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PERSPECTIVES

Acid glia provide a synaptic boost

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Astrocytes are intimately involved in synaptic transmission in the brain. Not only do they control glutamate signalling by the action of astrocyte-expressed glutamate transporters, they also release gliotransmitters such as ATP, adenosine and glutamate. These can act pre- or postsynaptically to affect short- and long-term synaptic modulation and plasticity (Santello *et al.* 2019). As the main brain neuro-transmitter, much focus has been on glutamate as a gliotransmitter. Notably, in brain areas associated with learning and memory such as the hippocampus and neocortex, this has led to the discovery of central roles for glutamate gliotransmitter in synaptic long-term potentiation and depression by its targeting of neuronal NMDA receptors. Despite this focus, there is much debate on the mechanism of glutamate release with studies presenting evidence for vesicular and channel-mediated release.

Now Beppu *et al.* (2021) describe a novel mechanism by which gliotransmitter glutamate release from Bergmann glia, a specialised type of astrocyte in the cerebellum boosts postsynaptic glutamate-mediated current. Recording from Purkinje neurons in the molecular layer of the cerebellum and stimulating parallel fibres that release glutamate to induce an AMPA receptor-mediated postsynaptic current, they found that optogenetic activation of ArchT expressed in Bergmann glia reduced the postsynaptic AMPA current, indicating that the glia were releasing glutamate during synaptic transmission.

To determine the mechanism, the authors used a combination of Glial and neuronal patch-clamp recordings, optogenetic stimulation and localised agonist uncaging.

Local glutamate application in the molecular layer elicited an inward, mostly AMPAR-mediated current in neurons as expected. Activation of ArchT in glia before glutamate activation led to a decrease in the neuron AMPAR response indicating that the AMPAR neuronal component was composed of a direct neuronal effect and a secondary effect mediated by glutamate release from glia. However, when specific agonist AMPA was locally applied in the same way, there was no additional glial-derived component. In contrast, D-aspartate application which is not an AMPA agonist resulted in neuronal inward current that was mediated via AMPA receptors. This nicely illustrates that glutamate is acting on different cellular targets; AMPA receptors in neurons, non-AMPA D-aspartate target in glia.

ArchT is a light-activated H^+/Na^+ exchanger that results in intracellular alkalisation (Beppu *et al.* 2014). Its effect when expressed in the Bergmann glia therefore implicated a pH change as a trigger for glutamate release. This was supported by the use of Channelrhodopsin-2 (ChR2), which upon light activation permeates H^+ into the glial cell leading to acidification. As would be predicted, glial ChR2 activation enhanced neuronal recorded glutamate current.

D-Aspartate is a substrate for glutamate uptake transporters and also an NMDA receptor agonist. Blocking NMDA receptors did not affect glial glutamate release; the authors therefore focused their efforts on a glutamate transporter mechanism. To aid precise temporal control of stimulation, D-aspartate uncaging was used. Recording from glia while uncaging D-aspartate resulted in an inward current. Glutamate transporters, in addition to mediating glutamate uptake, also comprise an anion channel component. Indeed, the glial D-aspartate current was partially inhibited by the transporter inhibitor TBOA, the remainder blocked by the broad spectrum anion channel inhibitor DIDS. Because of the pH link, the authors tested for HCO_3^- as a permeating anion candidate. Omitting extracellular HCO_3^- in order to deplete intracellular HCO_3^-, also abrogated the glial D-aspartate-induced current, suggesting HCO_3^- efflux as a mechanism for intracellular acidification.

To identify the mechanism of glutamate release from the Bergmann glia, they again used caged D-aspartate and direct ChR2 activation. It was found that glial-derived glutamate current in neurons was reduced by the volume-regulated anion channel (VRAC) antagonist DCPIB, while the D-aspartate-induced current in glia was not. This reveals that both the triggering, and the release of glutamate from glia are therefore dependent on anion channels, which are, however, different, and can be distinguished pharmacologically.

A critical question of course relates to the physiological role of this glial glutamate release. In synaptic stimulation experiments the glial-derived-glutamate component became apparent after a number of stimuli during high-frequency bursts. Importantly, the synaptic stimulation also revealed that glial glutamate activated neuronal metabotropic glutamate receptors to a greater extent than AMPA receptors. These receptors and the synaptic burst pattern used are known to induce long-term depression at this synapse. It therefore seems plausible that as for astrocytes in other brain areas, a Bergmann glial role may be to release glutamate to control plasticity in the cerebellum.

Research into astrocyte glutamate release often focuses on calcium dependence although channel-mediated mechanisms such as swelling-activated VRAC are now coming into focus (Yang *et al.* 2019). The novel glutamate transporter and pH-dependent mechanism described in the study by Beppu *et al.* (2021) extends our knowledge of glial glutamate release and presents the field with further questions: Does the mechanism exist in other brain areas apart from the cerebellum? Is the VRAC channel-gated directly by pH or via a secondary cell volume mechanism? What is the relationship between homeostatic HCO_3^- and pH regulation (Theparambil *et al.* 2017) and glutamate release?

Answering these questions will be important in understanding the underlying physiology and the perturbations leading to pathophysiology. Indeed, previous work from this group has shown that the pH-dependent release of glutamate is induced in oxygen glucose deprivation in an

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ischaemic stroke model (Beppu *et al*. 2014). In this respect is also important to confirm the identity of the glutamate-release anion channel. This may enable the development of specific antagonists as tools and to aid potential therapeutic developments.

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Additional information

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anion channel, astrocyte, gliotransmitter, glutamate transporter