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Hippocampal Slices and Their Electrophysiology in the Study of Brain Energy Metabolism

Avital Schurr

*Department of Anesthesiology & Perioperative Medicine,
University of Louisville School of Medicine, Louisville, KY,
USA*

1. Introduction

Dorland's Illustrated Medical Dictionary in its 24th Edition (1965), describes the term "Electrophysiology" as "The Science of physiology in its relations to electricity; the study of the electric reactions of the body in health." The ability of scientists to observe and record physiology's electrical phenomena long preceded the understanding of the membranous ionic processes that are responsible for them. Consequently, for a while, electrophysiology has been considered a subfield of physiology, aiming at improving our understanding of cellular, organ and bodily functions. With the advances made in molecular biology, genetics and neuroscience, the role of electrophysiology has shifted, where today it is being employed as one of the best, most accurate and least expensive real-time monitoring tools in basic science research and clinical studies and practice alike.

The discovery in the early 1950s that brain slices can sustain certain electrophysiological characteristics typical of the intact brain opened a wide range of possibilities for studying cerebral tissue and its electrophysiology *in vitro*. Obviously, the brain slice preparation affords the experimenter both the control and manipulation of the environmental conditions under which the neural tissue is maintained. Employing electrophysiological techniques allows a continuous monitoring/recording of the neural tissue's viability, its functions and its responses to environmental and other changes brought about by the experimenter's chosen manipulations.

Thousands of papers and several books have been published over the past 30 years, where brain slices were the topic itself or where studies employed them in combination with various techniques, including electrophysiological ones. The present chapter describes some important advances made over the past three decades using brain slices and their electrophysiology in our laboratory, advances that provided us with a better understanding of cerebral energy metabolism. All the experiments detailed herewith employed the rat hippocampal slice preparation, using a continuous extracellular, real-time monitoring of the electrically-evoked CA1 population spike (PS).

Of the different brain slice preparations available, the rat hippocampal slice preparation is without a doubt the most studied. Henry McIlwain and colleagues were the first to use thin brain sections for metabolic studies (McIlwain et al., 1951; McIlwain & Buddle, 1953;

Rodnight & McIlwain, 1954). McIlwain's laboratory was also the first to demonstrate that neurons in brain slices were in a healthy state, as was evidenced by the investigators' ability to record resting membrane potentials in slices that were more negative than -60 mV (Li and McIlwain, 1957). By the mid 1960s they have established that brain slices maintain their synaptic potential, exhibit synaptic plasticity and are capable of recovering their synaptic function following drug-induced depolarization (Gibson & McIlwain, 1965; Yamamoto & McIlwain, 1966).

2. Preparation of rat hippocampal slices, their maintenance and their electrophysiology

2.1 The preparation and its maintenance

In all the experiments described in this chapter, adult (200-350 g) male Sprague-Dawley rats were used. For each experiment one rat was decapitated and its brain rapidly removed, rinsed with cold (6-8°C) artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl, 124; KCl, 5; NaH₂PO₄, 3; CaCl₂, 2.5; MgSO₄, 2.4; NaHCO₃, 23; glucose, 10 or as indicated. Then, the hippocampi were dissected out and were sliced transversely at 400 µm with a McIlwain tissue chopper. The resulting slices were transferred to a dual linear-flow interface chamber (Schurr et al., 1985). Twelve to 15 slices were placed in each compartment of the dual chamber. Each compartment had its own supply of aCSF via a two-channel peristaltic pump (40 ml/h) and a humidified gas mixture of 95% O₂/5% CO₂, which was circulated above the slices that were supported on a nylon mesh. The temperature in the incubation chamber was maintained at 34±0.5°C. For each experiment, the slices in one compartment were used as 'control', while those in the other as 'experimental'. The great advantage of this *in vitro* system is the ability of the experimenter to control and change the environmental conditions under which the slices are maintained, including the concentration of any of the aCSF components, the supplied gas atmosphere composition and the incubation temperature. Moreover, additional chemicals can be added either to the aCSF or to the gas mixture.

2.2 Electrophysiological measurements

Continuous extracellular recording of evoked population responses in the stratum pyramidale of the hippocampal CA1 region were made from one slice in each compartment of the dual incubation chamber using borosilicate micropipettes filled with aCSF (impedance = 2-5 MΩ). A bipolar stimulating electrode was placed in the Schaffer collaterals (orthodromic stimulation) in one slice in each compartment from which the recording was made. Stimulus pulses of 0.1 ms in duration and amplitude of 8-10 V were applied once per minute. A two-channel preamplifier (x 100) and two field-effect transistor head-stages were used and their output was fed into a waveform analysis program to determine the amplitude of the evoked response. Although only the electrophysiological response from one slice in each compartment was recorded continuously throughout any given experiment, all slices were tested for the presence of such response i.e., a population spike (PS) amplitude of ≥3 mV (neuronal function) prior to the beginning of the experiment. Slices exhibiting a population spike amplitude <3 mV were discarded. Recordings were begun 90 min after the preparation of the slices, allowing them to fully recover from the process. At the end of each experiment all the remaining slices in both compartments of the chamber

were tested again for the presence of neuronal function. Those slices exhibiting a PS amplitude <3 mV were considered non-functional due to the experimental manipulation(s). Figure 1 is an illustration of a rat hippocampal slice showing the neuronal circuits within it and the synaptic connections between axons of one neuronal population and dendrites of another.

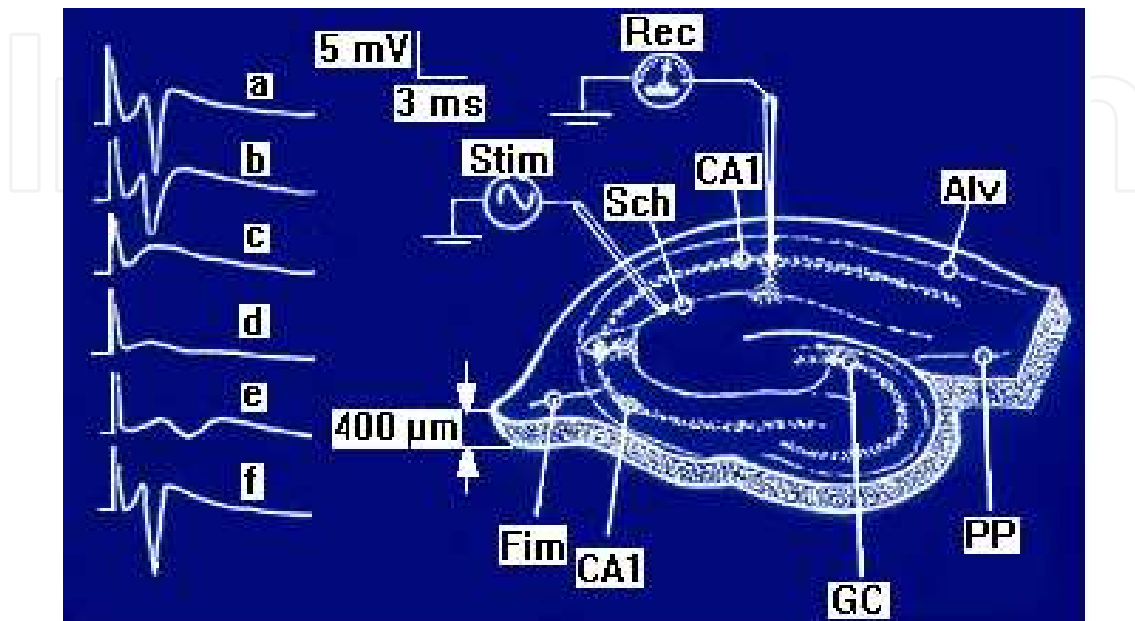


Fig. 1. A schematic illustration of a rat hippocampal slice. Shown are the typical positions where a stimulating (Stim) and a recording (Rec) electrodes were placed and a series of representative records (a-f) produced by stimulating the Schaffer (Sch) collaterals (orthodromic stimulation) to evoke a PS (neuronal function) in the CA1 region. When a given condition, which is necessary for normal maintenance of neuronal function, is removed and then replenished, the disappearance of the PS (b-c) and its recovery (d-f), respectively, is apparent. Similarly, the addition of a toxin and its removal may lead to the disappearance and the reappearance of the PS, respectively. Scales of amplitude (mV) and time (ms) are also shown. Additional abbreviations: CA1, cornu ammonis 1; CA3, cornu ammonis 3; Alv, alveus; PP, perforant path; GC, granule cells of the dentate gyrus; Fim, fimbria.

The presence of neuronal function (an orthodromically-evoked, CA1 PS) has been used as a sensitive and responsive signal to indicate both tissue viability and stability over time. Histological and morphological studies of hippocampal slices from which electrophysiological recordings were taken over time, confirmed that the tissue function was correlated with its structural integrity (Schurr et al., 1984). Hence, throughout all the experiments described in this chapter this neuronal function (PS amplitude ≥ 3 mV) was used to differentiate between functional (viable) and nonfunctional (damaged) hippocampal slices.

2.3 Statistical analysis

Each data point in every experiment described in this chapter was repeated at least three times. Values shown in the figures are mean \pm either SD (standard deviation of the mean) or

SEM (standard error of the mean). Statistical analysis for significant differences was performed using either the paired t test or the χ^2 test. A $P \leq 0.05$ was considered to be statistically significant.

3. A major dogma of ischemic brain damage sheds new light on brain energy metabolism

The 1981 inaugural issue of the Journal of Cerebral Blood Flow and Metabolism includes a review paper written by B.K. Siesjö, at the time, one of the leading researchers in the field of cerebral ischemia who studied the cellular and biochemical mechanisms of ischemic brain damage. Although Siesjö's article was, according to its title, "a speculative synthesis," it became one of the most cited papers in the field ever, receiving over 1,500 citations so far. This speculative synthesis was based, among others, on two research papers from Siesjö's own laboratory published in that very issue of the journal (Rehncrona et al., 1981; Kalimo et al., 1981). With the use of biochemical, neurophysiological and histopathological methodologies, Siesjö all but concluded that lactic acidosis is a major culprit in cerebral ischemia, responsible for the well-documented delayed neuronal damage observed post-ischemia. Quickly, the lactic acidosis hypothesis had immersed as the leading hypothesis in the field, highlighted in many other research papers, review articles, specialized books and textbooks. The hypothesis was accepted unopposed by almost all investigators in the field as the most plausible cellular mechanism that explains ischemic delayed neuronal damage. Although Siesjö's group was interested in the relationship between cerebral ischemic damage and acidosis for some years prior to the 1981 publications, a study by Myers and Yamaguchi (1977) was a major impetus for the formulation of Siesjö's speculative synthesis. Myers and Yamaguchi (1977) discovered that pre-ischemic hyperglycemia aggravates cerebral ischemia delayed neuronal damage, a finding that nestled nicely within the idea of 'higher blood [glucose] pre-ischemia = higher brain [lactic acid] during cerebral ischemia.' Consequently, to establish the hippocampal slice preparation as an adequate *in vitro* model for studying cerebral ischemia, we aimed to demonstrate that increased [glucose] in the aCSF prior to *in vitro* ischemia aggravates ischemic neuronal damage post-ischemia. Similarly, we also attempted to demonstrate that lactic acidosis aggravates ischemic neuronal damage in this model system, an approach difficult to duplicate *in vivo*. It is worth emphasizing that Siesjö's hypothesis was so entrenched in the annals of the field that hardly anyone questioned the paradoxical effect of elevated glucose concentration; why the only energy substrate the neural tissue is able to utilize in the absence of oxygen and which would avert ischemic damage, appears instead to increase the ischemic damage? Fittingly, this phenomenon has been thus termed "the glucose paradox of cerebral ischemia."

3.1 The effect of oxygen or glucose deprivation on neuronal function in hippocampal slices

Glucose is an essential component of the aCSF without which slices cannot survive. Early slicers have standardized an aCSF glucose concentration of 10 mM, twice the level considered to be isoglycemic *in vivo*. Since the slice preparation's supply of nutrients is dependent entirely on simple diffusion, this relatively high level of glucose in the aCSF

was established to assure ample supply of this energy substrate. Thus, when we first tested the sensitivity of neuronal function in hippocampal slices to O₂ deprivation (*in vitro* ischemia/hypoxia) we used the standard, 10 mM glucose in the aCSF. Oxygen deprivation or hypoxia in the slice preparation is achieved by simply replacing the supply of 95% O₂/5% CO₂ with 95% N₂/5% CO₂. Similarly, by exposing slices to aCSF depleted of glucose (0 mM glucose) an *in vitro* 'hypoglycemia' can be achieved. Fig. 2 shows the percentage of slices that exhibited recovery of neuronal function 30 min after depriving them for different durations of either O₂ or glucose (see also Schurr et al., 1989a). Neuronal function is clearly a sensitive measure of the effect of nutrient deprivation on the tissue. While O₂ was entirely depleted within 2 to 3 min from the moment it was replaced with N₂, an average 40-min was required for a complete depletion of glucose from the aCSF. Hence, the time slices were exposed to 0 mM glucose as shown in Fig. 2 is 40 + X min. It is obvious from the results shown in Fig. 2 that hippocampal slices are significantly more sensitive to 'hypoxia' than to 'hypoglycemia.' The data compiled in Fig. 2 are the percentages of slices that exhibited the presence of neuronal function after 30-min recovery period. A typical response of the PS continuously recorded from one slice during the experiment can be seen in Fig. 1, traces a-f. These traces represent normal neuronal function prior to O₂ deprivation (a), neuronal function at 3-min O₂ deprivation (b), at 5-min O₂ deprivation (c), at 10-min O₂ deprivation (d) at 10-min re-oxygenation (e) and at 30-min re-oxygenation. The results indicate that the hippocampal slice preparation is responsive to changes in both oxygen and glucose concentrations and was ready for its ultimate test that should establish whether or not this preparation is a worthy *in vitro* model of cerebral ischemia. Such a model should exhibit an aggravation of 'ischemic' neuronal damage with the elevation in [glucose] and a similar aggravation with increasing [lactic acid, pH]. These were our expectations based on the leading hypotheses of the time.

3.2 The effect of changing [glucose] on neuronal function in hippocampal slices post-hypoxia

In a series of experiments we then tested the effect of several glucose levels on the ability of hippocampal slices to recover their neuronal function from 10-, 15- or 20-min hypoxia. The results are shown in Fig. 3 (see also Schurr et al., 1987, 1989a). It is clear from these results that the higher the [glucose] in the aCSF, the longer the hypoxic period hippocampal slices tolerate. Whether the hypoxic period was short or long, elevating [glucose] had a neuroprotective effect against hypoxic damage, an effect completely opposite to the one expected and reported *in vivo* (Myers & Yamaguchi, 1977). Although understandably, we had some doubts about the slice preparation's suitability as an *in vitro* model of cerebral ischemia, at least from a biochemical stand point, increased [glucose] during hypoxia, when anaerobic glycolysis is the only metabolic pathway available for significant adenosine triphosphate (ATP) synthesis, should afford the tissue a longer survival time under such conditions. After all, an increase in glucose consumption under anaerobic conditions (Pasteur's Effect) suggests that the higher the glucose concentration, the longer the time it would last under anaerobic conditions. In light of this unexpected outcome, we were anxious to find out what would be the effect of lactic acidosis on neuronal function post-hypoxia.

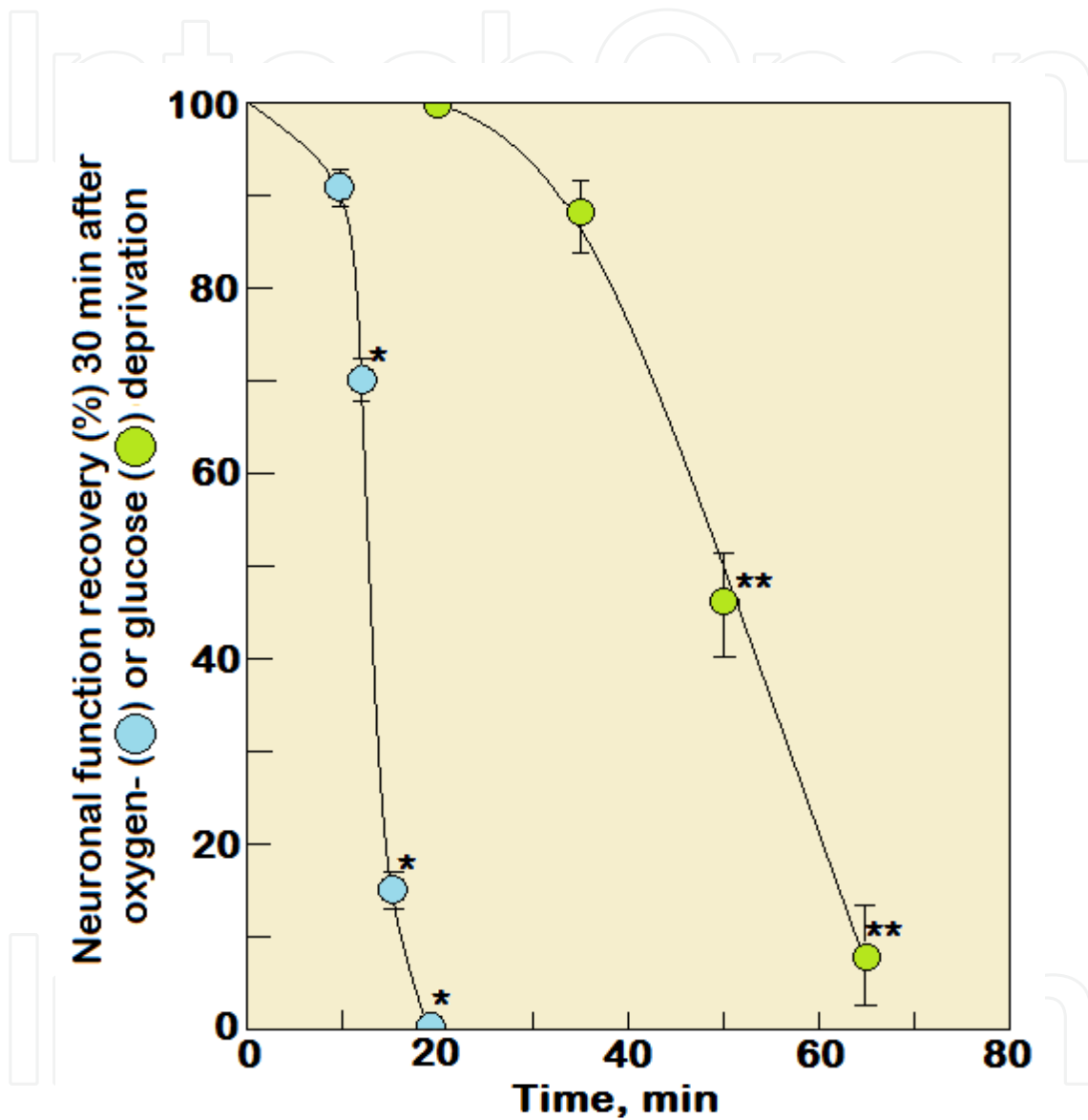


Fig. 2. The effects of different durations of O₂ or glucose deprivation on the recovery of neuronal function 30 min after the end of either deprivation in rat hippocampal slices. Neuronal function is much more tolerant to lack of glucose than to lack of oxygen (hypoxia). Data points are mean \pm SD; *significantly different from 10-min hypoxia ($P < 0.0005$); **significantly different from 20-min glucose deprivation ($P < 0.005$).

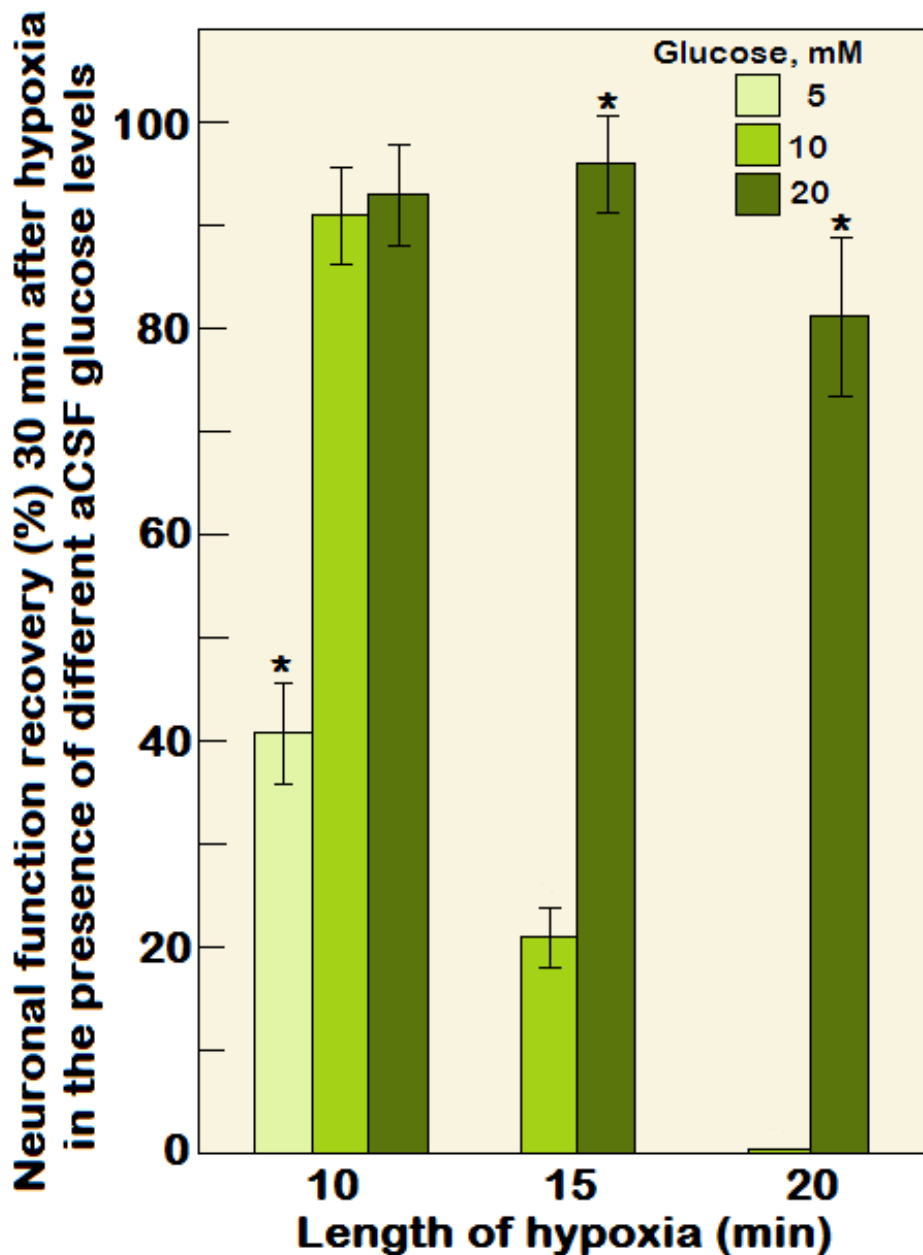


Fig. 3. The effect of elevated aCSF glucose levels during hypoxia on the ability of hippocampal slices to recover neuronal function 30-min post-hypoxia. The higher the glucose level during hypoxia, the better the recovery rate of neuronal function post-hypoxia. Data points are mean \pm SD; *significantly different from 10 mM glucose ($P < 0.0005$).

3.3 The effect of lactic acid on the recovery of neuronal function in hippocampal slices post-hypoxia

Hippocampal slices were first exposed to either 10 mM or 20 mM lactic acid for 30 min. The pH of the aCSF in each compartment of the dual chamber was measured shortly before slices were exposed to hypoxia (10, 12 or 15 min) in the presence of lactic acid. At the end of the hypoxic period, re-oxygenation ensued and the lactic acid aCSF was replaced with normal, glucose (10 mM) containing aCSF of physiological pH. At the end of a 30-min post-

hypoxic period, all slices were tested for the presence of neuronal function. The outcome of these experiments is summarized in Fig. 4. Surprisingly, lactic acidosis, at least at moderate pH values, appeared to be neuroprotective against hypoxic neuronal damage. Whether the hypoxic period was shorter (10 min) or longer (12 or 15 min), slices exposed to 30-min lactic acidosis (10 mM lactic acid, pH 6.8-6.9) prior to and during hypoxia, exhibited a decrease in the degree of neuronal damage compared to control slices (no lactic acid, 10 mM glucose, pH 7.30-7.34) or slices that were exposed to 20 mM lactic acid (pH 5.53-5.75). Thus, our *in vitro* model system of cerebral ischemia/hypoxia failed to demonstrate a detrimental effect of lactic acidosis on cerebral ischemic (hypoxic) neuronal damage (see also Schurr et al., 1988a). High lactic acid concentration (20 mM) seemed to only slightly worsen the post-hypoxic outcome when combined with 10-min hypoxia. Thus, at least where the lactic acidosis hypothesis of cerebral ischemic neuronal damage is concerned, the outcome of these experiments was unexpected. Also unexpected, although more plausible, were the results showing the beneficial effect of elevated glucose levels (section 3.2). In light of these results, we had to reevaluate not only the premise of the lactic acidosis hypothesis of cerebral ischemic damage, but also the usefulness of our *in vitro* system as a model of cerebral ischemia. The intriguing possibility that lactic acid is being utilized by neurons upon re-oxygenation, and thus, improved neuronal survival post-hypoxia, as the results in Fig. 4 suggested, led us to reconsider the fundamental principle of energy metabolism namely, that lactic acid (lactate) is an end-product of anaerobic glycolysis with no other function in energy metabolism (Huckabee, 1958). Consequently, our research branched into two avenues: one aiming at re-examining the role of lactate in cerebral energy metabolism, the branch with which this chapter deals, the other attempting to better understand the roles both glucose and lactate play in cerebral ischemic damage, if any.

3.4 Cerebral lactate – from a useless end product to a useful energy substrate

3.4.1 Hippocampal slices can utilize lactate as a sole energy substrate to support neuronal function

The results shown in Fig. 4, although hinting at the possibility that lactic acid could be utilized as an oxidative energy substrate, also indicate that an acidic pH is not optimal for such utilization. Thus, in all the experiments described henceforth, when indicated, Na-lactate, rather than lactic acid, was used as a component of the aCSF, while maintaining physiological pH (7.30-7.35). If neural tissue is capable of utilizing lactate as an energy substrate, the hippocampal slice preparation is probably one of the most suitable systems to test such capability. Fig. 5 illustrates a set of experiments performed with rat hippocampal slices, where their supply of normal, glucose-containing aCSF (10 mM glucose) was replaced with 0 mM glucose aCSF supplemented with 0, 1, 2, 5, 10 or 20 mM lactate. The osmolality of the aCSF was kept at 330 mOsm by adjusting the NaCl concentration. A lactate supplementation of 2 mM or higher was enough to maintain normal neuronal function in a great majority of the slices. This breakthrough demonstration that neural tissue is capable of maintaining normal function when lactate is the sole energy substrate, was published in detail almost a quarter of century ago (Schurr et al. 1988b). In that publication it was also demonstrated that oxidative lactate utilization by hippocampal slices is insensitive to the glycolytic inhibitor iodoacetic acid, which indicates that lactate enters the mitochondrial tricarboxylic acid (TCA) cycle directly via its conversion to pyruvate by lactate dehydrogenase, not through the glycolytic pathway. Needless to say, these findings have

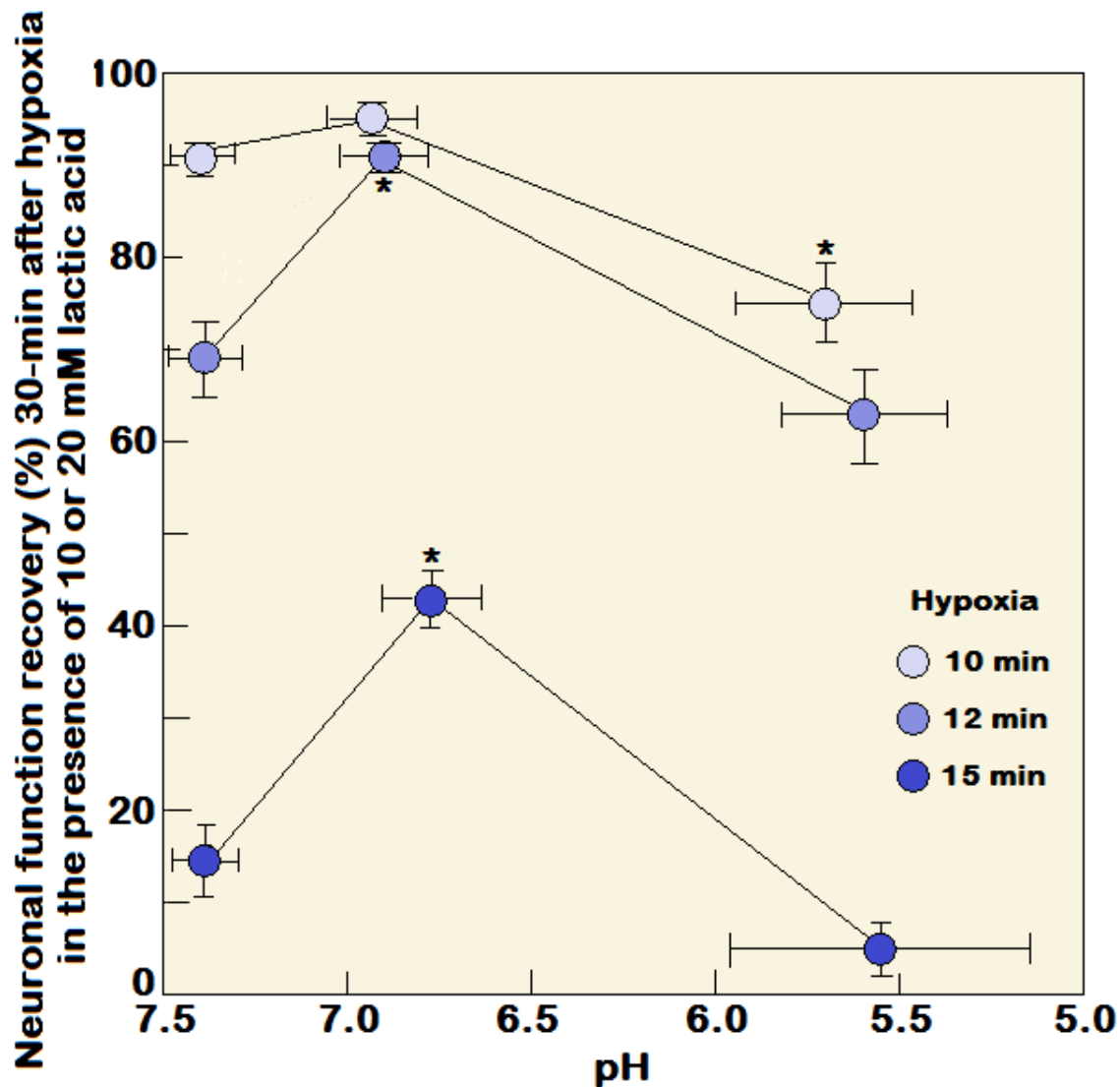


Fig. 4. The effects of lactic acidosis combined with hypoxia on the ability of hippocampal slices to recover their neuronal function 30 min post-hypoxia. At pH values approaching those found in the ischemic brain *in vivo* i.e., 6.5-6.9, lactic acidosis significantly improve the recovery of neuronal function at 12- and 15-min hypoxic durations. At lower pH values, values that are rarely reached *in vivo* (5.5-5.7) some aggravation of neuronal damage was observed, but only with the shorter (10 min) hypoxic period. Bars are means \pm SD; *significantly different from control slices supplied with normal, glucose-containing aCSF, pH 7.3-7.35 ($P < 0.0005$).

challenged the long-established dogma of lactate's fate in energy metabolism and thus have faced much skepticism and even ridicule. Clarke & Sokoloff (1994) actually recognized the ability of brain slices, homogenates or cell-free fractions to utilize lactate, pyruvate and other compounds as energy substrates, but have listed several reasons why they cannot be considered to be significant alternatives to glucose *in vivo*. Consequently, the prevailing notion at the time among the majority of scientists in the field had been that lactate utilization by brain slices is an *in vitro* oddity without any significant ramifications for the *in vivo* situation. Obviously, our findings were not, in any way, meant to challenge the leading role glucose plays in normal aerobic energy metabolism.

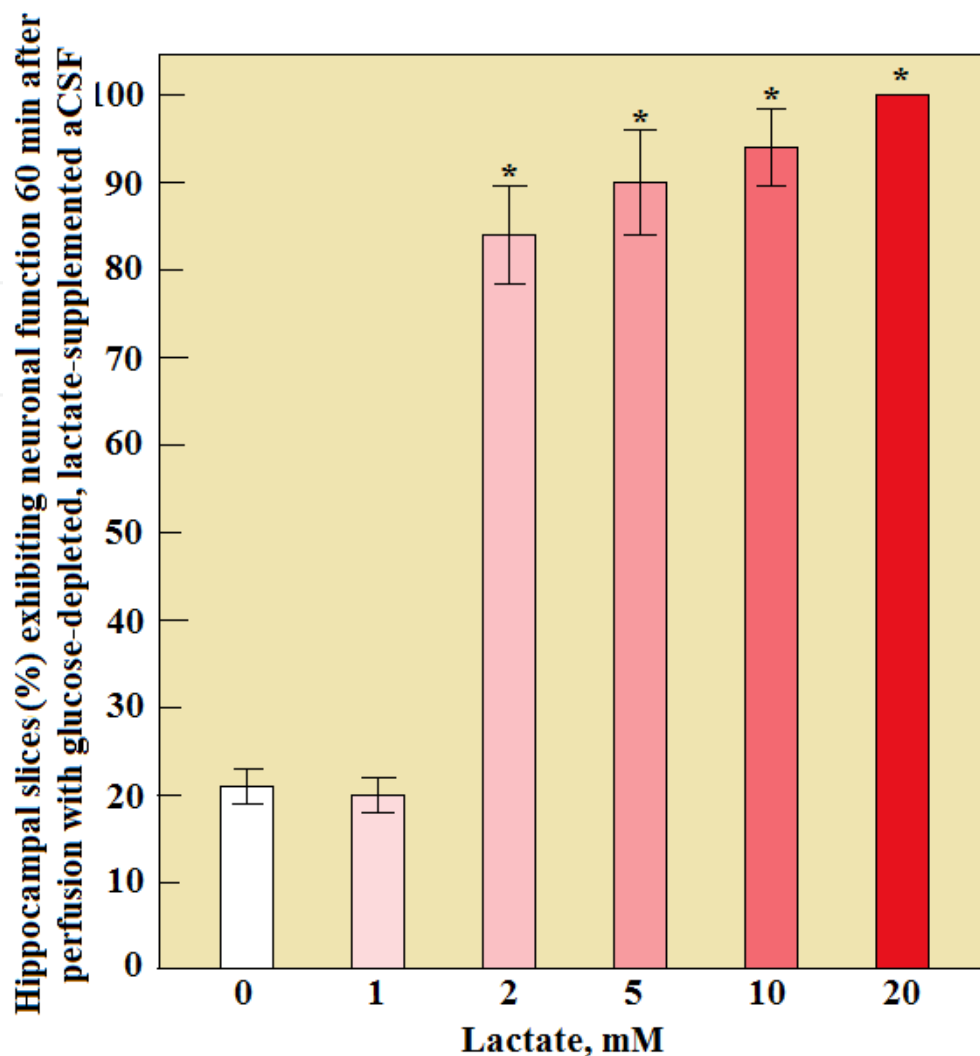


Fig. 5. The ability of hippocampal slices to maintain neuronal function in glucose-depleted, lactate-supplemented aCSF over a period of 60 min. Twenty percent of the slices supplemented with 0 or 1 mM lactate-aCSF still exhibited neuronal function after 60 min, but could not maintain it for an additional 30 min. Bars are means \pm SD; *significantly different from 0 mM lactate-aCSF ($P < 0.0005$).

3.4.2 Lactate utilization post-hypoxia is crucial and obligatory for recovery of neuronal function

The demonstration that neural tissue is capable of utilizing lactate aerobically, invited a look into the possible role that this monocarboxylate plays in the brain under conditions where glucose is limited or unavailable. One such possible condition is the utilization of lactate accumulating in cerebral tissue during hypoxia/ischemia, upon re-oxygenation, when tissue glucose and ATP levels are very low. The hippocampal slice preparation again proved itself to be a most adept system, allowing manipulations that are impossible *in vivo* to test the role lactate plays in the recovery of neuronal function upon re-oxygenation post-hypoxia. The picture that emerged from this set of experimental paradigms also confirmed the original finding that neural tissue can function normally when lactate is the sole energy

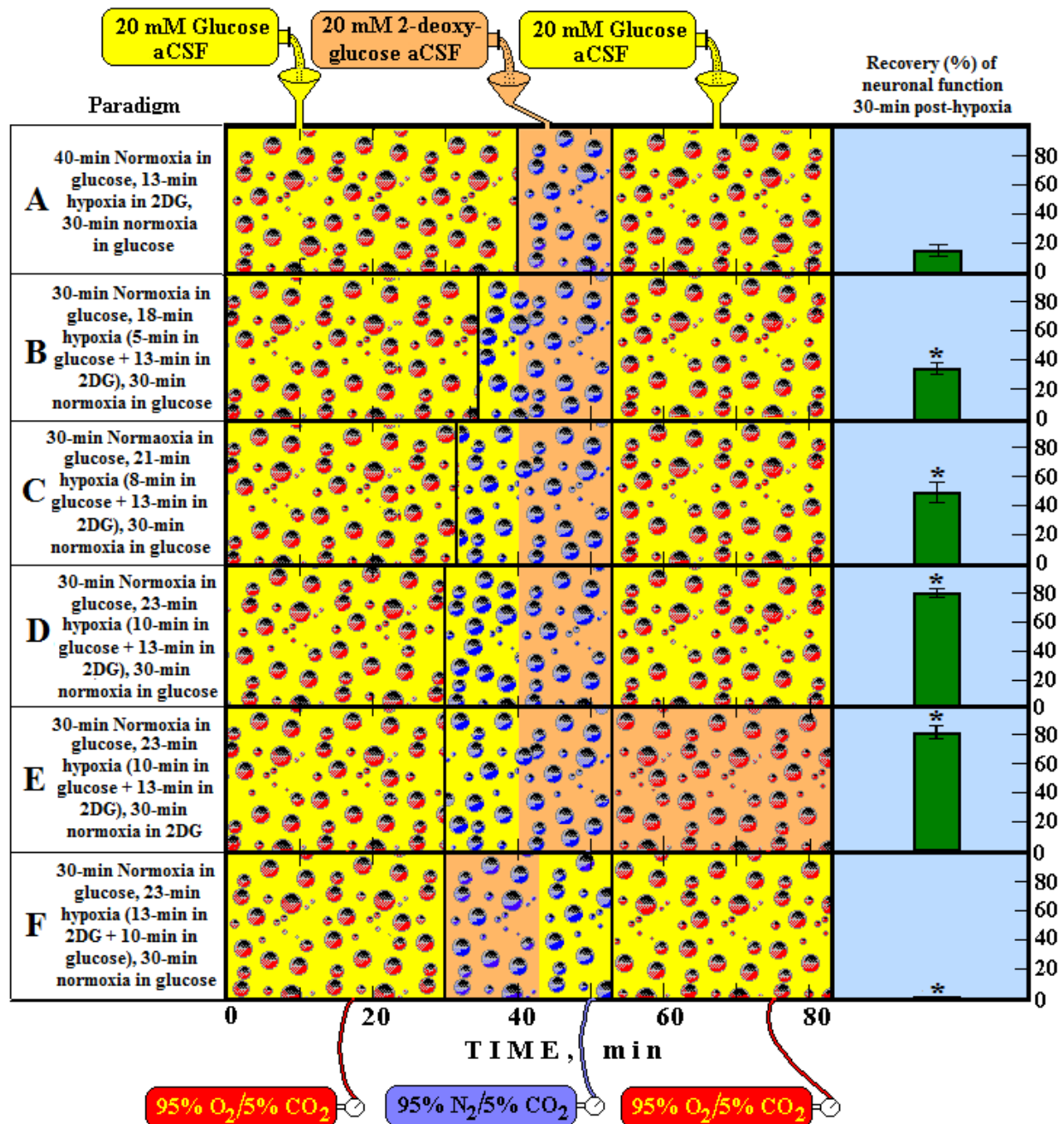


Fig. 6. A schematic representation of six different experimental paradigms using rat hippocampal slices and electrophysiological recording of CA1 evoked population spikes (PS, neuronal function). In each experimental paradigm, slices were supplied either with 20 mM glucose-aCSF (yellow bottle) or 20 mM 2-deoxy glucose (2DG)-aCSF (orange bottle) and the gas mixture bubbled through the dual incubation chamber and the aCSF was either 95% O₂/5% CO₂ (normoxia, red bubbles) or 95% N₂/5% CO₂ (hypoxia, blue bubbles). At the end of each experimental paradigm, all slices in each compartment of the dual chamber were tested for the presence of neuronal function. Functional slices are shown as percentage of the total number of slices present (green histograms on the right). Accordingly, by following the timeline from left to right, paradigm A is a protocol in which slices were incubated under normoxic conditions for 40 min, followed by 13-min hypoxia in the presence of 2DG and

then re-oxygenated for 30 min in the presence of glucose. Under these conditions less than 20% of the slices recovered their neuronal function at the end of the 80-min protocol. Similarly, each of the remaining paradigms (B-F) describes its corresponding protocol and its outcome in terms of percentage of slices exhibiting neuronal function. Bars are means \pm SD; *significantly different from paradigm A ($P < 0.0005$).

substrate (Fig. 5; Schurr et al., 1988b). In all six paradigms a relatively high glucose level (20 mM) was used that allowed a long enough hypoxic insult without causing a significant damage to the tissue (Fig. 3) yet, had produced measureable levels of anaerobic tissue lactate (Fig. 7). Supplying hippocampal slices with the glucose analog 2-deoxy glucose (2DG) during a 13-min hypoxia, an insult that slices incubated in 20 mM glucose aCSF can easily handle (Fig. 3), resulted in a dismal recovery of neuronal function (<20%, paradigm A). Such outcome would be expected, since the tissue was unable to produce glycolytic ATP anaerobically from 2DG, which meant that slices were actually suffering from a combined glucose-oxygen deprivation, an insult much harsher than the deprivation of one or the other. In addition, slices treated according to paradigm A were not able to produce much lactate during hypoxia (Fig. 7). In paradigm B slices were exposed to 18-min hypoxia, the first 5 min of which was continued to be supplied with 20 mM glucose aCSF before changing that supply to 20 mM 2DG aCSF for the remaining 13 min of the hypoxic period. Thus, slices in paradigm B produced lactate for 5 min before the replacement of glucose with 2DG. Although slices in paradigm B were exposed to hypoxia 38% longer than those in paradigm A, a significantly higher percentage of slices in the former recovered their neuronal function at the end of the 30-min re-oxygenation period than in the latter. Similarly, paradigms C and D included even longer hypoxic periods than the hypoxic period in paradigms A and B, yet, the recovery rate of neuronal function in paradigms C and D was significantly higher than that seen in paradigms A or B. Based on earlier experiments (Fig. 3) it would be expected that slices supplied with relatively high level of glucose (20 mM) would tolerate 20-min hypoxia almost unscathed. Considering the outcomes of paradigms B-D it is clear that most of the recovery of neuronal function observed in each of these paradigms was due to the ability of the incubated slices to produce lactate during hypoxia via the glycolytic pathway, which was utilized aerobically during the re-oxygenation period to bring about recovery of neuronal function. Moreover, the longer glucose-supplied slices were allowed to produce lactate during hypoxia, the higher was the recovery rate of neuronal function post-hypoxia. Thus, doubling the time slices were exposed to hypoxia in the presence of 20 mM glucose from 5 min (paradigm B) to 10 min (paradigm D) more than doubled the recovery rate of neuronal function, from 35% to 80%, respectively. The outcome in slices treated according to paradigm E demonstrates that even when glucose supply during re-oxygenation was not renewed (2DG supplementation continued throughout the re-oxygenation period), recovery of function was possible solely on lactate produced during the first 10 min of hypoxia. When the production of lactate was blocked by 2DG supplementation at the onset of hypoxia, none of the slices could recover neuronal function upon re-oxygenation (Paradigm F). Hence, the outcome of the above described experiments (Fig. 6) strongly suggests that, during hypoxia, anaerobic lactate production is crucial for the recovery of neuronal function post-hypoxia. While glucose is clearly an obligatory energy substrate during periods of oxygen deprivation, as glycolysis is the only pathway available for the production of enough ATP to assure the survival of ischemic/hypoxic neural tissue for at least a few minutes, the experiments above show that

the oxidative utilization upon re-oxygenation of the anaerobically produced lactate is obligatory for the recovery of the tissue post-hypoxia (Schurr et al., 1997a, b). On one hand, paradigm F in Fig. 6 clearly indicated that without glucose present during the initial stages of hypoxia, the neural tissue suffered a fatal damage and could not recover its neuronal function even when glucose was present during the latter part of the hypoxic period and the re-oxygenation period. On the other hand, paradigm E in Fig. 6 indicated that the presence of glucose during hypoxia in relatively high levels protected neural tissue from fatal damage, while the lactate produced during hypoxia allowed almost a full recovery of neuronal function in the absence of glucose during the re-oxygenation period. These conclusions are supported by results of lactate content analysis in hippocampal slices at several time points during their exposure to normoxic and hypoxic conditions in the presence of glucose or 2DG. Fig. 7 illustrates that the level of lactate produced in hippocampal slices during normoxia is minimal, but steady and similar to the *in vivo* situation. However, upon the initiation of hypoxia, the levels of tissue lactate immediately begin to rise as long as glucose is available, increasing more than 5-fold within 10 min (paradigm D). In the presence of 2DG, the rise in lactate level was insignificant (paradigm F). Concomitantly with the suggestion that lactate is an obligatory energy substrate for recovery of neuronal function post-hypoxia, we also postulated that following a severe hypoxic period that would completely exhaust glucose and ATP supplies, functional recovery would be entirely dependent on ATP that can be produced via a pathway that bypasses glycolysis, since glycolysis is a metabolic pathway that requires ATP investment if glucose is to be utilized. Lactate aerobic utilization via its conversion to pyruvate by lactate dehydrogenase (LDH) allows a direct entry of pyruvate into the mitochondrial tricarboxylic acid (TCA) cycle. This process does not require any ATP investment and can be resumed instantaneously upon re-oxygenation in the presence of lactate. As effective as 2DG was in blocking glucose utilization (Figs. 6 & 7), 2DG is ineffective in blocking or affecting in anyway the utilization of lactate. To expand our investigation into the possible role lactate may play in other cerebral functions, a “blocker” of lactate utilization was needed. The lactate transporter inhibitor, α -cyano-4-hydroxycinamate (4-CIN) is one such blocker (Halestrap & Denton (1975). Lactate and other monocarboxylates, such as pyruvate and β -hydroxybutyrate, are known to be transported via a facilitated diffusion process, similar to the one glucose is being transported across biological membranes. Although these processes always proceeds along the concentration gradient, they do involve the mediation of specific transporters and in the case of lactate a monocarboxylate transporter (MCT), of which several types have been identified (Garcia et al., 1994; Garcia et al., 1995; Gerhart et al., 1997; Broer et al., 1997; Volk et al., 1997). First, 4-CIN was able to block lactate-supplemented slices from exhibiting evoked CA1 population spike without affecting neuronal function of glucose-supported slices (Fig. 8). This simple experiment demonstrated the potential of 4-CIN in blocking the MCT responsible for transporting lactate into cells that utilize it aerobically for the production of ATP. Clearly, blockade of MCT does not interfere with glucose transport or its utilization, including any lactate that may be produced during glucose metabolism. This important ability of 4-CIN to discriminate between glucose and lactate utilization provided not only a great experimental tool, but also helped us in sorting several mechanistic issues, as will be discussed later. First, to assess 4-CIN potential in a familiar paradigm, slices were treated with the blocker in a set of experiments where hypoxia was used to induce accumulation of lactate, which we have shown to be used preferentially post-hypoxia. Fig. 9 illustrates the effects of 4-CIN on both the ability of hippocampal slices to recover their neuronal function after 10-min hypoxia

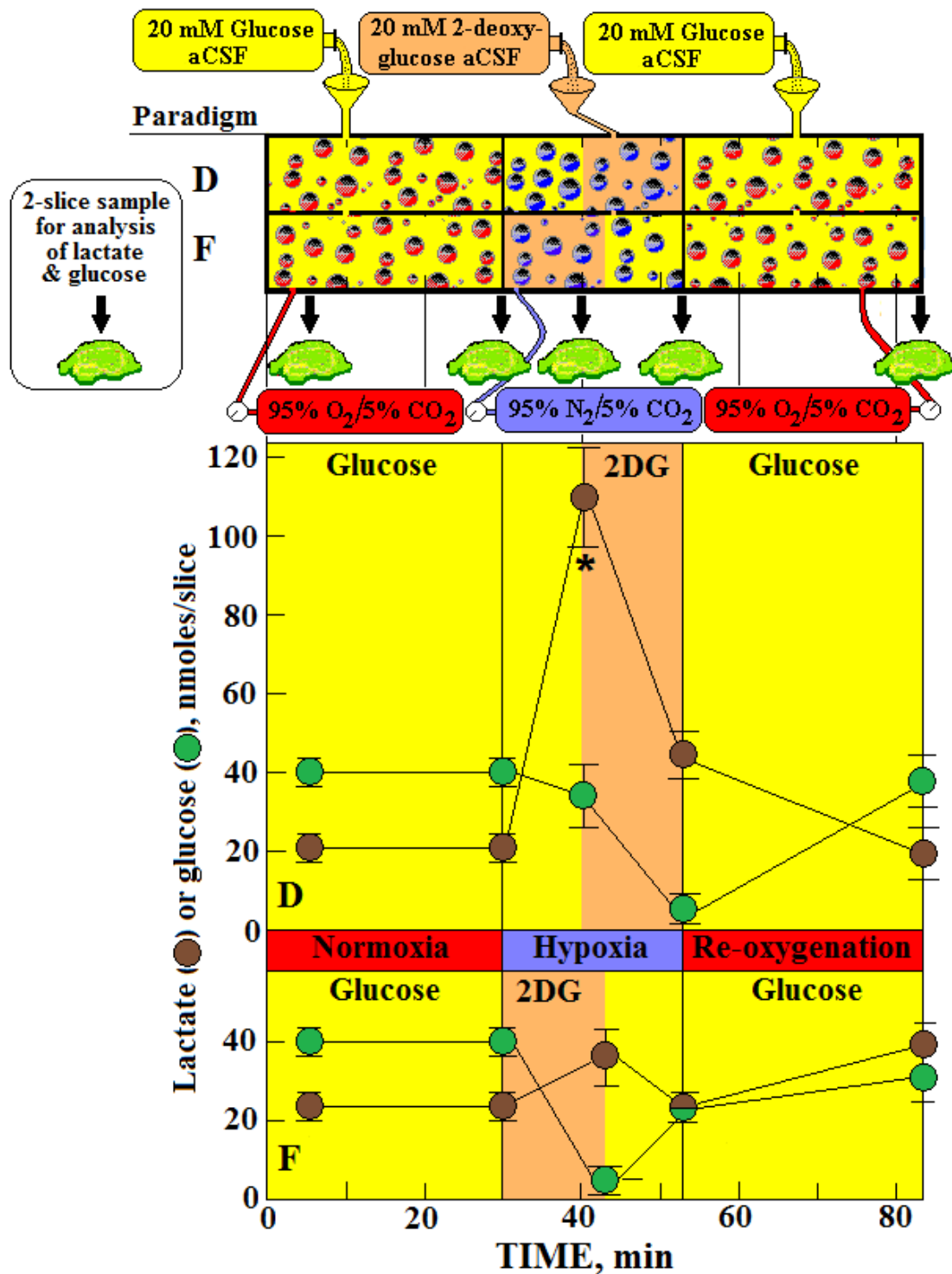


Fig. 7. The levels of lactate and glucose (nmoles/slice), as determined by using enzymatic kits (Schurr et al., 1997a), during the experimental paradigms D and F detailed in Fig. 6. Allowing slices to utilize glucose anaerobically during the first 10 min of a 23-min hypoxia resulted in an over 5-fold increase in tissue lactate content. Changing the supply of glucose to 2DG at the very beginning of a 23-min hypoxia blocked the ability of hippocampal slices to produce lactate via anaerobic glycolysis. Bars are means \pm SD; *significantly different from normoxic lactate level ($P < 0.05$).

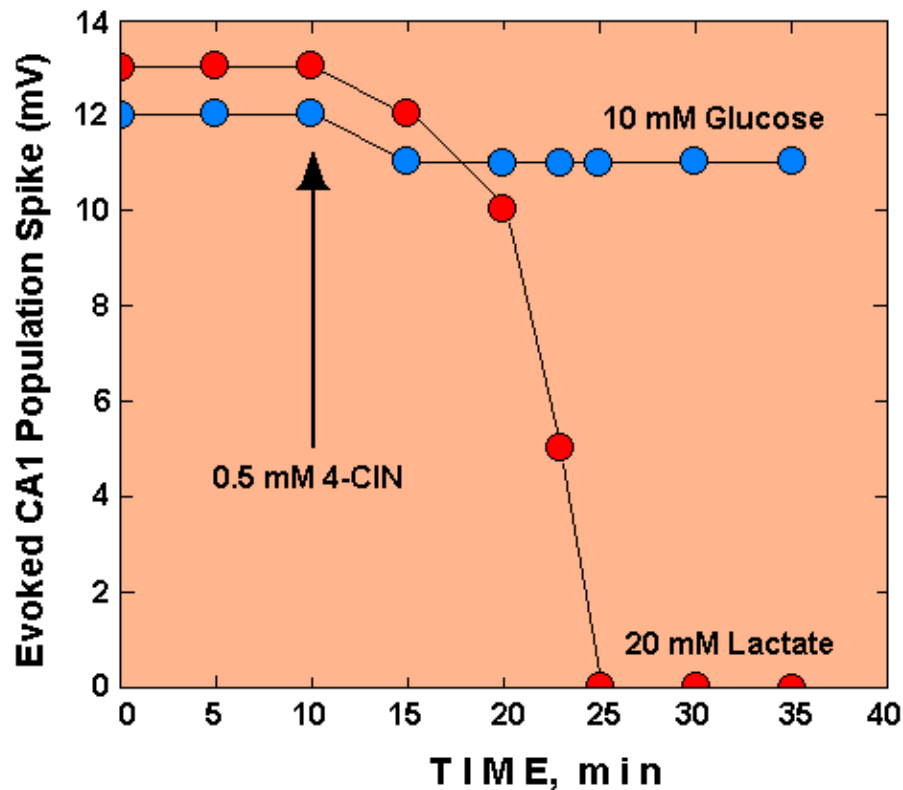


Fig. 8. The effect of adding 0.5 mM α -cyano-4-hydroxycinnamate (4-CIN) to the aCSF in which hippocampal slices were incubated on their ability to exhibit an evoked CA1 population spike. Slices incubated in 10 mM glucose aCSF were unaffected by 4-CIN. Slices incubated in 20 mM lactate could not maintain their evoked response in the presence of 4-CIN, as the inhibitor blocked the transport of lactate into neurons and/or out of astrocytes.

and on tissue levels of lactate and glucose at several time points during the experimental protocol. The inhibition of lactate transport by 4-CIN significantly attenuated the recovery of neuronal function post-hypoxia, most likely by blocking the entry of lactate, which accumulated during the hypoxic period, into cells and possibly mitochondria upon re-oxygenation. This blockade is evident in the post-hypoxic period by the higher lactate tissue content measured in slices treated with 4-CIN. The transporter blocker had no effect on the levels of tissue glucose at any time during the experimental protocol in comparison to control conditions (lack of 4-CIN). Although not shown, a CA1 evoked population spike was completely abolished, as expected, during the hypoxic period. Considering the results shown in both Fig. 8 and Fig. 9, it was concluded that 4-CIN could be used as a chemical tool in the investigation of additional possible roles lactate may play in neural tissue by potentially interfering with any cellular process that requires this monocarboxylate. In 1994, Pellerin & Magistretti hypothesized that the excitation of neurons by the excitatory neurotransmitter, glutamate (Glu), is coupled to its uptake by astrocytes, an uptake that induces aerobic glycolysis, which is responsible for the observed increase in glucose consumption during brain activation. They further hypothesized that the product of this elevated aerobic glycolysis in astrocytes is lactate, which is shuttled from astrocytes to neurons where it is aerobically consumed. This hypothesized transfer of lactate from the former to the latter was named the astrocytic-neuronal lactate shuttle hypothesis or ANLSH

(Pellerin & Magistretti, 1994). The hippocampal slice preparation is an excellent model system for testing the premise of the ANLSH.

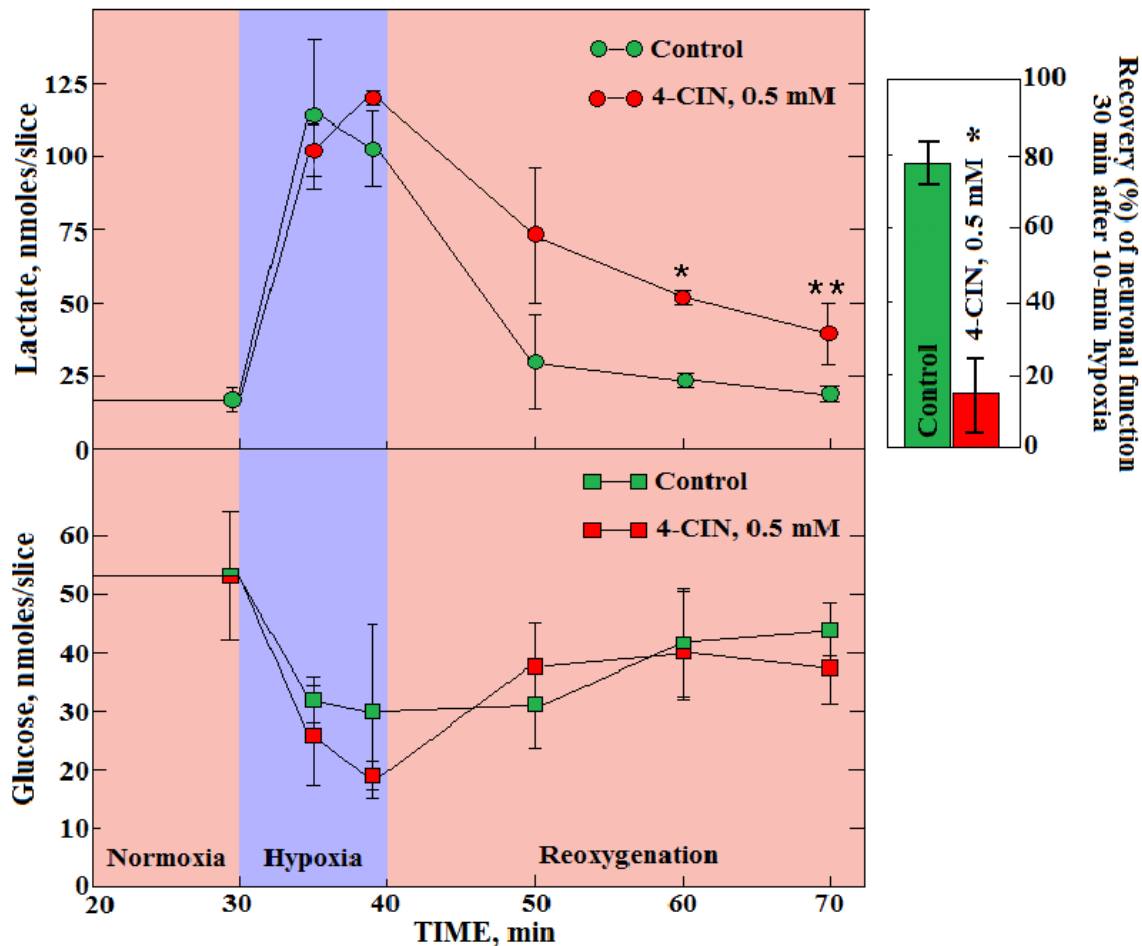


Fig. 9. The effects of α -cyano-4-hydroxycinnamate (4-CIN) on the recovery of neuronal function post-hypoxia in hippocampal slices and the levels of lactate and glucose in these slices at several times points along the experimental protocol. Glucose concentration in the aCSF was 10 mM. Normoxia was achieved by supplying slices with 95% O₂/5% CO₂. Hypoxia was created by replacing the normoxic atmosphere with 95% N₂/5% CO₂. Bars are means \pm SD; *significantly different from control ($P < 0.0005$); **significantly different from control ($P < 0.05$).

3.4.3 Lactate as an aerobic energy substrate for excited neurons

In testing the potential role of lactate in neuronal activation by glutamate, only relatively small changes were made in the experimental paradigms that already proved themselves useful when hypoxia was the insult applied to neurons (Figs 6, 7, 9). Supplying hippocampal slices with aCSF containing high, excitotoxic concentrations of Glu is similar in its detrimental effect on neurons to that of hypoxia. Actually, Glu's excitotoxicity has been suggested to be part of the hypoxic/ischemic mechanism of neuronal damage (Olney, 1969, 1990; Novelli et al., 1988, Choi, 1988; Siesjö, 1988; Schurr & Rigor, 1989; Henneberry, 1989; Cox et al., 1989; Choi & Rothman, 1990). Figure 10 summarizes the results of two sets of

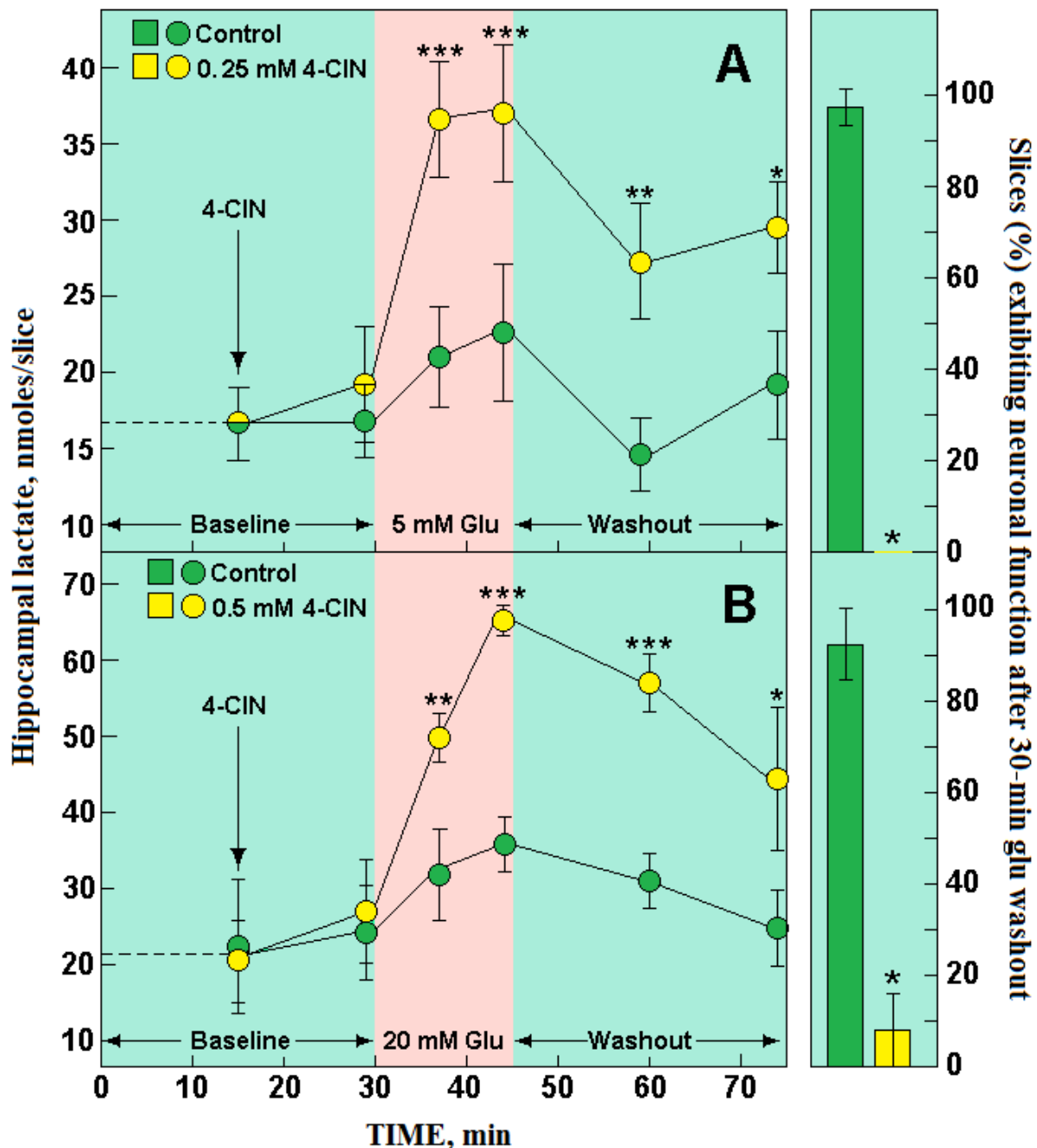


Fig. 10. The effect of 15-min exposure to glutamate (Glu) on the ability of hippocampal slices to recover their neuronal function following a 30-min Glu washout and on the content of tissue lactate at several time points during the two experimental paradigms used: (A) Perfusion of slices with 4 mM glucose aCSF for 30 min, followed by a 15-min exposure to 5 mM Glu, followed by a 30-min washout with 4 mM glucose aCSF, either in the presence of 0.25 mM 4-CIN (yellow symbols) or in the absence of 4-CIN (green symbols); (B) perfusion of slices with 10 mM glucose aCSF, followed by a 15-min exposure to 20 mM Glu, followed by a 30-min washout with 10 mM glucose aCSF in the presence of 0.5 mM 4-CIN (yellow symbols) or in the absence of 4-CIN (green symbols). Bars are means \pm SD; significantly different from control (* $P < 0.003$; ** $P < 0.01$; *** $P < 0.004$).

experimental paradigms in which exposure to Glu for a given period of time was used as an excitatory/excitotoxic event similar to the way hypoxia was used in earlier experiments described above. In paradigm A, glucose concentration in the aCSF (4 mM) was lower than in paradigm B (10 mM) and thus slices in the former were exposed to 5 mM Glu as compared with slices in the latter that were exposed to 20 mM Glu. Just as with hypoxia, the higher the glucose concentration in the aCSF, the higher concentration of Glu slices could tolerate. Moreover, the higher the glucose in the aCSF, the higher the level of lactate slices produced during exposure to Glu. In each paradigm, 4-CIN was used to block lactate transport by MCT. The blocker concentration was adjusted according to the concentration of glucose; 0.25 mM 4-CIN when glucose concentration was 4 mM, 0.5 mM 4-CIN when glucose concentration was 10 mM. Blockade of MCT by 4-CIN prevented the recovery of neuronal function after 15-min exposure to Glu in both paradigms. Although not seen in Fig. 10, it is important to mention that under the experimental conditions in both paradigms a CA1 population spike could not be evoked during the 15-min exposure to Glu regardless of the presence or absence of 4-CIN, similar to when slices were exposed to hypoxia. Glu at these levels is clearly excitotoxic, but the relatively short time of exposure (15 min) is not long enough to cause an irreversible damage, as over 90% of the slices under control conditions in both paradigms exhibited almost full recovery of neuronal function. However, in the presence of 4-CIN, none of the slices exhibited recovery of neuronal function when supplied with 4 mM glucose and exposed to 5 mM Glu for 15 min (Paradigm A). Less than 10% of the slices showed recovery of neuronal function when supplied with 10 mM glucose and exposed to 20 mM Glu for 15 min (paradigm B). While lactate content of control slices did not appear to be elevated much during Glu exposure in either paradigm, it is probably indicative of how fast lactate is being produced upon exposure to Glu in astrocytes and how quickly it is being utilized aerobically by neurons. The significant elevation in lactate tissue content of slices treated with 4-CIN during Glu exposure explains why lactate unavailability to neurons in these slices prevented recovery of neuronal function at the end of washout period in both paradigms. Since lactate could not be utilized in the presence of 4-CIN, its tissue content remained elevated throughout the washout period. As expected, the content of lactate in slices supplied with 10 mM glucose during Glu exposure in the presence of 4-CIN was almost twice as high as the content in slices supplied with 4 mM glucose. The results shown in Fig. 10 thus support the premise of the ANLSH and are schematically summarized in Fig. 11. The outcome of the experiments with Glu above (see also Schurr et al., 1999a, b) strongly indicates that the activation of neural tissue with an excitatory neurotransmitter increases the utilization of glucose via aerobic glycolysis and a large elevation in lactate production. The only difference between the effects of Glu and hypoxia on neural tissue is that in the former, lactate is produced in the presence of oxygen, while in the latter it is produced in the absence of oxygen. In both cases, glycolysis is the main pathway to provide the extra energy required to assure survival of the neural tissue during either insult. Moreover, as indicated by the effects of 4-CIN in the presence of Glu or hypoxia, the glycolytic product, lactate, is the oxidative energy substrate that secures the recovery of neuronal function post-Glu activation or post-hypoxia. In the debate that has ensued after Pellerin & Magistretti (1994) proposed the ANLSH (Tsacopoulos & Magistretti, 1996; Magistretti, 1999, 2000; Magistretti et al., 1999; Pellerin & Magistretti, 2003, 2004a, b; Chih & Roberts, 2003; Hertz, 2004; Schurr, 2006) the skeptics have tended to reject the hypothesis mainly on its premise that lactate is postulated to be a major oxidative energy substrate, not on the proposed lactate shuttle *per se*. The debate is not limited to the ANLSH;

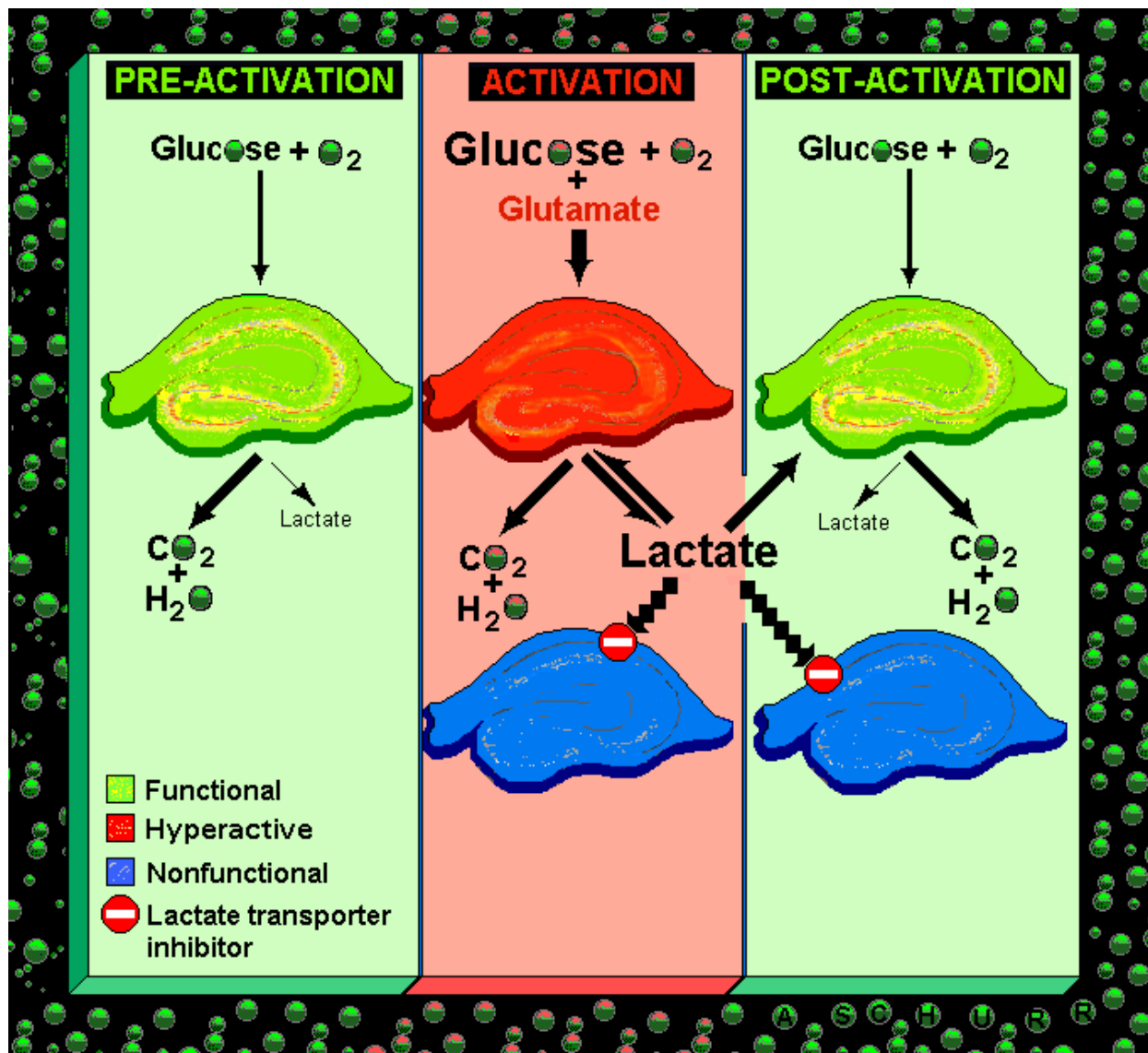


Fig. 11. A schematic representation of the biochemical events that took place in the experiments shown in Fig. 10. The first panel on the left (green) depicts a neuronally functional hippocampal slice (green-yellow) under resting baseline conditions, supplied with glucose and oxygen and produces mainly CO_2 , H_2O and a minimal amount of lactate. The middle panel (red) depicts the same hippocampal slice when exposed to Glu (red), which increases aerobic glucose utilization and lactate production. When this hippocampal slice is also treated with a monocarboxylate transporter inhibitor that prevents lactate from being transported from astrocytes to neurons, the slice cannot remain functional (blue). The panel on the right (green) depicts the hippocampal slice (green-yellow) of the upper middle panel from which Glu has been washed out, allowing its neuronal function to recover and its energy production to return to baseline conditions. Also shown is a slice (blue) that was treated with a monocarboxylate transporter inhibitor during the exposure to Glu and thus could not recover its neuronal function despite Glu washout.

it is still raging about a similar hypothesis of lactate shuttles in other tissues, which preceded the ANLSH and for the same reason (see Gladden, 2004, for review). The objection to the idea of a major role for lactate in energy metabolism beyond just being a waste

product or, at best, a minor player, is understandable. The dogma of glucose's obligatory role in energy metabolism in all tissues, organs and most aerobic organisms is an inseparable part of our understanding and acceptance of this process as formulated during the first half of the 20th century. However, experimental data have emerged over the past quarter of a century, all pointing to a major role for lactate in oxidative energy metabolism in brain, skeletal muscle, heart and probably many other mammalian tissues. These data (Brooks, 1985, 1998, 2000, 2002a, b; Schurr et al., 1988b, 1997a, b, 1999a, b; Larrabee, 1995, 1996; Hu & Wilson, 1997; Brooks et al., 1999; Mangia et al., 2003; Kassischke et al., 2004; Ivanov et al., 2011) in addition to several forgotten studies from the first half of the 20th century (Ashford & Holmes, 1929, 1931; Holmes, 1930; Holmes & Ashford, 1930; Flock et al., 1938), impelled me to hypothesize that lactate is the ultimate cerebral oxidative energy substrate in the brain (Schurr, 2006) and possibly in other organs and tissues. The electrophysiological experiments described here have contributed enormously to the formulation of this hypothesis. The ability to continuously monitor the function of neural tissue throughout each experiment and its many replications allowed us to confidently state what we understood to be the meaning of these experiments' outcome. The other studies cited here, each with its own methodology, only strengthen the foundation on which the interpretation of the experimental results was made and, consequently, the hypothesis herewith was postulated.

Cytosolic aggregate of glycolytic enzymes, substrates and products

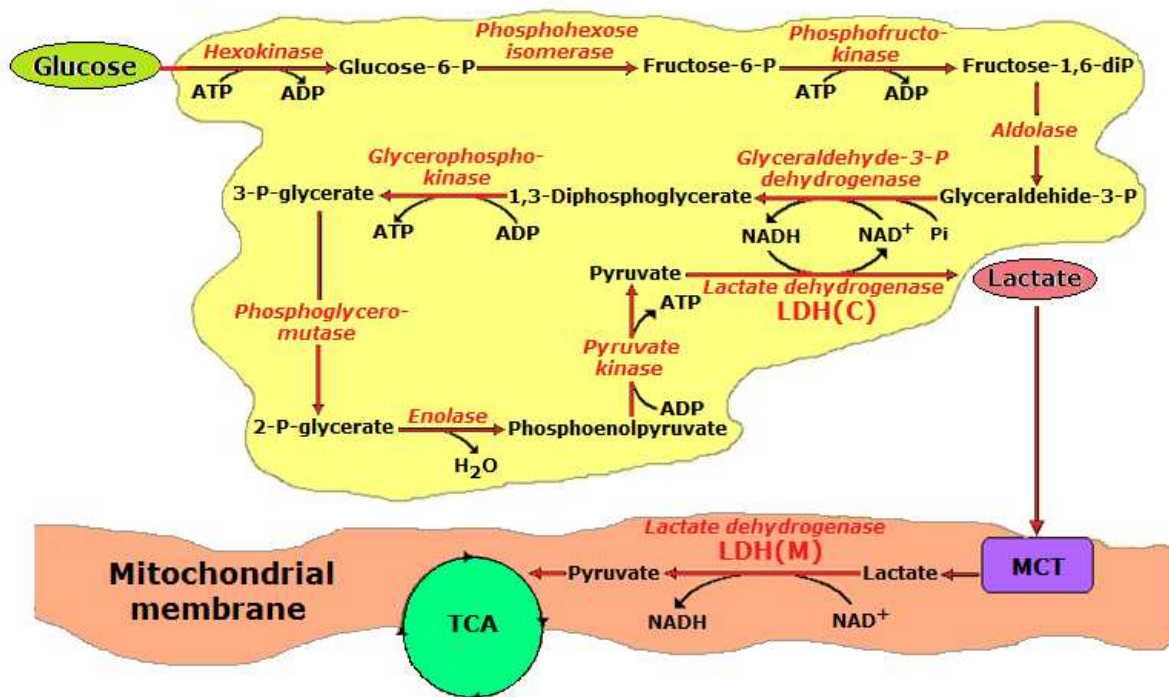


Fig. 12. A schematic illustration of a hypothesis first presented elsewhere (Schurr, 2006), postulating lactate, not pyruvate, to be the end product of aerobic glycolysis. This hypothesis is founded on thermodynamic and other considerations, including data from many studies spanning almost eight decades of research by scientists in laboratories all around the world. The illustration shows the glycolytic apparatus as an aggregate of the glycolytic enzymes, all in close proximity to each other, as required by such a pathway for a

maximized efficient output, with the entry of glucose into this pathway on the upper left side and an exit of lactate from the pathway on the right side through the action of cytosolic lactate dehydrogenase [LDH(C)]. Lactate is shuttled via an intracellular shuttle to the mitochondrial membrane, where it is converted back into pyruvate via mitochondrial lactate dehydrogenase [LDH(M)] and into the tricarboxylic acid (TCA) cycle.

3.4.4 Lactate, not pyruvate, is neuronal aerobic glycolysis end-product

Although the role of lactate in energy metabolism, both in the adult brain and other organs and tissues is controversial, more and more studies have produced an increasing amount of data in support of such a role, especially under certain conditions. Over the twenty odd years of using the hippocampal slice preparation and its electrophysiology and in consideration of the accumulated data from other studies during that period, the concept of lactate as a major player in brain energy metabolism seems to gather steam; not only under certain conditions, such as post-hypoxia or during neural activation, but as an integral intermediate of the normal cellular process of glucose conversion to ATP i.e., aerobic glycolysis and oxidative phosphorylation. Therefore, a hypothesis was put forward according to which in the brain (and possibly in other organs and tissues) lactate is the principal product of both aerobic and anaerobic glycolysis (Schurr, 2006). This hypothesis is schematically illustrated in Fig. 12. Additional evidence to support it could be provided by an efficient and specific lactate dehydrogenase inhibitor(s). The availability of enzymatic inhibitors in the study of the roles of enzymes, their substrates and products in any given metabolic pathway is paramount for the elucidation and understanding of these roles. For years, the absence of specific and efficient inhibitors for the two LDH-catalyzed reactions, the conversion of pyruvate to lactate in the cytosol by LDH(C), and the conversion of lactate to pyruvate by the mitochondrial form of the enzyme, LDH(M), has greatly impeded the assessment of lactate's role in energy metabolism. According to the prevailing dogma, pyruvate is the main end product of aerobic glycolysis. If specific and efficient LDH inhibitors exist, [X inhibitor for LDH(C) and Y inhibitor for LDH(M)], one could predict that, according to the prevailing dogma, glucose-supported neuronal function in a fully oxygenated hippocampal slice preparation would not be affected by either inhibitor, since LDH (either C or M form) does not participate in the conversion of glucose to pyruvate. However, if lactate, according to the above-mentioned hypothesis (Fig. 12), is the end product of aerobic glycolysis or if it is supplied exogenously as the sole energy substrate, neuronal function would be suppressed by inhibitor Y, but not by inhibitor X. Although the latter would inhibit lactate formation from glucose, it should not prevent glucose conversion to pyruvate and therefore, its utilization by mitochondria. Practically, all known LDH inhibitors, inefficient as they may be, exhibit both X and Y inhibitory capabilities, although with different potencies toward one reaction or the other. Hence, an inhibitor showing stronger Y than X inhibitory capability should be innocuous in inhibiting pyruvate-supported neuronal function yet, be able to suppress lactate-supported neuronal function. Moreover, if lactate is the end product of aerobic glycolysis, as the hypothesis postulates, then, glucose-supported neuronal function should be suppressed by an LDH inhibitor with $Y > X$ inhibitory capability to the same degree that it would suppress lactate-supported neuronal function, since it should inhibit the LDH(M) form. However, because such an inhibitor can also inhibit the LDH(C) form, although to a lesser and slower extent, one could expect that, after a while, pyruvate would begin to accumulate, becoming the main product

of glycolysis. Consequently, over time, pyruvate would, essentially, become the main glycolytic product and therefore the main substrate for the mitochondrial TCA cycle, an occurrence that should fully or partially relieve the initial suppression of neuronal function. Hence, the availability of a specific, efficient LDH inhibitor would clarify lactate's role in cerebral aerobic energy metabolism. Recently, malonate, a known competitive inhibitor of succinate dehydrogenase, has been shown to inhibit the conversion of lactate to pyruvate (Saad et al., 2006). Malonate is a dicarboxylate that is transported into neurons, astrocytes and mitochondria via the same transporter that transports succinate (Yodoya et al., 2006; Aliverdieva et al., 2006). With the availability of malonate we were able to perform a series of electrophysiological experiments aimed at testing the hypothesis that the end product of aerobic glycolysis in cerebral tissue is lactate (Schurr & Payne, 2007). In this set of experiments, we used much lower concentrations of the different energy substrates, as compared to other experiments described above, although sufficient for sustenance of normal neuronal function. It was verified by a separate set of experiments that 2.5 mM glucose, 5 mM lactate and 5 mM pyruvate (equicaloric concentrations) were all able to sustain similar and healthy evoked population spike (PS) amplitudes (data not shown). These "low" concentrations of the energy substrates that are closer to their *in vivo* levels were chosen in order to "sensitize" the hippocampal slice preparation to the effects of both malonate and Glu, while allowing also the use of relatively low malonate (10 mM) and Glu (2.5 mM) concentrations. As can be seen from Fig. 13, malonate (M) drastically attenuated the amplitude of lactate-supported evoked hippocampal CA1 PS, as can be seen from the two representative traces before (L) and after 75-min exposure to malonate (L + M). As predicted, lactate-supplemented slices were clearly susceptible to malonate, exhibiting a time-dependent diminishment in PS amplitude, while malonate was innocuous when slices were supplemented with pyruvate (P, P + M). Thus malonate appears to be an efficient inhibitor of the LDH(M) form, inhibition that slow down significantly the conversion of lactate to pyruvate, resulting in the suppression of neuronal function. Since pyruvate can directly enter the mitochondrial TCA cycle, as it is transported by the same transporter of lactate (MCT), pyruvate-supported neuronal function is unaffected by malonate. Furthermore, initially, glucose-supplemented slices, exhibited suppression of neuronal function by malonate (45', G + M), a suppression that was relieved later on (75', G + M). This outcome is the very scenario that was predicted above; malonate, being an LDH inhibitor with stronger inhibitory potency toward LDH(M) than toward LDH(C), would initially block mainly the conversion of lactate to pyruvate, strongly suggesting that lactate is eventually the end product of aerobic glycolysis, as expressed by the partial suppression of neuronal function (45', G + M). However, being also an inhibitor of LDH(C), over time, malonate would also inhibit that form of the enzyme, blocking the conversion of pyruvate to lactate in the glycolytic pathway, bringing about the accumulation of pyruvate and its utilization by the mitochondrial TCA cycle, as expressed by the recovery of neuronal function (75', G + M). Hence, these results support the hypothesis that postulates lactate to be the end product of cerebral aerobic glycolysis. These results also confirm that, for lactate to be utilized aerobically, it must first be converted to pyruvate via the LDH(M). One additional set of experiments was performed (Schurr & Payne, 2007), which provided further support for the major role lactate plays in cerebral energy metabolism (Fig. 14). This set tested the combined effect of Glu and malonate and emphasized the importance of lactate as a mitochondrial energy substrate for the maintenance of neuronal function during neural activation, whether lactate originates in neurons or astrocytes. That malonate is

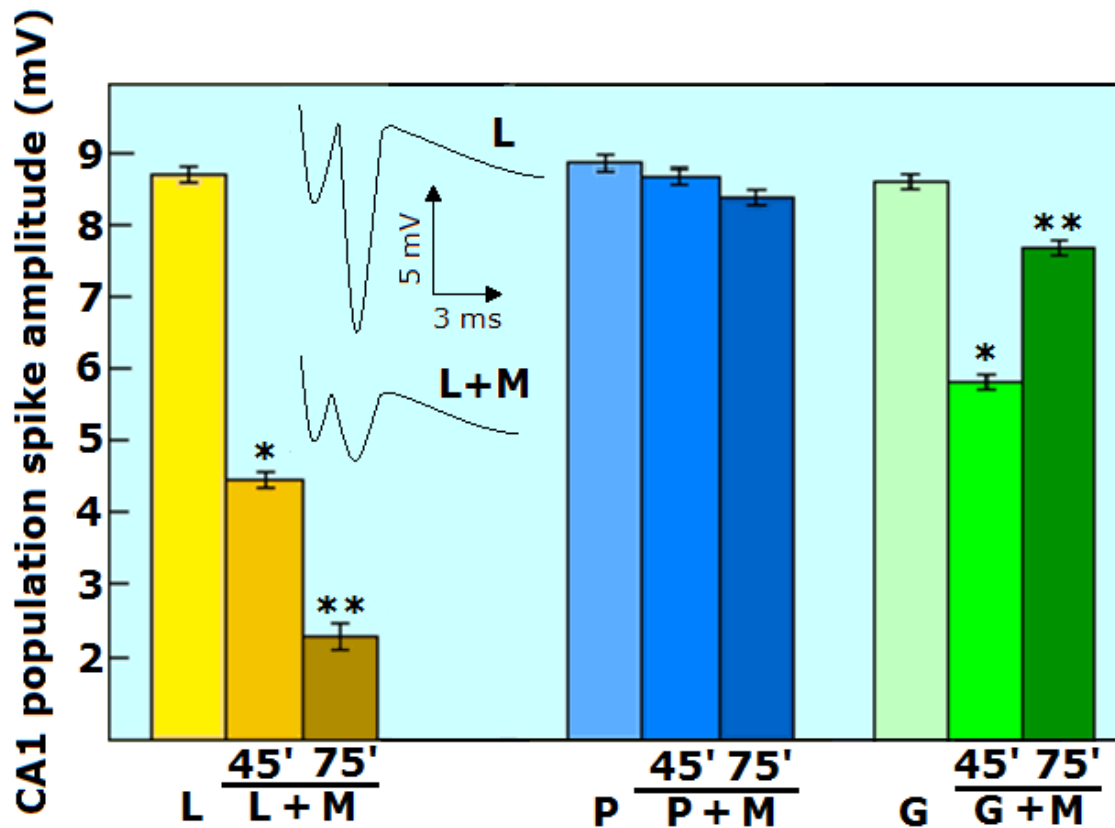


Fig. 13. The effect of the LDH inhibitor, malonate (M, 10 mM) on the evoked CA1 population spike amplitude (neuronal function) of rat hippocampal slices maintained in aCSF containing either lactate (L, 5 mM), pyruvate (P, 5 mM) or glucose (G, 2.5 mM). Malonate progressively inhibited lactate-supported neuronal function over time and was innocuous against pyruvate-supported neuronal function. Malonate initially inhibited glucose-supported neuronal function, inhibition that was later mostly relieved. Bars are means \pm SEM; * significantly different from energy substrate alone; **significantly different from energy substrate or energy substrate + malonate at 45 min ($P < 0.0001$).

detrimental to neuronal viability of glutamate-activated, glucose-supported hippocampal slices is apparent from the partial recovery (50%) of the energy-dependent PS amplitude (Fig. 14). This outcome indicates that lactate, whether neuronal or astrocytic in origin, is crucial for neuronal viability upon excitation. Alternatively, the outcome of these experiments may be explained by postulating an increase in astrocytic glucose consumption and lactate production in response to glutamate uptake, whereupon lactate becomes a major neuronal energy substrate (Pellerin & Magistretti, 1994; Schurr et al., 1999). Any interference with neuronal utilization of astrocytic lactate under this scenario i.e., LDH inhibition by malonate, would suppress normal neuronal function. However, such suppression should be overcome if astrocytes, while incapable of producing lactate in the presence of malonate, would produce enough pyruvate, which would be sufficient, upon Glu washout, to fuel neuronal function recovery, as observed in slices supplied with exogenous pyruvate (Fig. 14). Nevertheless, such recovery did not occur, indicating that astrocytic pyruvate, if it had been produced during exposure to Glu and malonate, is not readily available to neurons, in contrast to astrocytic lactate and neuronal pyruvate. Why is 2.5 mM glucose, in contrast to

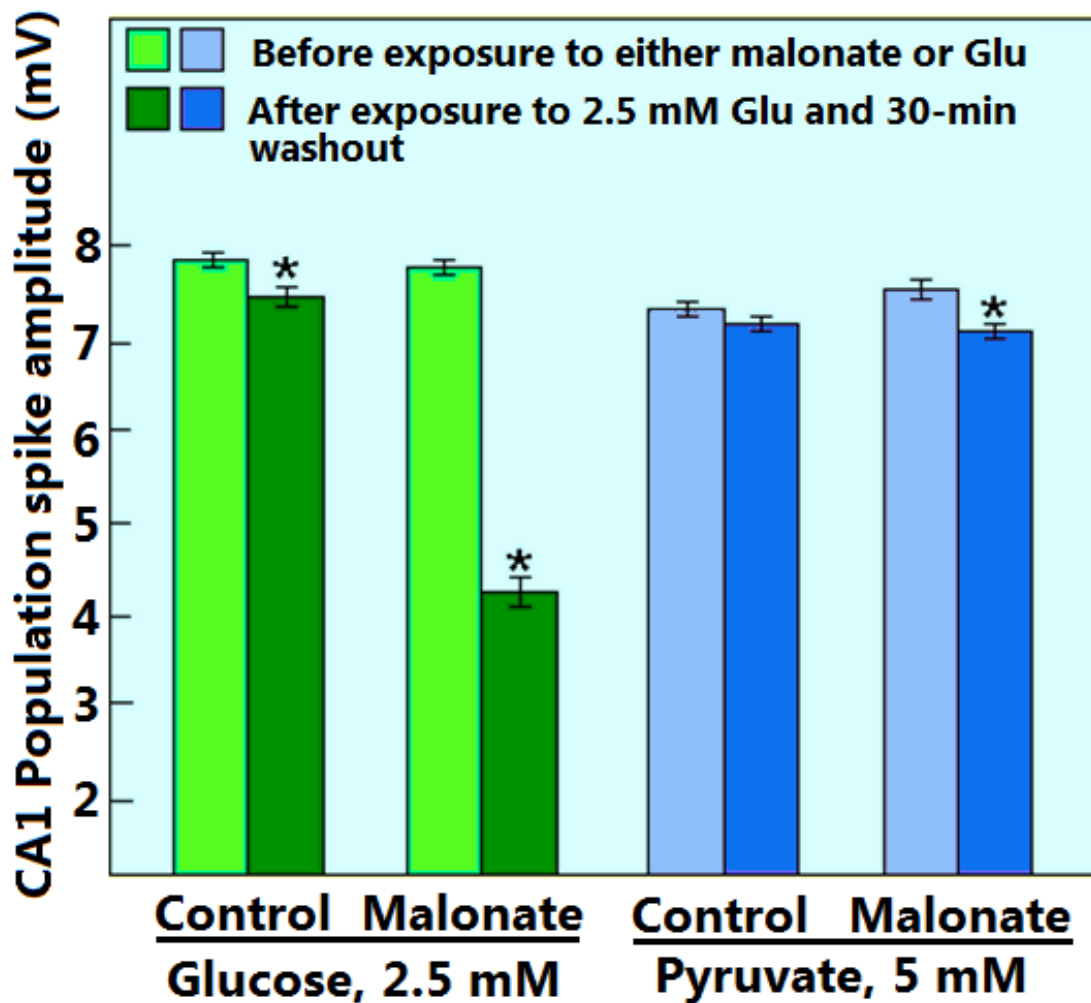


Fig. 14. The effect of 20-min exposure to glutamate (Glu, 2.5 mM) on hippocampal CA1 evoked population spike (PS, neuronal function) in the absence or presence of the LDH inhibitor, malonate (10 mM) when either glucose (2.5 mM) or pyruvate (5 mM) was the sole energy substrate. Slices maintained in glucose-aCSF could not recover their PS amplitude following Glu washout in the presence of malonate as compared to those maintained in the absence of malonate or those maintained with pyruvate whether malonate was absent or present. Bars are means \pm SEM; *significantly different from the mean values before exposure to either malonate or Glu ($P < 0.01$).

5 mM pyruvate, was unable to sustain neuronal viability in slices treated both with malonate and Glu? It is possible that not all the available glucose is converted to pyruvate under these conditions. However, a more compelling possibility is that glucose cannot increase the rate of mitochondrial respiration under aerobic conditions, while lactate, and most likely 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, an increase in lactate concentration did elevate mitochondrial oxygen consumption, plausibly allowing mitochondria to meet heightened energy demands. Consequently, glucose-supplemented and oxygenated hippocampal slices are incapable of increasing their mitochondrial respiration rate in response to activation by Glu, since they are unable to up-

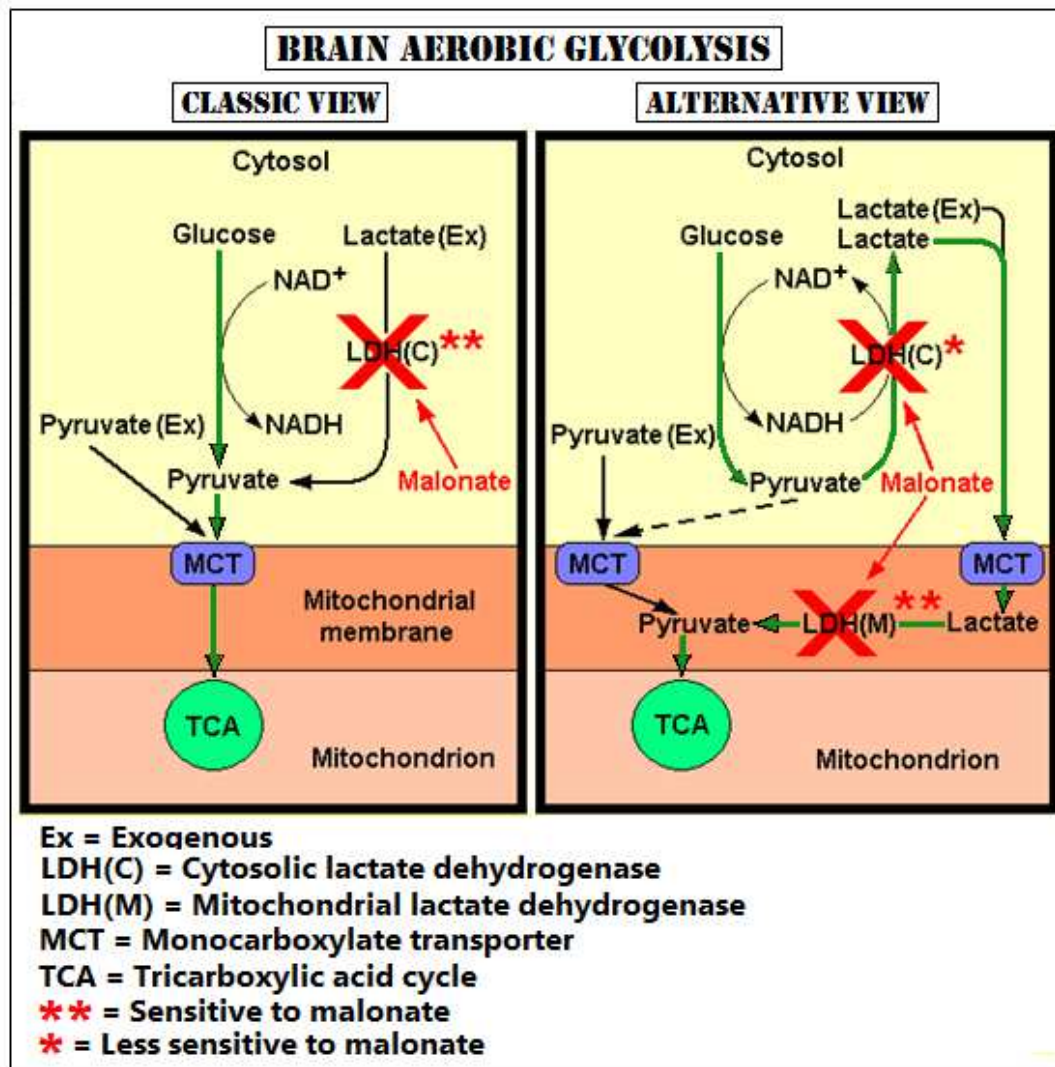


Fig. 15. Two views of aerobic glycolysis. The classic view depicts pyruvate as the glycolytic pathway's end product (green arrows, left panel) and thus as the pathway that should not be affected by an LDH inhibitor such as malonate in supplying pyruvate to mitochondria. The results shown in Figs. 13 & 14 cannot be explained by this view. The alternative view of the aerobic glycolytic pathway postulates lactate to be its end product (green arrows, right panel, Schurr, 2006). Since glycolytically-produced lactate must be converted to pyruvate to allow the latter to enter the TCA cycle, this alternative view explains the ability of malonate to interfere with glucose-supported neuronal function as shown in Fig. 13 (right side histograms). 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. 13).

regulate their glycolytic flux. More recently, Ivanov et al. (2011) have demonstrated that lactate can cover the energy needs of activated neonatal hippocampal slices and that lactate utilization augmented oxidative phosphorylation. Normally, both neuronal and astrocytic lactate produced from glucose would overcome this limitation (see Fig. 14, control glucose,

2.5 mM), but in the presence of malonate this avenue is unavailable (Fig. 14, malonate, glucose, 2.5 mM). Obviously, exogenous lactate would be useless in the presence of malonate, which is the reason why results with lactate-supported slices are not shown. Nonetheless, the results of the experiments that combined Glu and malonate in slices supplemented with 2.5 mM glucose (Fig. 14) suggest that under this condition there is an augmented shortfall in lactate (or pyruvate) supply. This outcome emphasizes the importance of lactate both at rest and during neural activation. These results (Fig. 14) cannot be explained by the classic depiction of aerobic glycolysis (Fig. 15, left panel) however, the hypothesis that lactate is the end product of aerobic glycolysis (schurr, 2006) could be a suitable explanation (Fig. 15, right panel). Therefore, it was concluded that the results, as shown in Figs. 13 & 14, support this hypothesis and signal that lactate is the most plausible mitochondrial energy substrate in the brain (and possibly in other organs and tissues), especially under neural activation.

4. Conclusion

The original intent of employing the rat hippocampal slice preparation and its electrophysiology in our laboratory was to establish an *in vitro* model system of cerebral ischemia/hypoxia. It has proved itself to be an excellent model system for that purpose. However, some key experiments have yielded unexpected results that have challenged conventional dogmas and “common knowledge.” These unexpected results, a repeating theme throughout the history of science and its major discoveries, have spurred debates and reexamination of those dogmas.

The present chapter describes the more provocative of those results, dealing with cerebral energy metabolism and the role lactate plays in these most important and basic cellular pathways. As important, considering the purpose of the book of which this chapter is a part, the electrophysiology of the hippocampal slice preparation has been its most important aspect in making it one of the most versatile *in vitro* systems in neuroscience today. The experiments described in this chapter demonstrate this versatility. The continuous electrophysiological monitoring of neuronal function offered an immediate assessment of the influence of experimental manipulations and the determination of the final outcome of each of these manipulations. Our journey began taking the necessary steps to establish this *in vitro* model adequacy for the purpose of answering outstanding questions in the field of cerebral ischemia/hypoxia. Naturally, the intention for any model is to produce the expected outcome based on the most established, yet current information that exists in the field. A failure to produce the expected outcome may question the usefulness of the model. In this respect, the hippocampal slice preparation had not always produced what was expected. Although where the study of cerebral ischemia/hypoxia has been concerned, the hippocampal slice preparation has led to some of the more important findings in this field, it has been the unexpected results produced by this system that have propelled it to its high place among all *in vitro* systems. The sheer number of papers and books written in which the brain slice preparation was used or where the preparation had been the topic described and discussed, attests to its scientific success. Nevertheless, without its electrophysiology, the preparation would have never inherited the place it occupies today in neuroscience research. The list below is a summary of the experiments described in this chapter, their outcome and their possible contribution to our understanding of cerebral energy metabolism:

1. Hyperglycemia does not worsen ischemic/hypoxic neuronal damage beyond the damage seen in isoglycemia, contrary to the perception advanced among researchers in the field in the last two decades of the 20th century. Moreover, increased tissue glucose concentration provided an increased tolerance against oxygen deprivation (Fig. 3). These experiments have led to the reexamination of the *in vivo* studies that reported glucose's detrimental effect (Myers & Yamaguchi, 1977), reexamination that found the interpretation of those results questionable (Schurr, 2001; Schurr et al., 2001; Payne et al., 2003).
2. Lactic acidosis, a hypothesized culprit in the worsening ischemic/hypoxic neuronal damage by hyperglycemia was found to be not only innocuous in this respect, but, just as it was shown for elevated glucose levels, lactic acid, too, was found to provide neuroprotection against the ischemic/hypoxic insult (Fig. 4). This unexpected outcome has presented strong evidence to refute the lactic acidosis hypothesis of cerebral ischemic damage and has been the driving force behind the renewed interest and active research in the field of cerebral energy metabolism.
3. Demonstrating that lactate is an excellent oxidative energy substrate for neural tissue has been a breakthrough discovery that can be specifically attributed to the employment of the hippocampal slice preparation and its electrophysiology in these experiments (Fig. 5). It took an additional six years for the published results (Schurr et al., 1988b) to receive significant confirmation (Pellerin & Magistretti, 1994). Since then, a large number of studies have been published that contain growing body of evidence in support of the original discovery.
4. Questioning the importance of neural tissue's ability to utilize lactate as the sole oxidative energy substrate to support its neuronal function, some have dismissed it as an *in vitro* aberration (Clarke & Sokoloff, 1994). Nevertheless, in a series of experiments (Figs. 6-11), while employing the glucose analog 2DG or the MCT inhibitor 4-CIN, the crucial role of endogenously-produced lactate in the recovery and/or sustenance of neuronal function post-hypoxia or during and after neuronal excitation has been demonstrated.
5. Lastly, a hypothesis was offered (Schurr, 2006) based on the experimental results described here and on many findings by others, according to which lactate, not pyruvate, is the end product of aerobic glycolysis (Fig. 12). The hypothesis was put to the test in a set of experiments illustrated in Figs. 13-15, using a newly discovered lactate dehydrogenase (LDH) inhibitor, malonate. The outcome of these experiments has provided support for the premise of this hypothesis (Schurr & Payne, 2007).

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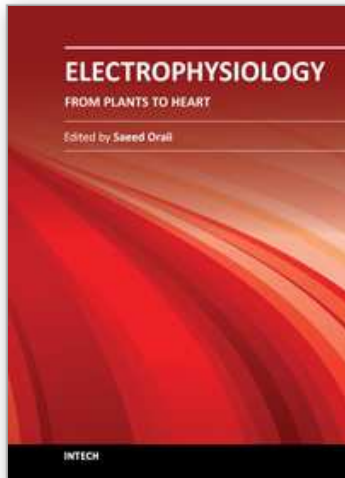
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University Campus STeP Ri
Slavka Krautzeka 83/A
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Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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