Neuroprotective Effect of Resveratrol Against Glutamate-Induced Excitotoxicity

Abstract
As the major neurotransmitter in the mammalian central nervous system (CNS), excessive extracellular glutamate (Glu) can activate the Glu receptors and neuronal calcium (Ca2+) overload, then produce neurotoxicity, which is a common pathway for neuronal injury or death, and is associated with acute and chronic neurodegenerative diseases. Therefore, it has been a therapeutic strategy to investigate neuroprotective effects against Glu-induced neurotoxicity for treating both acute and chronic forms of neurodegeneration. Resveratrol (Res), as a naturally occurring polyphenol mainly found in grapes and red wine, has shown a neuroprotective effect in a variety of experimental models for neurodegenerative diseases \textit{in vitro} and \textit{in vivo}. This review will focus on the neuroprotective effect of Res against Glu-induced excitotoxicity in neurodegenerative diseases by blocking different Glu receptors and Ca2+ ion channels (Adv Clin Exp Med 2015, 24, 1, 161–165).

Key words: resveratrol, glutamate, excitotoxicity, neuroprotective effect.

Glutamate (Glu) is the major excitatory neurotransmitter in the central nervous system (CNS), involved fast synaptic transmission, neuronal plasticity, outgrowth and survival, memory, learning and behavior [1]. Apart from its physiological role, excessive Glu leads to activation of the Glu receptors, particularly of N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainic acid (KA) subtypes; to excessive Ca2+ influx into neurons through ionic channels, and secondary excitotoxicity [2]; and finally to neuronal dysfunction, damage or even death [3]. As the final common pathway for neurologic disorders, Glu-mediated neurotoxicity has been shown to contribute to pathological consequences in several neuropathological disorders, particularly in Alzheimer’s disease, Parkinson’s disease, epilepsy, ischemic stroke and major depression [4]. For this reason, it is important to understand the various types of Glu receptors and the ion channels that they are capable of activating either directly or indirectly. Early work on Glu-induced excitotoxicity emphasized the importance of KA and its receptor in producing this type of neuronal damage [5]. Other studies have shown that a specific blockade of the NMDA receptor largely blocks the toxicity of exogenous Glu in cultured cortical neurons [3, 6]. The most relevant biochemical events in Glu-mediated neurotoxicity might be the acute influx of extracellular Ca2+ through cell membrane channels. However, other routes, including L- and
N-type voltage-gated Ca\(^{2+}\) channels, the Na\(^{+}\)/Ca\(^{2+}\) exchanger, and non-specific membrane leak, may also participate [7].

Resveratrol (Res) has been shown to exhibit various beneficial biological effects, such as protective effects against cardiovascular diseases [8], anti-inflammatory or anticarcinogenic activities [9]. Res has also been shown to pass the blood brain barrier and exert neuroprotective effect, and therefore its use for the prevention and treatment of neurodegenerative diseases, such as cerebral ischemia, Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral sclerosis, has been investigated [10]. For example, Res protected against neurotoxic effects and antagonized the [Ca\(^{2+}\)]i elevation produced by excess Glu concentrations in cerebrospinal fluid from amyotrophic lateral sclerosis patients, primary cultured rat cortical cells and the murine hippocampal cell line HT 22 [11–13]. Recent studies have reported that Res increased Glu uptake, glutathione content and S 100B secretion in primary cortical astrocytes [14], and reduced Glu-induced radical formation in murine brain cultures [15]. Res and capsaicin combined enhance neuroprotection against Glu-induced toxicity in mouse cerebral cortical neurons, implying a useful therapeutic method for treating neurodegenerative disorders [16]. A recent study confirmed that trans-Res significantly suppressed Glu-induced currents in rat hippocampal postsynaptic CA1 pyramidal neurons (which may be due to the L-type Ca\(^{2+}\) channel blockade and a subsequent reduction of Ca\(^{2+}\) influx) and had antioxidant action that ameliorated ischemic brain injury [17]. These findings support the use of Res in the treatment of diseases induced by Glu-mediated excitotoxicity in the future.

**Res-Mediated Neuroprotective Effects Against Glu-Induced Excitotoxicity**

Res inhibits the increase of extracellular Glu levels induced by stretch injuries by enhancing the Glu transporter, thus increasing Glu uptake, glutathione content, glutamine synthetase activity and S 100B (a neurotrophic cytokine) secretion in cortical astrocyte cultures and C6 glioma cells [14, 18, 19]. Moreover, Res could directly inhibit Glu release in rat cerebrocortical nerve terminals by decreasing mitogen-activated protein kinase activation and, subsequently suppressing voltage-dependent Ca\(^{2+}\) channel activity [20]. The key reason for Glu-induced excitotoxicity is that Glu markedly activate Glu receptors, and increase the concentration of intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]i) in neurons, and thus finally induce neuronal death. The major ionotropic receptors activated by Glu are commonly referred to as NMDA, AMPA and KA receptors.

**Res-Mediated Neuroprotective Effects Against Glu-Induced Excitotoxicity via NMDA Receptors**

Glu-induced excitotoxicity is closely related to the activation of NMDA receptors [21, 22]. The NMDA-mediated entry of excessive Ca\(^{2+}\) into the cytosol activates calcineurin, which induces apoptosis in both rat hippocampal neurons and glioblastoma cells [23, 24] via voltage-dependent Ca\(^{2+}\) channels and NMDA receptor-operated channels [25]. Therefore, current efforts to develop an effective therapy for hypoxic-ischemic neuronal injury are appropriately focused on NMDA antagonists and Ca\(^{2+}\) blockers [7]. The uncompetitive NMDA receptor antagonist memantine, is able to protect dissociated cortical neurons from Glu-induced excitotoxicity [26]. Res attenuates oxygen-glucose deprivation-induced neuron impairment and death in acute rat hippocampal slices by enhancing the activation of the large-conductance potassium channel and reducing the enhanced AMPA/NMDA receptor-mediated neuronal excitatory postsynaptic currents caused by oxygen-glucose deprivation [27]. Furthermore, some studies support that trans-Res inhibits the postsynaptic Glu receptors in hippocampal neurons, with NMDA receptors being more sensitive than AMPA receptors [17], which probably have an antioxidant effect that ameliorates ischemic brain injury. Res also inhibited NMDA-induced neuronal death, intracellular calcium increase and reactive oxygen species generation in rat cortical neurons, confirming the neuroprotective effect of Res [11, 28]. However, other studies have shown that pretreatment with Res did not alter the magnitude of the hypothalamus-pituitary-adrenal axis response to NMDA [29]. Furthermore, some studies have shown that treating neurons with Res did not protect them from Glu/NMDA-induced depletion of nicotinamide adenine dinucleotide (NAD\(^{+}\)), an important energy substrate and cofactor in multiple metabolic reactions, and death [30]. These findings suggest that Res protection
against Glu-induced excitotoxicity via NMDA receptors is still controversial, and further studies should be undertaken.

**Res-Mediated Neuroprotective Effect Against Glu-Induced Excitotoxicity via AMPA Receptors**

Res has been shown to reduce p 38 mitogen-activated protein kinase (MAPK) activation in cultured astrocytes following a stretch injury; the AMPA receptor is involved in p 38 MAPK activation following an injury [18]. Res also markedly reduced the amplitude and frequency of AMPA-mediated spontaneous excitatory postsynaptic currents in rat hippocampal pyramidal neurons [27]. Furthermore, trans-Res significantly suppressed Glu-induced currents in rat hippocampal postsynaptic CA1 pyramidal neurons, and NMDA and KA receptors were more sensitive than AMPA receptors to trans-Res [17].

**Res-Mediated Neuroprotective Effect Against Glu-Induced Excitotoxicity via KA Receptors**

Seizures induced by KA produced a marked increase in the free radical nitric oxide, causing oxidative stress and leading to depletion of energy stores [31]. Res or trans-Res has a neuroprotective effect against KA-induced convulsions and the attenuation of raised malondialdehyde levels, and markedly decreases spontaneous seizure frequency, suggesting that Res may be a potential anti-epilepsy agent, at least as an adjunct treatment in KA-induced epilepsy animal models [32, 33]. The chronic administration of Res significantly protects against KA-induced damage in the rat hippocampus and olfactory cortex, whereas the same treatment with Res does not play a protective role in an *ex vivo* hippocampal ischemia model [34]. Furthermore, KA-induced excitotoxicity has been implicated in increased oxidative stress. For example, KA-induced hippocampal neuronal damage and activation of astrocytes and microglial cells were significantly attenuated by treatment with Res, demonstrating that Res could act as free radical scavenger to protect against cell damage caused by excitotoxic insults [35, 36]. Furthermore, KA receptors have been reported to be involved in trans-Res-induced inhibition of Glu-induced currents in rat postsynaptic CA1 pyramidal neurons [17]. These studies suggest that Res may play an important role in protection from KA receptors-induced excitotoxicity.

**Discussion**

Generally, NMDA, AMPA and KA receptors can all be involved in Glu-induced excitotoxicity, separately or together, with varying sensitivity. For example, trans-Res significantly suppressed Glu-induced currents in rat postsynaptic CA1 pyramidal neurons, where KA and NMDA receptors were more sensitive to trans-Res than AMPA receptors [17]. Furthermore, Res induces opposite effects not only in different positions, but also at low vs high doses, indicative of the hormetic dose response [37]. For example, at a low concentration (25 mM), Res protected neurons from being killed by Glu and NMDA, whereas high concentrations of Res either had no effect or exacerbated excitotoxic neuronal death [30]. Some studies have shown that capsaicin or Res significantly increases cell viability, and the co-treatment with capsaicin and Res has a more significant impact than capsaicin or Res alone. Furthermore, the reactive oxygen species generation and the sequent apoptotic neuronal death induced by Glu are also significantly decreased by the co-treatment with both capsaicin and Res, suggesting that co-treatment with capsaicin and Res enhances neuroprotection against Glu-induced toxicity in the mouse cortex [16]. These data suggest that Glu-induced excitotoxicity via Glu receptors are still controversial, needing further exploration.

Glu-induced excitotoxicity has been clearly attributed to a massive influx of Ca2+ through NMDA and non-NMDA channels [38]. NMDA receptor-mediated elevations in [Ca2+]i have been shown to induce “epilepsy” in cultured hippocampal neurons [39]. Res can significantly reduce epileptiform discharges induced by Glu and reverse the increased discharges induced by Bay K 8644, the selective L-type Ca2+ channel agonist, which strongly suggests that the inhibitory effects of Res might be due to a blockade of Ca2+ influx [40]. Other studies have reported that Res protects against Glu-independent neurotoxic effects and antagonizes the [Ca2+]i elevation produced by cerebrospinal fluid from rat brain cortical motoneurons suffering amyotrophic lateral sclerosis [11].
Furthermore, Res does not protect against Glu/NMDA-induced NAD+ depletion and death in cultured neurons [30]. These studies may indicate the potential for Res to be used as a pharmacological tool for the treatment of diseases induced by the Glu excitotoxicity. However, there are still some controversies that need further exploration in the future.

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References

Neuroprotective Effect of Resveratrol


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