Göschl *et al.*: *Intrinsic Activity*, 2023; **11**(Suppl.1):A2.20
doi:10.25006/IA.11.S1-A2.20
published online: 22 September 2023

INTRINSIC ACTIVITY

27th Scientific Symposium of the Austrian Pharmacological Society

Vienna, 29–30 September 2023

MEETING ABSTRACT

A2.20

The role of glutamate metabolism in neuronal excitotoxicity

Vanessa GÖSCHL[1], Matej HOTKA[1], Stefan BOEHM[1], Andrey V. KOZLOV[2], Helmut KUBISTA[1,*]

[1]*Department of Neurophysiology and Neuropharmacology, Medical University of Vienna, Austria;* [2]*Ludwig Boltzmann Institute for Traumatology in the AUVA Research Center, Vienna, Austria*

Background: Glutamate excitotoxicity is a cell death mechanism triggered by accumulation of glutamate in the extracellular space. It is involved in a variety of brain pathologies including traumatic brain injury (TBI) and epilepsy. Hence, strategies leading to neuroprotection from the toxic effects of excess glutamate are needed. The TCA-cycle enzyme 2-oxoglutarate dehydrogenase complex (OGDHC) acts as a branching point enabling both glutamate synthesis and its consumption, and may thus provide a promising target for neuroprotection. OGDHC activity can be modulated experimentally by succinyl phosphonate (SP; inhibitor) and thiamine (TH; co-factor promoting OGDHC activity). Hence, we used these compounds to address the potential role of OGDHC activity in glutamate excitotoxicity.

Methods: We performed propidium iodide-based viability assays on primary hippocampal neurons (co-cultured with glial cells) exposed to excitotoxic stimulation (exposure to Mg^{2+}-free solution). Before this, neurons were pre-incubated for 36–48 hours in presence of either 1 mM TH or 200 µM SP. The percentage of dead neurons was determined after 6 hours in Mg^{2+}-free solution. Furthermore, to gain insight into possible mechanisms of action, the glutamate content of synaptic vesicles was determined via sucrose-shock release using whole-cell patch-clamp electrophysiology. Perforated patch-clamp recordings were performed to test the neuronal response to glutamate as well as to probe NMDA receptor-mediated and AMPA receptor-mediated membrane currents. Additionally, viability was also assayed in SP- and TH-pretreated neurons after exposure to exogenously applied glutamate (30 µM, 1.5 hours).

Results: Viability assays performed after induction of excitotoxicity in Mg^{2+}-free solution indicated that promotion of OGDHC activity by 1 mM TH had a neuroprotective effect, while its inhibition with 200 µM SP enhanced cell death. However, glutamatergic current induced in neurons by sucrose shock was increased by TH and decreased by SP. Electrophysiological experiments demonstrated altered voltage responses to exogenously applied glutamate, which were augmented in SP-treated neurons and reduced in TH-treated neurons. In line with this observation, the neuroprotective effect of TH and the neurotoxic effect of SP could also be demonstrated when application of glutamate was used as the excitotoxic stimulus. Measurement of glutamate receptor currents using increasing concentrations of AMPA and NMDA provided evidence of changes of NDMA receptor-mediated signaling (*i.e.* potentiation by SP and reduction by TH), whereas AMPA receptor mediated signaling remained unaltered.

Discussion: Our data demonstrate that interfering with OGDHC activity alters glutamate-dependent excitotoxicity. The decrease by TH or increase by SP of excitotoxicity was not matched by corresponding changes of the vesicular content of glutamate. Hence, these effects could not be explained by synaptic release. Instead, we identified changes in NMDA-receptor signaling that we currently interpret as secondary (possibly mGlu-receptor-dependent) effects arising in the course of altered metabolism of glutamate.

Acknowledgements: This work was funded by the Austrian Science Fund FWF (grants P33799 and P36145).

Keywords: 2-oxoglutarate dehydrogenase complex (OGDHC) – glutamate – excitotoxicity

*Corresponding author e-mail: *helmut.kubista@meduniwien.ac.at*