

Inhibition of Glutamine Synthetase Induces Critical Energy Threshold for Neuronal Survival

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A current hypothesis is that reactive oxygen species, damage to mitochondrial DNA, and decreased rates of oxidative phosphorylation (OXPHOS) contribute to the selective neuronal death in Alzheimer's disease (AD).¹⁻³ We propose that age-related inhibition of the glial enzyme glutamine synthetase (GS), whose inhibition is further increased in AD,⁴ ultimately results in a decreased rate of neuronal OXPHOS and a decreased rate of release of neurotransmitter glutamate.⁵ We argue from published data that the gradual reduction in astrocytic GS activity, in combination with the rate of glutamate release, can determine a critical energy threshold for neuronal viability. In other words, we suggest that an important cause of impaired energy metabolism in AD may reside in astrocytic rather than in neuronal metabolism.

BACKGROUND

Most investigations into the cause of the suspected impairment of mitochondrial energy metabolism in Alzheimer's disease (AD) have focused on alterations of neuronal enzyme activity.¹⁻³ However, the interdependence of neurons and astrocytes with respect to exchange of intermediates of the citric acid cycle (CAC), glutamate and glutamine, is well established (FIG. 1).⁶⁻⁸ In addition to the classical glutamate-glutamine cycle (FIG. 2), the following exchanges occur as well: Astrocytes release several intermediates of the citric acid cycle, namely malate, citrate and α -ketoglutarate (FIG. 1).^{9,10} Neurons take up malate, α -ketoglutarate, possibly lactate,¹¹ but apparently not citrate.¹⁰ The oxidation of acetyl CoA by the CAC is sufficient to maintain the activity of the CAC because the equivalent of the two carbons of acetyl CoA are released as CO₂ by the actions of the CAC during a single "turn" of the cycle. In other words, consumption of acetyl CoA does not add to or remove from the mass of intermediates of the CAC.¹²

Continued glutamate neurotransmission requires resynthesis of glutamate, whose carbon atoms may be taken from the intermediates of the CAC via α -keto-

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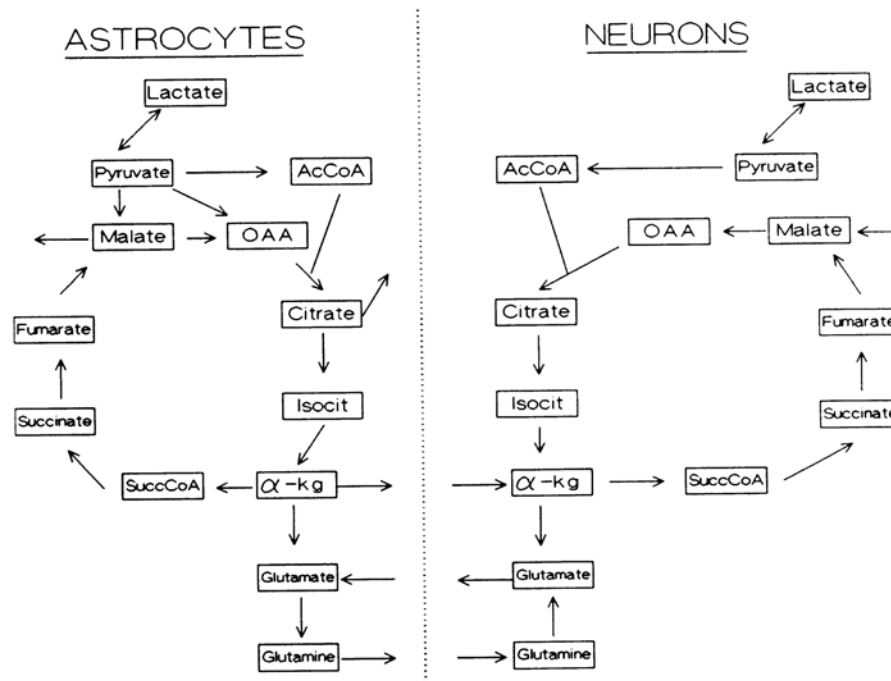


FIGURE 1. Normal metabolic flow and intercellular traffic between intermediates of the citric acid cycle (CAC) in astrocytes and glutamatergic neurons. *Arrows* indicate direction of movement of carbon skeletons (metabolic flow) and of molecules (intercellular traffic). Astrocytes and neurons convert pyruvate to acetyl CoA and CO_2 , a reaction catalyzed by pyruvate dehydrogenase complex. Acetyl CoA is required for citrate synthase and is sufficient for the maintenance of the CAC, by replacing the two carbon atoms lost as CO_2 during each traversal of the cycle. *In vitro*, neurons take up α -ketoglutarate and malate as well as glutamine from the extracellular fluid. Astrocytes in culture release malate, citrate, α -ketoglutarate, and glutamine.

glutamate (FIG. 2). Replenishment of metabolic intermediates of the CAC is accomplished by means of the "anaplerotic" enzymes (e.g., pyruvate carboxylase and malic enzyme) that do not belong to the CAC, but whose products are metabolic intermediates of the CAC. In brain, the anaplerotic enzymes are found in astrocytes, but not in neurons.^{13,14} The differential localization of anaplerotic enzymes implies that maintenance of a steady rate of neuronal oxidative metabolism during periods of glutamate neurotransmission requires neuronal uptake of glutamine (the classical glutamate–glutamine cycle) and/or intermediates of the CAC. In particular, neuronal metabolism of glucose, pyruvate, or lactate cannot fill up the pools of the CAC to compensate for carbons released in glutamate molecules.

Next we explore the possible consequences of the decreases in activity of glutamine synthetase that have been observed in frontal cerebral cortex of older adults (average age, 70 years) compared to younger adults (average age, 29 years) (decrease of 45%) and in patients with AD compared to cognitively normal elderly persons (decrease of 40%).⁴

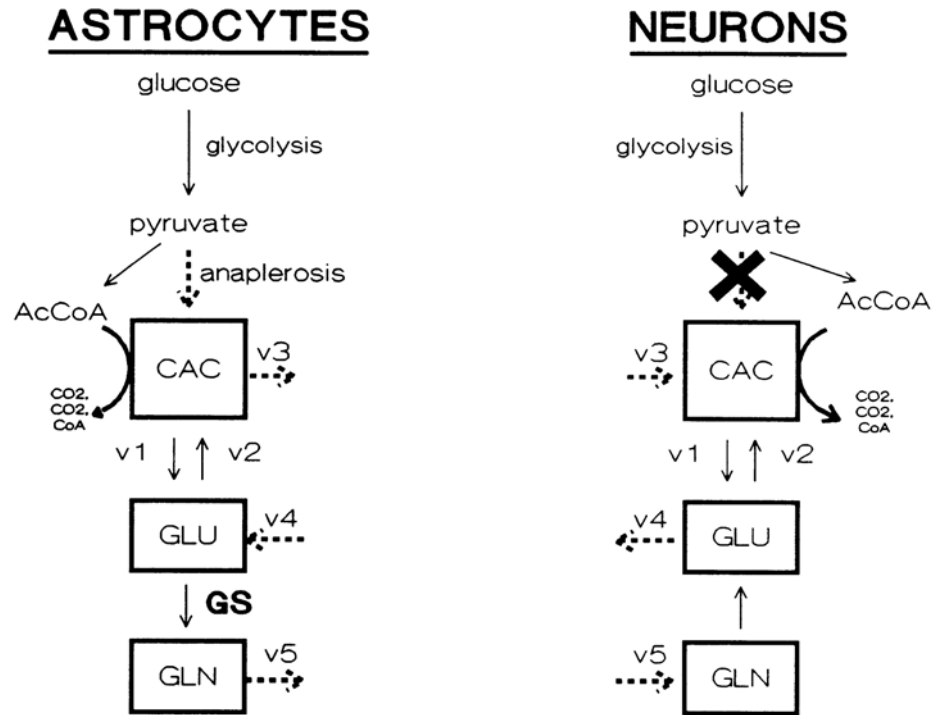


FIGURE 2. Schematic description of balance of carbons within the citric acid cycle (CAC) of astrocytes and glutamatergic neurons. Astrocytes but not neurons possess enzymes (pyruvate carboxylase or malic enzyme) capable of performing anaplerosis, i.e., increasing the mass of the pools of the CAC. Glutamate (GLU) exchanges carbons with the CAC via α -ketoglutarate by reactions catalyzed by aminotransferase enzymes or by glutamate dehydrogenase (v1, v2). Synthesis of glutamine (GLN) from glutamate is catalyzed by glutamine synthetase (GS), which is found almost exclusively in glia. Exchange of molecules among astrocytes and neurons occurs predominantly through the classical glutamate–glutamine cycle (v4, v5) as well as by release of intermediates of the CAC (e.g., malate) by astrocytes for uptake by neurons (v3).

Using the principle of conservation of mass, the net rate of increase in the total metabolites of the CAC, glutamate, and glutamine can be derived in terms of the chemical fluxes shown in *dotted* lines. For astrocytes, the formula is the following:

$$\text{anaplerosis} + v4 - (v3 + v5),$$

where v3 equals the rate of release of intermediates of the CAC, v4 equals the rate of uptake of glutamate, and v5 equals the rate of release of glutamine. For neurons, the net rate of increase of mass of the metabolites of the CAC, plus glutamate and glutamine is

$$v3 + v5 - v4,$$

where v3 equals the rate of uptake of intermediates of the CAC, v4 equals the rate of release of neurotransmitter glutamate, and v5 equals the rate of uptake of extracellular glutamine.

Consider the inactivation of GS in normal aging or in AD, which decreases the rate of conversion of glutamate to glutamine catalyzed by GS. The rate of release of glutamine from astrocytes (v5) will decrease, and at some point, the rate of uptake of glutamine in neurons (v5) will also decrease. In order to maintain the rate of the neuronal CAC (which depends

on the mass of metabolic intermediates), our formula (above) indicates that the decrease in v_5 must be compensated by an increase in $v_3 - v_4$. In other words, decreased neuronal uptake of glutamine requires decreased rate of glutamate release by neurons and/or increased rate of neuronal uptake of intermediates of the CAC. When the rate of glutamate neurotransmission exceeds the rate of uptake of extracellular molecules by the neuron, it may reduce the mass of metabolites of its CAC (i.e., when $v_1 > v_2 + v_3$ in neurons). Reduction of the pools of the CAC lowers the rate of neuronal oxidative energy metabolism. Thus inactivation of GS may result in defining a limit to the rate of energy production by the CAC in neurons.

PREDICTION: EXISTENCE OF A CRITICAL NEURONAL ENERGY THRESHOLD DETERMINED BY ASTROCYTIC METABOLISM

Given that the activity of cerebral glutamine synthetase (GS) decreases with age and to a greater degree in AD, what might be the long-term consequences? Because glutamine synthetase (GS) consumes 98% of the ammonia entering the brain from the blood or cerebrospinal fluid,¹⁵ we suggest that gradual decrease in activity of GS will increase the concentration of ammonia (mostly present as ammonium ion)¹⁵ and require alternative means of disposing of extracellular glutamate (to avoid excitotoxic damage). This decline in activity of GS may be accelerated in specific regions of the brain by the increased concentration of ammonia, as has been observed in rats during chronic exposure to elevated levels of ammonia.¹⁶ Indeed, ammonia has been proposed as a contributor to the pathogenesis of AD.^{17,18}

The consequences of the metabolic interdependency of neurons and astrocytes during inhibition of GS can be summarized as follows (FIG. 2).⁵ Faced with a decrease in rate of uptake of glutamine, neurons will need to either substitute uptake of intermediates of the CAC (e.g., malate, α -ketoglutarate) or decrease the rate of release of glutamate. As the capacity for glial release of glutamine diminishes, a critical point will be reached at which continued release of glutamate will decrease the content of intermediates of the neuronal CAC, thereby lowering the rate of neuronal energy production. Increases in neuronal demand for oxidative metabolism will not be matched by increased oxidative phosphorylation, and neuronal viability will be compromised. Magnetic resonance spectroscopic measurements of patients with probable AD indicate a modest (-20%) deficit of cerebral glutamate + glutamine,¹⁹ which is consistent with this prediction.

It is important to note that the maximum activity of GS (determined *in vitro*) far exceeds (by a factor of 20–50) the actual activity of GS as measured in hyperammonemic rats *in vivo*.²⁰ At present, no one has reported any impairment of the rate of conversion of glutamate to glutamine in the brains of patients with AD *in vivo* compared to age-matched cognitively normal individuals.

ACKNOWLEDGMENT

We thank Dr. Caleb E. Finch for helpful discussions and for critiquing an earlier version of this paper.

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