

Neuronal-astrocytic and cytosolic-mitochondrial metabolite trafficking during brain activation, hyperammonemia and energy deprivation

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Abstract

A novel concept is described, according to which both neurons and astrocytes are capable of metabolizing glucose all the way to CO₂ and water, but in addition interact metabolically in a process generating glutamate from glucose, and subsequently, metabolizing excess glutamate to CO₂ and water Hertz, L., Dringen, R., Schousboe, A., Robinson, S.R., 1999. Astrocytes: Glutamate producers for neurons (Journal of Neuroscience Research 57, 417–428). The proposed metabolic degradation of glucose via glutamate serves the purpose of adjusting transmitter pools of glutamate to the demands for glutamatergic transmission, and it must account for a major fraction of glucose utilization. Evidence in favor of this concept is presented and a multitude of in vivo data are interpreted in the context of metabolic trafficking between neurons and astrocytes. In addition, intracellular trafficking occurs between cytosol and mitochondria during synthesis of transmitter glutamate, partly explaining a robust quantitative correlation between glutamine synthesis, as a measure of release of transmitter glutamate, and glucose utilization, reported by several authors. Both intracellular and intercellular metabolic trafficking may be affected during pathological conditions, as evidenced by effects of hyperammonemia (mimicking hepatic encephalopathy) and energy deprivation (mimicking stroke). It is suggested that neuronal-astrocytic interactions may also be impaired during degenerative dementing diseases. © 2000 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

It has long been known that glutamate is the most abundant amino acid in brain (Cutler and Dudzinski, 1974), the principal excitatory transmitter (Fonnum, 1984; Orrego and Villanueva, 1993) and a major metabolite of glucose (Gaitonde and Richter, 1966). It

is, however, only recently that the interrelationship between the different roles of glutamate has begun to be unraveled. In this communication we will describe a novel concept (Hertz and Fillenz, 1999), according to which both neurons and astrocytes are capable of metabolizing glucose all the way to CO₂ and water (Peng et al., 1994a), but in addition interact metabolically in a process generating glutamate from glucose and subsequently metabolizing excess glutamate to CO₂ and water (Hertz et al., 1999). The proposed metabolic degradation of glucose via glutamate serves the purpose of adjusting transmitter pools of gluta-

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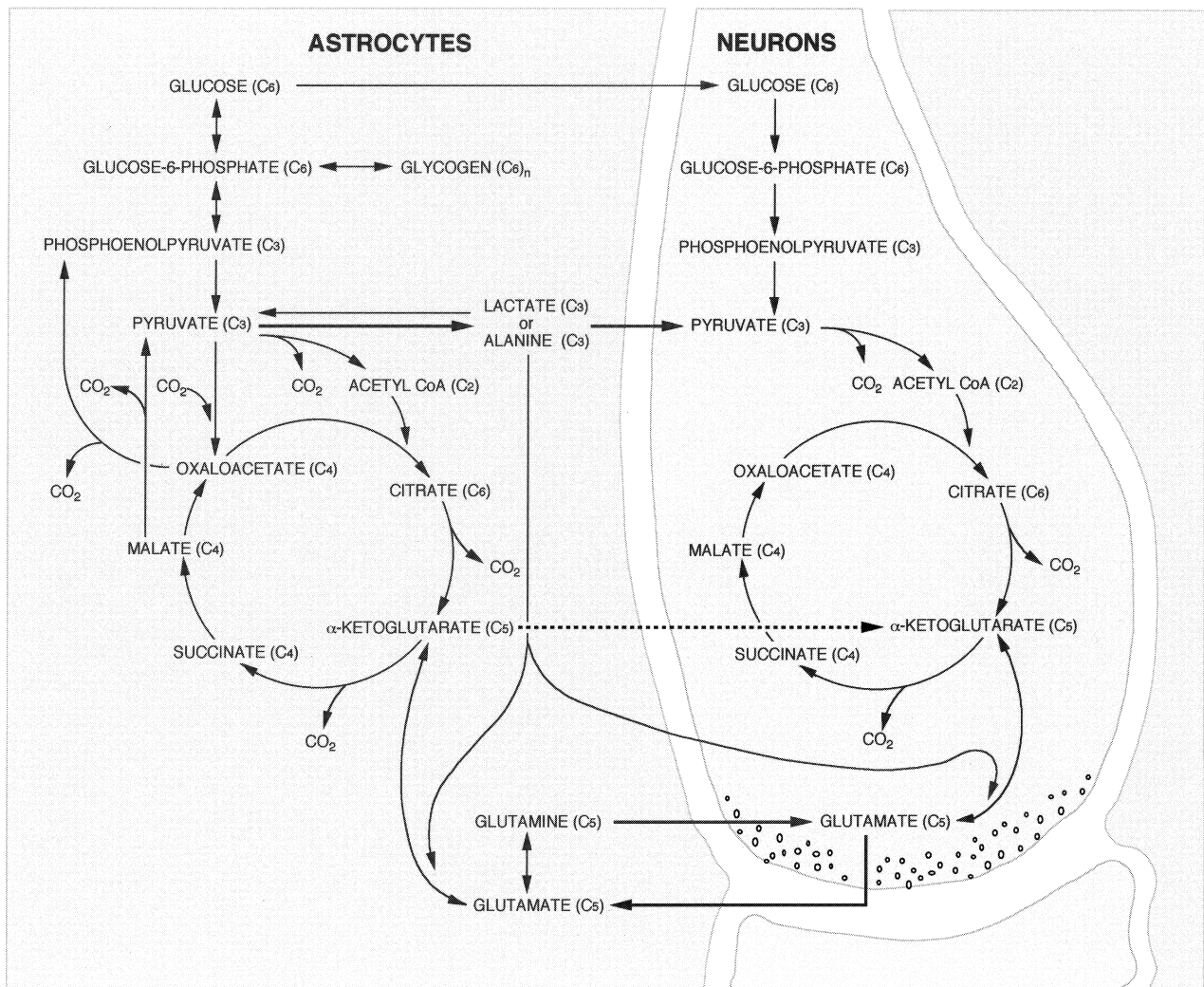


Fig. 1. Metabolic interactions that occur at glutamatergic synapses between neurons and astrocytes that ensheath them. The cartoon shows the cellular compartmentation in de novo formation of glutamate, its disposal by oxidative metabolism, and its return to neurons in the "glutamate-glutamine cycle". The number of carbon atoms in each intermediate is indicated in parentheses. Astrocytes and neurons are both capable of metabolizing glucose all the way to CO_2 and water, by initial glycolysis to pyruvate (not all intermediates are shown); subsequent formation of acetyl coenzyme A (acetyl CoA), releasing one molecule of CO_2 ; condensation of acetyl CoA with oxaloacetate in the tricarboxylic acid (TCA) cycle to form citrate; oxidation through different intermediates (which are not all shown) in the TCA cycle, producing 2CO_2 molecules per acetyl CoA, and regenerating one molecule of oxaloacetate, replacing the oxaloacetate molecule consumed during the initial formation of citrate, and ready for condensation with another molecule of acetyl CoA. During the oxidation of glucose a vast amount of energy is created (as ATP), but there is no net synthesis of any TCA cycle intermediate. Net synthesis of TCA intermediates is essential to replace leakage from the cycle and, especially, to synthesize transmitter glutamate. For this purpose, a molecule of oxaloacetate is generated de novo from pyruvate by addition of CO_2 (pyruvate carboxylation). This process cannot occur in neurons, which do not express pyruvate carboxylase activity, but it occurs readily in astrocytes. The newly generated molecule of oxaloacetate condenses with acetyl CoA, generating one molecule of citrate from one molecule of glucose (2 molecules of pyruvate) and, by further cycling, other TCA cycle intermediates. The TCA cycle intermediate α -ketoglutarate can be transaminated with alanine, formed from pyruvate, to form glutamate. It is not known whether this transamination occurs in astrocytes or in neurons. If it occurs in neurons, α -ketoglutarate is released from astrocytes and accumulated in neurons (stippled line in the figure). If it occurs in astrocytes, the generated glutamate is amidated by the astrocyte-specific glutamine synthetase to glutamine, which has no transmitter activity and can be safely released to the extracellular space, taken up in neurons and deamidated to glutamate. Since net amounts of glutamate are produced in the CNS and glutamate cannot leave the CNS across the blood-brain barrier, there must be mechanisms for disposal of excess glutamate. This is by formation of α -ketoglutarate from released transmitter glutamate after its accumulation in astrocytes; cycling in the astrocytic TCA cycle to malate, which can exit from mitochondria; formation of pyruvate or oxaloacetate, which is further converted to phosphoenolpyruvate. Pyruvate is either oxidized via acetyl CoA in the astrocytes where it was generated or it is released, mainly after conversion to lactate, to the extracellular space and taken up and metabolized in neurons. Phosphoenolpyruvate can be used for synthesis of glycogen (in astrocytes), and glucose, which is again oxidized (in astrocytes or neurons). Although much more released transmitter glutamate is taken up by astrocytes than by neurons, it is not always oxidized. Another pathway is the formation of glutamine in astrocytes and return of glutamine to neurons, as discussed above, in a glutamate-glutamine cycle. From Hertz et al. (1999).

mate to the demands for glutamatergic transmission, and it accounts for a major fraction of glucose utilization.

2. Glutamate formation, recycling and degradation pathways

2.1. Glutamate synthesis from glucose

Glutamate does not readily cross the blood-brain barrier (Oldendorf, 1971; Hawkins et al., 1995), and all CNS glutamate is formed from glucose within the CNS itself (Gruetter et al., 1994), requiring de novo synthesis of tricarboxylic acid (TCA) cycle constituents. Glutamate synthesis and contents are significantly enhanced by functional activation of neuronal activity, but return to normal after cessation of the stimulation (Dienel et al., 1997; Madsen et al., 1995a). Net synthesis of TCA cycle constituents requires participation of the enzyme pyruvate carboxylase, which is absent in neurons but abundant and functionally active in astrocytes (Yu et al., 1983; Shank et al., 1985; Kaufman and Driscoll, 1992). During pyruvate carboxylation, one of the two molecules of pyruvate formed from one glucose molecule is carboxylated to oxaloacetate (i.e., a new molecule TCA cycle constituent is formed),

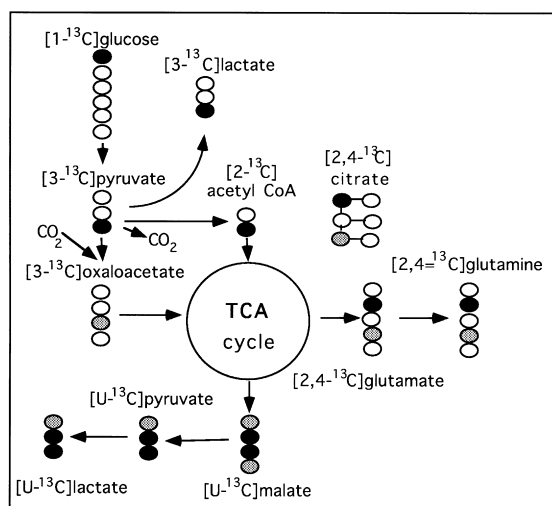


Fig. 2. Labeling of selected TCA cycle constituents and their derivatives during the first turn of the cycle after administration of $[1-^{13}\text{C}]$ glucose as the substrate and after introduction of the generated pyruvate into the TCA cycle by either formation of acetyl CoA, catalyzed by pyruvate dehydrogenase (PDH) and shown by black circles, or formation of oxaloacetate, catalyzed by pyruvate carboxylase (PC) and shown as gray circles. Note specifically that the position of the labeled carbon(s) in α -ketoglutarate, glutamate and glutamine provide unequivocal evidence of the route of synthesis and that lactate formed directly from glucose is labeled differently than lactate formed from glucose-derived glutamate. The reasons for the indicated labeling can be understood by consulting a textbook of Biochemistry. Modified from Sonnewald et al. (1993b).

whereas the second pyruvate molecule follows the conventional route for oxidative metabolism of glucose, i.e., formation of acetyl coenzyme A (acetyl CoA) (Fig. 1). Subsequently, oxaloacetate and acetyl CoA condense to form citrate. This process is different from utilization of oxaloacetate circulating in the TCA cycle during oxidative degradation of glucose via acetyl CoA, when one molecule oxaloacetate is consumed and another molecule regenerated at the end of each turn of the cycle.

During exposure to ^{13}C -labeled compounds, the labeling of individual carbon atoms in citrate and its derivatives is different after pyruvate carboxylation and after pyruvate metabolism via acetyl CoA (although scrambling of the labeling eventually occurs after continued circling in the TCA cycle), because the pyruvate molecule is incorporated differently (Fig. 2). This difference is extremely important for the interpretation of NMR spectroscopic findings (Sonnewald et al., 1993b; Bachelard, 1998; Gruetter et al., 1998). Since pyruvate carboxylase activity is absent in neurons but abundant in astrocytes, metabolic flux via pyruvate carboxylation is an unequivocal indication of astrocytic involvement. In addition, astrocytic metabolism in the intact brain has been followed by administration of labeled acetate which after conversion to acetyl CoA is metabolized in the TCA cycle. Since acetate is accumulated much more effectively in astrocytes than in neurons, it will mainly or exclusively be metabolized in these cells (O'Dowd, 1995; Waniewski and Martin, 1998).

Citrate is not transferred from neurons to astrocytes, but gets converted to isocitrate (not indicated in Fig. 1), in a reaction which is inhibited by fluoroacetate (Fonnum et al., 1997). Like acetate, fluoroacetate is preferentially accumulated in astrocytes, and in suitable concentrations it inhibits the astrocytic TCA cycle without adverse effects on neurons. Therefore, susceptibility to appropriate doses of fluoroacetate is also an indication of astrocytic involvement. Fluoroacetate prevents glutamate production from glucose by interfering with formation of α -ketoglutarate in the astrocytic TCA cycle (Swanson and Graham, 1994), it inhibits glutamatergic impulse transmission in brain slices (Keyser and Pelmar, 1994), it reduces glutamate and glutamine levels in the retina and prevents a specific constituent of the electroretinogram (Bull and Barnett, 1999), and it abolishes retention of one-trial aversive learning (a glutamate dependent process [Ng et al., 1997]) in newly hatched chicks (Hertz et al., 1996). Thus, it can be concluded that astrocytic TCA cycle activity is indispensable for glutamatergic transmission.

Glutamate is synthesized from α -ketoglutarate by transamination with either alanine (Fig. 1) or one of the branched chain amino acids leucine, isoleucine and valine (Yudkoff, 1997). The latter are essential amino

al., 1997; Izquierdo and Medina, 1997; Ungerer et al., 1998).

Glutamate production from glutamine is catalyzed by phosphate-dependent glutaminase (PAG), but synthesis of transmitter glutamate from glutamine in glutamatergic neurons appears to be more complex than generally envisaged. In the glutamatergic cerebellar granule cell neurons, it is blocked by the transamination inhibitor amino oxyacetic acid (AOAA), as well as by phenylsuccinate, an inhibitor of the ketodicarboxylate carrier, transporting malate and α -ketoglutarate across the mitochondrial membrane (Palaiologos et al., 1988). These characteristics and recent immunocytochemical observations (Laake et al., 1999) suggest that glutamate, formed by PAG-dependent hydrolysis of glutamine on the outer side of the inner mitochondrial membrane, is transported across the inner membrane in exchange with aspartate, transaminated in the matrix to α -ketoglutarate, which by aid of the ketodicarboxylate carrier is transferred to the cytoplasm (Palaiologos et al., 1989; Svarna et al., 1996), and retransaminated to glutamate (Fig. 3). Cytosolic glutamate is accumulated in vesicles, from which it can be released in a Ca^{2+} -dependent manner (Fonnum et al., 1998; Ozkan and Ueda, 1998).

The proposed transmembrane pathway for synthesis of glutamate from glutamine in glutamatergic neurons is identical to the malate-aspartate shuttle (MAS), with the exception that glutamate molecules in the MAS circle between the intramitochondrial space and the cytosol. In MAS there is no de novo generation of glutamate from glutamine and no removal of glutamate from the cytosol into vesicles, but it is the same molecule of glutamate which is generated in the cytosol and transported back into the mitochondrion. In both MAS and the “pseudo-MAS” operating during synthesis of transmitter glutamate from glutamine two transaminations between glutamate/oxaloacetate and α -ketoglutarate/aspartate are involved, one intramitochondrial and the other cytosolic, reflected by the both intramitochondrial and cytoplasmic localization of aspartate aminotransferase (Fonnum, 1968). The continuous conversion of one molecule oxaloacetate to malate in the cytosol for each molecule of glutamate (Fig. 3) requires supply of stoichiometric amounts of NADH, which is provided by conversion of glyceraldehyde 3-phosphate to 1,3-biphosphoglycerate during glycolysis. MAS is not restricted to glutamatergic neurons but is an ubiquitous metabolic shuttle serving the purpose of re-oxidizing NADH to NAD^+ in the cytosol and transferring a reducing equivalent to the mitochondrial matrix for oxidation. Without such a process glycolysis can only proceed with regeneration of NAD^+ by formation of lactate from pyruvate, because the amount of NAD^+ is limited and NAD^+ and NADH do not cross the mitochondrial membrane.

Thus, generation of one molecule transmitter glutamate from glutamine by the proposed mechanism is correlated with glycolysis of one half of a molecule neuronal glucose. However, no energy is being consumed at this step, since each turn of such a “pseudo-MAS shuttle” during formation of transmitter glutamate replaces one turn of MAS, and malate is oxidized intramitochondrially. In contrast, synthesis of glutamate from α -ketoglutarate occurs by transamination in the cytosol (Fig. 3) and requires no utilization of NADH (Palaiologos et al., 1989).

2.3. Glutamate degradation

It has long been known that isolated or cultured brain cells oxidize glutamate and glutamine (Yu et al., 1982; Tildon and Roeder, 1984), and more recently, evidence has been obtained that glutamate and glutamine are oxidized in normal brain in vivo (Zielke et al., 1998). In order to completely oxidize glutamate or glutamine they must be taken out of the TCA cycle and re-introduced via acetyl CoA (Fig. 1). Cerdan and coworkers, using 1- ^{13}C acetate, were the first to demonstrate recycling of TCA cycle constituents to pyruvate/lactate in brain (Cerdan et al., 1990; Kunnecke et al., 1993). This observation has been corroborated in a series of important studies in cultured astrocytes and intact brain by the Sonnewald group (Schousboe et al., 1993; Sonnewald et al., 1993a; Sonnewald et al., 1993b; Sonnewald et al., 1996; Sonnewald et al., 1997; Haberg et al., 1998a; Haberg et al., 1998b). Oxidative degradation of glutamate is essential to remove excess glutamate, from the CNS, since de novo synthesis of glutamate takes place within the brain, and glutamate does not readily cross the blood-brain barrier. At the same time it preserves most, although not all, of the chemical energy which originally resided in glucose before its conversion to glutamate (Hertz and Fillenz, 1999). Surplus glutamine can be metabolized in a similar manner after hydrolysis to glutamate (Sonnewald et al., 1996).

Since only little label was incorporated from 1- ^{13}C acetate into glutamine, Cerdan et al. (1990) suggested that formation of pyruvate from malic acid occurs in neurons. However, the absence of glutamine formation is no strong argument in favor of a neuronal localization, since the complete process from the generation of acetyl CoA from acetate to pyruvate formation from malate can take place in astrocytes, without involvement of glutamine synthetase activity (Fig. 1). Moreover, it is the cytosolic malic enzyme that catalyzes the formation of pyruvate from malate and this enzyme is reportedly astrocyte-specific (McKenna et al., 1993; Kurz et al., 1993; Vogel et al., 1998). However, the cellular location of pyruvate formation may not be not a major issue functionally,

since the presence of different isozymes of the lactate dehydrogenase and subtypes of the monocarboxylate transporter (a high-affinity MCT2 and a low-affinity MCT1) in astrocytes and neurons suggest that pyruvate in astrocytes to a considerable extent may be converted to lactate, released from astrocytes and accumulated in neurons for oxidative degradation via pyruvate (Bittar et al., 1996; Broer et al., 1997; Broer et al., 1999). This might especially apply to malate-derived pyruvate, since pyruvate generated directly from glucose can only be used for net synthesis of new TCA cycle intermediates and their derivatives by metabolism in the astrocytic TCA cycle. Moreover, metabolic trafficking of lactate in the mammalian CNS may be more complex than an ubiquitous transfer of lactate from

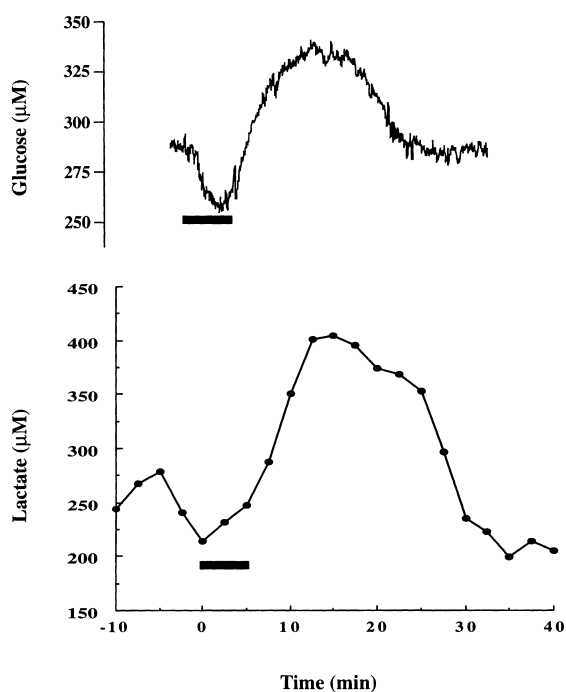


Fig. 4. Representative tracings showing the effect of a 5 min tail pinch (indicated by solid bar and known to be accompanied by glutamate release) on brain tissue glucose and lactate in freely moving rats. Concomitant with the stimulation, there were increases in tissue oxygen and cerebral blood flow, but these had subsided immediately after the end of the stimulation (not indicated in the figure). Extracellular glucose, monitored using a glucose oxidase-based biosensor, shows a decrease during the stimulation, followed by a prolonged increase, beginning at the end of the stimulation. Lactate was measured by microdialysis, and the concentrations are those in the dialysate. It begins to rise during the stimulation, but most of the increase occurs after the end of the stimulation, and the return to baseline is slow. As discussed in the text, by Hertz and Fillenz (1999) and Hertz et al. (1999) it has been hypothesized that the delayed increases in lactate and glucose originate from the metabolism of glutamate, via α -ketoglutarate, malate and pyruvate to lactate, or via oxaloacetate to phosphoenolpyruvate, glycogen and glucose (see Fig. 1). The tracings were obtained in experiments by M. Fillenz and co-workers, and were published in Hertz and Fillenz (1999), from which this figure is modified.

astrocytes to neurons, since extracellular lactate can also be accumulated in astrocytes by the high affinity MCT2 (Gerhart et al., 1998, 1999).

Recently, Hertz and Fillenz (1999) have suggested that the increases in lactate and glucose, which have repeatedly been observed by Fillenz and her coworkers (Fray et al., 1996; Lowry and Fillenz, 1997) during an extended period (Fig. 4) after brain activation in awake and freely moving rats represent glutamate-derived lactate and glucose. They proposed that newly synthesized glutamate, present in excess after discontinuation of functional activity, is cleared from the CNS by formation of α -ketoglutarate, circling in the TCA cycle to malate, exit of malate to generate pyruvate, which is then either oxidatively degraded in the astrocytes or converted to lactate, which may be transferred to neurons, re-converted to pyruvate and oxidized (Fig. 1). The oxidation of glutamate-derived pyruvate requires a large amount of oxygen without concomitant utilization of exogenous glucose (Fig. 5). Alternatively, malate could be further metabolized to oxaloacetate (probably in the cytosol, although this for graphical reasons is not indicated in Fig. 1) and metabolized to phosphoenolpyruvate, which in a gluconeogenic process can be converted to glycogen (Dringen et al., 1993b; Wiesinger et al., 1997) and glucose (Haberg et al., 1998a). Although cultured astrocytes reportedly are unable to release glucose from glycogen, this is probably not the case in the brain in situ (Forsyth, 1996). Most of the glucose increase in brain following functional activation appears to be generated by glycogenolysis (a process virtually restricted to astrocytes), since the usual postactivation peak in glucose is abolished (and in some cases even reverted) in the presence of propranolol, an inhibitor of glycogenolysis (Fillenz and Lowry, 1999); in contrast, propranolol has no effect on a similar increase in glucose evoked by hyperoxia. Accordingly, the postactivation increase in brain glucose has a cellular and more specifically astrocytic origin, whereas the increase in glucose during hyperoxia is the result of a diminished rate of glycolysis, resulting from enhanced oxidative metabolism of available oxidizable substrates, which are not limited to glucose (Yu and Hertz, 1983), but include amino acids like glutamate and glutamine (Yu et al., 1982; Tildon and Roeder, 1984; Zielke et al., 1998). However, in accordance with the astrocytic accumulation of transmitter glutamate before its degradation, the post-stimulation increase in lactate is inhibited by glutamate uptake blockers (Demestre et al., 1997).

The hypothesis proposed by Hertz and Fillenz (1999) may explain the increase in cerebral blood flow and glucose utilization without a corresponding quantitative increase in oxygen consumption during functional activation of the brain. This metabolic

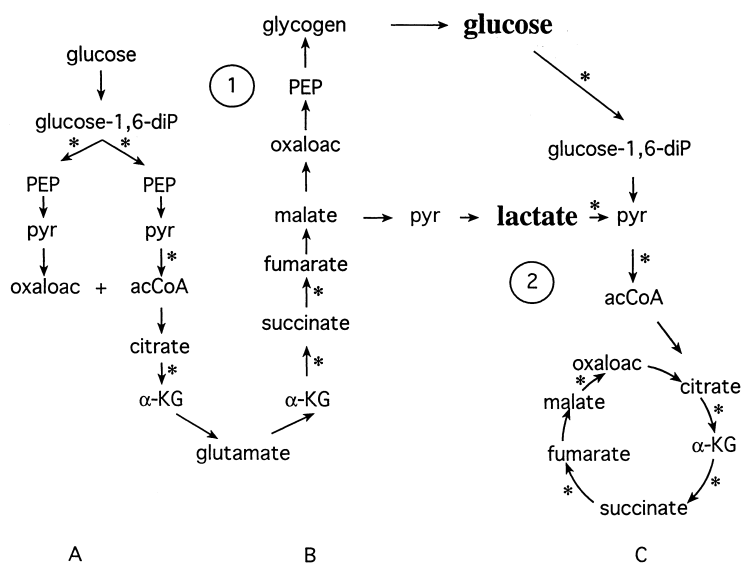


Fig. 5. Complete metabolic pathway proposed for glucose oxidation via glutamate during and following neuronal activation. The figure follows the metabolic fate of a single molecule of glucose, used to generate a single molecule of glutamate during the stimulation (panel A); after the stimulation, one molecule of glutamate is converted to either one molecule of lactate or one half of a molecule of glucose (panel B); finally lactate or glucose, formed from glutamate, is oxidatively degraded (panel C). In panel A, one molecule of glucose (six carbon atoms [C]) is converted to two molecules of pyruvate (3C), via glucose-1,6-diphosphate (glucose-1,6-diP), phosphoenolpyruvate (PEP) and additional glycolytic intermediates which are not shown; oxaloacetate (oxaloac) is formed from one molecule of pyruvate and acetyl CoA (acCoA) from the other; oxaloacetate and acCoA condense to form citrate (6C), which is metabolized in the initial part of the tricarboxylic acid (TCA) cycle (see panel C) to the 5-C metabolite α -ketoglutarate (α -KG); α -KG is taken out of the TCA cycle and converted to glutamate (5C). In panel B, glutamate is re-converted to α -KG, which is decarboxylated to succinate (4C), and from there metabolized in the TCA cycle to malate (4C), which can leave the TCA cycle and in the cytosol either be decarboxylated to pyruvate (3C), or form oxaloacetate (4C), which can be decarboxylated to PEP (3C); PEP can be converted via glycogen to glucose (pathway 1), and pyruvate converted to lactate (pathway 2). Since there are only 3C left from the glutamate molecule, either one molecule lactate or one half of a molecule glucose is formed from each individual glutamate molecule. However, since many glutamate molecules are metabolized at the same time, both lactate and glucose can be produced. These two metabolites, shown in bold, may account for the delayed increases in extracellular glucose and lactate shown in Fig. 4. In panel C, pyruvate is formed from either glucose or lactate and, via acCoA, oxidatively degraded in a complete TCA cycle without any need for exogenous oxaloacetate, since no net synthesis of a TCA cycle constituent is involved, and acCoA is regenerated in the turn of the TCA cycle. All reactions are conventional biochemical processes (Stryer, 1995), known to occur in brain. Utilization of each atom (1/2 molecule) oxygen is indicated by an asterisk, with the exception of oxidative processes during formation of oxaloacetate and pyruvate in B, which are counterbalanced by subsequent reductions of pyruvate to lactate and between PEP and glycogen. It can be seen that four oxygen atoms, or two oxygen molecules are utilized in A and eight oxygen atoms or four oxygen molecules in B plus C. The total utilization of oxygen in A, B and C is six molecules for each molecule of glucose, the same oxygen/glucose ratio as in direct oxidative metabolism of glucose. From Hertz and Fillenz (1999).

“mismatch” was first reported by Fox and Raichle (1986), and it is now exceedingly well established (Bari-naga, 1997; Raichle, 1998). As it can be seen from Fig. 5, synthesis of glutamate from glucose requires large amounts of glucose (one molecule per glutamate molecule), but relatively little oxygen (for details see legend of the figure). From the experiments by the Fillenz group, it can not be concluded that oxygen consumption is increased, relative to utilization of exogenous glucose, at the time when the increased glucose and lactate levels normalize. However, as shown in Table 1, such an increase in relative utilization of oxygen has recently been observed by two different groups (Madsen et al., 1998, 1999). Occasionally, the increased glucose utilization continues for a considerable length of time after the end of the stimulation (Madsen et al., 1995b), before the reversal finally occurs (Madsen et al., 1999). This might be connected

with the establishment of memory, a process which in many different models has been found to involve glutamatergic activity (Fonnum et al., 1995; Kaczmarek,

Table 1
Cerebral oxygen/glucose ratios ($\text{mmol} \times \text{l}^{-1} / \text{mmol} \times \text{l}^{-1}$) reported by Madsen et al. (1998^a, 1999^b)

	Madsen et al., 1998	Madsen et al., 1999
Baseline conditions	5.5	6.1
Activation	4.2	5.0
Post-activation	6.4	7.7

^a Ratio between cerebral uptake rates for oxygen and glucose was calculated from cerebral arterial-venous differences in awake rats; activation was induced by opening the sheltering box during 6 min.

^b Ratio between cerebral uptake rates for oxygen and glucose was calculated from cerebral arterial-venous differences in awake rats; activation was induced by sensory stimulation during 6 min.

1993; Izquierdo and Medina, 1997) and to require glutamate synthesis at specific stages (Hertz et al., 1996; Ng et al., 1997).

2.4. Metabolic fluxes

Although a wealth of information is available about transport kinetics and metabolic fluxes in cultured brain cells, they are of little value for the evaluation of corresponding rates in the intact brain. Apart from the possibility of differences between cultured and non-cultured cells, there is the huge problem that volumes and surface areas of different cell constituents in the intact brain have not been accurately determined. It has been known for decades that glutamate is taken up much more avidly in cultured astrocytes than in cultured neurons (Hertz et al., 1978; Yu and Hertz, 1982; Drejer et al., 1982; Schousboe and Westergaard, 1995), but this question has only recently been directly approached in studies of intact brain, using knock-out mice deficient in an astrocyte specific glutamate carrier (Rothstein et al., 1996) or histochemical determination of glutamate in individual cells (Danbolt et al., 1992; Chaudry et al., 1995; Majkowska et al., 1995; Gundersen et al., 1996; Kojima et al., 1999). These studies have shown that a large fraction of the released glutamate is taken up by astrocytes. Moreover, the Na^+ current which is associated with glutamate uptake in astrocytes faithfully reflects release of transmitter glutamate (Bergles and Jahr, 1998).

Recently, impressive advances have been made in determination of quantitative aspects of brain metabolism in the brain *in vivo* by the aid of NMR spectroscopy, which allows the investigator to determine which individual atoms in a molecule become labeled after exposure to a ^{13}C -labeled substrate, e.g., glucose. Rate of incorporation of labeled carbon atoms from glucose into glutamate can be used as an indication of total metabolic rate (Mason et al., 1995; Gruetter et al., 1998), due to a very fast isotope exchange between α -ketoglutarate and glutamate. Since citrate and its derivatives are differently labeled after pyruvate carboxylation and after flux through acetyl CoA (Fig. 2), it is possible to determine rates of pyruvate carboxylation, a process occurring in astrocytes, but not in neurons. By aid of this methodology (in some cases with additional refinements) the rate of pyruvate carboxylation in mammalian brain, including the human resting brain, has been determined to correspond to 10–16 percent of total TCA cycle activity, measured as incorporation of label into glutamate (Lapidot and Gopher, 1994; Aureli et al., 1997; Gruetter et al., 1998). Since two pyruvate molecules are required for formation of one TCA cycle intermediate or its derivatives, and only one of these is carboxylated (Figs. 1 and 5), a pyruvate carboxylation rate of 10–16 percent of the

turnover rate in the TCA cycle means that 20–32 percent of total glucose metabolism occurs as net synthesis of TCA cycle constituents and their derivatives, in all probability mainly glutamate.

Using ^{15}N -labeled substrates, NMR spectroscopy can be used to determine the rate of glutamine formation as an approximate measure of glutamatergic transmitter activity (Sibson et al., 1997; Gruetter et al., 1998). Since the “resting” extracellular concentration of glutamate is exceedingly low, this is a legitimate approach provided (i) released transmitter glutamate is accumulated in astrocytes, not in neurons; (ii) glutamate accumulated in astrocytes is converted to glutamine, not metabolized to CO_2 and water; and (iii) glutamate transferred in the glutamate-glutamine cycle is released transmitter glutamate, not glutamate being generated from recently synthesized α -ketoglutarate. None of these prerequisites is completely satisfied, but the errors might to some extent cancel each other. In the “resting” human brain, the rate of glutamine formation is four times higher than the rate of pyruvate carboxylation (Gruetter et al., 1998). Assuming that the latter is a measurement of *de novo* synthesis of glutamate, it can be concluded that turnover rate in the glutamate-glutamine cycle is three–four times higher than *de novo* synthesis of glutamate; it is also almost as fast as glucose utilization.

Shulman and coworkers (Sibson et al., 1998; Shen et al., 1999), comparing metabolism during light and deep anaesthesia in rats, or measuring “resting” brain metabolism in humans, have similarly reported that the rate of glutamine synthesis amounts to ~80% of the rate of glucose oxidation (a glutamine/glucose ratio of 0.8). They postulated that the reason for the close quantitative correlation between glucose utilization and glutamine formation is that glutamate uptake and subsequent glutamine synthesis in astrocytes require glycolytically derived energy and provide the driving force for energy metabolism in brain (Magistretti et al., 1999). This hypothesis is in disagreement with several experimental observations, including that astrocytic glutamate uptake and glutamine synthesis do not depend upon glycolytically derived energy (Hertz et al., 1998; Bakken et al., 1998; Di Monte et al., 1999), and that glutamate uptake in salamander Muller cells is fueled by oxidation of glutamate (Poitry et al., 2000). However, glutamate uptake can, if necessary, be driven by energy obtained from glycolysis (Huang et al., 1993; Swanson et al., 1994; Hertz et al., 1999).

A simpler explanation of the robust quantitative correlation between glucose oxidation and glutamate release is that formation of one molecule transmitter glutamate, as discussed above, requires one molecule of cytosolic NADH, and thus, is correlated with utilization of half a molecule of glucose (a glutamine/glu-

cose ratio of 2.0). Moreover, approximately one fourth of all molecules of transmitter glutamate are formed de novo from glucose (see above). If this newly synthesized glutamate has been transferred to neurons without participation of the glutamate-glutamine cycle, a glutamine/glucose ratio of zero will decrease the total glutamine-glucose ratio towards 1.2; if the glutamate-glutamine cycle is involved in the transfer, this molecule of glutamate will be generated with a glutamine/glucose ratio of one, and the combined effect of recycling by the glutamate-glutamine cycle and de novo synthesis will be a glutamine/glucose ratio of 1.6, i.e., in any case glutamine synthesis exceeds glucose utilization. The consistent observation that the rate of glutamine synthesis is close to that of glucose utilization or slightly lower (Sibson et al., 1998; Gruetter et al., 1998; Shen et al., 1999) accordingly indicates that glucose utilization exceeds the rate necessary for synthesis of transmitter glutamate, i.e., that glutamate transmission is not triggering energy metabolism. However, the rate of glucose oxidation is at most two times higher than the rate that would be necessary for production of sufficient NADH to maintain synthesis of transmitter glutamate, attesting to the importance of glutamatergic neurotransmission.

The glucose utilization correlated with the supply of NADH for formation of transmitter glutamate from glutamine must occur in glutamatergic neurons, which accordingly must have a glycolytic rate of at least one half of the glutamate turnover in the glutamate-glutamine cycle, or approximately 40% of the total glycolytic rate. The glucose utilization required for de novo synthesis of glutamate by pyruvate carboxylation must occur in astrocytes, which probably are responsible for approximately one third of the total glycolytic rate, with the remainder occurring in other cell types. This conclusion is compatible with a high density of glucose transporters on both neurons and astrocytes (Vannucci et al., 1997). The characteristics of lactate carriers (Dringen et al., 1993a, 1993b; Broer et al., 1997, 1999; McKenna et al., 1998) and of lactate dehydrogenase isozymes (Bittar et al., 1996) suggest that there may be a net transfer of lactate from astrocytes to neurons; however, with a substantial amount of obligatory glycolytic activity in neurons during synthesis of transmitter glutamate, the magnitude of such a transfer is likely to be much smaller than envisaged by Magistretti et al. (1999). Moreover, astrocytes also express the carrier which was suggested by these authors to be involved in uptake of extracellular lactate (Gerhart et al., 1998; Gerhart et al., 1999)

3. Hyperammonemic conditions

Acute exposure to very high levels of ammonia

($\text{NH}_3 + \text{NH}_4^+$) causes convulsions and targets mainly neurons, primarily by stimulation of NMDA receptors (Marcaida et al., 1992). Chronic exposure to ammonia concentrations in the high micromolar or low millimolar range causes, in contrast, a predominantly astrocytic pathology; by down-regulating NMDA receptors, chronic exposure to relatively low levels of ammonia may actually protect against NMDA receptor overactivation by subsequent exposure to highly elevated ammonia concentrations (Marcaida et al., 1995). One reason for NMDA receptor activation (and downregulation during chronic exposure to ammonia) is that the extracellular concentration of glutamate is increased during liver failure or exposure to pathophysiologically relevant concentrations of ammonia (de Knecht et al., 1994), and that glutamate uptake is inhibited (Mena and Cotman, 1985; Oppong et al., 1995; Norenberg et al., 1997), possibly secondary to an ammonia-induced increase in production of an endogenous ouabain-like compound in astrocytes (Kala et al., 2000). This may also explain the observation by Sugimoto et al. (1997) that the extracellular concentration of K^+ is increased.

Reduced cellular uptake of glutamate together with an enhanced formation of glutamine in astrocytes

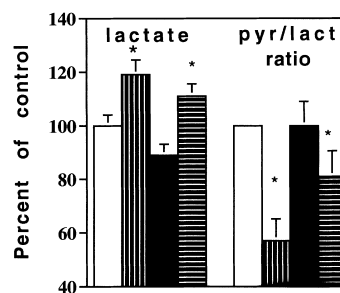


Fig. 6. Effect of 0.3 mM ammonia on lactate production and on pyruvate/lactate ratio in primary cultures of mouse astrocytes in the absence and presence of 50 μM glutamate in the incubation medium. The cultures were incubated for 60 min under control conditions (white columns), in the presence of 0.3 mM added NH_4Cl (vertically striped columns), in the presence of 50 μM glutamate (black columns) or in the presence of 0.3 mM NH_4Cl plus 50 μM glutamate (horizontally striped columns). Lactate and pyruvate were measured in the incubation media (very small amounts remain in the cells under the conditions used) and expressed as percentages of the content in control cultures from the same batches. The left part of the figure shows that ammonia increases lactate production significantly ($P < 0.05$ in ANOVA, followed by Fisher's PLSD test for the difference between the values in the presence and absence of ammonia), and that the magnitude of the ammonia-induced increase in lactate production is unaffected by the addition of glutamate to the medium; the right part shows a profound ammonia-induced decrease in pyruvate/lactate ratio in the absence of glutamate in the medium and significantly less ($P < 0.05$ in student's *t*-test for the difference between the two columns marked by asterisks) reduction in the presence of glutamate. Results are means \pm SEM from seven to nine individual experiments, using cultures from three different batches. Modified from Kala (1991).

during chronic exposure to ammonia leads to a rapid decrease in intracellular glutamate content, both in brain and in cultured astrocytes (Huang et al., 1994; Lapidot and Gopher, 1997). The glutamate deficit might affect the malate-aspartate shuttle (MAS), which transports reducing equivalents from the cytosol to the mitochondria and requires a glutamate/aspartate exchange together with a malate/oxaloacetate exchange, similar to those occurring during the synthesis of transmitter glutamate (Fig. 3). A reduction of the glutamate concentration to a level that does not allow normal function of MAS may be the reason for some, but not all, effects of ammonia on astrocytic metabolism. Thus, elevated ammonia concentrations increase lactate production in astrocytes, whereas the pyruvate/lactate ratio is reduced (Fig. 6). Addition of glutamate to the medium causes a slight decrease in lactate production, but has no effect on the ammonia-

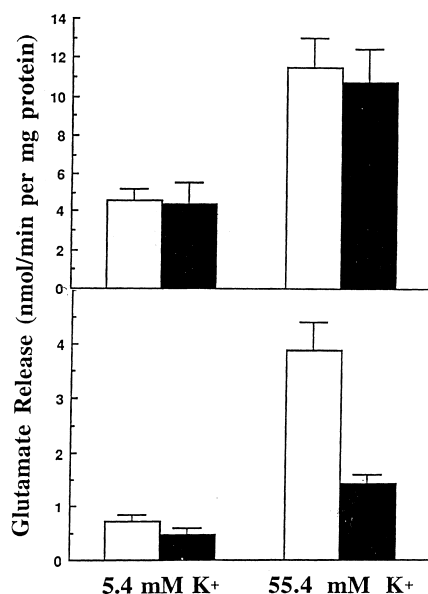


Fig. 7. Release of transmitter glutamate (nmol/min per mg protein) from cerebellar granule cell neurons in primary cultures, a glutamatergic preparation, under control conditions (white columns) and during acute (30–60 min) treatment with 3 mM NH₄Cl (black columns). The upper panel shows the release in the presence of abundant supply of glutamate precursor (0.8 mM glutamine), and the lower panel the release without any exogenously added glutamate precursor (note the different scales). The releases are the averages of three superfusate fractions (each collecting superfusate during 45 s) from each culture obtained during superfusion with a control concentration of K⁺ (5.4 mM) and another three fractions from the same culture during exposure to an elevated K⁺ concentration (55.4 mM), and results were pooled from three individual cultures under control conditions and another three cultures from the same batches treated acutely with NH₄Cl. The elevated K⁺ concentration significantly stimulated glutamate release both in the presence and absence of glutamine in the medium; ammonia significantly ($P < 0.05$) reduced the K⁺-induced glutamate release in the absence of glutamine, but not in its presence. From Kala (1991).

induced increase in lactate production, suggesting that it is a direct effect of ammonia on glycolytic enzymes (Ratnakumari and Murthy, 1993). However, the addition of glutamate counteracts the effect of ammonia on the pyruvate/lactate ratio, perhaps by supplying enough glutamate to maintain MAS activity. This hypothesis is supported by the observation that administration of AOAA, an inhibitor of transaminase activity, and thus of MAS, mimics the ammonia effect on pyruvate/lactate ratio (Kala, 1991).

Both acute and chronic exposure to ammonia causes a substantial reduction in TCA cycle activity in astrocytes in primary cultures, as indicated by decreased production of labeled CO₂ from 1-[¹⁴C]glutamate and 2-[¹⁴C]pyruvate (Yu et al., 1984; Fitzpatrick et al., 1988). This might either reflect an ammonia-induced impairment of enzyme activity in the TCA cycle (Butterworth, 1998) or be the result of ammonia-induced MAS inhibition. The inhibition of 1-[¹⁴C]glutamate oxidation in the presence of ammonia might also represent a mass action effect, resulting from the alteration in the equilibrium between ammonia plus α -ketoglutarate on one hand and glutamate on the other. An increase in the concentration of glutamate counteracts the inhibition of CO₂ production from 1-[¹⁴C]glutamate (Kala, 1991), but this could either be on account of the altered ratio between glutamate and ammonia + α -ketoglutarate or because the inhibition of MAS is alleviated by administration of exogenous glutamate.

Ammonia has no effect on the excitatory mechanisms involved in the stimulated release of glutamate from cerebellar granule cell neurons, a glutamatergic cell type, when glutamine is abundantly available as a glutamate precursor (Fig. 7); however, when intracellular reserves of glutamate and/or glutamine are the source of stimulated release, acute exposure (30–60 min) to 3 mM ammonia substantially reduces the stimulated release of glutamate, possibly because inhibition of glutamate uptake has reduced intracellular stores. Thus, even concentrations of ammonia which mainly affect astrocytic functions may have direct actions on neurons, which contribute to the inhibition of glutamatergic activity, characteristic of hyperammonemic conditions (Raabe, 1987; Albrecht, 1998; Butterworth, 1998; Norenberg, 1998). In addition, the pronounced effect of moderate increases in the ammonia concentration on astrocytes will, in the intact brain, secondarily affect neuronal functions. One reason for this is the elevated concentrations in extracellular K⁺ and glutamate, but a blockade of the neuronal-astrocytic interactions shown in Figs. 1 and 5 by decreased glutamate release from neurons, decreased glutamate uptake in astrocytes and decreased oxidative metabolism of glutamate in astrocytes may have even more deleterious effect by pre-

venting an appropriate metabolic response in situations stimulating neuronal activity.

4. Effects of energy deprivation

In most cases of energy deprivation in the CNS, the supplies of both oxygen and glucose are reduced by blockade of an artery, causing focal ischemia, which is characterized by an ischemic core and a surrounding penumbra. In spite of complete blockade, some perfusion may still occur in the stricken area due to collateral perfusion; in the core of the ischemic region, which is destined for infarction, cerebral blood is reduced to or below ~20% of its normal rate; blood flow to the penumbra, which may partly survive if circulation is restored in time, is between ~20 and ~40% of normal blood flow (Ginsberg et al., 1996; Hossmann, 1994; Back, 1998; Kato and Kogure, 1999). In this situation, it is important to realize that the amount of oxygen carried by blood (~200 ml/l or 8–9 mM) is less than that required for complete oxidation of the content of glucose (6 mM), since one molecule of glucose reacts with six molecules of oxygen. Accordingly, glycolysis may continue relatively unhindered, or in some cases even at an increased rate (Ginsberg et al., 1996; Belayev et al., 1997; Haberg et al., 1998b), albeit at the expense of a build-up of lactate in the tissue during the blockade. The maintenance of glycolytic activity may generate sufficient energy to maintain some activities, in spite of the fact that glycolysis produces only a small fraction of the energy created by complete oxidative degradation of glucose.

In contrast to neurons and oligodendrocytes, astrocytes can survive on glycolytically derived energy (Yu et al., 1989; Sochocka et al., 1994; Huang et al., 1997; Jelinski et al., 1999). Astrocytes can therefore, survive brain ischemia and carry out some of their functions for a considerable length of time. These functions include uptake of glutamate, which can be metabolically supported by either glycolysis or oxidative phosphorylation (Huang et al., 1993; Swanson et al., 1994). Accordingly, the primary reason for an immediate increase in extracellular glutamate concentration during brain ischemia (Benveniste et al., 1984; Caragine et al., 1998) and ensuing neurotoxicity (Olney, 1971; Choi, 1988), is not a deficient glutamate uptake, but rather an increased formation of glutamate from glutamine in injured glutamatergic neurons and subsequent release of the newly synthesized glutamate (Goldberg et al., 1988; Huang and Hertz, 1994; Newcomb et al., 1997). Glutamate production and release are further increased if extracellular K^+ is simultaneously increased (Huang and Hertz, 1995a; Huang et al., 1997).

Phenylsuccinate, acts as an inhibitor of the dicarbox-

ylate carrier (see above) and inhibits synthesis of transmitter glutamate from glutamine. By doing so it prevents excess formation and release of glutamate in cultured glutamatergic neurons during energy deprivation (Huang and Hertz, 1995b), and thus, reduces neuronal death (Fig. 8). However, phenylsuccinate does not prevent cell death during exposure to glutamate (Huang and Hertz, 1995b). It does also not prevent the increase in extracellular glutamate concentration during protracted energy deprivation in the brain in vivo (Christensen et al., 1991). This is because only the glutamate release during the first few minutes of ischemia represents transmitter glutamate, whereas the remainder originates from cytosolic pools, by reversal of uptake processes (Mitani et al., 1994), phospholipase-triggered impairment of membrane integrity (Phillis and O'Regan, 1996) or swelling-induced opening of anion channels (Phillis et al., 1997).

An increased glutamate content in astrocytes has been demonstrated histochemically after ischemia in vivo or combined hypoxia and substrate deprivation in vitro (Storm-Mathisen et al., 1992; Torp et al., 1993; Aas et al., 1993; Gajkowska et al., 1995; Ottersen, 1996). This is due to a relatively well preserved glutamate uptake in astrocytes (utilizing glycolytically derived energy), whereas the subsequent oxidation of glutamate is impaired by lack of oxygen, as reflected by the observation that especially intramitochondrially glutamate is elevated (Torp et al., 1991). Following reperfusion or re-establishment of the supply of oxygen and energy substrate, glutamate in astrocytes may normalize both in vivo (Torp et al., 1993), and in brain

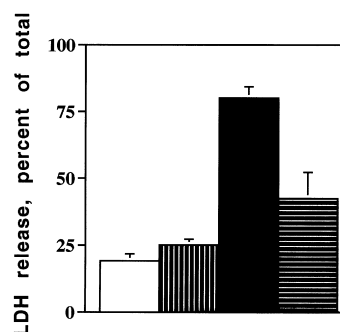


Fig. 8. Neuronal cell death, determined by release of lactate dehydrogenase (LDH) in cerebellar granule cells, incubated for 30 min in tissue culture medium with 7.5 mM glucose and 2.0 mM glutamine during control conditions (white column); during exposure to 5 mM phenylsuccinate, an inhibitor of the dicarboxylate carrier and thus of the formation of transmitter glutamate from glutamine (vertically striped column); during anoxia, i.e., exposure to 95% nitrogen and 5% CO_2 in a special anoxic incubator (black column); and during anoxia in the presence of 5 mM phenylsuccinate (horizontally striped column). Modified from Huang and Hertz (1995b). The difference between anoxia in the presence and absence of phenylsuccinate is statistically significant ($P < 0.05$).

slices (Aas et al., 1993), suggesting that the accumulated glutamate is oxidatively metabolized along the pathways indicated in Figs. 1 and 5. It is in agreement with this concept that considerable oxidation of glutamate has been demonstrated in rat brain following shortlasting ischemia (Pascual et al., 1998). Utilization of glutamate and of accumulated lactate may contribute to the reduction of glucose consumption after ischemia demonstrated by Ginsberg and coworkers (Belayev et al., 1997), since glutamate potently competes with glucose for oxidative degradation in astrocytes (Fig. 9), and lactate has a similar effect (Taberner et al., 1996). Thus, a reduced deoxyglucose-utilization after re-oxygenation is not per se an indication of metabolic damage. Nevertheless, there is a clear correlation between rate of glucose utilization 1 h after re-perfusion following a 2 h long occlusion of the middle cerebral artery and eventual histological outcome, as will be discussed below.

Fig. 10 shows the detailed distribution of glycolytic activity (deoxyglucose-6-phosphate retention), demonstrated by three-dimensional autoradiographic image analysis at a specific level of the previously ischemic area 1 h after a 2 h long occlusion of the right middle cerebral artery in the rat. The distribution is bimodal, with a major peak corresponding to 22 $\mu\text{mol}/\text{min}$ per 100 g wet wt, and a minor peak around 35 $\mu\text{mol}/\text{min}$ per 100 g wet wt, which is only slightly less than the normal rate of glucose utilization in this area (approximately 40 $\mu\text{mol}/\text{min}$ per 100 g wet wt). From the correlation between blood flow during the occlusion and rate of glucose utilization after re-perfusion, it can be concluded that the major peak corresponds to the ischemic core and the minor peak to the penumbra. The lower, continuous line in the figure indicates the areas that with certainty will survive the ischemic insult, as shown by detailed histological examination

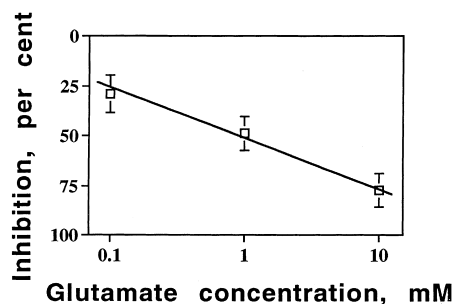


Fig. 9. Concentration-response relationship between the concentration of exogenously added glutamate and inhibition of $^{14}\text{CO}_2$ production from $U\text{-}^{14}\text{C}$ labeled glucose during incubation of primary cultures of mouse astrocytes for 60 min in tissue culture medium containing 7.5 mM glucose. Results are averages of six individual experiments with SEM values indicated by vertical bars.

at a later time of precisely the same areas in a different group of animals. It can be seen that higher rates of glucose utilization soon after the insult are correlated with better survival.

The surviving cells may either be those that for some reason are metabolically more robust, or they might be cells that have retained less oxidizable substrates (glutamate and lactate) during the ischemic period, either because they have had better access to remaining oxygen, have been exposed to less extracellular glutamate, or have suffered fewer energy-consuming episodes. A major reason for bouts of increased energy demand in the penumbra is triggering of waves of spreading depression, resulting from perinfarct depolarizations (Hossmann, 1994). A considerable amount of energy is required for re-establishment of cellular ion homeostasis after the passage of such waves, which can be prevented or reduced by glutamate receptor antagonists. This may be one reason that treatment with such agents as MK 801, an antagonist of the NMDA receptor, in animal experiments can reduce infarct size, even when administered after the ischemic insult. Another reason may be that a secondary elevation of extracellular glutamate can occur at the onset of re-perfusion (Caragine et al., 1998), possibly because glutamine is re-introduced and may reach glutamatergic neurons due to damaged blood-brain barrier function. It is consistent with this point of view that glutamine perfusion of brain tissue injured by ischemia leads to a considerable increase in extracellular glutamate (Newcomb et al., 1998). The fact that some areas survive, although they showed low rates of glucose utilization (Fig. 10) should not be taken as an indication of survival of parts of the ischemic core, but is a reflection of a wide distribution of post-occlusion glycolytic rates in the penumbra.

In some cases (e.g., insulin overdose) isolated hypoglycemia occurs. In the complete absence of glucose, glycolysis is abolished when glycogen pools have been depleted which takes only few minutes. However, TCA cycle activity can continue to a moderate degree by conversion of glutamate to aspartate, i.e., benefiting from the energy derived by conversion of α -ketoglutarate to succinate (Fig. 1). Evidence for such a process has been obtained both in brain tissue (Szerb and O'Regan, 1987) and in astrocytes (Sonnewald et al., 1997), and isolated hypoglycemia may lead to vesicular release of aspartate (Auer and Siesjö, 1993; Gundersen et al., 1998). In addition, tissue culture experiments have suggested that astrocytes are capable of generating oxidizable amino acids by breaking down glutathione (Juurlink et al., 1996) and endogenous proteins (D. Dunlop and L. Hertz, unpublished results). Nevertheless, ion homeostasis at the cellular level is compromised (Auer and Siesjö, 1993), which is consistent with the observation that cellular uptake of excess extra-

cellular K^+ partly depends upon glycolysis (Raffin et al., 1992).

5. Concluding remarks

This review has focused on the concept of a metabolic interaction between neurons and astrocytes proposed by Hertz and Fillenz (1999). According to this concept, intense formation of glutamate from glucose during neuronal activation involving enhanced glutamatergic activity explains the apparent discrepancy between large increases in cerebral blood flow and glucose utilization with no correspondingly large increase in oxygen utilization, followed by a post-activation period during which surplus glutamate is oxidatively degraded, causing an increase in oxygen consumption with no corresponding utilization of exogenous glucose.

A multitude of experimental observations support the proposed neuronal-astrocytic interactions. Thus, the apparent discrepancy between glucose and oxygen utilization, which was first demonstrated by Fox and Raichle (1986), has been overwhelmingly well demonstrated under many different forms of functional stimulation of neuronal activity, and the reversal, a higher rate of oxygen consumption than of utilization of exogenous glucose has been convincingly demonstrated by two groups during the last

year (Madsen et al., 1998; Madsen et al., 1999); Dienel and his coworkers have provided direct experimental evidence that glutamate production from glucose is increased during neuronal activation; Cerdan and associates and Sonnewald and coworkers have shown that glutamate is used by brain cells, including astrocytes, as a substrate for metabolic degradation; and events occurring during brain ischemia have supported the concept of glucose degradation via glutamate by showing that glutamate oxidation in astrocytic mitochondria is arrested during ischemia, but under suitable conditions recovers following re-perfusion.

A robust quantitative correlation between rates of glucose utilization and of glutamine synthesis, used as an estimate of release of transmitter glutamate, was explained as a result of the complex pathway followed during synthesis of transmitter glutamate from glutamine in glutamatergic neurons. This pathway involves “pseudo MAS” activity and therefore depends upon one molecule cytosolic NADH, which can be generated by glycolysis of half a molecule of glucose per molecule transmitter glutamate. Utilization of glucose in this manner does not represent any increased expenditure of energy, since malate produced from oxaloacetate and NADH is oxidized in the mitochondria. In contrast, interference with MAS (or “pseudo MAS”) activity resulting from glutamate depletion during exposure to elevated ammonia concentrations may

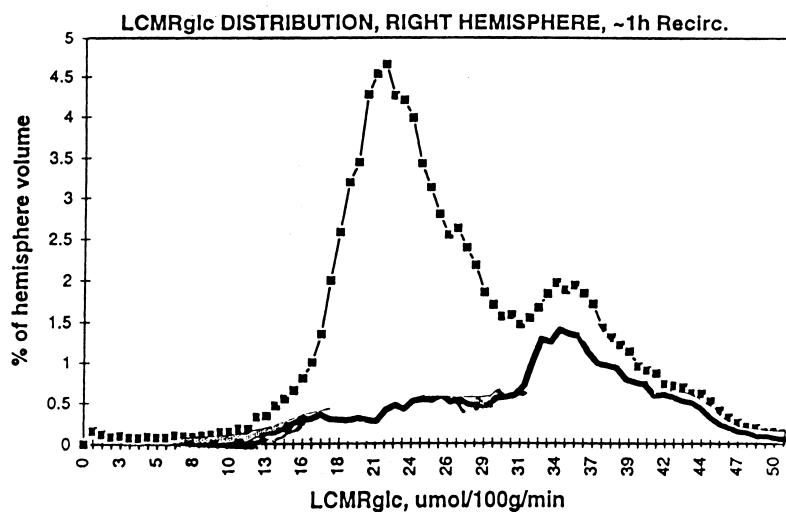


Fig. 10. *Upper curve with data points*: Distribution of rates of deoxyglucose phosphorylation, LCMRglc (as a measure of glucose utilization, but not of oxidative metabolism) in different pixels (“microareas”) at a specific coronal level of the right hemisphere subjected to occlusion of the middle cerebral artery for 2 h and subsequent recirculation for ~1 h. The curve is clearly bimodal, with one peak at 22 $\mu\text{mol}/100 \text{ g}/\text{min}$, another around 35 $\mu\text{mol}/100 \text{ g}/\text{min}$ and considerable overlap between the two distributions. By comparison with measured rates of cerebral blood flow (not shown) it can be concluded that the distribution peaking at 22 $\mu\text{mol}/100 \text{ g}/\text{min}$ represents the ischemic core and the distribution peaking at 35 $\mu\text{mol}/100 \text{ g}/\text{min}$ the penumbra. *Lower solid curve*: Surviving pixels in eight out of eight rats (100% survival), determined histologically at a later state in exactly the same pixels by three-dimensional analysis in a different series of animals from the same group, shown as a function of their rate of glucose utilization. Note that the surviving cells correspond to the distribution peaking around 35 $\mu\text{mol}/100 \text{ g}/\text{min}$ and, under these specific experimental conditions, corresponds to most of the peak representing the penumbra. Modified from Belayev et al. (1997), to whom can be referred for further details.

severely impair energy metabolism and production of transmitter glutamate.

Not all neuronal activation leads to the series of events described above, which focuses on glutamatergic transmission. During at least some types of sensory stimulation a very brief (<2 s) and very modest initial decrease in oxygen tension (<5%) has been convincingly demonstrated (Vanzetta and Grinvald, 1999). This effect was interpreted as an increase in oxygen utilization, probably as a direct result of sensory stimulation as such, and it was rapidly converted to the longer lasting and much more pronounced increase in oxygen tension, characteristic of enhanced blood flow and utilization of glucose without corresponding increase in oxygen consumption. Also, seizures have long been known to cause a large increase in lactate production, accumulation and release (Bolwig and Quistorff, 1973). Similar phenomena are caused during spreading depression (Cruz et al., 1999). This type of functional activation is not focused on provision and subsequent degradation of glutamate, but on cellular re-accumulation of extracellular K^+ in neurons and glial cells. Both glycolytically derived and oxidatively derived energy is necessary for cellular uptake of K^+ , perhaps with larger emphasis on the glycolytically derived energy initially, when the extracellular K^+ concentration is still greatly increased (Raffin et al., 1992). Under these conditions, the production of lactate may become so large that there is an overflow of lactate to circulating blood or to non-stimulated brain areas (Cruz et al., 1999). This is a genuine dissociation between utilization of glucose and of oxygen in the brain, rather than the temporal “tying up” of chemical energy by de novo synthesis of glutamate from glucose, most of which is recovered when glutamate eventually is oxidatively degraded (Hertz and Fillenz, 1999).

A large and important question has not been dealt with, i.e., to what extent is neuronal glucose degradation via glutamate impaired in degenerative brain disorders? Only little exact information is available in this field, although a decreased glutamate content (Lowe et al., 1990) has been observed in brain biopsies from patients suffering from Alzheimer's disease. A decrease in glutamine synthetase expression and activity has also been observed by some investigators in postmortem brain tissue from victims of Alzheimer's disease (Le Prince et al., 1995; Smith et al., 1991), whereas other authors found no correlation between Alzheimer's disease and glutamine synthetase expression (Jorgensen et al., 1990). Similarly, a decreased uptake of D-aspartate (which is accumulated by glutamate transporters) and a reduced expression of the glial glutamate transporter GLT (EAAT-2) have been reported in postmortem brain tissue from Alzheimer's patients by some authors (Procter et al., 1988; Li et

al., 1997), whereas others found considerable interindividual differences in expression of both GLT and the astrocyte-specific GLAST, but no significant correlation with Alzheimer's disease (Beckstrom et al., 1999). In spite of these divergent results, it is likely that deficiencies in glutamatergic activity may contribute to the impairment of memory in Alzheimer patients (Fønnum et al., 1995; Myhrer, 1998), since glutamatergic activity is so important for the establishment of memory.

The preliminary study illustrated in Table 2 shows no significant change in glutamine synthetase activity, but a distinct decline in pyruvate carboxylase activity in postmortem tissue from the frontal and temporal lobes of Alzheimer brains, compared to age-matched control tissue from the same areas. These observations should be interpreted with caution, both because the number of patients is small and on account of postmortem decline in the activities of the two enzymes, which in the controls are only 10–20% of those found in the rat brain. This difference is far too large to be accounted for by species differences. Moreover, it is unthinkable that pyruvate carboxylase activity was abolished in vivo, although clinical conditions are known in which reduced expression or biotinylation of pyruvate carboxylase occur, concomitant with severe psychomotor retardation (Wallace et al., 1998). However, the distinct difference between the two glial-specific enzymes seems of sufficient interest to warrant a more detailed investigation of possible impairment of pyruvate carboxylase activity and pyruvate carboxylation in dementing degenerative brain disease. NMR spectroscopy has lately been used for in vivo measurements of specific compounds in Alzheimer's patients (Pfefferbaum et al., 1999; Rose et al., 1999). Although the procedures which were used in these studies are simpler than those required for dynamic studies of metabolism by NMR, it might become possible to use

Table 2

Activities ($\text{nmol} \times \text{mg}^{-1} \text{ protein} \times \text{min}^{-1}$) of pyruvate carboxylase (PC) and glutamine synthetase (GS) in postmortem, previously frozen samples of brain tissue from Alzheimer patients and age-matched controls as well as in fresh mouse brain

Tissue	PC	GS
Alzheimer's brains ($n = 3$)	-0.02 ± 0.01^a	7.2 ± 1.0^a
Control human brain ($n = 4$)	$0.11 \pm 0.02^{b,c}$	$5.0 \pm 0.1^{b,d}$
Mouse brain ($n = 4$)	1.24 ± 0.07^e	18.3 ± 2.6^f

^a Average plus minus S.E.M. of five tissue samples (three from frontal and two from temporal lobes).

^b Average plus minus S.E.M. of eight tissue samples (four from frontal and four from temporal lobes).

^c Significantly different from control ($P < 0.005$) in student's *t*-test.

^d Not significantly different from control.

^e From Yu et al. (1983).

^f From Hertz et al. (1978).

NMR techniques to establish whether malfunction of neuronal-astrocytic metabolite trafficking in the human brain *in vivo* contribute to the pathophysiology of dementing disorders.

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