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# LACTATE, NOT PYRUVATE, IS NEURONAL AEROBIC GLYCOLYSIS END PRODUCT: AN *IN VITRO* ELECTROPHYSIOLOGICAL STUDY

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**Abstract—For over 60 years, a distinction has been made between aerobic and anaerobic glycolysis based on their respective end products: pyruvate of the former, lactate of the latter. Recently we hypothesized that, in the brain, both aerobic and anaerobic glycolysis terminate with the formation of lactate from pyruvate by the enzyme lactate dehydrogenase (LDH). If this hypothesis is correct, lactate must be the mitochondrial substrate for oxidative energy metabolism via its oxidation to pyruvate, plausibly by a mitochondrial LDH. Here we employed electrophysiology of the rat hippocampal slice preparation to test and monitor the effects of malonate and oxamate, two different LDH inhibitors, and glutamate, a neuronal activator, in experiments, the results of which support the hypothesis that lactate, at least in this *in vitro* setting, is indeed the principal end product of neuronal aerobic glycolysis.** 

**Key words: glucose, glutamate, glycolysis, hippocampus, lactate dehydrogenase, pyruvate.**

The role lactate plays in brain energy metabolism is controversial. The dogma according to which lactate is a useless end product of anaerobic glycolysis prevailed unabated until two decades ago. Since then, evidence has accumulated indicating that lactate is an important cerebral oxidative energy substrate (Schurr et al., 1988, 1997a,b, 1999; Fellows et al., 1993; McKenna et al., 1993, 1994; Izumi et al., 1994, 1997; Pellerin and Magistretti, 1994; Larrabee, 1995, 1996; Tabernero et al., 1996; Wada et al., 1997; Hu and Wilson, 1997; Qu et al., 2000; Bliss and Sapolsky, 2001; Takeda et al., 2001; Smith et al., 2003; Bouzier-Sore et al., 2003; Dalsgaard et al., 2004; O'Brien et al., 2006; Levasseur et al., 2006; Zielke et al., 2007). It has been suggested that in skeletal muscle, glycolytically-produced pyruvate is predominantly converted to lactate, not to acetyl-coenzyme A (acetyl-CoA), while lactate, when supplied exogenously, is preferentially metabolized to acetyl-CoA (Brooks, 1985, 1998, 2000, 2002a,b; Szczesna-Kaczmarek, 1990, 1992; Brooks et al., 1999). In the brain, at least in the hypothalamus, it has been shown that glucose and triglyceride-rich lipoproteins sensing requires the conversion of glucose to lactate, which stimulates pyruvate metabolism (Lam et al., 2005, 2007). Recently we hypothesized that, in the brain, lactate is the principal product of glycolysis, whether or not oxygen is present (Schurr, 2006).

The absence of specific and efficient inhibitors of the two lactate dehydrogenase (LDH)-catalyzed reactions, namely, the conversion of pyruvate to lactate and vice versa, has greatly impeded the assessment of lactate's role in energy metabolism. Such inhibitors could facilitate the resolution of several outstanding questions regarding lactate's role in brain energy metabolism. Postulating lactate to be the end product of aerobic glycolysis requires, by definition, that: a) pyruvate conversion to lactate be executed glycolytically by cytosolic LDH3-5 isoforms (see O'Brien et al., 2006) and b) that lactate conversion back to pyruvate be catalyzed by another group of isoforms, LDH1-3, which have been suggested to be located, at least where skeletal muscle is concerned, in the mitochondrion (Brooks, 1998). For the present study, we considered the rat hippocampal slice preparation as a model system capable of differentiating the actions of specific inhibitors of the LDH isoforms, since neuronal function in this preparation can be sustained either with glucose, lactate or pyruvate as the sole substrate of energy metabolism (Schurr et al., 1988, 1997a,b, 1999; Schurr and Rigor, 1998).

Allowing for pyruvate to be the main end product of aerobic glycolysis, one could predict that glucose-supported neuronal function in fully oxygenated hippocampal slices would be affected neither by an LDH3-5 inhibitor (inhibitor X) nor by an LDH1-3 inhibitor (inhibitor Y), since LDH does not participate in the conversion of glucose to pyruvate. Moreover, neither inhibitor should affect neuronal function supported by exogenously supplied pyruvate. However, if lactate is the end product of aerobic glycolysis or if it is being supplied exogenously, neuronal function should be suppressed by inhibitor Y, but not by inhibitor X. Although the latter would inhibit lactate formation from glucose, it should not prevent pyruvate formation from the sugar and its utilization by mitochondria.

In reality, all known LDH inhibitors, inefficient as they may be, exhibit both X and Y inhibitory capacities, although with different potencies toward one reaction or the other. Thus, an LDH inhibitor with a stronger Y than X inhibitory activity should be innocuous in inhibiting pyruvate-supported neuronal function, but able to suppress lactate-supported neuronal function. Considering our hypothesis that lactate is the aerobic brain's glycolytic end product (Schurr, 2006), glucose-supported neuronal function should be suppressed by an LDH inhibitor with Y>X inhib-

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*Abbreviations:* acetyl-CoA, acetyl-coenzyme A; aCSF, artificial cerebrospinal fluid; LDH, lactate dehydrogenase; MMA, methylmalonate; PS, population spike; TCA, tricarboxylic acid cycle.

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itory activity to the same extent that it would suppress lactate-supported neuronal function. However, such an inhibitor is expected, over time, to exert its X inhibitory activity and to inhibit the conversion of glucose to lactate by LDH3-5 (cytosolic). Since this inhibitor should not suppress pyruvate-supported neuronal function, predictably, its initial suppression of glucose-supported neuronal function would be partially or completely relieved over time, as pyruvate becomes the main glycolytic end product and the mitochondrial substrate in the presence of that inhibitor.

The hippocampal slice preparation and its electrophysiology have been extensively used over the years to elucidate basic cellular mechanisms of cerebral ischemia/hypoxia and excitotoxicity. One drawback of the model that has been overlooked is the traditionally high concentration of glucose (10 mM), believed to be necessary for maintenance of healthy tissue and its neuronal function. However, the use of "hyperglycemic" concentrations of glucose is avoidable. We have reduced the glucose concentration in the artificial cerebrospinal fluid (aCSF) used to maintain our slices from 10 mM to 2.5 mM without affecting the robustness of the evoked neuronal response. This lower, physiologically relevant, glucose concentration also allows us to use the preparation to model conditions (hypoxia, excitotoxicity) that are also more relevant to the *in vivo* situation.

Various studies have suggested that malonate and/or its metabolite, methylmalonate (MMA) inhibit either succinate dehydrogenase activity, respiratory chain complex II or tricarboxylic acid cycle (TCA) components (Takeda et al., 1967; Davis, 1968; Koeppen and Riley, 1987; McKenna et al., 1990; Okun et al., 2002). Notwithstanding, a study asserts that MMA is not an inhibitor of the mitochondrial respiratory chain (Kölker et al., 2003), while Takeda et al. (1967) showed that malonate inhibits incorporation of $^{14}C$ from 1-$^{14}C$-pyruvate into lactate. Curiously, Takeda and colleagues did not consider malonate to be an LDH inhibitor. Saad et al. (2006) have demonstrated that malonate and its metabolite, MMA are relatively efficient competitive inhibitors of LDH and that malonate is a more potent inhibitor than MMA in inhibiting the conversion of lactate to pyruvate. Malonate is a dicarboxylate that is most likely transported into neurons, astrocytes and mitochondria via the dicarboxylate transporter responsible for succinate transport (Yodoya et al., 2006; Aliverdieva et al., 2006).

Oxamate and oxalate are the better known LDH inhibitors. Relatively high concentrations (25–50 mM) of these inhibitors have been used to achieve only a partial inhibition of LDH activity (Chretien et al., 1995; Brooks et al., 1999; Cortes et al., 2001). Oxamate is a competitive inhibitor of LDH with respect to pyruvate and fits the description of the X>Y inhibitor in our introductory remarks (stronger inhibitory activity against the conversion of pyruvate to lactate), while oxalate is an uncompetitive inhibitor with respect to pyruvate, fitting an LDH inhibitor with Y>X (stronger inhibitory activity against the conversion of lactate to pyruvate).

In the present study we evaluated the effects of malonate and oxamate on the ability of equicaloric concentrations of each of the three energy substrates, lactate, pyruvate and glucose to sustain neuronal function in rat hippocampal slices under standard conditions and upon activation with glutamate. Oxalate, as we found out very quickly, cannot be used in our system due to its calcium chelating properties.

## EXPERIMENTAL PROCEDURES

Adult (200–350 g) male Sprague–Dawley rats were used. Rats were housed in the facility at the University of Louisville School of Medicine, maintained on a 12-h light/dark cycle at 22 °C and had unlimited access to food and water. All protocols were approved by the University of Louisville School of Medicine Institutional Animal Care and Use Committee and were consistent with the guidelines of the U.S. National Institutes of Health (NIH). All efforts were made to minimize the number of animals used and their suffering. For each experiment, one rat was decapitated under ether anesthesia, its brain rapidly removed and rinsed with cold (6–8 °C) aCSF (see composition below) and dissected. Isolated hippocampi were sliced transversely at 400 $\mu$m with a McIlwain tissue chopper and the resulting slices (10–12 slices per hippocampus) were placed in a dual linear-flow incubation/recording chamber (Schurr et al., 1985). Each of the two compartments of the chamber was supplied with a humidified gas mixture (95% $O_2$/5% $CO_2$) through separate flow meters (2 l/min) and aCSF via a dual peristaltic pump (1 ml/min). The aCSF composition was (in mM): NaCl 124; KCl 5; $NaH_2PO_4$ 3; $CaCl_2$ 3; $MgSO_4$ 2; $NaHCO_3$ 23; D-glucose 10. The aCSF had a pH of 7.3–7.4 and an osmolality of 300±5 mOSM. The temperature of the incubation chamber was held at 34±0.3 °C. After 90 min of incubation, the 10 mM glucose–aCSF was replaced either with 2.5 mM glucose–, 5 mM lactate– or 5 mM pyruvate–aCSF. Oxamate (15 mM), malonate (10 mM) and/or glutamate (2.5 mM) were also supplemented when indicated.

Continuous extracellular recordings of electrically evoked population spikes (PS, neuronal function) in the stratum pyramidale of the hippocampal CA1 region were made from one slice in each compartment of the dual chamber using borosilicate micropipettes filled with aCSF (impedance 2–5 MΩ). A two-channel preamplifier (×100) and two field-effect transistor head stages were used. Bipolar stimulating electrodes were placed in the Schaffer collaterals (orthodromic stimulation) and stimulus pulses (0.1 ms in duration) of an amplitude twice threshold (~10 V) were applied once/min to evoke a response. At the end of each experiment the rest of the slices (nine to 11) in each compartment were tested for the presence and amplitude of their PS by stimulating the Schaffer collaterals and recording the evoked response in the CA1 cell body layer. This test lasted 10–15 min. Acquisition, analysis and storage of data were done using the CED 1401 mk II data acquisition system with Spike 2 software package (Cambridge Electronic Design Corporation, Cambridge, UK). All chemicals were of analytical grade (Sigma Chemical, St. Louis, MO, USA). Each experiment (condition) was repeated three times (30–36 slices). Separate experiments (not shown here) were carried out to determine the optimal concentration of glucose, lactate, pyruvate, malonate and glutamate used in this study. The three energy substrates, at the equicaloric concentrations used, were able to sustain similar PS amplitudes. Statistical analysis was performed using the paired, two-sample for means *t*-test, comparing control slices to treated slices (malonate and/or glutamate) within each of the different energy substrate-supplemented groups.

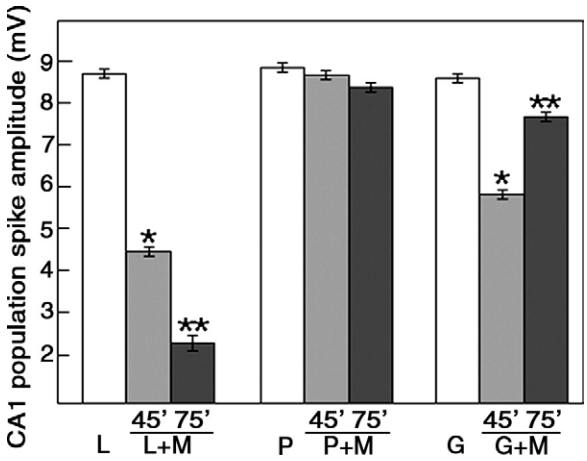


**Fig. 1.** The effect of the LDH inhibitor, malonate (M, 10 mM), on CA1 PS (neuronal function) amplitude in rat hippocampal slices maintained in aCSF containing either lactate (L, 5 mM), pyruvate (P, 5 mM) or glucose (G, 2.5 mM). M progressively inhibited L-supported neuronal function over time and was innocuous against P-supported neuronal function. The LDH inhibitor initially inhibited G-supported neuronal function, inhibition that later was relieved. These results suggest that aerobically, G is metabolized glycolytically to L, not to P. Bars are means±S.E.M.; * significantly different from energy substrate alone; ** significantly different from energy substrate alone or energy substrate+M at 45 min ($P<0.0001$).

## RESULTS

Malonate (10 mM) suppressed lactate-supported neuronal function in rat hippocampal slices by approximately 50% of its baseline value within 45 min after the introduction of the inhibitor and by 75% after 75 min of incubation with the inhibitor (Fig. 1). Pyruvate-supported neuronal function was unaffected by malonate regardless of the length of the period slices were exposed to the inhibitor. Similar results were found with oxamate (15 mM), where lactate-supported neuronal function was inhibited by 78% after 30 min of incubation with the inhibitor and by 86% after 60 min exposure to oxamate. Oxamate, similar to malonate, was ineffective in inhibiting pyruvate-supported neuronal function (Fig. 2). These findings verify that lactate-supported neuronal function requires LDH-catalyzed conversion of lactate to pyruvate. Inhibition of this conversion either by malonate or oxamate prevented lactate utilization and thus suppressed lactate-supported neuronal function. Expectedly, pyruvate was found to be as efficient a substrate as lactate in supporting neuronal function in rat hippocampal slices, a function insensitive to either malonate or oxamate. Malonate effect on glucose-supported neuronal function is also illustrated in Fig. 1. Initially (after 45 min of exposure), malonate suppressed glucose-supported neuronal function by 32%. However, by 75 min of incubation with malonate, the initial suppression was almost completely relieved (only 10% inhibition). Oxamate, in contrast with malonate, only slightly inhibited glucose-supported neuronal function (15% inhibition) after 30 min of exposure to the inhibitor (Fig. 2), suppression that was not removed as time of exposure to the inhibitor was prolonged. This outcome was also predicted by the introductory considerations, as oxamate is an LDH inhibitor with X>Y inhibitory activity. In other words, oxamate is expected to inhibit pyruvate conversion to lactate faster and stronger than the conversion of lactate to pyruvate and, therefore, to have only a slight effect on glucose-supported neuronal function. When we originally planned this study, oxalate was our choice of an LDH inhibitor of the Y>X type, expected to exhibit a similar biphasic inhibitory effect on glucose-supported neuronal function to that produced by malonate. However, we could not carry out any experiments with oxalate because of its metal chelating properties. When added to the aCSF, oxalate immediately chelates $Ca^{2+}$, a cation necessary for expression of normal neuronal function in brain tissue.

In the present experiments we supplied slices with an aCSF containing a relatively low glucose concentration (2.5 mM), a concentration similar to the *in vivo* brain levels of the carbohydrate. Consequently, a 20-min exposure to a relatively low concentration of the excitatory neurotransmitter glutamate (2.5 mM) compromised the ability of rat hippocampal slices to maintain "normal" PS amplitude (Fig. 3, glucose, control). In the presence of malonate (10 mM), glutamate's compromising effect on the PS amplitude was significantly augmented despite the fact that glutamate was washed out at the end of 20 min exposure. Slices supplemented with exogenous pyruvate recovered their PS amplitude to the pre-glutamate exposure level regardless of the presence or absence of malonate.

## DISCUSSION

For the purpose of interpreting the present results, we have postulated that malonate (Saad et al., 2006) is inhib-

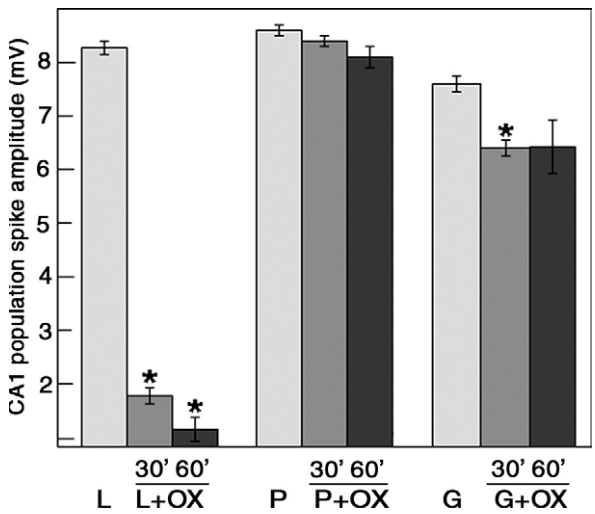


**Fig. 2.** The effects of the LDH inhibitor oxamate (OX, 15 mM) on CA1 PS (neuronal function) amplitude in rat hippocampal slices maintained in aCSF containing either lactate (L, 5 mM), pyruvate (P, 5 mM) or glucose (G, 2.5 mM). OX strongly and progressively inhibited L-supported neuronal function, but was harmless against P-supported neuronal function. OX exerted a weak inhibition of G-supported neuronal function throughout the duration of the experiment (60 min). Bars are means±S.E.M.; * significantly different from energy substrate alone ($P<0.001$).

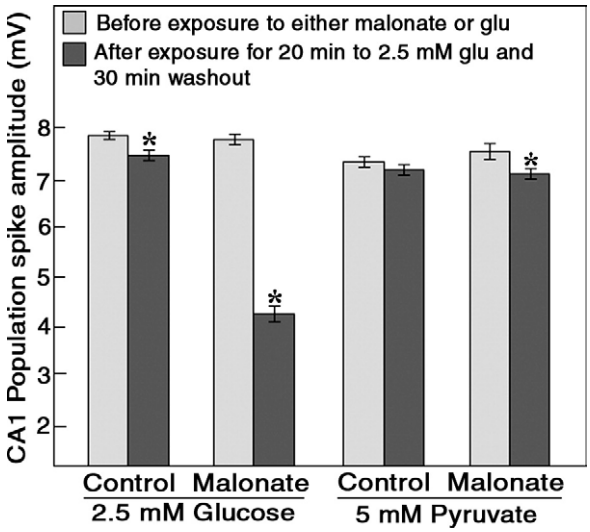


**Fig. 3.** The effect of 20 min exposure to glutamate (glu, 2.5 mM) on CA1 PS amplitude (PS, neuronal function) in the absence or presence of the LDH inhibitor, malonate (10 mM) when either glucose (2.5 mM) or pyruvate (5 mM) was the sole energy substrate in the aCSF in which rat hippocampal slices were maintained. Slices that were maintained with glucose could not recover their original PS amplitude after glu washout in the presence of malonate as compared with glucose-maintained slices without malonate or slices maintained with pyruvate in the presence or absence of malonate. This outcome indicates that glycolysis and its production of lactate is crucial for the preservation of neuronal viability during excitation with glu. Bars are means±S.E.M.; * significantly different from the mean values before the exposure to either malonate or glu ($P<0.01$).

iting lactate conversion to pyruvate more efficiently than the reverse reaction. Our results indicate that this postulate is correct.

Obviously, the insensitivity of pyruvate-supported neuronal function to malonate (Fig. 1) indicates that the inhibitor, at least in hippocampal slices, is innocuous where respiratory chain complex II, succinate dehydrogenase and any TCA cycle component are concerned, in contrast to the suggestion by several studies (Takeda et al., 1967; Davis, 1968; Koeppen and Riley, 1987; McKenna et al., 1990; Okun et al., 2002). Inhibition of any of these three sites should be equally effective in suppressing pyruvate-supported neuronal function. Both malonate and oxamate were effective in inhibiting lactate supported neuronal function, inhibition that grew stronger over time (Figs. 1 and 2). Similarly to malonate, oxamate was innocuous against pyruvate-supported neuronal function.

The initial suppression of glucose-supported neuronal function by malonate (Fig. 1) indicates that the neuronal cytosolic LDH (LDH3-5) reaction, which converts pyruvate to lactate, is the terminal step of aerobic glycolysis, not the pyruvate kinase reaction, which converts phosphoenolpyruvate to pyruvate. Hence, lactate formed by aerobic glycolysis must be converted back to pyruvate, most likely by a mitochondrial LDH (LDH1-3) (Brooks 1985, 1998, 2000, 2002a,b; Szczesna-Kaczmarek, 1990, 1992; Brooks et al., 1999), a reaction inhibited by malonate. This inhibition initially limits the supply of pyruvate from (neuronal glycolytic) lactate to mitochondria and thus ATP production necessary to support neuronal function. Eventually, malonate also inhibits cytosolic LDH3-5 in a somewhat weaker and delayed fashion compared with its effect on LDH1-3. Nevertheless, over time, malonate inhibits LDH3-5 and the glycolytic conversion of pyruvate to lactate, making pyruvate the main product of glucose metabolism, which fuels the observed, almost complete (~90%) recovery (relief of suppression) of glucose-supported neuronal function. If the terminal product of aerobic glycolysis were pyruvate, malonate would not exert any suppression of glucose-supported neuronal function. In addition, the cytosolic LDH3-5-catalyzed conversion of pyruvate to lactate continuously supplies $NAD^+$ for glyceraldehyde-3-phosphate dehydrogenase reaction, assuring the recycling nature of the glycolytic pathway. Therefore, it is likely that the cytosolic LDH isoforms (LDH3-5) execute the conversion of pyruvate to lactate, while the LDH isoforms responsible for the conversion of glycolytically-produced lactate back to pyruvate (LDH1-3) are located on or in close proximity to the outer mitochondrial membrane, as suggested by others (Brooks 1985, 1998, 2000, 2002a,b; Szczesna-Kaczmarek, 1990, 1992; Brooks et al., 1999).

An alternative explanation of malonate effect on glucose-supported neuronal function relies on the astrocytic–neuronal lactate shuttle hypothesis (ANLSH, Pellerin and Magistretti, 1994), which surmises that a portion of neuronal mitochondrial ATP production is fueled by astrocytic lactate. Accordingly, malonate would inhibit the utilization of astrocytic lactate by neuronal mitochondria, leading to the observed partial (32%) suppression of glucose-supported neuronal function. Yet, the inhibition of glucose-supported neuronal function by oxamate amounted to only ~15% without any observable biphasic effect (Figs. 1 and 2). Hence, at rest, it seems that astrocytes do not contribute more than 15% of the lactate utilized by neurons. Moreover, one could posit that upon LDH inhibition, astrocytic glycolysis would produce pyruvate instead of lactate and that the former would be available to support recovery of neuronal function despite the presence of oxamate. Such recovery was not observed with oxamate in contrast to the ~90% recovery observed with malonate (Fig. 1). This degree of neuronal function recovery is very similar to the level that oxamate suppressed glucose-supported neuronal function, pointing again to our earlier conclusion that, at rest, astrocytes contribute about 10–15% of the lactate consumed by neuronal mitochondria. Additionally, if at rest astrocytes metabolize glycolytically significant amounts of glucose to produced lactate for neuronal utilization, then, LDH inhibition by either malonate or oxamate should not prevent these cells from continuing to produce glycolytic pyruvate capable of supporting neuronal function. There are several possibilities why astrocytic pyruvate is incapable of fueling any recovery of the oxamate-inhibited, glucose-supported neuronal function or a 100% recovery of the malonate-inhibited, glucose-supported neuronal function. It could be that pyruvate cannot be shuttled to neurons (unlikely) or that it is only produced in small quantities or that astrocytes themselves consume most of it. However, the most likely explanation is that astro-

cytic LDH inhibition would slow down glycolysis due to a shortage in $NAD^+$ production. Thus, it is possible that the partial, yet significant recovery (~90%) seen in the presence of malonate is fueled by neuronally-produced pyruvate.

The results of the combined glutamate–malonate experiments (Fig. 3) emphasize the important role lactate plays as a mitochondrial energy substrate for maintenance of neuronal function, whether lactate originates in neurons or astrocytes. That malonate is detrimental to neuronal viability of glutamate-activated, glucose-supported hippocampal slices is apparent from the partial recovery (50%) of the energy-dependent PS amplitude. This outcome suggests that lactate, whether neuronal or astrocytic in origin, is crucial for neuronal viability upon excitation.

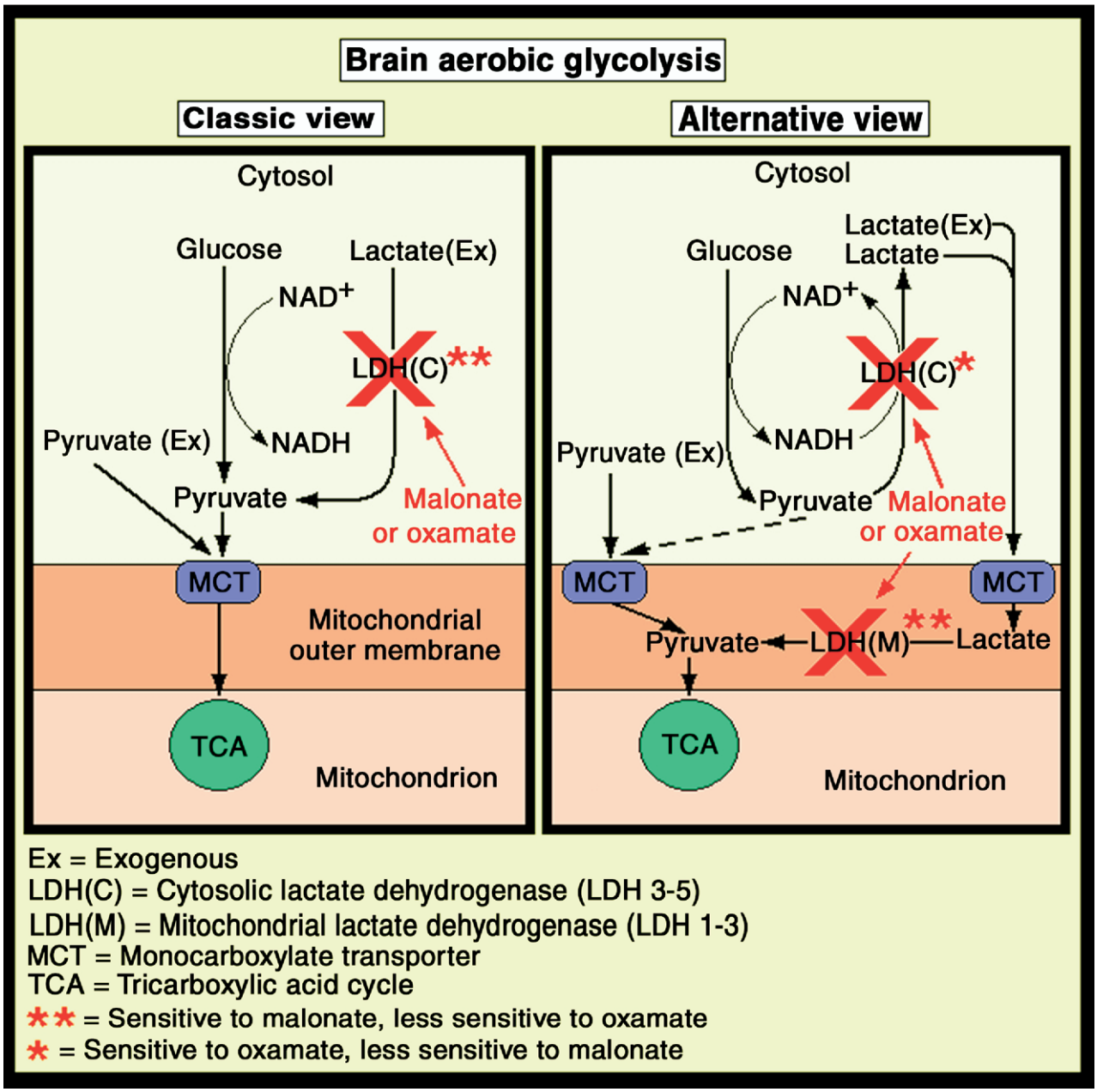


**Fig. 4.** Two views of the aerobic glycolytic pathway. The classic view depicts pyruvate as the pathway's end product and thus as a pathway that should not be affected by an LDH inhibitor such as malonate. The results of the present study cannot be explained by this view. The alternative view of the aerobic glycolytic pathway postulates lactate to be its end product. Since glycolytically-produced lactate must be converted to pyruvate to allow the latter to enter the TCA, this alternative view explains the ability of malonate to interfere with glucose-supported neuronal function as depicted in Fig. 1. However, over time, malonate's weaker inhibiting activity of the conversion of pyruvate to lactate will shift the glycolytic conversion of glucose from lactate to pyruvate, at which time, the latter could become the main glycolytic end product and the mitochondrial substrate (broken arrow, right panel), relieving the suppression of neuronal function observed earlier when glucose is the energy substrate (Fig. 1).

Alternatively, the outcome of these experiments may be explained by postulating astrocytic increase in glucose consumption and lactate production due to glutamate uptake, whereupon lactate becomes a major neuronal energy substrate (Pellerin and Magistretti 1994; Schurr et al., 1999). Under such a scenario, any interference with neuronal utilization of astrocytic lactate i.e. LDH inhibition with malonate, would suppress normal neuronal function. However, this suppression should be overcome if astrocytes, while incapable of producing lactate in the presence of malonate, would produce enough pyruvate, which upon glutamate washout, should fuel neuronal function recovery, similar to the recovery observed in slices supplied with exogenous pyruvate (Fig. 3). But, such recovery did not occur, indicating again that astrocytic pyruvate is not readily available to neurons, in contrast to either astrocytic lactate or neuronal pyruvate. The fact that slices supported by pyruvate (5 mM) were able to withstand glutamate excitotoxicity, while slices supported by an equicaloric amount of glucose (2.5 mM) lost 50% of this ability, may suggest that astrocytes are themselves consumers of pyruvate during glutamate activation or that astrocytic pyruvate, unlike lactate, is less available to neurons. Why is 2.5 mM glucose, in contrast to 5 mM pyruvate, unable to sustain neuronal viability in slices treated with both glutamate and malonate? It is possible that not all the available glucose is converted to pyruvate under these conditions. A more compelling possibility is that glucose cannot increase mitochondrial respiration under aerobic conditions, while lactate (and probably pyruvate) can. Levasseur et al. (2006) have demonstrated that glucose sustains mitochondrial respiration at low, “fixed” rate, since despite increasing the glucose concentration nearly 100-fold oxygen consumption was not up-regulated. In contrast increasing lactate concentration elevated mitochondrial oxygen consumption, plausibly allowing mitochondria to meet heightened energy demands. Consequently, oxygenated, glucose-supplemented hippocampal slices are incapable of increasing their mitochondrial respiration rate in response to activation by glutamate, since they are unable to up-regulate their glycolytic flux. Normally, both neuronal and astrocytic lactate would overcome this limitation, but in the presence of malonate this avenue is lost. Obviously, exogenous lactate would be useless in the presence of malonate, which is the reason why lactate could not be tested under the paradigm shown in Fig. 3. Nonetheless, the results of the combined glutamate–malonate experiments suggest an augmented shortfall in lactate (or pyruvate) supply, emphasizing the importance of the monocarboxylate both at rest and in activated conditions.

It is our contention that the results of the present study cannot be explained by the classic depiction of aerobic glycolysis (Fig. 4, left panel). However, our recently proposed alternative version of this pathway (Schurr, 2006) could provide a plausible explanation (Fig. 4, right panel). Therefore, we conclude that our present results support the hypothesis, according to which aerobic neuronal glycolysis, at least *in vitro*, proceeds to form lactate. This conclusion leads us to speculate that lactate is the most plausible mitochondrial energy substrate and that LDH isoforms that catalyze lactate conversion to pyruvate most probably reside within or closely attached to the outer mitochondrial membrane. Under activated conditions, astrocytes may play a greater role in providing for neuronal energy metabolism, mainly in the form of shuttled lactate and any interference in neuronal lactate utilization could compromise energy metabolism and result in neuronal damage.

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