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Review

Role of nonsynaptic GluN2B-containing NMDA receptors in excitotoxicity: Evidence that fluoxetine selectively inhibits these receptors and may have neuroprotective effects

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ABSTRACT

In acute ischaemic brain injury and chronic neurodegeneration, the primary step leading to excitotoxicity and cell death is the excessive and/or prolonged activation of glutamate (Glu) receptors, followed by intracellular calcium (Ca^{2+}) overload. These steps lead to several effects: a persistent depolarisation of neurons, mitochondrial dysfunction resulting in energy failure, an increased production of reactive oxygen species (ROS), an increase in the concentration of cytosolic Ca^{2+} $[Ca^{2+}]_i$, increased mitochondrial Ca^{2+} uptake, and the activation of self-destructing enzymatic mechanisms. Antagonists for NMDA receptors (NMDARs) are expected to display neuroprotective effects, but no evidence to support this hypothesis has yet been reported. A number of clinical trials using NMDAR antagonists have failed to demonstrate neuroprotective effects, either by reducing brain injury or by preventing neurodegeneration. Recent advances in NMDAR research have provided an explanation for this phenomenon. Synaptic and extrasynaptic NMDARs are composed of different subunits (GluN2A and GluN2B) that demonstrate opposing effects. Synaptic GluN2A-containing and extrasynaptic GluN2B-containing NMDARs have different co-agonists: D-serine for synaptic NMDARs and glycine for extrasynaptic NMDARs. Both co-agonists are of glial origin.

The mechanisms of cell destruction or cell survival in response to the activation of NMDAR receptors depend in part on $[Ca^{2+}]_i$ and the route of entry of this ion and more significantly on the subunit composition and localisation of the NMDARs. While synaptic NMDAR activation is involved in neuroprotection, the stimulation of extrasynaptic NMDARs, which are composed of GluN2B subunits, triggers cell destruction pathways and may play a key role in the neurodegeneration associated with Glu-induced excitotoxicity. In addition, it has been found that synaptic and extrasynaptic NMDA receptors have opposing effects in determining the fate of neurons. This result has led to the targeting of nonsynaptic GluN2B-containing NMDARs as promising candidates for drug research. Under hypoxic conditions, it is likely that the failure of synaptic glutamatergic transmission, the impairment of the GluN2A-activated neuroprotective cascade, and the persistent over-activation of extrasynaptic GluN2B-containing NMDARs lead to excitotoxicity. Fluoxetine, a drug widely used in clinical practice as an antidepressant, has been found to selectively block GluNR2B-containing NMDARs. Therefore, it seems to be a potential candidate for neuroprotection.

Contents

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1. Introduction

The presynaptic nerve terminal, which is equipped with a number of different receptors, is a regulatory site for neuronal activity-mediated chemical transmission (Vizi, 1979; Vizi and Labos, 1991; MacDermott et al., 1999; Vizi and Lendvai, 1999; Lendvai and Vizi, 2008). The activation of ionotropic receptors localised on pre-terminals such as GABA-gated Cl channels, presynaptic nicotinic acetylcholine receptors (nAChRs) and presynaptic Glu receptors may result in the modulation of transmitter release. If these receptors are localised on the postsynaptic site, they may influence the efficacy of transmission.

The $[Ca^{2+}]_i$ can be increased through the activation of voltage-dependent Ca^{2+} channels, through the opening of NMDAR channels and through impaired activity of the membrane Na^+/Ca^{2+} exchanger. The over-activation of postsynaptic NMDARs has been hypothesised to play a pivotal role in inducing neuronal death (Bullock et al., 1992; Zhou and Baudry, 2006a,b) by increasing the $[Ca^{2+}]_i$, which activates nucleases, cytosolic proteases and kinases (Favaron et al., 1990; Mills and Kater, 1990), resulting in destruction of the cell. Extracellular concentrations of Glu and aspartate in the rat hippocampus are increased during transient cerebral ischaemia (Benveniste et al., 1984). *In vitro* experiments have also shown that oxygen–glucose deprivation is associated with a large increase in the extracellular Glu concentration (Benveniste et al., 1984). Under physiological conditions, Glu that is released into the synaptic region or the extracellular space is taken up by transporters (Fig. 1A). In contrast, under ischaemic conditions, Glu is released by the reversed operation of the transporter (Fig. 1B). Both the early swelling and the subsequent neuronal degeneration that are produced by ischaemic conditions can be blocked by the addition of NMDAR antagonists but not by the AMPA/kainate receptor antagonist 6-cyano-7-dinitroquinoxaline-2,3-dione (CNQX), by dihydropyridines nifedipine (nimodipine), or by tetrodotoxin (TTX).

If the cell becomes depolarised, NMDARs are released from the Mg^{2+} block and can be activated by synaptic Glu, resulting in further Ca^{2+} entry into the cell. The osmotic balance of the cell is also disturbed during persistent depolarisation due to the increase in intracellular Na^+ concentration (Kiedrowski et al., 1994). The entry of Na^+ is followed by an increase in the $[Na^+]_i$ and passive entry of Cl^- ions and water, which causes a reversal of transporter activity, an increase in the cell volume (osmotic swelling) and the release of cell contents into the extracellular space.

Cell swelling occurs during anoxia and/or ischaemia (Franco et al., 2008), and the reverse operation of transporter activity may result in an excessive $[Ca^{2+}]$-independent release of transmitters, including catecholamines (Milusheva et al., 1992, 2003; Uchihashi et al., 1998) and Glu, from neurons and astrocytes (Benveniste et al., 1984; Katayama et al., 1990; Szatkowski et al., 1990; Fellin et al., 2004). During ischaemia, when oxygen and glucose supply are either limited or completely blocked, synaptic transmission is impaired, but Glu is released in relatively high concentrations by means of the reverse operation of transporters (Fig. 2). Hence, during ischaemia, synaptic NMDARs do not become activated as a result of axonal activity. Glutamate that is released into the extracellular space mainly from glial processes (Fellin et al., 2004) may result in the persistent activation of extrasynaptic GluN2B receptors, which are of high affinity and are sensitive to low concentrations of Glu (Vizi, 2000). This activation then induces neuronal damage (Hardingham et al., 2002) (see Fig. 2). Ischaemia has been shown to be accompanied by a reduction in the volume of the extracellular space (Sykova and Vargova, 2008), which results in a further increase in the concentrations of extrasynaptic Glu and other substances, such as catecholamine and ATP. It has also been shown that GluN2B receptors are involved in neuropathic pain (Abe et al., 2005; Zheng et al., 2012) and in alcohol dependence (Nagy, 2004; Nagy et al., 2004).

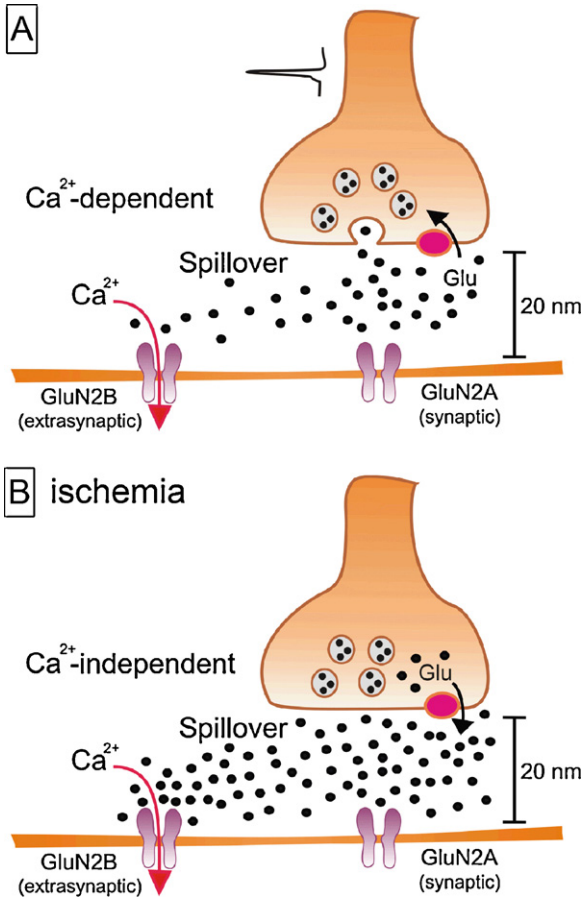

Fig. 1. Synaptic (GluN2A) and extrasynaptic (GluN2B) localisation of NMDARs. Release of glutamate (Glu) under normal (A) and ischaemic (B) conditions. The role of Glu uptake into nerve and glial cells is to keep the extracellular [Glu] low. In response to the excessive and prolonged activation of glutamatergic neurons, Glu released into the synapse spills over and may activate extrasynaptic GluN2B-containing NMDARs (A). During ischaemia, there is no vesicular release (Vizi et al., 2010), and there is a non-vesicular $[Ca^{2+}]_o$-independent release of Glu from glial cells and neurons (Szatkowski et al., 1990). Under these conditions, Glu is released by the reverse operation of transporters.

These findings have prompted pharmacological studies and clinical trials of NMDAR antagonists to examine their effects on neurological diseases and to determine whether they prevent neuronal loss after stroke and head trauma.

2. Role of extrasynaptic GluN2B-containing and synaptic GluN2A-containing NMDA receptors in excitotoxicity and neuroprotection

Calcium entry through synaptic GluN2A-containing NMDARs induces activity of cAMP response element binding protein (CREB)