ABSTRACT

The brain encompasses several types of cells of which neurons and astrocytes are the major players. Neurons conduct electrical signals and communicate with each other via release of neurotransmitters, thus forming the basis for brain activity and function. Astrocytes ensheath synapses and are essential neuronal counterparts. Neurons as well as astrocytes rely heavily on continuous supply of glucose and oxygen to maintain proper brain function. Although glucose is the primary fuel, cerebral cells are capable of metabolizing alternative energy substrates. In this context, astrocytes are of particular interest. These cells ensure rapid uptake of glutamate from the synaptic cleft subsequent to glutamatergic neurotransmission. Glutamate taken up by astrocytes is returned to neurons in the form of glutamine and subsequently converted to glutamate in the neuronal compartment. Additionally, astrocytes perform oxidation and *de novo* synthesis of glutamate. Glutamate dehydrogenase (GDH) is in this regard an intriguing enzyme. GDH catalyzes the anaplerotic entry of glutamate into the tricarboxylic acid (TCA) cycle via oxidative deamination to α-ketoglutarate and the reverse reaction (i.e. reductive amination) forming glutamate from α-ketoglutarate. *De novo* synthesis of glutamate from glucose involves the astrocyte-specific enzyme pyruvate carboxylase (PC), which enables introduction of a TCA cycle intermediate, i.e. an anaplerotic pathway. For the TCA cycle to remain operational, anaplerosis must be balanced by cataplerotic mechanisms allowing carbon units to exit the TCA cycle. Among other enzymes, malic enzyme (ME) facilitates cataplerosis by catalyzing decarboxylation of the TCA cycle intermediate malate to pyruvate. Pyruvate may either be converted to lactate or alanine or reenter the TCA cycle via acetyl-CoA. These pathways are traditionally referred to as partial or full pyruvate recycling, respectively. Complete oxidation of the glutamate- and glutamine-derived carbon backbones can only occur via full pyruvate recycling.

The constant need for energy dictates mechanisms ensuring energy equivalents at a proper time and place. In this context, the metabolic master switch AMP-activated protein kinase (AMPK) is of considerable interest. This energy sensor is activated physiologically by low cellular energy status and in turn upregulates energy-producing pathways and downregulates energy-consuming pathways. However, the role of AMPK in astrocytes has until now received modest attention.

The goal of the current study was to investigate how AMPK and GDH are implicated in brain energy metabolism, with particular focus on astrocytes. Hippocampal slices and cultured astrocytes prepared from NMRI mice were employed to investigate the role of AMPK, while brain slices and cultured astrocytes from a CNS-specific GDH knockout mouse model of C57BL/6J mouse strain were

used to investigate the role of GDH in brain energy metabolism. Hippocampal slices from the GDH knockout mice were likewise used to investigate a possible link between GDH and AMPK. Metabolic mapping was obtained using a variety of ^{13}C-labeled substrates (i.e. [U-^{13}C]glucose, [^{13}C]bicarbonate, [1,2-^{13}C]acetate, [U-^{13}C]glutamate and [U-^{13}C]glutamine) in combination with gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) analyses. In addition to ^{13}C metabolic mapping experiments, supplemental analyses of glucose uptake and respiratory and glycolytic rates were performed in the part of the study where AMPK was investigated. Pharmacological activation of AMPK was brought about using the compound 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR).

Activation of AMPK led to increased glucose uptake and pyruvate carboxylation in cultured astrocytes from NMRI mice. Furthermore, our results pointed towards increased glucose oxidation and potentially increased glycolysis in hippocampal slices from NMRI mice in response to AMPK activation. Additionally, AMPK activation increased TCA cycle oxidation of glutamine in hippocampal slices from NMRI mice. While TCA cycle oxidation of glutamate was unaltered, partial and full pyruvate recycling of the carbon backbone of this amino acid was upregulated in response to AMPK activation in hippocampal slices from NMRI mice. Full pyruvate recycling was likewise increased by AMPK activation in hippocampal slices from C57BL/6J mice possessing GDH activity. Furthermore, our results revealed that GDH is important for oxidation of glutamate in hippocampal slices and cultured astrocytes from C57BL/6J mice. The reduced capacity for glutamate oxidation, due to loss of GDH activity, might be compensated for by increased flux through glycolysis and upregulated flux through alanine aminotransferase (ALAT). The increased flux through ALAT may provide a way of introducing the glutamate carbon backbone to the TCA cycle when GDH activity is absent. Moreover, in hippocampal slices from C57BL/6J mice, GDH was central in maintaining a compartment of TCA cycle intermediates used for complete oxidation of the glutamate-derived carbon backbone. Interestingly, upregulated flux through full pyruvate recycling, induced by AMPK activation, seemed to depend on GDH.