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CROSSTALK

CrossTalk opposing view: lack of evidence supporting an astrocyte-to-neuron lactate shuttle coupling neuronal activity to glucose utilisation in the brain

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In 1993, Dringen *et al.* concluded that 'glycogen in astrocytes can be considered as a store for lactate rather than for glucose', and suggested that lactate derived from the breakdown of glycogen in astrocytes may serve the energetic needs of neighbouring cells. The following year, Pellerin and Magistretti (1994) published their now famed astrocyte-to-neuron lactate shuttle hypothesis in which the transfer of lactate from astrocytes to neurons, in this case derived from extracellular glucose rather than glycogen, is coupled to uptake of neurotransmitter glutamate (i.e. neuronal activity). According to this hypothesis, glycolysis and lactate production are astrocytic phenomena while oxidative metabolism of lactate takes place in neurons. The astrocyte-to-neuron lactate shuttle hypothesis as proposed by Pellerin and Magistretti (1994) has gained widespread acceptance, and its popularity is not surprising due to its conceptually simple and compelling idea of an activity-based coupling between neuronal synaptic activity and astrocyte metabolism. We will argue that the biochemical and physiological evidence for the existence of a unidirectional flow of lactate from astrocytes to neurons, as proposed, is lacking. However, before we get to that, let us briefly explore why this subject is even interesting to physiologists.

Why is this issue worth a CrossTalk debate?

First of all, the extensive acceptance of the astrocyte-to-neuron lactate shutte means that many researchers use this hypothesis as a master template on which they interpret their data, thus ignoring alternative explanations and hence creating a bias in the literature. Besides this, and of course the pure scientific desire to know how the brain operates, the cellular site of glucose metabolism in the brain is important for interpreting fluorodeoxyglucose (FDG) positron emission tomography (PET) results, a method extensively used for both research and diagnostic purposes. In the following, we will focus on two key issues that we believe severely contest the existence of a lactate shuttle from astrocytes to neurons, as proposed: (1) neurons express glucose transporters and metabolise glucose in an activity-dependent manner; and (2) the distinct cellular isoform expression of lactate transporters and lactate dehydrogenase, the enzyme forming lactate from pyruvate, cannot be employed as an argument for a directional flow of lactate.

Neurons metabolise glucose in an activity-dependent manner suggesting that glucose is an important neuronal energy substrate during activation

In support of the lactate shuttle hypothesis, it has been proposed that neurons do not metabolise glucose in an activity-dependent manner and that lactate is their preferred substrate (e.g. Bouzier-Sore *et al.* 2003). Contesting this view, we know that neurons express transport systems for glucose both *in vitro* and *in situ* (Simpson *et al.* 2007), and others and we have repeatedly shown that both cultured neurons and synaptosomes (an *ex vivo* preparation of presynaptic neuronal terminals typically obtained from rodent brain) avidly take up and metabolise glucose in an activity-dependent manner (e.g. Bak *et al.* 2006; Patel *et al.* 2014). Further, Lundgaard *et al.* (2015) and Diaz-Garcia *et al.* (2017), employing a near-infrared 2-deoxyglucose probe and redox biosensors, respectively, showed that glucose is metabolised by neurons in an activity-dependent manner *in situ* in awake mice. As discussed further below there are no good biochemical reasons why neurons should primarily consume lactate during activation; indeed, neurons *in vitro* are, not surprisingly, able to *produce* lactate upon activation and thus neurons may contribute to the extracellular surge in lactate associated with brain activation (Prichard *et al.* 1991; Hu & Wilson, 1997; Bak *et al.* 2009; Contreras & Satrustegui, 2009). To be fair, it is important to note that neurons do metabolise lactate if present (Bak *et al.* 2009), and in the words of a long-time opponent of the lactate shuttle hypothesis, Gerry Dienel, lactate is an 'opportunistic' substrate that, if present, indeed will serve to support energy metabolism (Dienel, 2012).

Distinct isoform expression of lactate transporters and lactate dehydrogenase in neurons and astrocytes does not predict directionality of any shuttling of lactate

Monocarboxylate transport (MCT) systems, facilitative transporters allowing lactate or pyruvate to cross the plasma membrane, are present on both neurons and astrocytes and they differ in their kinetic profiles, i.e. their transport capacity and binding affinities for lactate (Simpson *et al.* 2007). These differences have been

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employed as arguments for a unidirectional flow of lactate from astrocytes to neurons (e.g. Bittar *et al.* 1996). However, regardless of the kinetic parameters of a facilitative transport system, the flow of substrate in either direction is governed by the prevailing intra- to extracellular concentration gradient, i.e. in this case the production *vs.* disappearance or consumption of lactate. Thus, an extensive, activity-dependent astrocyte-to-neuron gradient of lactate needs to be established for the lactate shuttle to work as suggested. Mächler *et al.* (2016) recently investigated this and we will get to that shortly. First, the preferential synthesis of lactate in astrocytes and consumption in neurons have been argued to be possible due to distinct cellular expression of isozymes of lactate dehydrogenase (LDH) having dissimilar kinetic parameters, e.g. in terms of binding constants for lactate (e.g. Laughton *et al.* 2000). However, regardless of their kinetic parameters, enzymes influence the speed at which the thermodynamic equilibrium is obtained but do not change the equilibrium of a chemical reaction. Thus, a distinct cellular distribution of LDH isozymes with different kinetic parameters does not predict which cells are producing and which are consuming lactate (please see Bak & Schousboe, 2017 for a detailed discussion). Further, Quistorff & Grunnet (2011*a*, *b*) argue that the differences in kinetic parameters determined for LDH at room temperature are not present at body temperature. Thus, the distinct kinetic parameters of LDH employed as an argument in favour of the shuttle may not be real. So, how can an extensive lactate gradient be formed? The only way that is possible is if astrocytes are relentlessly outpacing neurons in terms of glycolytic flux and lactate production during activation. As alluded to above, Mächler *et al.* have investigated if there is such a lactate gradient between astrocytes and neurons. Employing anaesthetised mice expressing a lactate biosensor specifically in neurons or astrocytes they show that both neurons and astrocytes take up lactate when present in the blood in excessive amounts consistent with the concept of lactate being an 'opportunistic' substrate. By measuring the rate of biosensor saturation in the presence of ammonium chloride to inhibit mitochondrial ATP production, and thus boost glycolysis and lactate production, they estimate that neurons have a lower baseline level of lactate than do astrocytes. While this an interesting observation, it does not tell us if astrocytes outpace neurons in lactate production during activation.

Final thoughts

In our minds, the current literature and the biochemical design of neurons and astrocytes are largely consistent with a situation according to which both neurons and astrocytes contribute to the surge in extracellular lactate during brain activation; either cell type may then consume lactate when available or the lactate may be dispersed and metabolised elsewhere or even leave the brain (Madsen *et al.* 1999; Hertz *et al.* 2014; Satrustegui & Bak, 2015). The cellular location and timing of lactate synthesis and consumption in the brain in health and disease largely remains an open question that deserves to be investigated with an open mind.

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Additional information

Competing interests

None declared.

Author contributions

Both authors have contributed to the conception or design of the work and drafting the work or revising it critically for important intellectual content. Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Supporting information

The following supporting information is available in the online version of this article.

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