduce large neuron cell death when injected into the cord after a mild unilateral contusion injury (Ferguson *et al.*, 2008b; also Fig. 2).

Further support for the role of AMPAR trafficking in other CNS disorders

Data from other laboratories and other disease models support the importance of AMPAR modulation in the interactions between neuroinflammation and excitotoxicity. There has been considerable attention to the role of cytokines, including TNF, in the production of glial and axonal cell death in multiple sclerosis (e.g. (Matute *et al.*, 2001). Recently, Centonze *et al.* (2009) have shown that, in EAE in mice, TNF is released from microglia in the striatum, and this changes AMPAR expression and phosphorylation and leads to synaptic degeneration that can be prevented by AMPAR antagonists. In yet another demonstration of the complexity of the interacting elements in secondary degeneration, Lebrun-Julien *et al.* (2009) have shown that NMDA-induced retinal degeneration is mediated by NMDAR-induced release of TNF from Muller glia and this, in turn, alters Ca-permeable AMPARs and kills retinal neurons *in vivo* via an increase in surface Ca^{++}-permeable AMPARs.

These data underscore the importance of glia as a source of the TNF that regulates AMPAR trafficking. Identifying the relative roles of specific glial subtypes in modulating AMPAR trafficking represents an area for further research as both astrocytes and microglia have been shown to release TNFα in culture (Sierra *et al.*, 2007; Su *et al.*, 2009). In addition, astrocyte-conditioned media induces rapid AMPAR trafficking in neurons (Beattie *et al.*, 2002). However, astrocyte cultures are often contaminated by small numbers of microglia (Li *et al.*, 2008; Watkins *et al.*, 2008). As microglia are prolific producers of TNF, it is not clear to what extent microglial contamination could contribute to TNF release by astrocyte cultures. Activated microglia as well as infiltrating peripheral macrophages have a well-known role in cell death after CNS injury *in vivo* (Beattie, 2004; Kigerl *et al.*, 2009), and it is possible that TNF release by these cells plays a role in tipping the balance of AMPAR trafficking from a physiological level toward a pathological outcome. Further, as microglia and astrocytes coexist *in vivo* it may be difficult to extrapolate TNF concentrations produced from pure cell cultures to the tissue microenviroment levels in the injured CNS. Nevertheless it is clear that glial-derived TNF may have an impact in a variety of pathological states.

Blockade of TNF has been shown to reduce the development of neuropathic pain in a peripheral nerve inflammation model (Svensson *et al.*, 2005) and, in an elegant immunogold electron microscopic study, Larsson & Broman (2008) have shown changes in both GluR1 labeling and the GluR1/GluR2-3 ratios in lamina II dorsal horn postsynaptic sites innervated by primary afferents that have been sensitized by capsaicin, within 20–25 min of the sensitization stimulus. This short-term change in the surface expression of GluR2-lacking AMPARs is strikingly similar to that demonstrated in the spinal cord after injury or TNF application (Ferguson *et al.*, 2008b). Further, TNFα and IL-1β modulate AMPA-induced excitotoxicity in mouse hippocampal slice (Bernardino *et al.*, 2005), but there is a critical dose–response relationship between TNF, neurodegeneration and neuroprotection, related to the different TNFRs. Higher concentration of TNF, affecting TNFR1, appeared to produce toxicity, whereas activation of the p55 TNFR2 appeared to be neuroprotective. The role of TNFR1 in the AMPAR toxicity in these studies is consistent with the findings of Stellwagen *et al.* (2005), who identified TNFR1 as the mediator of TNF-induced AMPAR movement to the plasma membrane. Thus, the data from a series of related experiments in different neuronal systems point to the recruitment of Ca^{++}-permeable AMPARs to the plasma membrane as an important aspect of secondary injury after CNS trauma, and highlights the role of TNF and possibly other inflammatory cytokines including IL-1β in driving this process.

Further implications for neuroinflammation and neurodegeneration

While the above studies provide perhaps the most direct evidence of AMPAR-trafficking effects in cell death, a number of recent studies from a variety of CNS injury models provide continuing evidence that TNF and other cytokines can combine with glutamatergic receptor mechanisms to drive excitotoxicity, perhaps also by means of AMPAR trafficking. Some also provide reminders of the complexity of the secondary injury process and the caveat that, even if this mechanism is a crucial one, its occurrence within the elaborate context of the multiple cellular interactions leading to secondary damage and repair may make it a difficult therapeutic target. For example, seizure susceptibility in mouse models of epilepsy is altered in mice lacking TNFRs, due in part to alterations in AMPAR and NMDAR subunit composition (Balosso *et al.*, 2009). The role of TNF in excitability extends beyond postsynaptic neurons and includes effects on astrocytes. TNF is permissive for the physiological release of glutamate from astrocytes, and at higher concentrations drives constitutive release of glutamate from astrocytes via endocytosis (Domercq *et al.*, 2006; Jourdain *et al.*, 2007). This same group has shown that, in contrast, TNF-mediated glutamate release from astrocytes is impaired in a mouse model of Alzheimer's disease (Rossi *et al.*, 2005). Further, peripheral inflammation is associated with increased central excitability, and this also appears to be related to microglial activation and TNF (Riazi *et al.*, 2008). These and many more examples of modulation of excitability by apparent interactions between inflammatory cytokines and AMPARs suggest that regulation of glutamate receptor trafficking may play a nodal role in the elaboration of acute, and perhaps chronic, neurodegenerative changes after injury.

This hypothesis comes with a list of important caveats: first, it is well established that NMDAR and mGluR types of glutamate receptors are also important in cell death (Choi, 1992; Agrawal & Fehlings, 1997). Alterations of glutamatergic signaling aimed at excitotoxicity need to take these receptors into account. Further, the intracellular pathways mediating TNF and other ligand-mediated changes in AMPARs are not yet clear. There is clear evidence for TNF-mediated CAMKII signaling in LTD and LTP (e.g. Derkach *et al.*, 2007), but the studies directed specifically at TNFR1-mediated changes in AMPAR surface expression implicate PI3K–akt pathways (e.g. Stellwagen *et al.*, 2005). Thus, therapeutics aimed at downstream pathways will require more information on these mechanisms.

AMPARs are not the only receptors involved in plasticity or degeneration that alter trafficking in response to various stimuli. Other receptors may be involved as well. For example, Marsden *et al.* (2007) have shown that NMDA receptor activation results in exocytosis of $GABA_A$ receptors, with a consequent increase in inhibitory transmission in hippocampal neurons *in vitro* (Marsden *et al.*, 2007). In theory, this could act as a neuroprotective mechanism in response to glutamate stimulation and, indeed, our laboratory has found a similar increase in $GABA_A$ receptors on spinal cord neurons 90 min after injury (Ferguson, A.R., Gensel, J.C., Christensen, C.R.N., Tovar, C.A., Miller, B.A., Bresnahan, J.C., & Beattie, M.S., unpublished observations). Perhaps an especially important caveat involves the timing of events that lead to cellular damage or repair after injury. Early inflammation, TNF release and consequent rapid increases in Ca^{++}-permeable AMPARs would seem to be deleterious, but it is clear that other longer-term reparative events may depend upon TNF or other cytokine actions (e.g. Bruce *et al.*, 1996). Such effects may be partially mediated by preferential activation of TNFR2 over TNFR1 at lower doses of TNF, which have been found to be protective (Bernardino *et al.*, 2005). Further, longer-term alterations in the differential production and surface expression of AMPARs with different subunit compositions could well be involved in repair and recovery, especially with respect to neuroplasticity associated with recovery and rehabilitation after CNS damage (e.g. Edgerton *et al.*, 2004; Wolpaw, 2006; Ferguson *et al.*, 2008a; Baumbauer *et al.*, 2009). There is clear evidence for chronic alterations in AMPAR composition and expression after injury (e.g. Wrathall *et al.*, 1996; Grossman *et al.*, 2001a,b; Yu *et al.*, 2002), and how these alterations play onto the effects of more rapid modulation of AMPAR trafficking during injury and repair are not well understood. One hypothesis is that CNS injuries result in a leftward shift of the dose–response function for TNF, so that doses that fall within the normal physiological range come to elicit cell death. This idea is consistent with observations that glutamate levels are increased in a number of pathological conditions (Choi, 1992). In the presence of increased levels of glutamate in the CNS parenchyma, even slight increases in TNF-induced AMPAR trafficking could become toxic. However, at chronic timepoints, when glutamate levels return to normal, regulation of AMPAR physiology by TNF may be expected to facilitate recovery by contributing to physiological control of AMPAR trafficking. Thus attempts to alter AMPAR trafficking as a means to reduce injury and improve outcomes will need to be designed with these provisions in mind.

Summary and therapeutic implications

Nevertheless, interfering with the signaling mechanisms that regulate this particular aspect of neuroinflammatory–excitotoxicity synergy may represent a powerful new therapeutic approach for CNS disorders. In support of this concept, TNF inhibitors have recently been reported to increase functional performance (Genovese *et al.*, 2006) and reduce neuropathic pain (Marchand *et al.*, 2009) after spinal cord injury. Similar promising findings are beginning to emerge from the literature on other neurological disorders including Alzheimer's disease (Tobinick & Gross, 2008) and epilepsy (see Kleen & Holmes, 2008, for a review). Glutamate-receptor trafficking appears to be essential in normal synaptic plasticity and homeostasis. Rapid interference with TNF-induced changes in AMPAR trafficking could provide an effective means to prevent the hijacking of this critical mechanism by the pathophysiological events following CNS injury.

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Abbreviations

AMPAR	*α*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
CNS	central nervous system
EAE	experimental allergic encephalomyelitis
IL	interleukin
KA	kainic acid
LTD	long-term depression
LTP	long-term potentiation
NMDA	*N*-methyl-D-aspartate
NMDAR	NMDA receptor
NTS	nucleus of the solitary tract
SCI	spinal cord injury
sTNFR1	soluble TNF-receptor 1 protein
TNF	tumor necrosis factor alpha

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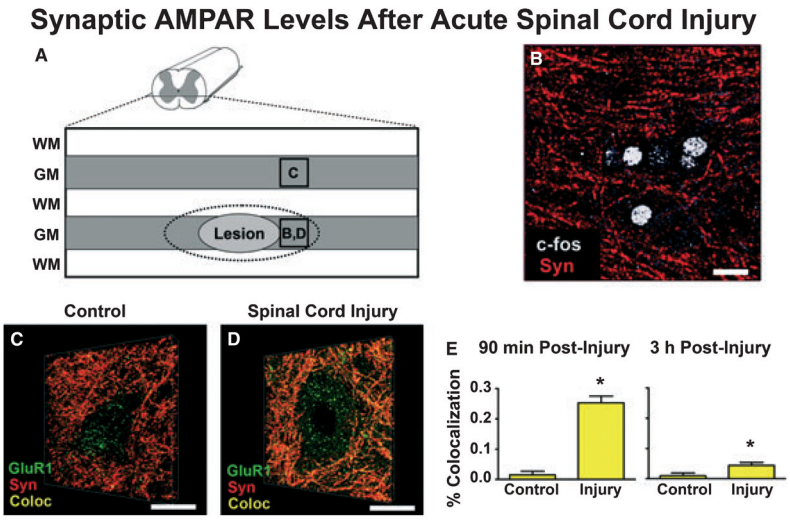

Fig. 1.

AMPAR trafficking to the synaptic membrane after spinal contusion injury. (A) Schematic representation of a horizontal section through the ventral horn of the spinal cord after unilateral contusion injury. Neurons were sampled in the lesion penumbra (dashed ellipse) just beyond the site of frank lesion. The contralateral side served as a control. (B) C-fos labeling in the lesion penumbra defined a region of interest for high-resolution confocal microscopy. (C and D) Spinal cord injury induced a marked increase in synaptic AMPAR levels relative to the contralateral uninjured tissue as revealed by laser scanning confocal microscopy coupled with 3-D blind deconvolution. (E) Automated image analysis revealed a significant increase in synaptic AMPAR puncta at 90 min and 3 h after injury, although the effect was more pronounced at the acute time point. *$P < 0.05$.

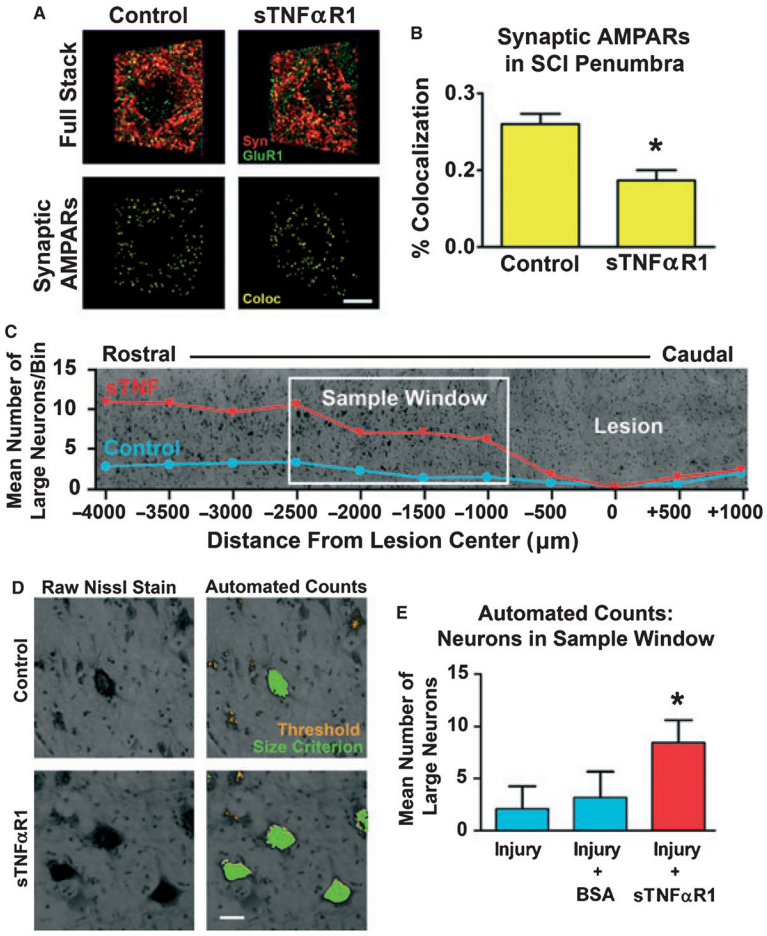

Fig. 2.

Reduction in AMPAR trafficking and excitotoxicity following spinal cord injury after treatment with the TNF inhibitor soluble TNF receptor 1 (sTNFR1). (A) Representative confocal images depict the partial reversal of injury-induced increases in synaptic AMPAR levels. (B) Automated image analysis revealed a significant decrease in AMPAR puncta within the synaptic membrane. (C) Survival of neurons increased in the ventral gray matter as a function of distance from the lesion epicenter. (D) Automated cell counting algorithm revealed greater numbers of surviving large-diameter (> 40 μm) neurons within the *a priori* sample window within the lesion penumbra. (E) Quantitative comparisons revealed a significant increase in surviving neuron numbers in the sTNFR1 group relative to either injury alone or BSA vehicle treatment. *$P < 0.05$.