A commentary on

Brain slice studies in the Research Topic “The link between brain energy homeostasis and neuronal activity”

In “The link between brain energy homeostasis and neuronal activity” two papers discuss the importance of optimum energy metabolism for neuronal spike activity in brain slices incubated in glucose-containing media, with one demonstrating benefits of lactate supplementation. A third demonstrates effects of succinate and γ-hydroxybutyrate on ATP-mediated [Ca²⁺]i gradients in astrocytes, and a fourth discusses whether lactate is the glycolytic end product and exerts neuroprotection. This commentary discusses the quantitative importance of oxidative metabolism in astrocytes, importance of their [Ca²⁺]i, and role(s) of lactate.

Metabolic brain slice studies were initiated by Warburg et al. (1924). During the 1930s several such studies showed lactate release to incubation media and stimulation of respiration by high K⁺ concentrations, initially by ~65% (Ashford and Dixon, 1935; Dickens and Greville, 1935). Electrical stimulation acted similarly (McIlwain, 1951, 1955). Glutamate caused neuronal depolarization (Gibson and McIlwain, 1965), and slices displayed synaptic activity (Yamamoto and McIlwain, 1966). Hertz and Schou (1962) and Weiss et al. (1972), using Warburg equipment with rapidly oscillating tissue chambers or an oxygen electrode inserted into intensely aerated flasks, reported O₂ uptake rates similar to Ivanov and Zilberter’s (2011) and Ca²⁺-dependence and procaine-inhibition of the K⁺-mediated stimulation. The center and both surfaces of slices showed marked cell swelling under all conditions, but especially at high extra-cellular K⁺ concentrations (Møller et al., 1974). Elevated K⁺ increased (Franck, 1970; Lund-Andersen and Hertz, 1970), and electrical stimulation decreased (Cummins and McIlwain, 1961) intracellular K⁺ content. Electrical pulses evoked transition from a more oxidized to a more reduced phase in NAD(P)H and cytochromes, blockable by tetrodotoxin, whereas elevated extracellular K⁺ caused a more oxidized redox state (Cummins and Bull, 1971; Galeffi et al., 2011). In ¹³C-NMR studies, using labeled glucose and the astrocyte-specific substrate acetate, Badar-Goffer et al. (1992) concluded that the high K⁺-mediated increase in O₂ consumption occurred in glial cells. This may reflect a normally occurring active astrocytic uptake of K⁺ released from neurons (Somjen et al., 2008; Hertz, 2011) and depolarization-induced increase in [Ca²⁺]i, stimulating astrocytic metabolism. Electrical stimulation of brain slices also increase astrocytic [Ca²⁺], (Filosa et al., 2004).

Recently, several groups have measured tricarboxylic acid (TCA) cycle activity in the living, functioning brain in humans and rats using ¹³C-NMR (reviewed by Hertz, 2011) and tabulated in Table 1. In awake rats total pyruvate fluxes after glycolytic conversion of glucose to pyruvate followed by pyruvate dehydrogenase (PDH-) mediated entry into the TCA cycle (in both neurons and astrocytes) together with flux mediated by the astrocyte-specific pyruvate carboxylase (PC) amount to ~1.67 μmol/min/g wet wt (Öz et al., 2004; Table 1). With a pyruvate/O₂ ratio of 3.0, this equals 300 μmol of O₂/h/g wet wt, close to the upper limit cited by Ivanov and Zilberter (2011). As noted by them, ourselves, and Okada and Lipton (2007), this rate is substantially higher than that of oxygen uptake in brain slices. However, under anesthesia in vivo, respiration becomes more comparable to that in brain slices (see Choi et al., 2002; Table 1). Thus, the enhanced rates of oxygen consumption in slices during neuronal stimulation shown by Ivanov and Zilberter (2011), discussed by Kann (2011), and quantitated by Galeffi et al. (2011), are functionally the most meaningful. Moreover, determinations of average metabolic rates in neurons (PDHn) and astrocytes (PFHg + PC) separately (lower two lines of Table 1) shows that astrocytic O₂ consumption equals one quarter of total brain energy metabolism in vivo, indicating that per volume astrocytes consume O₂ at least at the same rate as neurons. Additional ¹³C-NMR studies in brain slices during different types of neuronal activation would be useful to evaluate neuronal and astrocytic responses.

Astrocytes are the topic of the non-metabolic study by Molnár et al. (2011). It describes astrocytic [Ca²⁺], responses to ATP and modulation of a subset of astrocytic ATP receptors by succinate and γ-aminobutyrate. Besides illustrating the high density of functioning ATP receptors, even in the young astrocytes studied, and the localization of the succinate-affected receptors to vascular-associated astrocyte processes, the study emphasizes important effects of succinate beyond its role as a TCA cycle constituent. Succinate is present in serum and its concentration is increased in diabetes, which may be of considerable importance in diabetic nephropathy (Deen and Robben, 2011), and raises the possibility of involvement of succinate and astrocytes in diabetic effects on the brain. The Molnar paper is also of interest in connection with that by Zilberter (2011), and it supports that the roles of astrocytes in brain metabolism may be underestimated in the Venkateswaran et al. (2012) paper.

Observations in brain slices by Takagaki and Tsukada (1957) that lactate sustains similar rates of oxygen consumption as glucose have been repeatedly confirmed. The Schurr and Gozal (2011) paper suggests important physiological (mitochondrial lactate oxidation) and pathological (neuroprotection) roles of lactate. However, most authors agree that lactate dehydrogenase activity in mitochondria is unlikely (Sahlin et al., 2002; Yoshida et al., 2007), and lactate cannot prevent anoxic depolarization in rat hippocampal slices, when gly-
Table 1 | Representative values for metabolic fluxes in human and rat brain in vivo.

| Reference                  | Species | PDH_n | PC  | % PC  | PDH_g | % PDH_g |
|----------------------------|---------|-------|-----|-------|-------|---------|
| Aureli et al. (1997)       | Rat*    |       |     |       |       |         |
| Cruz and Cerdán (1999)     | Rat*    | 1.0   |     | 10.0  |       |         |
| Shen et al. (1999)         | Human*  | 0.71  | 0.04| 4.9   | 0.06  | 7.4     |
| Gruetter et al. (2001)     | Human*  | 0.57  | 0.09| 12.5  | 0.06  | 8.3     |
| Blüml et al. (2002)        | Human*  | 0.70  |     | 13.6  | 0.13  | 15.7    |
| Lebon et al. (2002)        | Human*  | 0.80  |     | 2.5   | 0.14  | 16.7    |
| Choi et al. (2002)         | Rat*    | 0.41  | 0.04| 9.8   | 0.28  | 38      |
| Öz et al. (2004)           | Rat*    | 1.19  | 0.18| 10.8  | 0.30  | 18      |
| Xu et al. (2004)           | Rat*    |       |     | 0.15  |       |         |
| Patel et al. (2005)        | Rat*    | 0.62  | 0.06| 10.3  |       |         |
| Mason et al. (2007)        | Human*  |       |     | 0.02  |       |         |
| Deelchand et al. (2009)    | Rat*    |       |     |       | 0.49  |         |
| Patel et al. (2010)        | Rat*    |       |     |       | 0.37  |         |
| Lanz et al. (2010)         | Rat*    | 0.76  |     | 14.6  |       |         |
| Lanz et al. (2012)         | Rat*    |       |     | 14.6  |       |         |
| Average                    |         |       |     | 9.9 ± 1.0| 18.5 ± 3.6 |         |
| Average, awake brain       |         |       |     | 9.6 ± 1.6| 15.9 ± 3.2 |         |

Absolute values are μmol/min per gram wet wt. PDH_n, pyruvate dehydrogenase-mediated pyruvate flux in neurons (equals flux in neuronal TCA cycle); PDH_g, pyruvate carboxylase-mediated flux (astrocyte-specific);% PC, pyruvate carboxylase-mediated flux as a percentage of total measured pyruvate metabolism; % PDH_g, pyruvate dehydrogenase-mediated pyruvate flux in glial cells (astrocytes); % PDH_n, pyruvate dehydrogenase-mediated pyruvate flux in astrocytes as percent of total measured pyruvate metabolism. *Awake, unanesthetized subjects; **anesthetized subjects. Slightly modified from Hertz (2011) where more details can be found.

colysis is completely inhibited (Allen et al., 2005). Techniques used during preparation of slices are important for subsequent metabolic effects of glucose and lactate (Dienel and Hertz, 2005; Okada and Lipton, 2007; Dienel, 2011). Lactate serves as a partial substrate for brain metabolism during intense exercise (when its blood concentration is increased), but this does not indicate any need for lactate in addition to glucose in brain function, including ongoing activity, since during rest there is a small lactate exit from brain (Quistorff et al., 2008). Nevertheless, if serum lactate is increased, lactate is preferentially oxidized (van Hall et al., 2009). In brain slices the question is complex, because of simultaneous lactate release. Could simple replacement of this lactate restore optimum glucose metabolism? Can results in astrocyte cultures (Sotelo-Hitschfeld et al., 2012) be similarly explained?

In conclusion, a considerable part of oxidative glucose metabolism in brain is astrocytic, exogenous lactate is not a necessary brain fuel in vivo, and past history of metabolic brain slice experiments may inspire future studies.

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