Neurons eat glutamate to stay alive

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Neurons are thought to primarily rely on glucose to fuel mitochondrial metabolism. In this issue, Divakaruni et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201612067) show that neurons are also happy to use glutamate. When neurons use this neurotransmitter, its concentration drops, thus protecting against glutamate-induced excitotoxic stress.

Glutamate is the most abundant excitatory neurotransmitter, but too much of it causes toxicity by overactivating postsynaptic receptors. When postsynaptic receptors are activated, the post-synaptic cytoplasmic calcium concentration rises, activating proteases, lipases, and endonucleases, causing cellular damage and cell death. Given the broad implications of this process in numerous neurodegenerative diseases and stroke, considerable effort has focused on devising strategies to remove glutamate from the synaptic cleft, to block receptor activation, or to limit the rise of intracellular calcium. In a series of exciting experiments using in vitro cell culture and acute hippocampal slices, Divakaruni et al. not only show that neurons are capable of switching to a glutamate-fueled mitochondrial metabolism but also that when they do so, the concentration of this amino acid is lowered sufficiently such that excitotoxic stress is avoided.

Classical "textbook" neuronal energy production involves the oxidation of glucose: pyruvate produced from glucose enters mitochondria to fuel an oxidative TCA cycle. Unlike neurons, it is well established that nonneuronal cells adapt to nutrient availability and switch from using glucose to using alternative nutrients, including amino acids (Elia and Fendt, 2016). However, neurons were thought to depend on glucose to fuel energy production (Bélanger et al., 2011) and to use glutamate as a neurotransmitter. The doctrine is that there is a strict division of labor (Fig. 1 A). Neuronal terminals produce glutamate from glutamine that enters neurons from the surrounding glial cells that soak up excess glutamate from the synaptic cleft. In nonneuronal cells, both glutamate and glutamine can be readily oxidized to produce energy, but is this not so in neurons?

Divakaruni et al. (2017) revisited the dogma that neurons depend on glucose to fuel their mitochondrial metabolism by performing ^{13}C tracer analyses. This methodology allows determining the fate of ^{13}C-labeled nutrients by following the labeled carbons through the metabolic network. Strikingly, they found that, even in glucose-rich conditions, neurons use alternative nutrients for mitochondrial energy production, such as the amino acid leucine and β-hydroxybutyrate. Having established that neurons are able to use alternative nutrients in their mitochondrial metabolism, Divakaruni et al. (2017) next asked how neurons respond when inhibiting the entry of pyruvate into the mitochondria, thus largely precluding the use of glucose. Pyruvate is the major downstream product of glucose and is transported into the mitochondria via the mitochondrial pyruvate carrier (MPC). Blocking this transporter excludes glucose and any other glycolytic carbon source, such as lactate, as a nutrient to fuel mitochondrial metabolism (Vacanti et al., 2014). The results were surprising, because inhibiting the MPC did not affect mitochondrial energy production and neurons seemed to seamlessly switch to glutamate oxidation as an alternative to glucose (Fig. 1 B). Although the data are compelling in neurons in culture, the effects in rat brain slices were less pronounced. However, as Divakaruni et al. (2017) explain, many other cell types may be masking the effects. Nonetheless, it would also be interesting to test such metabolic flexibility in vivo by infusing ^{13}C-labeled glutamate to determine its in vivo use and by isolating specific neuronal cell types from an intact brain. Moreover, it would be interesting to investigate what other nutrients beyond the ones discovered by Divakaruni et al. (2017) can sustain the mitochondrial metabolism of neurons.

Another interesting issue that Divakaruni et al. (2017) touch upon is the advantage for neurons to switch specifically to glutamate rather than increase their use of leucine or β-hydroxybutyrate. The latter two substrates were already known as substrates for energy production in neurons, but, unlike pyruvate that refills mitochondrial metabolites via pyruvate carboxylase, the use of leucine or β-hydroxybutyrate only allows sustaining of energy production (Hassel and Bråthe, 2000). In contrast, glutamate, similar to pyruvate, allows for both energy production and the refilling of mitochondrial metabolites. Thus, when neurons switch to using glutamate, they are able to produce energy as well as maintain the production of mitochondrial metabolites. Divakaruni et al. (2017) reasoned that if glutamate is oxidized, less glutamate would be available for neurotransmitter release. In line with this idea, when the MPC was inhibited, less glutamate was indeed released upon neuronal stimulation. Using isotope tracing, the researchers were also able to show that this released glutamate had an altered composition that was consistent with MPC inhibition and glutamate/glutamine oxidation. Less glutamate release could be protective to excitotoxic stress that is elicited when glutamate activates postsynaptic receptors. Consistent with this model, when Divakaruni et al. (2017) simulated neurons in which pyruvate entry in mitochondria was blocked, they observed less cell death than in
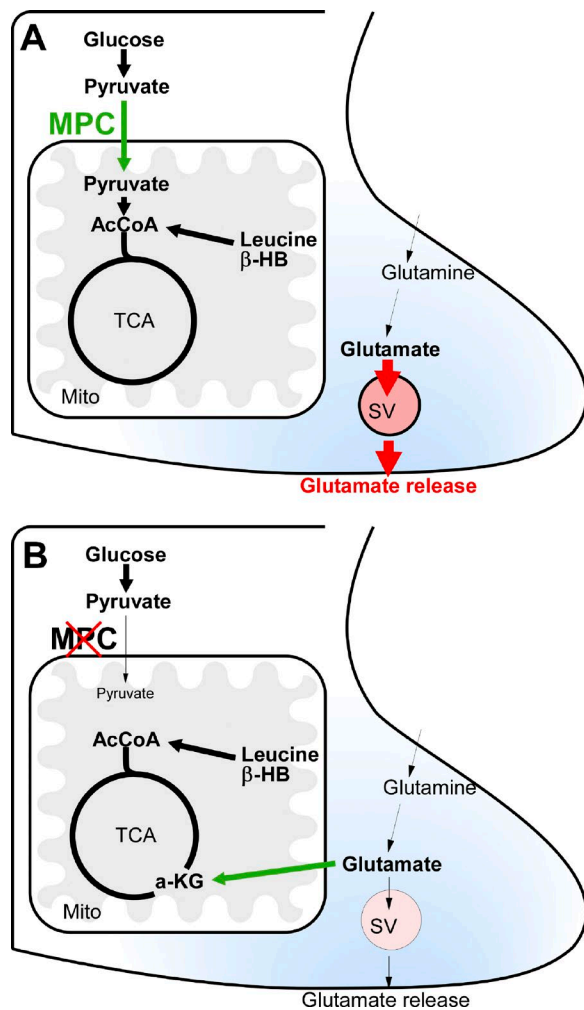
Figure 1. **Glutamate balances between serving as a neurotransmitter and fueling neuronal mitochondrial metabolism.** (A) Synaptic terminal depicting mitochondrial metabolism (Mito) and glutamatergic neurotransmission from synaptic vesicles (SV). Pyruvate enters mitochondria using the MPC and is used in the TCA to produce energy and mitochondrial metabolites. Leucine and β-hydroxybutyrate (β-HB) are also used but only for energy production. Most glutamate is used for transmitter release and high glutamate concentrations may trigger excitotoxicity. (B) With MPC inhibited, pyruvate cannot enter mitochondria. Under these conditions, glutamate fuels the TCA cycle to produce energy and mitochondrial metabolites. Less glutamate is available to neurotransmission, preventing excitotoxicity. aKG, α-ketoglutarate.

stimulated neurons in which the MPC was not inhibited. From a clinical perspective, it will be interesting to test if blocking pyruvate entry in mitochondria in vivo is also protective in disease models characterized by excitotoxic stress.

Neurons are extremely compartmentalized and cell bodies are most often located at considerable distances from the presynaptic terminals. This is interesting because glutamate is released specifically from presynaptic terminals. Given that metabolic switching protects against glutamate excitotoxicity, likely because it lowers the glutamate concentration, this process must therefore at least be active within presynaptic terminals as well. The experiments by Divakaruni et al. (2017) do not yet make the distinction, but an exciting future topic of research will be to decipher whether there are differences in metabolic fitness in the neuronal cell body versus axons or synapses. Interesting in this context are previous findings that presynaptic function

during intense stimulation requires glucose-dependent glycolysis and the concomitant deployment of glucose transporters to synapses (Rangaraju et al., 2014; Ashrafi et al., 2017). Moreover, glycolytic enzymes associate with synaptic vesicles (Ikemoto et al., 2003), and, in *Caenorhabditis elegans*, glycolytic enzymes appear to relocalize during activity to form an ad hoc compartment, a "glycolytic metabolon" (Jang et al., 2016). These findings suggest that synaptic compartments can regulate and need glycolysis in response to neuronal activity. One possibility is that neurons need glycolysis during acute bouts of stimulation, but metabolic switching may serve the chronic need for sustained energy and metabolite supply. Additional electrophysiological studies in the context of the described metabolic switch and in the context of the need for glycolysis as well as further studies on the compartment specificity of these processes are needed. Ultimately, such studies will aid in understanding how these metabolic processes each contribute to the energy and metabolite demands of the synapse and the synaptic vesicle cycle.

There is considerable evidence that neuronal activity promotes glycolysis and mitochondrial function. It will be interesting to determine whether neurons—similar to cancer cells (Christen et al., 2016)—use metabolic regulation, which is a passive adaptation mechanism, to switch to other carbon substrates or whether they possess mechanisms that control metabolic switching to nonglucose substrates. If they do, what are the triggers? Understanding such pathways and regulatory mechanisms will be important because they could potentially be targeted to lower the effects of excitotoxic stress that is implicated in numerous neuronal diseases. At least in the context of refractory forms of epilepsy, diets low in carbohydrates appear to be beneficial (Giménez-Cassina et al., 2012). Such observations are consistent with the idea that if neurons switch from using glucose to alternative carbon sources like glutamate, this is protective, but definitive proof is lacking. It is nonetheless clear that changes in metabolism in neuronal clusters in the context of disease have an effect on network activity and likely on neuronal survival as well. Further refinement in measuring the metabolic state of specific and defined neuronal subclasses in the brain as well as compartment-specific effects will undoubtedly yield even greater insight into disease mechanisms and the ways to tackle them.

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