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PERSPECTIVE

Fractalkine: multiple strategies to counteract glutamate receptors activation leading to neuroprotection

Glutamate (Glu) is the main excitatory amino acid in the brain and plays a pivotal role in many neurophysiological functions. Nevertheless, an excess and prolonged exposure to Glu determines the overactivation of glutamate receptors (GluRs) with consequent impairment of cellular calcium (Ca^{2+}) homeostasis, leading to the dysregulation of intracellular pathways and resulting in neuronal dysfunction and death, a process called excitotoxicity.

In the last two decades, excitotoxic mechanisms have been proposed to explain the neuronal cell death characteristic of neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's diseases, including increase of intracellular Ca^{2+}, accumulation of oxidizing free radicals, impairment of mitochondrial function and activation of apoptotic and autophagic programs. Since excitotoxicity is implicated in a variety of neuropathological conditions, it represents a common pathogenic pathway for neurodegenerative diseases with distinct genetic etiologies and it appears to be important in determining the extent of tissue damage.

The excitatory effects of Glu are exerted by the activation of three types of ionotropic receptors that are N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainic acid (KA) receptors, and several classes of G-protein coupled metabotropic receptors (mGluRs).

In the attempt to counteract excitotoxic insult, damaged neurons respond by releasing soluble factors that might be sensed by surrounding cells to induce a wide range of cellular responses leading to neuroprotection and tissue damage repair. Among these factors there is fractalkine (CX3CL1), a chemokine constitutively expressed on neuronal membrane (Harrison et al., 1998) that is upregulated, cleaved and released upon excitotoxic insult (Chapman et al., 2000).

The peculiarity of CX3CL1 is that, contrary to almost all chemokines, it binds a unique receptor, CX3CR1, that in the brain is expressed only by microglia (Harrison et al., 1998; Cardona et al., 2006): thus the CX3CL1/CX3CR1 pair represents an intriguing communication system between neuronal cells and microglia in order to maintain brain homeostasis and to fully guarantee central nervous system (CNS) functions.

Another soluble factor, whose extracellular level is enhanced after noxious brain stimuli, is adenosine, a cellular metabolite that binding different G-protein coupled receptors (adenosine receptor type 1 (A_1R), $A_{2A}R$, $A_{2B}R$, and A_3R) is able to modulate neurotransmission and neuroprotection.

In our laboratory, in the last decade, we have highlighted the neuroprotective activity of CX3CL1 that acting in a synergistic way with adenosine is able to contrast AMPA or NMDA receptors activity with different molecular mechanisms, leading to reduced excitotoxic neuronal cell death.

CX3CL1 protects neurons from Glu excitotoxicity both in *in vitro* and *in vivo* models with mechanisms fully dependent on A_1R (Lauro et al., 2008, 2010; Cipriani et al., 2011) and partially dependent on A_3R (Rosito et al., 2014). Indeed CX3CL1, acting on microglia, is able to increase extracellular adenosine derived from released adenosine triphosphate (ATP), since this effect is abolished in the presence of specific inhibitor of ectonucleotidases (AOPCP) but not by the inhibitor of equilibrative transport (NBTI) (Lauro et al., 2008, 2010). Upon Glu toxic challenge, we have shown that CX3CL1 is able to increase Glu removal from synaptic cleft by enhancing excitatory amino acid transporter GLT-1 expression and function, specifically on astrocytes, with a mechanism that depends on A_1R activation (Catalano et al. 2013). Moreover, CX3CL1 can modulate AMPA receptor activity by reducing AMPA current, *via* A_1R action (Lauro et al., 2008): this might lead to a reduction of Ca^{2+} flow through AMPA receptor and AMPA-mediated NMDA receptor activation.

CX3CL1 effect against Glu excitotoxicity requires also the activity of A_3R, whose genetic or pharmacological inactivation is able to reduce CX3CL1 neuroprotection. This mechanism is independent from GluRs activity, but represents another aspect of the neuroprotective mechanism driven by CX3CL1 that involves microglia-astrocytes crosstalk and that leads to the release of neuroprotective molecules such as CXCL16 and CCL2 (Rosito et al., 2012, 2014).

Experimental evidence suggests that NMDARs, due to their high Ca^{2+} permeability and conductance properties, are mainly responsible for uncontrolled increase of neuronal intracellular Ca^{2+} and consequent cell death. However, depending on subcellular distribution and receptor subunits composition, NMDARs stimulation may exert opposite effects on neurons: the activity of synaptic NR1/NR2A/NR2B-containing NMDARs provides neuroprotection while the activation of extrasynaptic NR1/NR2B-containing NMDARs is responsible for neurotoxicity. In particular, the activation of synaptic NR2A containing NMDAR induces cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) activity with a concomitant increased expression of neuroprotective brain-derived neurotrophic factor (BDNF); in contrast, the overactivation of extrasynaptic NR2B containing NMDAR stimulates a dominant CREB shut-off pathway, which inhibits BDNF expression and leads to excitotoxicity (Hardingham and Bading, 2010).

Interestingly, our recent work shows that CX3CL1 modulates NMDA-mediated synaptic transmission in the hippocampal CA1 region through the activation of $A_{2A}R$ and the consequent release from glial cells of D-serine, the co-agonist of NR2A/NMDAR (Scianni et al., 2013). Thus, we decided to investigate the action of CX3CL1 on NMDA-mediated neurotoxicity and, differently from what reported for Glu excitotoxicity, we found that CX3CL1 is able to contrast specific NMDA induced excitotoxicity with a mechanism that depends on $A_{2A}R$ activity and extracellular D-serine. In line with these results, D-serine is able to counteract NMDA, but not Glu excitotoxicity. Moreover, both CX3CL1 and D-serine are able to phosphorylate cyclic-AMP response element-binding protein (CREB) with a mechanism involving

the expression of $A_{2A}R$ (Lauro et al., 2015).

This is the first demonstration of a functional interaction between NMDAR and $A_{2A}R$ with the purpose of modulating protective action. CX3CL1 modulates NMDAR effects in an $A_{2A}R$-dependent way, by potentiating neurotransmission and by counterbalancing the neuronal death induced by NMDAR activation through the selective potentiation of synaptic D-serine sensitive NR2A/NMDARs, thus contributing to the enhancement of "CREB on" activation pathway. Nevertheless, in hippocampal cultures obtained from mice that do not express $A_{2A}R$, D-serine alone is not sufficient to induce neuroprotection or CREB phosphorylation, suggesting that in CX3CL1 neuroprotection $A_{2A}R$ also acts by regulating the activity of NMDAR independently of D-serine. It should be considered that different populations of $A_{2A}R$ have different localizations and functions: one possibility is that that $A_{2A}Rs$ expressed by glial cells are mostly responsible for the release of D-serine and those expressed on post-synaptic neurons modulate NMDARs activity. Moreover, when D-serine is degraded by the enzyme D-amino acid oxidase (DAAO), there is only a partial block of CREB phosphorylation, suggesting that other pathways triggered by CX3CL1 act synergistically with $A_{2A}R$ to counteract NMDA-induced excitotoxicity. One of these pathways might be the BDNF/TrkB signaling: we showed that CX3CL1 increases BDNF expression and TrkB phosphorylation in hippocampal cultures, both events possibly linked to CREB phosphorylation and neuroprotection (Lauro et al., 2015).

All together, these data corroborate the idea that CX3CL1 engages different AR subtypes on neighboring cells to counteract excitotoxicity, depending on the GluRs activation; nevertheless, it has to be considered that both in acute and chronic neurodegenerative disorders, high level of Glu in the brain leads to the activation not only of NMDA receptors but also of other GluRs such as AMPA and mGlu receptors. In this contest, it might be interesting to analyze if CX3CL1 is able to activate different pathways in case of overactivation of specific AMPA or mGlu receptors to better clarify its role as a neuroprotective and neuromodulator molecule. What is clear is that CX3CL1, after the direct action on CX3CR1 expressed on microglia, counteracts GluRs activation by promoting an intense dialogue among neurons, microglia and astrocytes that cooperate to promote neuroprotective mechanisms. I conjectured that, upon excitotoxic insults, damaged neurons release CX3CL1 which acts locally, by inducing the release of different soluble factors, including adenosine, that in turn act on cells in close proximity in order to orchestrate a broad spectrum of protective activities. In this view, I want to highlight the role played by CX3CL1 as as a modulator of the GluRs, that adopting alternative strategies, drives neuroprotective action and represents an interesting endogenous self-protective mechanism initiated from neurons to counteract brain damage.

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