Hypothesis Article

Lactate is the ultimate oxidative energy substrate in brain and elsewhere

Avital Schurr

Abstract

Now-a-days, the focus on lactate is due to its being an oxidative substrate for energy metabolism in brain (and other tissues), rather than a useless end product of anaerobic glycolysis. Mounting evidence indicates that lactate does play a major role in aerobic energy metabolism in the brain, the heart, skeletal muscle and possibly in any other tissue and organ. Nevertheless, this evidence has challenged the old concept of lactate being an anaerobic waste product and ignited a fierce debate between the supporters of glucose as the major oxidative energy substrate and those who support lactate as a possible alternative to glucose under certain conditions. While researchers working on energy metabolism in skeletal muscle have taken great strides toward bridging between these two extreme positions, accepting lactate role as an oxidative energy substrate, neuroscientists appear to be somewhat more emotional about their differences and less agreeable. In this paper I have employed findings from research on skeletal muscle along with the existing old and new data on cerebral energy metabolism, to postulate that lactate is the only major product of cerebral (and other tissues) glycolysis, whether aerobic or anaerobic, neuronal or astrocytic, under rest or during activation. Accordingly, lactate is a major, if not the only, substrate used by the mitochondrial tricarboxylic acid cycle. If proven true, this hypothesis should provide a better understanding of the biochemistry and physiology of (cerebral) energy metabolism and hold important implications where neuroimaging is concerned.

Keywords: Cerebral energy metabolism, glucose, glycolysis, lactate, tricarboxylic acid-cycle

Introduction

For many years, most scientists agreed that lactate is a useless end product of anaerobic energy metabolism, which at times can become harmful. These notions have manifested, among others, the concept that lactate accumulation in muscle tissue is responsible for muscle fatigue, a concept that has recently been refuted [1]. In the brain, lactate has been promoted as a major exacerbating factor of cerebral ischemic damage. Such bad reputation would explain why an important body of research on the oxidation of this monocarboxylate in the brain has all but ignored and especially why recent evidence that indicates lactate to be an important cerebral oxidative energy substrate has met with great skepticism. A hypothesis proposed by Magistretti and colleagues [2], known as the astrocyte-neuron lactate shuttle hypothesis (ANLSH), has added fuel to the ongoing debate about which of the two, glucose or lactate is the more important energy substrate during brain activation [3-6]. Recently, the ANLSH triggered another old controversy regarding the role of hypotheses in scientific research [7,8].

Regardless, the present paper puts forward a new hypothesis on the role lactate plays in cerebral (and other tissues) energy metabolism. In piecing together the hypothesis, I have used old and new studies that were conducted both in brain and other tissues. 'De facto,' there is much agreement among scientists today on the role of lactate in energy metabolism. The division, at least among neuroscientists, appears to focus mainly on whether or not lactate plays as important a role in cerebral oxidative

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energy metabolism as does glucose. If one is to accept that lactate is not a significant cerebral oxidative energy substrate then, any hypothesis which promotes lactate as such substrate, including the ANLSH, would be unacceptable. It is my belief that the debate over 'which is more important' has distracted many from realizing a more significant possibility, namely, that both glucose and lactate are integral and important entities of energy metabolism.

Historical considerations

Much of the current knowledge on the formation and fate of lactate has originated from early studies on muscular exercise [9]. The authors of those studies also coined the majority of the terminology associated with tissue lactate and are, eventually, responsible in large part for the conceptions and misconceptions about lactate. Thus, as late as 1958, lactate is described as the end-product of reduction in the LDH (lactate dehydrogenase) system, which has no other function in metabolism [10]. Moreover, LDH is described as unique in being dead-end system where endogenous lactate does not participate in any other reaction which would be affected by its accumulation in the cell [10].

However, many papers on brain energy metabolism that were published in the late 1920s and the early 1930s had been completely ignored over the years. Upon reviewing these papers, one must take into account the fact that at the time of their publication neither the mitochondrial tricarboxylic acid (TCA) cycle nor the glycolytic pathway had proposed. Nevertheless, the fact that the TCA-cycle was formulated in 1937 (Krebs and Johnson), three years prior to the formulation of the glycolytic pathway, could contribute, a priori, to the prevailing concepts about the roles of certain substrates and products of the glycolytic pathway (see below). The most active investigators in the area of nervous system metabolism during that early period were Holmes and Ashford. The two published several studies on the oxidation of glucose and especially lactate in brain tissue. In an early study they concluded that brain tissue possesses two mechanisms of lactate formation, one, involving glucose, which is quantitatively the more important and is independent of phosphate, the other, which is much smaller, involves glycogen and depends on the availability of phosphate [11]. Later, they published detailed studies on the oxidation of lactic acid in the brain [12,13]. In these studies they demonstrated that there is no synthesis of carbohydrate from the portion of lactic acid which disappears but is not accounted for by O_2 uptake. Moreover, they found that oxygen uptake in the presence of glucose was inhibited by NaF, while such uptake in the presence of lactic acid was insensitive to fluoride [14]. Thus, they were the first to demonstrate aerobic utilization of lactate in brain tissue i.e., a correlation between lactic acid disappearance and oxygen consumption. They also demonstrated that the respiratory quotients, both of brain tissue with and without lactate supplementation, are close to unity, even when animals from which the brain tissue was prepared were rendered hypoglycemic by insulin injection [13]. They also concluded that lactate oxidation is unlikely to spare the utilization of another substrate [13]. One may find this conclusion somewhat bewildering, if one is to assume that lactate utilization replaces that of glucose. However, *in vivo*, lactate originates from glucose and the oxidation of the former should drive the utilization of the latter, rather than sparing it. Sparing of glucose may occur only when lactate (or pyruvate) is supplied exogenously in high enough levels directly into the brain, bypassing the liver. In addition, these investigators were not equipped with the luxury of experimenting with two different substrates (glucose and lactate) concomitantly in order to directly assess whether or not the addition of lactate to their preparation could spare the utilization of glucose or vice versa. Thus, their conclusion should be looked upon mainly as an estimate.

Obviously, Holmes and Ashford were not aware of the, yet nonexistent, notion that under aerobic conditions glucose is supposed to be reduced to pyruvate, not to lactate. The concept of pyruvate as the end product of aerobic glycolysis was formulated with the introduction of the glycolytic pathway. The most plausible explanation for the decision of the pathway formulators to place pyruvate as its aerobic end product had to do with the introduction of the TCA-cycle three years earlier, where pyruvate was proposed to play a central role in the cycling formation of citrate from oxaloacetate and acetyl-SCoA [15]. Clearly, many biochemists of the period were already familiar with the TCA-cycle requirement for pyruvate as its key substrate. Glycolysis was, and still is, the best source for pyruvate in sufficient quantities. While there were isolated reports about lactate conversion to pyruvate [16], most studies had demonstrated the opposite reaction, namely, the production of lactate from glucose, fructose, hexosediphosphate or pyruvate [17-22].

Thus, more than 60 years after glycolysis was introduced, an event that launched the era of modern biochemistry, it is described in all text books as a pathway that begins with the phosphorylation of glucose and ends with the formation of lactate (reaction I and **Figure 1**).

I. Glucose+2ATP+4ADP+2Pi+2NAD$^+$ ⟶ 2lactate+2ADP+4ATP+2NAD$^+$

cated that lactate, produced during exercise, is taken up and metabolized by the brain.

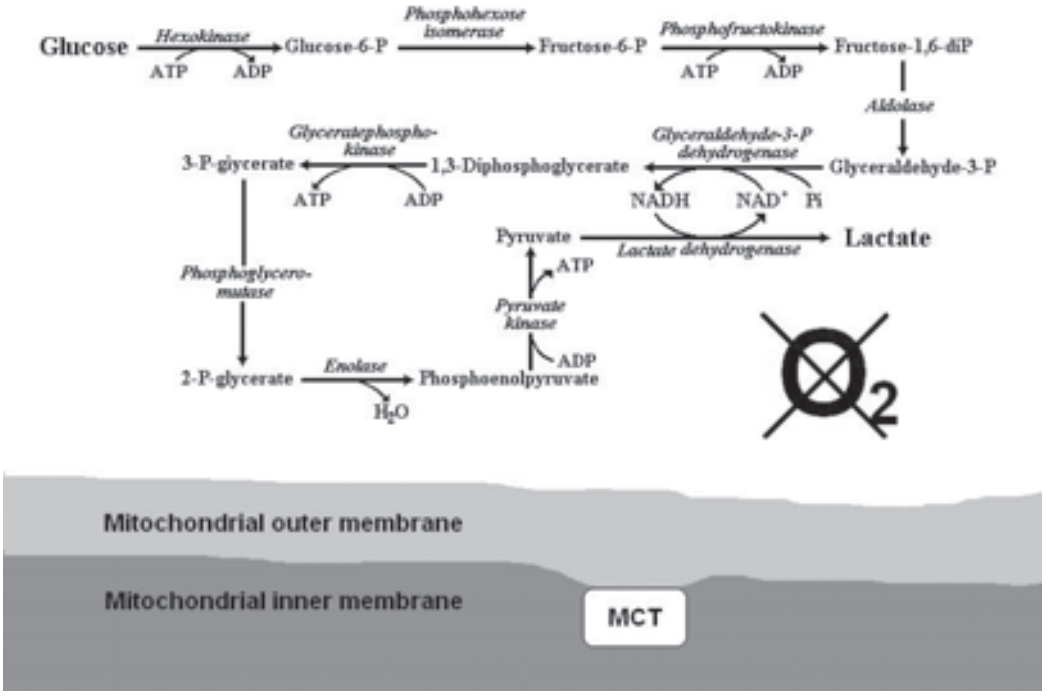


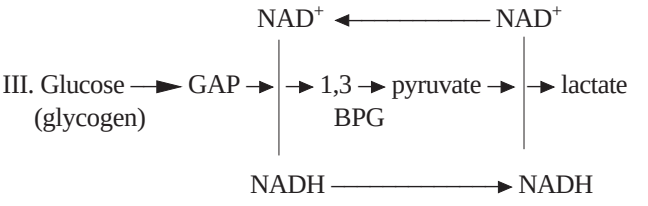
FIGURE 1. A SCHEMATIC ILLUSTRATION OF THE ANAEROBIC GLYCOLYTIC PATHWAY. AS DEPICTED IN MOST TEXT BOOKS, GLUCOSE IS THE SUBSTRATE OF GLYCOLYSIS, WHICH THROUGH A SERIES OF ENZYMATIC REACTIONS, IS CONVERTED TO LACTATE. THIS SERIES OF REACTIONS REQUIRES THE INVESTMENT OF TWO MOLES OF ATP FOR EACH MOLE OF GLUCOSE AND PRODUCES TWO MOLES OF LACTATE AND FOUR MOLES OF ATP.

This description changes when oxygen is present, in which case glycolysis stops short of its last enzymatic step, namely, lactate dehydrogenase (LDH), ending with pyruvate, the hallmark substrate of the mitochondrial TCA-cycle (reaction II and **Figure 2**).

II. Glucose+2ATP+4ADP+2Pi+2NAD$^+$ ⟶ 2pyruvate+2ADP+4ATP+2NADH

While exceptions to the aerobic sequence have been documented, where glycolysis proceeds to form lactate, such as in red blood cells, heart muscle and retina, the prevailing notion is that, under ample oxygen supplies, pyruvate, not lactate, is the final product of aerobic glycolysis. Nevertheless, over the past 50 years, evidence has been accumulated, which indicates lactate to be a suitable aerobic energy substrate for brain tissue [23-43]. This evidence should have raised doubts in regard to the notion that lactate is a dead-end product. Moreover, a recent human study by Dalsgaard et al. [44] has indi-

A critical examination of the widely accepted dogma that aerobically, pyruvate, not lactate, is the final product of glycolysis (reaction II and Figure 2) suggests that it is both chemically and thermodynamically flawed. First, thermodynamically, glycolysis should proceed to completion i.e., lactate formation, since pyruvate conversion to lactate yields free energy ($\triangle G^{0'}$ = -6 kcal/mole). Second, chemically, glycolysis should proceed to form lactate, since the LDH reaction regenerates NAD$^+$ by oxidizing the NADH formed during the glyceraldehyde phosphate (GAP) dehydrogenase reaction, assuring the cyclical nature of glycolysis (reaction III and Figure 1).



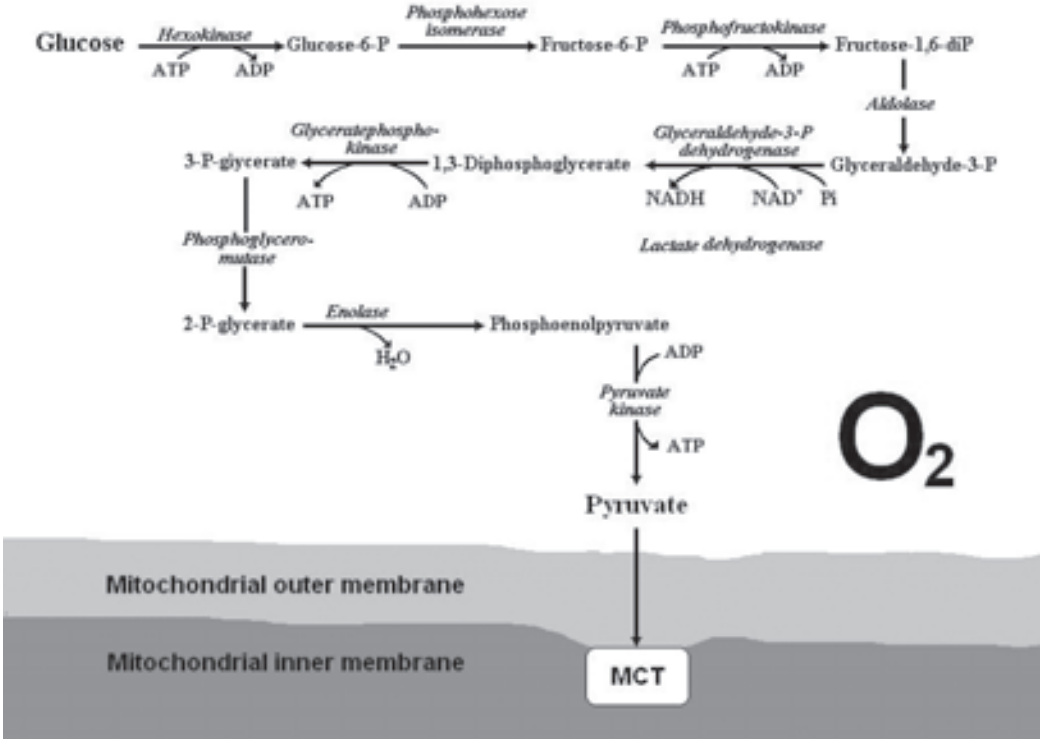


FIGURE 2. A SCHEMATIC ILLUSTRATION OF THE AEROBIC GLYCOLYTIC PATHWAY. IT IS GENERALLY ACCEPTED THAT IN THE PRESENCE OF OXYGEN, THE GLYCOLYTIC PATHWAY METABOLIZE GLUCOSE TO PYRUVATE, SKIPPING THE LAST STEP OF THE PATHWAY, THE CONVERSION OF PYRUVATE TO LACTATE BY LACTATE DEHYDROGENASE (LDH). PYRUVATE INSTEAD IS TRANSPORTED INTO THE MITOCHONDRION VIA A MONOCARBOXYLATE TRANSPORTER (MCT) TO BE UTILIZED AS SUBSTRATE FOR THE TRICARBOXYLIC ACID (TCA)-CYCLE.

Moreover, the assumption that under aerobic conditions pyruvate conversion to acetyl-SCoA, a reaction that takes place in the mitochondrion and requires NAD^+, is somehow thermodynamically preferable over pyruvate conversion to lactate, is unsubstantiated. This assumption would have some basis if the glycolytic enzymes, substrates and products were, as was believed for many years, floating freely in a cytosol that behaves like an aqueous medium.

Lehninger [45] generalized on aerobic glycolysis stating that either lactate or pyruvate is oxidized to CO_2 and H_2O. However, in dealing with the intracellular organization of the glycolytic system he stated that the eleven enzymes catalyzing the glycolytic sequence exist free in solution in the soluble portion of the cytoplasm, at least in most cells. Clearly, it did not occur to many great biochemists that the assumption about the glycolytic sequence of reactions and enzymes as existing in free solution may not necessarily reflect the *in situ* situation. Thus, many even today, hold that the glycolytic reactants and their enzymes flow freely in the cytosol. It is only such notion that would explain why glycolytic pathway is represented as a branched chain i.e., where pyruvate, in the presence of oxygen, does not behave according to the rules of thermodynamics and is changing direction in search of a mitochondrion and not proceeding through its natural last step in the pathway, the LDH reaction.

Nonetheless, Lehninger's notion as cited above, is somewhat surprising, since six years prior to its publication questions had been raised regarding the validity of the concept that the glycolytic pathway exists free in solution. Green et al. [46] concluded from their studies of red blood cells and yeast that in the intact cell the complete glycolytic complex of enzymes is associated with the plasma membrane and is not in free solution. Schrier [47], concomitantly working on erythrocytes, reached the same conclusion. By 1978, Knull, who worked on rat brain tissue, showed that the glycolytic enzymes are all associated with particulate fractions of lysed nerve endings [48]. Clegg opined that there is an intimate connection between cellular architecture and most, and possibly all, of the metabolic machinery. He also concluded that these relations are dynamic and under tight control, and their disruption leads to malfunction and loss of regulation [49].

Hence, lactate formation from pyruvate takes place within an aggregate of glycolytic enzymes, while pyruvate conversion to acetyl-SCoA, the initial step in the TCA-cycle, takes place in the mitochondrion, a conversion that first requires the transport of the monocarboxylate into this organelle via its inner membrane. It is also important to take into consideration that mitochondrial LDH existence has been established and that lactate has been shown to be as good as, if not better than, pyruvate as a substrate for the mitochondrial TCA-cycle [50-52].

Brandt et al., in their study of mitochondrial preparation from different tissues, placed a large part of the mitochondrial LDH inside the mitochondria [51]. They distinguished it from cytosolic LDH, characterized by them as LDH5 (see also below), which preferentially adheres to the outer mitochondrial membrane. Of all the different tissues from which these investigators isolated mitochondria, including heart, kidney, liver lymphocytes and brain, only the latter's preparations were somewhat contaminated with three glycolytic enzymes, phosphoglucomutase, phosphoglucose isomerase and 3-phosphoglycerate kinase [51].

Thus, aerobic glycolysis, when coupled to the mitochondrial TCA-cycle, is better described by the following reactions:

IV. Glucose +2ATP + 4ADP + 2Pi + $2NAD^+$ ⟶ 2lactate + 2ADP + 4ATP + $2NAD^+$
(a cascade of reactions that occurs within the glycolytic aggregate)

V. 2lactate + $2NAD^+$ ⟶ 2pyruvate + 2NADH
(*a conversion that occurs within the mitochondrial inner membrane after lactate is transported through the outer mitochondrial membrane by a monocarboxylate transporter [MCT]*).

Therefore, one may conclude that glycolysis proceeds through the conversion of pyruvate to lactate under both anaerobic and aerobic conditions simply because it is the most feasible path thermodynamically, chemically and spatially.

One must also considers the matter of tissue levels of lactate and pyruvate. In the early years following the formulation of the glycolytic pathway, many investigators studied the two determining their ratio in human blood at rest, during exercise, after a meal, following the administration of pyruvate, lactate or glucose and in various pathological states [10,18,19,53-55]. In all studies pyruvate levels were always found to be significantly lower than those of lactate and thus the measured ratio [lactate]/[pyruvate] was anywhere from 7.0 to 25.0, depending on the conditions under which measurements were made. The low end of this ratio was usually associated with pathological situations.

Thus, Flock et al. [17] demonstrated the conversion of perfused pyruvate to lactate in the dog. Huckabee [10] studied the relationships of pyruvate, lactate and oxygen and suggested that they can be written as follows:

$$[\text{Lactate}] = [\text{Pyruvate}] \times K \frac{[\text{NADH}]}{[\text{NAD}^+]}$$

The above equation expresses the dependence of lactate formation on pyruvate and a constant K x [NADH/NAD^+]. Hence, under fully oxygenated conditions, when the ratio [NADH]/[NAD^+] is held, more or less, constant, a change in pyruvate concentration should bring about a corresponding change in lactate concentration i.e., an increase in [pyruvate] would follow immediately by a similar increase in [lactate] without any change in oxygen consumption. Of course, these relationships hold true as long as blood pH remains unchanged. Consequently, an increase in glycolytic flux under fully oxygenated conditions should result in increased pyruvate and a concomitant increase in lactate concentration, keeping the ratio [lactate]/[pyruvate] constant. According to the above equation and the high ratios of [lactate]/[pyruvate] that are normally maintained, it is clear that even under fully aerobic conditions, lactate is the end-product of glycolysis.

Interestingly, Huckabee [10] did not consider in his calculations two important factors: a) localized changes in oxygen concentration, which could lead to changes in [NADH/NAD^+] and b) a decrease in the concentration of lactate that would decrease [NADH]. I elaborate on these two factors later.

Some of the above considerations in relation to energy metabolism of skeletal and heart muscle and lactate's putative role in this metabolism are detailed in an excellent review by Gladden [56].

The hypothesis

Based on the preceding considerations, it is hypothesized that in the brain, and most likely in many other tissues, whether at rest (does the brain ever rest?) or during activation, both under aerobic and anaerobic conditions, glycolysis always proceeds to its final step, the LDH reac-

tion in which pyruvate is converted to lactate. Thus, this hypothesis postulates that lactate, not pyruvate, is the oxidative substrate for the mitochondrial TCA-cycle in situ (**Figure 3**).

ANLSH. Rather, it aims at providing a plausible explanation to the overwhelming amount of data that indicates that lactate is an important oxidizable energy substrate in the brain.

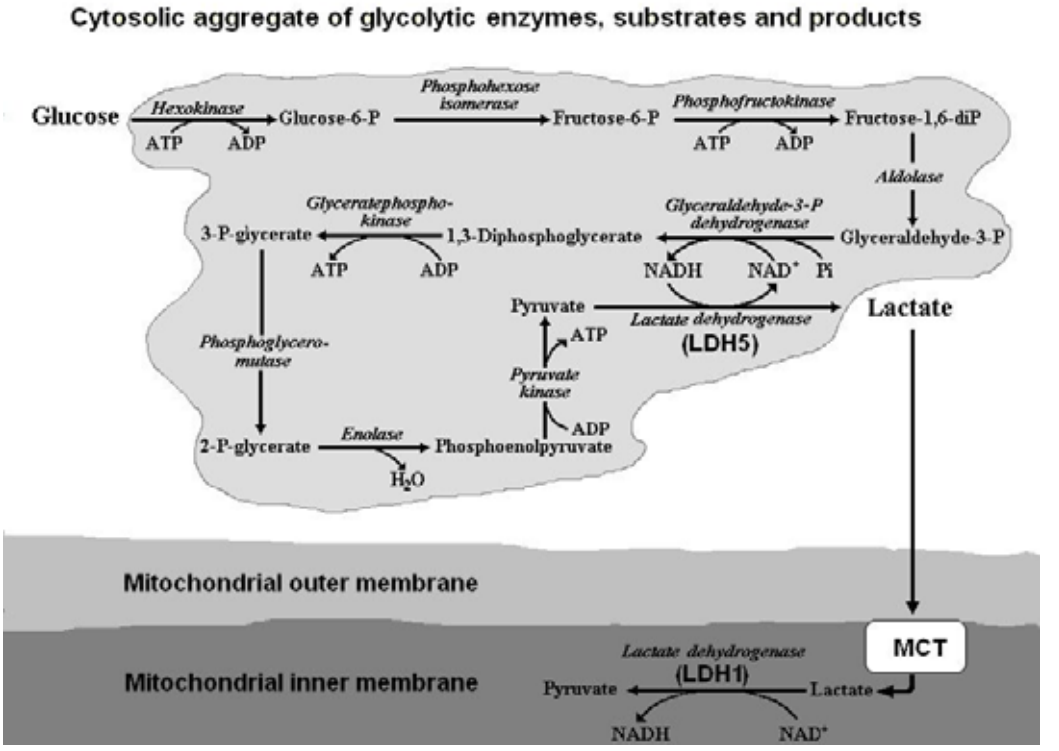


FIGURE 3. A SCHEMATIC DEPICTION OF A HYPOTHETICAL ARRANGEMENT OF THE GLYCOLYTIC PATHWAY AGGREGATE WITH ALL ITS ENZYMES, SUBSTRATES AND PRODUCTS. ACCORDING TO THE HYPOTHESIS PUT FORWARD IN THIS PAPER, GLYCOLYSIS, WHETHER AEROBIC OR ANAEROBIC, ALWAYS PROCEEDS TO ITS LAST STEP, THE FORMATION OF LACTATE, THE REAL *IN SITU* SUBSTRATE FOR MITOCHONDRIAL RESPIRATION. THE LAST ENZYMATIC STEP IN THE CYTOSOLIC GLYCOLYSIS IS LACTATE DEHYDROGENASE COMPOSED GENERALLY OF THE ISOFORM THAT CATALYZES THE CONVERSION OF PYRUVATE TO LACTATE (LDH5). IN CONTRAST, THE ISOFORM THAT CONVERTS LACTATE TO PYRUVATE (LDH1) IS THE FIRST ENZYME OF THE MITOCHONDRIAL OXIDATIVE PATHWAY, SUPPLYING PYRUVATE TO THE TRICARBOXYLIC ACID CYCLE.

It is important and fair to point out that researchers of energy metabolism in skeletal and heart muscles have considered the idea that lactate is the product of aerobic glycolysis and the one being shuttled either intracellularly or extracellularly from its place of formation to be oxidized in the mitochondrion [52,56-60].

Testing the hypothesis

Gladden [56] lists some key queries about the ANLSH. While these queries concern mainly the validity of the ANLSH per se, one major issue that emerges from many of them is whether or not lactate is a major oxidizable energy substrate in the brain. The purpose of hypothesis advanced here is not to validate or discredit the

Since the ANLSH has become a central issue in the debate over the role of lactate in energy metabolism, a brief description of the ANLSH for those who are not familiar with the ongoing debate is in order. The ANLSH hypothesizes that, upon neuronal activation by glutamate, astrocytes take up the presynaptically-released glutamate from the synaptic cleft via specific Na^+-dependent glutamate transporters. The resulting intracellular increase in astrocytic [Na^+] induces Na-K-ATPase activity aimed at pumping out the extra Na^+. The increased demand for ATP by this pumping activity induces astrocytic glycolytic activity and thus lactate production. The mounting astrocytic lactate levels are transported through MCTs that are found in the membranes of both astrocytes and neurons, first to the extracellular space and then into neighboring neurons, where it is

oxidized in the mitochondria. Moreover, it has been suggested that neurons exclusively contain the LDH1 isoform, while astrocytes contain both LDH1 and LDH5 [2,61-66].

Pellerin and Magistretti have recently revised the ANLSH (see below) and thus not all the details in the brief description above are updated. Nevertheless, it is worth emphasizing again that much of the criticism of the ANLSH, whether in its original or revised format, has been directed toward the notion of lactate as a major aerobic energy substrate. Consequently, any hypothesis, including the one proposed here, that promotes lactate as a major oxidative energy substrate would be similarly criticized.

The relevancy of the distribution of the two LDH isoforms, LDH1 and LDH5, between neurons and astrocytes, respectively, has been questioned by the ANLSH critics, since the LDH reaction is near-equilibrium and thus the isoform that carries out this reaction may have only a small effect on the reaction's flux in vivo [67](Newsholme, 2003). And thus, while the relevancy of the LDH isoforms distribution between neurons and astrocytes is yet to be determined, their intracellular distribution, where LDH1 is the mitochondrial isoform and LDH5 is the cytoplasmic one, could be an important part of and relevant to the premise of the present hypothesis. Chih and Roberts, two avid critics of the ANLSH, clarify in their criticizing paper [4] that "*glycolysis* refers to the conversion of glucose to pyruvate under aerobic conditions and *anaerobic glycolysis* refers to the conversion of glucose to lactate," the classical definition of glycolysis. They reject lactate as a significant oxidative energy substrate *in situ* because of what they term "thermodynamic feasibility for conversion of lactate to pyruvate." According to Chih and Roberts [4], in the initial stages of neural activation, as the glycolytic flux increases, pyruvate levels rise and the cytosolic ratio $NADH/NAD^+$ increases. These changes arguably drive the LDH-catalyzed reaction toward lactate production rather than lactate use, making lactate's oxidative utilization unlikely. However, this argument contradicts their description of aerobic glycolysis. Actually, their argument is in full agreement with the present hypothesis that the increased glycolytic flux due to higher energy demands would result in increased aerobic lactate production. More importantly, if one accepts the premise of the present hypothesis that the glycolytic (cytosolic) LDH (5) always catalyzes the conversion of pyruvate to lactate, while the mitochondrial LDH (1) is the enzyme that converts lactate to pyruvate then, there should be no conflict between glycolytic lactate formation and concomitant mitochondrial lactate utilization, whether the lactate originates intracellularly or extracellularly. Moreover, there should not be any problem with the ratio $NADH/NAD^+$, since the cytosolic LDH-catalyzed formation of lactate also assures the oxidation of NADH and the cyclical nature of glycolysis. NAD^+ reduction to NADH during lactate oxidation takes place in the mitochondrion and should have negligible effect on cytosolic glycolysis. The work of Brooks and his colleagues [52,57,58,68-70], using isotopic tracers, strongly implies that glycolytically produced pyruvate is predominantly converted to lactate, not to acetyl-CoA, while lactate, supplied exogenously, is preferentially metabolized to acetyl-CoA. Brooks was the first to propose the existence of an intracellular lactate shuttle [57], which by no means supports or refutes the ANLSH.

Additionally, there is no reason that increases in lactate production would result in an observable lactate accumulation if one considers the postulate that at the onset of activation every molecule of lactate produced is being transported to and oxidized by the mitochondria. Nevertheless, examination of the study of Hu and Wilson [38] indicates that immediately upon neuronal stimulation there is a dip (increased utilization) in all three cerebral energy substrates, glucose, oxygen and lactate. If astrocytes are the cells responsible for clearing the excitatory neurotransmitter from the synaptic cleft then, this clearing activity is the signal that induces the increased flux of the glycolytic pathway in astrocytes. As has been shown by Hu and Wilson [38], the increase in extracellular lactate levels upon stimulation is significant (160-200%) and is accompanied by a measurable decrease in extracellular glucose levels without a significant change in tissue oxygen concentration [38]. Since glutamate is the most prominent excitatory neurotransmitter in the brain, the role of astrocytes in its uptake is most probably central in the above-mentioned signaling. Studies using hippocampal slices have clearly demonstrated that any increase in tissue lactate levels due to activation by glutamate is hardly detectable, unless lactate transport is inhibited [30]. Inhibition of lactate transport, either from astrocytes or the extracellular space, into neurons should cause its accumulation either in the astrocytes themselves or extracellularly. In addition, N-methyl-D-aspartate (NMDA), an analogue of glutamate not recognized by astrocytic glutamate transporters, while being able to excite neurons, could not mimic glutamate-induced accumulation of lactate in the presence of MCT inhibition [30]. This finding supports the premise of the ANLSH that most of the production of lactate during neural activation takes place in astrocytes. Moreover, since glycolysis is the first

responder to energy shortage, one may expect that neuronal glycolysis would react similarly to astrocytic glycolysis i.e., increased lactate production upon disturbance in ion homeostasis. Such a scenario agrees well with the argument of Chih & Roberts [4], although they claim that an increase in extracellular lactate levels must occur if lactate is to fuel the elevation in oxidative metabolism seen with increased neural activity. To discredit the role of lactate as an oxidative fuel during neuronal activation, these critics cite several studies showing that increases in extracellular lactate *in situ* lag behind neural activity and sometimes is not seen until after that activity has ended. However, the measurements of Hu and Wilson [38] strongly suggest that neural stimulation induces an immediate short-lived fall in extracellular lactate levels concomitant with a similar short-lived drop in glucose and oxygen levels. It is plausible that the increased glycolytic flux would continue for sometime after neural excitation has terminated, resulting in detectable lactate accumulation, as has been demonstrated by Hu and Wilson [38]. It is important to emphasize that according to the present hypothesis the only route by which glucose can be utilized for oxidative energy metabolism is via its conversion to lactate, not pyruvate, both in astrocytes and in neurons. The difference between astrocytic and neuronal glycolysis is only quantitative as astrocytes outnumber neurons 10 to 1 [71]. Astrocytic pumping activity of Na^+ while clearing the synaptic cleft from glutamate should continue sometime after neuronal activation has ended. This continuing pumping activity is probably responsible, at least in part, for the cessation of neuronal excitation and for the increase in extracellular lactate, which is available for aerobic utilization by neurons. That should not minimize the importance of neuronal ion pumping, which is probably responsible for the increase in neuronal energy demands and thus, aerobic mitochondrial utilization of lactate. The critics of the ANLSH argue that oxygen use during neural activity *in situ* rises rapidly within the first 1 to 3 seconds and that the slow rise in extracellular lactate probably cannot fuel these rapid responses. Hu and Wilson [38] have demonstrated very clearly that it is lactate that fuels those responses. Hertz [6], on the other hand, claims that although, lactate does rise upon activation, this rise is relatively small and there is uncertainty as to what it signifies; an increase in the level of a static lactate pool or an increase in glycolytic flux. However, if lactate is the oxidative substrate, then the fact that the increase in its extracellular level is small and lags behind the increase in oxygen consumption indicates that lactate is the substrate being oxidized. Regardless, one should find the argument of the ANLSH critics that lactate levels have to rise concomitantly with the onset of activation somewhat odd. Do these critics expect glucose or oxygen tissue levels to rise upon activation? On the contrary, the levels of both these substrates, as indicated by the critics themselves, are actually declining upon activation, which they interpreted as a sign of increased utilization. Hu and Wilson [38] describe the expected biphasic change in lactate levels following activation, where extracellular levels of the monocarboxylate dip within the first 10-12 s of activation followed by an overshoot that lasts 60 s or so. A more recent paper by Mangia et al. [72] describes the same phenomenon using ^{1}H-NMR in humans. The authors have interpreted their results as an early use of lactate for the purpose of replacing glucose when glucose is not available in sufficient amounts. Yet, these authors still argue that once blood flow increases, neurons can use glucose as the main metabolic substrate. The assumption that glucose concentrations are less than sufficient, compared to those of lactate in the normal brain prior to activation, does not agree with normal resting cerebral concentrations of these two substrates. More likely, lactate is the real substrate both during the initial phase of activation and in its later stages; any glucose consumption should result in additional lactate production and its oxidation, as was shown by Hu and Wilson [38]. The initial dip in lactate concentration could be explained by the simple reasoning of higher consumption than production of this monocarboxylate. As to the issue of increased levels of substrates, there are ample examples of increases in glucose levels of brain tissue post-activation or post-trauma due to a drop in its utilization [38,73-75]. It is reasonable to assume that any rise in lactate levels post-activation indicates that the rate of lactate production exceeds its rate of utilization. Additionally, when lactate levels increase significantly, as in the case of an anaerobic period [28-30,76] or upon continuous stimulation [38], lactate does become the oxidative substrate of choice upon reoxygenation or termination of stimulation. Moreover, in a recent paper, Kasischke et al. [77], using NADH fluorescence measurements in brain slices, have demonstrated that upon activation of neural tissue there is an early oxidative metabolism that is entirely neuronal, as indicated by a short-lived (~10 s) dip in NADH fluorescence localized in neuronal dendrites, followed by an overshoot in NADH fluorescence localized in astrocytes and signaling glial glucose uptake and increased lactate-producing glycolytic activity lasting for ~60 s. The observed initial dips in the concentration of both lactate and NADH [38,71,77] are in excellent correlation with the observed dip in oxygen levels upon acti-

vation as reported by Malonek and Grinvald [78] and Hu and Wilson [38]. All these observations can be simply explained by the hypothesis advanced here.

The report by Kasischke and colleagues [77] drove Pellerin and Magistretti to revise the ANLSH [79]. Accordingly, the increase in energy demands upon neural activation is supported by neuronal oxidative lactate utilization, indicated by dips in both dentritic mitochondrial NADH [77] and extracellular lactate levels [38] followed by a longer lived overshoot in astrocytic NADH and lactate concentrations. Hence, it is now clear that the original notion of the ANLSH, postulating that astrocytic glutamate uptake is the signal coupling neural activity to glucose consumption and increased energy metabolism, was wrong. It is more likely that neuronal ionic movement is the signal that induces the increase in dendritic mitochondrial substrate oxidation (oxygen, lactate and NADH dips) that follows an elevation in astrocytic glucose consumption (lactate and NADH overshoot).

Hertz, another critic of the ANLSH, rejects the notion that lactate is predominantly a neuronal oxidative energy substrate [6]. To support his claim, he employs several studies where either [^{14}C] or [^{13}C] labeled glucose or lactate was used. Thus, Itoh et al. [80] found that unlabeled glucose did not inhibit $^{14}CO_2$ production from labeled lactate in cortical neurons. However, both Itoh et al. [80] and Bouzier-Sore et al. [43] have demonstrated that unlabeled lactate strongly reduces the production of $^{14}CO_2$ from labeled glucose. Itoh et al. [80] explained their results by assuming that lactate conversion to pyruvate also reduces NAD^+ to NADH, minimizing the availability of the former for the oxidation of glyceraldehyde-3-phosphate during glycolysis. Hertz [6] argues that if this were the mechanism by which lactate inhibits glycolytic glucose utilization, then pyruvate, which is not involved in oxidation-reduction reactions of NAD^+/NADH, should be less efficient an inhibitor of $^{14}CO_2$ production from labeled glucose than lactate. Hertz [6] then presents results of experiments from his laboratory that indicate pyruvate to be as good an inhibitor of $^{14}CO_2$ production from labeled glucose as is lactate. Although, these results may refute the explanation given by Itoh et al. [80] for the possible mechanism of inhibition of $^{14}CO_2$ production from labeled glucose, Hertz's very experiments provide strong support for the present hypothesis, namely, that lactate (and pyruvate), when supplied exogenously, is an oxidative substrate for the mitochondrial TCA-cycle. Consequently, abundant exogenous supplies of either lactate or pyruvate would be expected to inhibit the glycolytic utilization of glucose, since the oxidative utilization of any of these monocarboxylates results in the production of ~15 moles of ATP per mole of monocarboxylate without the need for ATP investment when glucose is the substrate. In addition, no shortage of NAD^+ would be expected when lactate is the substrate, since the enzyme that oxidizes it to pyruvate is not the glycolytic LDH (5), rather it is the mitochondrial LDH (1).

It is interesting that those who dismiss lactate as an oxidative energy substrate on the grounds that its levels do not rise sufficiently to support the energy needs of the activated neuron, do not hold pyruvate to the same standard: none of the critics of the ANLSH expect pyruvate levels to rise significantly during activation although, according to their predominant notion, pyruvate is the aerobic glycolytic product and thus its levels must also rise if it is to support the higher energy demands.

Nonetheless, it should be emphasized that 'lactate as the ultimate oxidative energy substrate' hypothesis has not been constructed merely to support or refute the ANLSH. This hypothesis aims at furnishing a framework to explain the results of numerous studies of the past two decades on cerebral (and other tissues) energy metabolism that have challenged one of the most enduring dogmas in biochemistry.

Conclusion

There are many parallels between brain and skeletal muscle tissues. In both, there are at least two types of neighboring cells that influence each other, especially during heightened activity; neurons and astrocytes in the brain and type I and II fibers in the skeletal muscle. Many of the features of skeletal muscle fibers, so elegantly elaborated on by Brooks [52,57,58], the first investigator to coin the term "lactate shuttle," and Van Hall [59], are features that could easily be applied to brain tissue. The hypothesis put forward here postulates that lactate is the main glycolytic product in brain (and other tissues), whether under aerobic or anaerobic conditions, in neurons or astrocytes. It provides the necessary bridge between the two factions in the debate on which is more important, glucose or lactate, for oxidative energy metabolism. Accordingly, both are: glucose as the substrate of the glycolytic pathway and lactate as the substrate of the mitochondrial TCA-cycle.

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