# Astrocyte metabolism following focal cerebral ischemia

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**Anna Thorén**

Fakultetsopponent:
Prof. Gary Fiskum
University of Maryland School of Medicine,
Baltimore, USA

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**I.** Thoren AE, Helps SC, Nilsson M, Sims NR. (2005). Astrocytic function assessed from [1-$^{14}$C]acetate metabolism following temporary focal cerebral ischemia in the rat. *Journal of Cerebral Blood Flow and Metabolism,* 25 (4 ): 440-450.

**II.** Thoren AE, Helps SC, Nilsson M, Sims NR. (2006). The metabolism of $^{14}$C-glucose by neurons and astrocytes in brain subregions following focal cerebral ischemia in rats. *Journal of Neurochemistry*, 97 (4): 968-978.

**III.** Thoren AE, Sørbø J-G, Holen T, Moe S-E, Bergersen, LH, Ottersen O-P, Nilsson M, Nagelhus EA. Specialized membrane domains for lactate transport at the blood-brain and blood-retinal interfaces: enrichment of MCT4 in glial endfeet membranes. *Manuscript.*

Sahlgrenska akademin
VID GÖTEBORGS UNIVERSITET
Institutionen för neurovetenskap och fysiologi

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**Anna Thorén**

*Institute of Neuroscience and Physiology, the Sahlgrenska Academy, Göteborg University, Sweden*

## Abstract

Stroke is one of the leading causes of disability and death. Most often, stroke results from blockage of an artery in the brain leading to tissue infarction within the perfusion territory of the affected vessel. Despite the severity of the insult, many cells are not irreversibly damaged within the first few hours and can be rescued by early restoration of blood flow or other interventions. Astrocytes, the most numerous cells in the brain, normally perform many functions that are essential for neuronal viability. Thus, stimulation of key astrocytic properties in ischemic or post-ischemic brain could potentially contribute to neuroprotection. However, at present, there is very little understanding of either the response of astrocytes to cerebral ischemia or the extent to which these cells can recover function if blood flow is restored.

The main aim of the project was to assess key metabolic properties in astrocytes during early reperfusion following unilateral occlusion of the middle cerebral artery (MCA) in rats. Astrocytic oxidative metabolism was assessed from the incorporation of radiolabel from [1-$^{14}$C]acetate into glutamine, an activity that is essentially specific for these cells. Striatal tissue from the hemisphere subjected to ischemia showed substantial decreases in $^{14}$C-glutamine production at 1 hour of reperfusion following either 2 or 3 hours of ischemia. In contrast, this activity was almost fully preserved for at least 4 hours in parts of the cerebral cortex that had been subjected to more moderate ischemia, even when the duration of ischemia was sufficient to induce infarction in this region. The production of $^{14}$C-glutamine was also not significantly affected in cortical tissue exposed to more severe ischemia but this measure was much more variable between animals. These findings demonstrate regional differences in the response of astrocytes to focal ischemia and provide evidence that most cortical astrocytes remain viable and metabolically active for many hours, even in tissue destined to become infarcted.

To further evaluate metabolic recovery in the post-ischemic brain, the production of $^{14}$C-glutamate and $^{14}$C-glutamine from [U-$^{14}$C]glucose was assessed. Neurons are responsible for most of the $^{14}$C-glutamate generation whereas $^{14}$C-glutamine is produced in astrocytes from glutamate of neuronal and astrocytic origin. Marked reductions in the labeling of both amino acids were observed in all regions of the MCA territory during early reperfusion after either 2 or 3 h ischemia irrespective of whether the tissue would become infarcted. These results provide evidence for widespread depression of glucose metabolism in neurons and altered metabolic interactions with astrocytes. Interestingly, this reduction in glucose metabolism was not associated with substantial changes in tissue phosphocreatine content and ATP:ADP ratio suggesting that energy requirements were reduced by the ischemia-reperfusion.

Increases in lactate content were detected during early reperfusion in tissue regions that would develop infarcts. This finding coupled with previous evidence for deleterious effects of lactic acid suggests that accumulation of this metabolite might promote cell death. An impairment of pyruvate oxidation or reduced clearance of lactate could contribute to the increased lactate. The mechanisms by which excess lactate is cleared from the brain are not known. We hypothesized that MCT4 is involved in the removal of lactate as this transporter isoform is responsible for lactate export from other tissues. Using immunogold cytochemistry, MCT4 was found to be densely expressed in the endfeet of glial cells facing blood capillaries and pial surface of the brain, suggesting an important role in the removal of excess lactate from the CNS. In future studies, the expression of MCT4 will be examined following ischemia to resolve whether an altered expression of this transporter may be one reason for the elevated lactate levels in the brain.

***Key words:*** *Astrocyte, metabolism, focal cerebral ischemia, reperfusion, infarct, [1-$^{14}$C]acetate, [U-$^{14}$C]glucose, glutamine, glutamate, ATP, ADP, lactate, MCT4, immunogold cytochemistry.*

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