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CHAPTER 21

Glutamate as an energy substrate for neuronal-astrocytic interactions

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Introduction

Glutamate is the most plentiful amino acid and the major excitatory neurotransmitter in adult CNS (Watkins and Evans, 1981; Fonnum, 1984; Shank and Aprison, 1988). Obviously, it plays more roles than neurotransmission alone. Glutamate participates in the synthesis of proteins, peptides and fatty acids, and in the control of osmotic or anionic balance; it is a constituent of at least two important co-factors, glutathione and folic acid; it contributes along with glutamine to the regulation of ammonia levels; and it serves as precursor for GABA and various tricarboxylic acid (TCA) cycle intermediates.

There is no doubt that glutamate is released from neurons in large amounts. Uptake studies demonstrated that both neurons and astrocytes take up glutamate (Yu and Hertz, 1982; Schousboe et al., 1988). The major part of this glutamate is accumulated in astrocytes (McLennan, 1976; Yu and Hertz, 1982; Hertz et al., 1983). The uptake of glutamate into astrocytes seems to represent a net transfer of carbon skeleton from neurons to astrocytes (Hertz et al., 1983; Schousboe et al., this volume). Three probable roles for this uptake process are: (1) to remove the glutamate from extracellular space and synaptic clefts as a means of termination of the transmitter activity; (2) to form glutamine during the detoxification of ammonia; and (3) to serve as a metabolic substrate for astrocytes. This chapter will focus on the role of glutamate as a metabolic substrate. Other aspects of glutamate metabolism are described in other chapters in this volume.

Cerebral tissue is complex, with many different cell types that makes it difficult to determine the relevant mechanisms and the type of cell involved in in vivo studies. We have used primary cell cultures of rat cerebral cortical astrocytes and neurons to partly circumvent the problem of complexity (Yu et al., 1986, 1989). This system allows us to study cellular metabolism and to examine factors known to be involved in CNS injury in culture enriched in a single cell population.

Glutamate as an energy substrate

The use of glutamate as a substrate for metabolic oxidation is supported by the observation that cultured astrocytes maintained their rate of oxygen uptake better in a medium containing glutamate but no glucose than in a substrate-free medium (Yu and Hertz, 1983). Exogenous glutamate is taken up by both astrocytes and neurons (Yu et al., 1982). Metabolic studies have shown that a part of the glutamate taken up by astrocytes is metabolized to CO_2 and another part to glutamine, the latter of which then can be returned to neurons as a precursor for glutamate and GABA (Yu et al., 1982; Hertz et

al., 1983; Waniewski and Martin, 1986; Hertz and Peng, this volume). Glutamate taken up by neurons is metabolized to CO_2 to a lesser extent (Hertz et al., 1988a). We have shown that glutamate enters the TCA cycle mainly through the action of glutamate dehydrogenase, and not through glutamate transaminase (Yu et al., 1982; Hertz et al., 1983, 1988a). This is important because incorporation of the glutamate carbon skeleton into the TCA cycle mainly reflects a net conversion of glutamate to α-ketoglutarate, which is then decarboxylated to succinyl CoA, which can be used to produce CO_2 and ATP. The available evidence suggests that the activity of glutamate dehydrogenase is sufficient to allow glutamate to serve as a substrate for astrocyte energy metabolism (Hertz, 1982; Yu et al., 1982; Hertz et al., 1988a).

Glutamate seems to exert a regulatory effect on glycogen metabolism in astrocytes. Under constant metabolic demand, the entry of glutamate-derived α-ketoglutarate into the TCA cycle decreases the entry of glucose-derived pyruvate into the cycle. If this is the case, glucose metabolism will favor glycogenesis. The finding of a doubling of glycogen content in primary cultures of astrocytes in the presence of L-glutamate supports this hypothesis (Swanson et al., 1990). Other amino acids and the glutamate receptor antagonist kynurenic acid did not exert a similar effect or influence the glutamate effect. The effect was completely blocked by the glutamate uptake inhibitor threo-3-hydroxy-D,L-aspartate, or by the removal of Na^+ from the medium. This observation again suggests that glutamate is closely linked to energy production mechanisms in astrocytes.

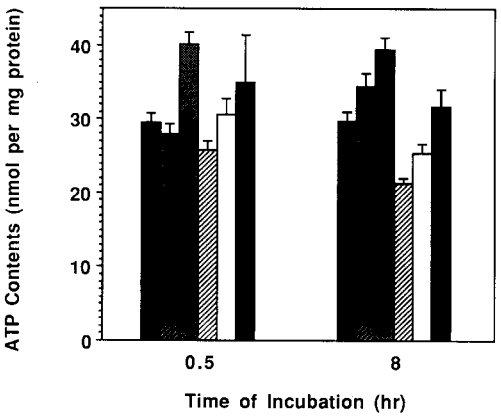

Fig. 2. ATP content in primary cultures of rat cerebral cortical astrocyte were measured 0.5 and 8 h after treatment with glutamate and/or K^+. Cultures were at least 4 weeks old with no dibutyryl cAMP treatment before use for the experiment. Control, ■; 1 mM glutamate, ▨; 5 mM glutamate, ▩; 55 mM K^+, ▨; 55 mM K^+ + 1 mM glutamate, □; 55 mM K^+ + 5 mM glutamate, ■. Data were adapted from Eng et al. (1992).

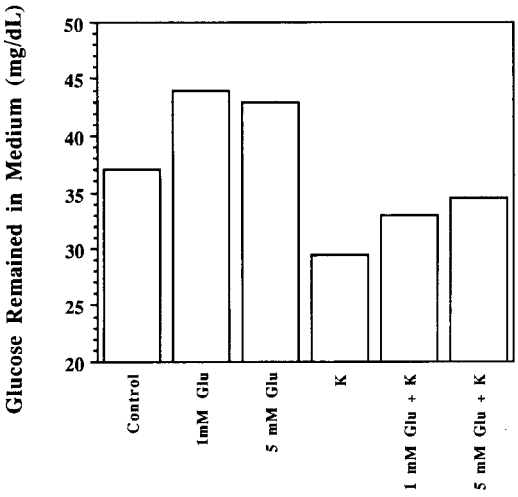

Fig. 1. Glucose content remaining in medium of astrocyte culture after 16 h of incubation with 1 or 5 mM glutamate and/or 55 mM K^+. The medium glucose content at 0 h was 63.07 mg/dl. Cultures were prepared from cerebral cortex of newborn rats and used for experiment when they were at least 4 weeks old with no dibutyryl cAMP treatment. Glucose was measured by a Kodak Ektachem 700 machine based on the method described by Trinder (1969). Data were adapted from Eng et al. (1992).

Glutamate also affects glucose utilization in astrocytes (Swanson et al., 1990; Eng et al., 1992). Changes in glucose content in medium of primary culture astrocytes were studied in the presence and absence of glutamate (Fig. 1). After incubation with 3.5 mM glucose (63 mg/dl) and 1 or 5 mM glutamate for 16 h, the glucose contents in culture medium were higher than in the control culture medium, indicating less glucose utilization. The role of glutamate as an energy substrate in the presence of glucose was further studied by measurement of

ATP content in astrocytes after exposure to glutamate (Eng et al., 1992). A normal culture of astrocytes contains 30.1 ± 0.8 nmol of ATP per mg protein. The results showed that cultures incubated with glutamate contained a higher cellular ATP content (Fig. 2). A 30 min incubation with 1 mM glutamate did not cause an observable increase in ATP content, but the increase in ATP content was apparent after 8 h. Cultures treated with 5 mM glutamate contained a significantly higher ATP content than those with 1 mM after 0.5 h of incubation. The ATP content was maintained at the same level when measured at 8 h. These observations support the concept that glutamate can serve as an immediate energy substrate for astrocytes. Therefore, glutamate, when present in the medium or extracellular space, may be preferentially metabolized by astrocytes in place of glucose. This conclusion was also suggested by the work of Hertz et al. (1988a) who compared the relative rates of oxidation of glutamate and glucose in astrocyte cultures.

Pathological conditions

It is now well-known that glutamate plays an important role in the pathogenesis of various neurologic diseases and insults (Choi, 1988, 1990). Increased extracellular glutamate is one of the biochemical events that result in structural and functional damage to neural cells. Other biochemical events include the degradation of membrane phospholipids, leading to the release of polyunsaturated fatty acids (PUFAs), especially arachidonic acid and docosahexaenoic acid; an increase in extracellular content of K^+; and a depletion of high-energy phosphate. It is now recognized that brain damage resulting from several different insults share in common a perturbation of cellular energy metabolism (Siesjö and Wieloch, 1985). Elucidation of such common features may shed light on general mechanisms and the relative importance of factors such as glutamate, K^+, PUFAs and energy level in cell damage resulting from hypoxic and ischemic insults.

Potassium

Neuronal activity can readily lead to elevations of extracellular K^+. During epileptogenesis, K^+ levels may be three to four times higher than normal (Katzman and Grossman, 1975). During oxygen deficiency, the extracellular K^+ concentration increases from its normal level of 3 mM to levels well above 50 mM (Hansen, 1985; Hossmann, 1985). The fluctuation of extracellular levels of K^+ during normal and abnormal conditions is known to affect energy metabolism and fluid distribution in brain slices (Hertz, 1981, 1990). It has consistently been found that high K^+ causes an immediate but relatively transient increase in the respiration rate of astrocytes (Hertz, 1981, 1982; Yu et al., 1983). Along similar lines, Holtzman and Olson (1983) have found that astrocyte metabolism is stimulated by dinitrophenol to a much larger extent than neuronal oxygen uptake. These observations suggest that the elevation of extracellular K^+ concentration resulting from neuronal release or injury can directly trigger an increase in astrocyte metabolism.

In primary culture of astrocytes, glucose uptake is increased by elevated concentrations of K^+ (Hertz, 1982; Yu and Hertz, 1983). We studied the K^+ effect on glucose metabolism in astrocytes by measuring the glucose content of the culture medium incubated with 50 mM K^+ (Eng et al., 1992; Fig. 1). In cultures with high K^+, the medium glucose content was lower than in the controls (Fig. 1). This indicates that glucose consumption in K^+-treated astrocytes is higher, reflecting an increased aerobic glycolysis and/or an increased oxidative metabolism. An increased aerobic glycolysis has been observed by Walz and Mukerji (1988). Consistent with our previous findings, high K^+ appeared to also stimulate oxidative metabolism, as suggested by an increase in pyruvate/lactate ratio in cultures treated with high K^+ (0.092 compared to 0.08 in normal culture at 6 h, $P < 0.005$) (Yu et al., 1990; for further discussion, see also Hertz, 1992). Glutamate added to these cultures slowed down glucose utilization as indicated by a higher amount of glucose remaining in the medium (Fig. 1).

We have measured the ATP content of cultured astrocytes in the presence of K^+ and glutamate (Fig. 2). The ATP content of astrocyte was substantially reduced by elevated K^+ concentrations. The

effect was observable after 0.5 h of exposure. ATP was further reduced at 8 h of exposure. A K^+-induced reduction of ATP content in astrocytes has been reported by others (Hertz, 1982). Again, this may be an astrocytic phenomenon as the ATP content of cultured neurons is not affected by high K^+. However, the decline in ATP content was relatively small, suggesting that an elevated ADP/ATP content may not be the only stimulus for the increase in oxidative metabolism in astrocytes (Hertz, 1992).

Addition of glutamate to these cultures seems to reverse the reduction of ATP content by K^+ (Eng et al., 1992; Fig. 2). In the presence of either 1 or 5 mM glutamate, the ATP content of the culture incubated with high K^+ remained in the normal range during the first half hour. At 8 h of incubation, ATP in culture treated with 1 mM glutamate was lower than the control, but the ATP level was still significantly higher than in cultures with high K^+ without addition of glutamate. The ATP level in high K^+-treated cultures with addition of 5 mM glutamate remained normal through the 8 h experimental period. Glucose alone is not enough to satisfy the energy demand of astrocytes when there is an increase in extracellular K^+. Therefore, glutamate may serve as a key supplemental energy substrate for astrocytes under these circumstances.

Polyunsaturated free fatty acids

It is known that complete ischemia leads to a rise in tissue PUFA concentrations (Bazan and Tureo, 1980; Yoshida et al., 1982). The normalization of the PUFA concentration during recirculation and reoxygenation is relatively slow (Yoshida et al., 1982). As originally shown by Bazan (1970), the PUFAs showing the largest relative increases are arachidonic acid and docosahexaenoic acid. It has been reported that arachidonic acid and its radical metabolites are key determinants of membrane injury in astrocytes (Chan et al., 1988). Furthermore, arachidonic acid is a precursor to prostaglandins, thromboxane, and leukotrienes, which are known to play a role in various insults (Moncada, 1983; Barkai and Bazan, 1989).

Polyunsaturated free fatty acids inhibited

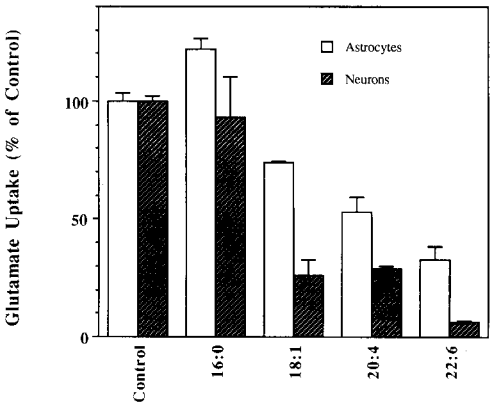

Fig. 3. Effects of fatty acids (0.5 mM) on the uptake rate of [U-^{14}C]glutamate in primary culture of rat cerebral cortical astrocytes and neurons. The uptake was measured after 90 min exposure to the fatty acids. Controls were cultures without exposure to fatty acids. Values are in percentage of the uptake rate of the control ± S.E.M. For details, see Yu et al. (1986).

glutamate uptake in both astrocytes and neurons (Yu et al., 1986; Fig. 3). The inhibitory effect was both dose- and time-dependent. Other PUFAs, such as docosahexaenoic acid, affected amino acid uptake in a manner similar to arachidonic acid in both astrocytes and neurons. However, saturated fatty acids, such as palmitic acid, exerted no effect. Studies with primary cultures of cerebellar granule cells (a glutamatergic neuronal preparation) (Yu et al., 1987) showed that the glutamate uptake was equally sensitive to arachidonic acid as that in primary cell cultures of cortical neurons.

PUFA-inhibited glutamate uptake would lead to an extracellular accumulation of this excitotoxic compound. It has been shown that high concentration of extracellular glutamate can induce depolarization of astrocytes (Bowman and Kimelberg, 1984). The toxic effects caused by failure of the glutamate uptake system may also be metabolically related. As mentioned above, astrocytes accumulate and convert glutamate to α-ketoglutarate and subsequently to CO_2 and succinyl CoA as metabolic substrate (Yu et al., 1982; Hertz et al., 1983; Yu and Hertz, 1983; Hertz and Peng, this volume). Neurons take up extracellular

glutamate as one way to replenish the loss of this compound during neurotransmission (Hertz et al., 1983, 1992). Therefore, the inhibition of glutamate uptake induced by PUFAs in astrocytes and neurons would cause a deficiency in the supply of glutamate as a metabolic fuel to astrocytes. This may be fatal in the situation of increased extracellular K^+, which may occur during injury and neuronal transmission. It would also be detrimental for the replenishing mechanisms in neurons (see Hertz and Peng, this volume; Schousboe et al., this volume).

There is evidence that extracellular arachidonic acid and glutamate are synergistic in inducing cell damage (Yu and Chan, 1988). Astrocytes were incubated with glutamate (1 mM) and/or arachidonic acid (2 mM) and the amount of lactate dehydrogenase (LDH) released was measured as an index of cell injury (Yu et al., 1989; Fig. 4). Glutamate at 1 mM did not cause any LDH release. At 2 h in 2 mM arachidonic acid, astrocytes began to release LDH. When the culture was incubated in the presence of 1 mM glutamate and 2 mM arachidonic acid, LDH was detected as early as after 1 h of exposure. LDH release was always higher in cultures treated with both compounds, indicating

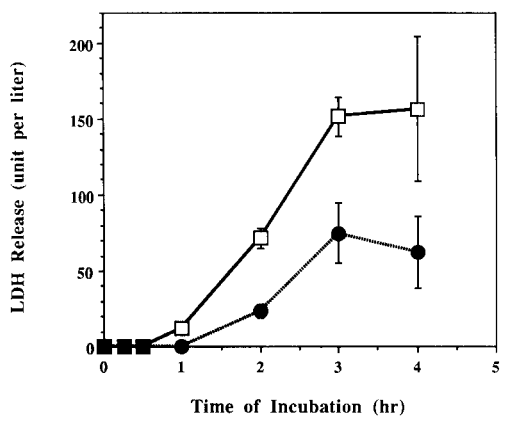

Fig. 4. Lactate dehydrogenase release from primary culture of rat cerebral cortical astrocytes after treatment of glutamate (1 mM) and/or arachidonic acid (2 mM). LDH was measured as described in Yu et al. (1989). Arachidonic acid, ●; glutamate and arachidonic acid, □. Glutamate alone did not induce release of LDH (data not shown).

the two compounds were working synergistically in damaging astrocytes. The mechanisms involved in the process of injury seem to differ between glutamate and arachidonic acid. Cultures treated with both glutamate and arachidonic acid produced the same amount of malondialdehyde (MDA) as cultures with arachidonic acid alone (Yu et al., 1989). An increase of MDA content indicates lipid peroxidation, a process closely related to free radical formation and release of PUFAs (Yu and Chan, 1988). This indicated that lipid peroxidation is one of the mechanisms involved in arachidonic acid-induced injury. As glutamate alone does not cause MDA formation in culture of astrocytes, other mechanisms may contribute in producing the synergistic effect.

Hypoxia-ischemia

The causes of injury under ischemia are multifactorial, including severe hypoxia, substrate deprivation, and failure to remove toxic metabolic products. During global ischemia, a 7-fold stimulation of brain glycolytic activity may be due to an increase in intracellular Na^+ and extracellular K^+ (Shanker and Questel, 1972). The effects of raised K^+ concentration, as mentioned above, are to a large extent exerted on astrocytes, suggesting that K^+ may be a key factor determining astroglial reactions to ischemia-hypoxia. The stimulation of glycolysis and a reduction of glutamate uptake into partly depolarized cells may magnify the depletion of metabolic intermediates and ATP during ischemia. Glutamate has been shown to protect the integrity of the oxidative respiratory system during anoxia (Phizackerley and Fixter, 1973). Such protection may not exist if PUFAs are released from the tissue and inhibit the uptake of glutamate.

We have shown that severe hypoxia causes morphological changes, injury and metabolic dysfunctions, including glutamate uptake, in primary culture of astrocytes (Yu et al., 1989). Evidence for the injury includes a time-dependent loss of LDH activity and an increase of MDA content. Inhibition of glutamate uptake may be caused by an energy failure of hypoxic astrocytes, or by PUFAs released

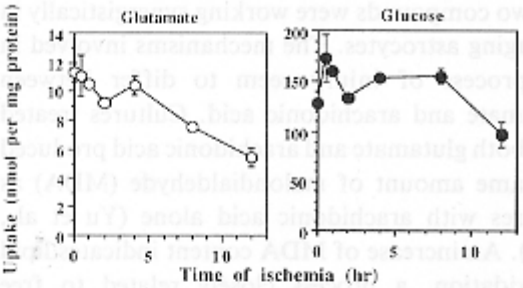

Fig. 5. [U-^{14}C]Glutamate and [U-^{3}H]glucose uptake were measured in primary cultures of rat cerebral cortical astrocytes (4-week-old without dibutyryl cAMP treatment) as a function of time of ischemia. The total uptake time was 5 min. The concentrations of glutamate and glucose were 50 μM and 3.5 mM, respectively. For details, see Yu et al. (1992).

from the disrupted cell membrane. The observed inhibition of glutamate uptake agrees with the findings of others, that during hypoxia and many other pathological conditions, uptake of this neurotransmitter amino acid is inhibited, but its release is enhanced (Arnfred and Hertz, 1971; Benveniste et al., 1984; Hirsch and Gibson, 1984; Globus et al., 1988). We have shown that glutamate uptake was inhibited by the presence of arachidonic acid and other PUFAs (Yu et al., 1986). The defect of glutamate uptake in astrocytes would lead to an accumulation of this excitotoxic amino acid in the extracellular space and subsequent receptor-mediated neuronal cell death (Olney, 1983; Meldrum, 1985; Rothman and Olney, 1986; Choi, 1988, 1990). Under hypoxia, the concentration of glucose in the astrocytic culture medium declines progressively and the pyruvate/lactate ratio is decreased (Yu et al., 1990). Under similar conditions, a decrease in ATP content was observed which correlated well with the release of LDH into the culture medium and inhibition of the glutamate uptake (Gregory et al., 1990).

We have studied the uptake of glutamate and glucose in astrocyte cultures under ischemia (Yu et al., 1992). The ischemic condition was created by sealing the culture with a layer of mineral oil after the culture medium had been drained. The glutamate uptake in ischemic cultures was inhibited as expected (Fig. 5). The rate of glucose uptake was slightly higher than the control throughout the first 8 h ischemia. Such higher uptake may be metabolically related. It is known that the glucose utilization rate of astrocytes increases during hypoxia, as reflected by an increase in glucose consumption and lactate production (Hertz, 1981; Yu et al., 1990). The lower glucose uptake after 8 h of ischemia may indicate a leakage of cell membrane or cell death.

Cerebral uptake of glucose has been shown to gradually increase to a three-fold higher level than the pre-ischemic level between 1 and 3 h post-ischemia (Nemoto, 1978). A similar result was observed in cultures of astrocytes during a post-ischemic period. Using the same mineral oil ischemia model (Yu et al., 1992), we measured the uptake of glutamate and glucose in astrocyte cultures 5 and 12 h after 30 min of ischemia (Fig. 6). The uptake of glutamate was increased by 23% at 5 h and 35% at 12 h in the post-ischemic period. The uptake of glucose was also increased by 23 and 26%. Such increases in glutamate and glucose uptake during post-ischemic incubation were observed in all astrocyte cultures under ischemia for less than 8 h. These data suggested that during post-ischemia,

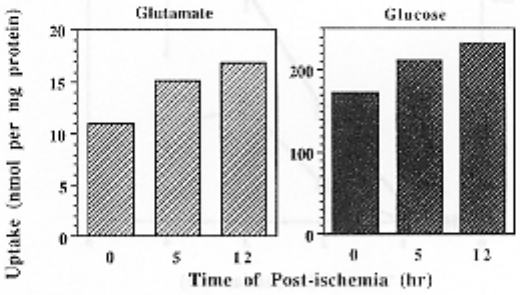

Fig. 6. [U-^{14}C]Glutamate and [U-^{3}H]glucose uptake were measured in primary cultures of rat cerebral cortical astrocytes (4-week-old without dibutyryl cAMP treatment) 5 and 12 h after 30 min of ischemia. The total uptake time was 5 min. The concentrations of glutamate and glucose were 50 μM and 3.5 mM, respectively. For details, see Yu et al. (1992).

astrocytes are active in taking up energy substrates for the replenishment of depleted intracellular compounds. Cultures under ischemia for longer than 8 h did not show an increase in uptake of either substrate. This indicates that the injury to the cells beyond 8 h of ischemia was such that no biochemical uptake of energy substrates could be performed.

Concluding remarks

Based on in vivo studies, it has been thoroughly established that the brain's only significant substrate for energy metabolism under normal circumstances is glucose (see Sokoloff, this volume). Through the overall pathways of glucose metabolism, glucose carbon has been shown to incorporate into many compounds, including intermediates of glycolysis and the TCA cycle. It has been shown that brain slices, homogenates, cell-free fractions, and cultured neural cells can utilize glutamate, arginine, glycine, ϱ-aminobenzoate, succinates, malate, lactate, pyruvate, acetate and ketone bodies as energy substrates (Robinson and Williamson, 1980; Yu and Hertz, 1983; Lopes-Cardozo et al., 1986; Edmond et al., 1987; Hertz et al., 1988b, 1992). Oxidation of these compounds as energy substrate is consistent with glucose as a primary energy substrate because the latter metabolites are derived from the glucose carbon skeleton. Such an alternative capability provides much flexibility and efficiency to the energy metabolism system beyond the blood-brain barrier, especially in the interactions between neurons and astrocytes.

Inhibition of glutamate uptake was observed in most injurious conditions in the CNS. This is a derangement of one of the most important biochemical functions of astrocytes. There is no doubt that glutamate released from neurons, to a large extent, is taken up and metabolized by astrocytes. The accumulated glutamate undergoes oxidative deamination to α-ketoglutarate followed by decarboxylation to CO_2 and succinyl CoA. This process will ultimately increase ATP content in astrocytes. During neuronal activity, there is an elevation of K^+ which, in turn, induces a depletion of astrocyte ATP content. Such ATP depletion in astrocytes has been shown not to be replenished by glucose alone. Under such conditions, astrocytes need to metabolize other substrates, such as glutamate, in place of or together with glucose. This conclusion has been further supported by the observation of the lack of ATP depletion under high K^+ when glutamate is present. Such preference during periods of rapid neuronal release of glutamate may not only facilitate clearance of glutamate from the extracellular space, but also spare the available extracellular glucose for neuronal metabolism. Most important, oxidation of the exogenous glutamate provides an extra source of energy substrate for astrocytes during neuronal excitation.

Inhibition of glutamate uptake resulting from released toxins and/or dysfunction of astrocytes will result in an excessive accumulation of synaptic glutamate and subsequent loss of calcium homeostasis in the post-synaptic neurons. Strong evidence points to the activated NMDA receptor/channel as an important route of calcium entry (Choi, 1988, 1990). This subject is beyond the scope of this chapter. Interestingly, this NMDA-related component of damage was, at least under certain conditions, also energy-dependent, since hypoxic neuronal death was prevented by glucose at concentrations above 1 mM (Tombaugh and Sapolsky, 1990). Protection was observed even when glucose was elevated to hyperglycemic levels, suggesting that storage of intracellular energy substrates is an important factor in regulating ischemic pathology. The glucose utilization rate in astrocytes increases during hypoxia. Simultaneously, the energy substrate transport system is impaired. These consequences could conceivably propagate a spiral of energy failure by increasing tissue energy demand and decreasing energy supply (Kaplan et al., 1987). By no means are we trying to imply that glutamate is the primary energy substrate under these circumstances, but its special role in neurotoxicity and participation in the neuropathogenesis of various neural diseases and injury certainly distinguish it from the others.

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