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Review

^{13}C-labelled microdialysis studies of cerebral metabolism in TBI patients

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ABSTRACT

Human brain chemistry is incompletely understood and better methodologies are needed. Traumatic brain injury (TBI) causes metabolic perturbations, one result of which includes increased brain lactate levels. Attention has largely focussed on glycolysis, whereby glucose is converted to pyruvate and lactate, and is proposed to act as an energy source by feeding into neurons' tricarboxylic acid (TCA) cycle, generating ATP. Also reportedly upregulated by TBI is the pentose phosphate pathway (PPP) that does not generate ATP but produces various molecules that are putatively neuroprotective, antioxidant and reparative, in addition to lactate among the end products.

We have developed a novel combination of ^{13}C-labelled cerebral microdialysis both to deliver ^{13}C-labelled substrates into brains of TBI patients and recover the ^{13}C-labelled metabolites, with high-resolution ^{13}C NMR analysis of the microdialysates. This methodology has enabled us to achieve the first direct demonstration in humans that the brain can utilise lactate via the TCA cycle. We are currently using this methodology to make the first direct comparison of glycolysis and the PPP in human brain.

In this article, we consider the application of ^{13}C-labelled cerebral microdialysis for studying brain energy metabolism in patients. We set this methodology within the context of metabolic pathways in the brain, and ^{13}C research modalities addressing them.

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1. Introduction

1.1. Need for better understanding of energy metabolism in the injured human brain

Energy metabolism in the human brain is incompletely understood, even in the normal uninjured state. Moreover, following traumatic brain injury (TBI), a complex (and variable) sequence of pathological processes arises, in which cerebral energy perturbations appear to play a major role. A well-recognised feature is elevation of brain extracellular lactate and the lactate/pyruvate (L/P) ratio, associated with unfavourable clinical outcome (Timofeev et al., 2011). TBI-induced pathologies evolve over the scale of hours and days, and, despite treatment, may lead to a range of clinical outcomes from good recovery to varying degrees of disability or even death (Kolias et al., 2013). Historically, attention has focussed on ischaemia, leading to a deficiency in supply of oxygen, glucose and other blood-borne nutrients, falling short of the metabolic demands of the injured brain. Much has been learned in neuro-critical care management of patients, so that ischaemia is minimised by adopting modern protocol-driven therapy designed to maintain adequate cerebral perfusion whilst keeping intracranial pressure below a critical threshold. Survival rates, and quality of survival, have thus improved. Despite these advances, there is a growing recognition that, in some circumstances, diffusion barriers (Menon et al., 2004) or increased metabolic demands, e.g. by spreading depression (Parkin et al., 2005), may adversely affect the balance between substrate supply and demand, thus resulting in tissue hypoxia or reduced glucose availability. In other settings, despite seemingly adequate provision of metabolic fuels and oxygen, the injured brain is unable to efficiently utilise these substrates to generate cellular energy. This has sometimes been termed 'mitochondrial dysfunction' although the exact basis of this process is not understood. Increased reliance on glycolysis, which produces a low yield of ATP per molecule of glucose, followed by conversion of pyruvate to lactate, is often regarded as a consequence of mitochondrial dysfunction. Also, alternative pathways that consume glucose may become upregulated, such as the pentose phosphate pathway (PPP) that does not generate ATP but is potentially reparative. There is therefore a need to improve our understanding of the fundamental processes of brain energy metabolism, refine our clinical interventions to minimise this energy failure and, ultimately, generate specific strategies to optimise clinical outcome.

Until recently, understanding of energy metabolism has arisen through laboratory investigations, and the concepts applied to humans. Advances in technology are now enabling direct, detailed exploration of metabolism in man. Techniques available include microdialysis, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and *in vivo* magnetic resonance spectroscopy (MRS).

In this article, we consider the application of the novel technique of ^{13}C-labelled cerebral microdialysis that we have developed for studying brain energy metabolism in patients (Gallagher et al., 2009). We set this methodology within the context of metabolic pathways in the brain, and ^{13}C research modalities addressing them.

1.2. Fundamental biochemistry of energy metabolism

Energy metabolism is the overall process through which living systems acquire and utilise the energy they need to carry out various functions. Humans, like other chemoorganotrophs, obtain this energy by oxidising organic compounds, notably carbohydrates, lipids and proteins. This energy is coupled to endergonic reactions resulting in the synthesis of high-energy phosphate compounds, specifically adenosine triphosphate (ATP). The primary organic compound utilised by humans is glucose. ATP is generated by a sequence of three well-recognised processes (Fig. 1), as follows:

(a) *Glycolysis:* This is the linear pathway (also termed Embden–Meyerhof–Parnas pathway, or the Embden–Meyerhof pathway) by which glucose is converted (via several intermediates) to pyruvate, thereby generating 2 molecules of ATP per molecule of glucose. Glycolysis does not involve molecular oxygen. After glycolysis, under aerobic conditions, pyruvate is converted to acetyl CoA and enters the tricarboxylic

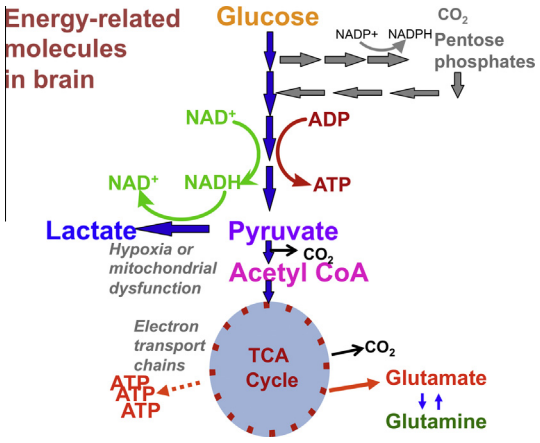

Fig. 1. Simplified schematic of major energy pathways in the brain include glycolysis, which takes places in the cytosol and produces pyruvate, which enters mitochondria and is converted into acetyl CoA that enters the TCA cycle. Alternatively, pyruvate can stay in the cytosol and is converted into lactate that is exported out of the cell. The pentose phosphate pathway (PPP) takes place in the cytosol and is an alternative energy pathway that can be up-regulated after injury; it is an important source of NADPH used to produce the reduced form of glutathione (GSH) for preventing oxidative stress.