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BRAIN RESEARCH

Research Report

Glucose metabolites in the striatum of freely behaving rats following infusion of elevated potassium

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ABSTRACT

The outcome of patients with traumatic brain injury (TBI) can be predicted by the extracellular potassium concentration and the change in energy homeostasis. In this study, the authors investigated the effects of high potassium concentrations on extracellular levels of glucose, pyruvate and lactate in the rat striatum. Applying artificial cerebrospinal fluid (ACSF) enriched with 120 mM potassium by reverse microdialysis leads to an increase in lactate and reduction in glucose and pyruvate. Consequently, the lactate to pyruvate ratio was also increased. These data are discussed in the context of recent studies on lactate/pyruvate conversion and the potential mechanisms whereby high potassium could affect this equilibrium. We conclude that ischemic-like events are unlikely to explain these K^+-induced changes.

1. Introduction

Traumatic brain injury (TBI) is associated with an increase in extracellular potassium (Doppenberg et al., 1999; Goodman et al., 1999; Reinert et al., 2000a,b; Yamamoto et al., 1999) and a change in energy homeostasis (Bentzer et al., 2000; Hutchinson et al., 2000; Meixensberger et al., 2001; Vespa et al., 2003), with both alterations predictive of outcome (Glenn et al., 2003). Efforts to understand the relation between energy homeostasis and potassium levels could lead to better management of post-traumatic neurotoxic risk. Thus, the focus of this study is to determine the changes in extracellular glucose, lactate and pyruvate levels induced by a local increase of potassium. The striatum was examined since it is a subcortical structure known to be functionally affected in models of brain injury and TBI patients (Grossman et al., 2003; Hattori et al., 2003; Hoshino et al., 2003; Paschen et al., 2004).

Under physiological conditions, brain energy demand is met primarily by the consumption of glucose. Thus, increases in pyruvate, the product of glycolysis, reflect an increase in brain metabolic activity. Subsequently pyruvate can be metabolized anaerobically into lactate or undergo oxidative phosphorylation with a balance between aerobic and anaerobic metabolism maintained in order to optimize ATP production and to limit lactate production. Further, increases in the lactate to pyruvate ratio suggest that there is a relative increase in anaerobic metabolism.

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The technique of microdialysis has been used in both basic science and clinical research to monitor various extracellular compounds (for a review, see Ungerstedt, 1997) including energy metabolites. Monitoring extracellular glucose, lactate and pyruvate, and thus also the ratio of lactate/pyruvate within a given region can thereby provide in vivo information on local glucose availability and utilization. Indeed, these parameters have been used previously to characterize metabolic alterations in the clinic (Nordstrom et al., 2003; Roslin et al., 2003), and in various in vivo models of neuropathology (Darbin et al., 2000, 2003; Kawamata et al., 1995; Lonjon et al., 2001; Roslin et al., 2003; Stahl et al., 2001).

The purpose of this study was to investigate changes in central nervous system metabolism during an increase in extracellular K^+. Specifically, microdialysis coupled with an enzymatic assay was used to simultaneously monitor extracellular levels of glucose, lactate, and pyruvate before, during, and after perfusion of KCl-enriched artificial cerebrospinal fluid (ACSF) into the striatum of freely moving rats.

2. Results

The in vitro recovery of the probes ($n=6$) for glucose, lactate, and pyruvate were 0.18 (0.16–0.21), 0.20 (0.18–0.24), and 0.18 (0.17–0.21) respectively. Under control conditions (i.e., following post-implantation recovery and before infusing elevated K^+) normalized dialysate levels were 2.64 mM glucose (2.62–2.75), 0.96 mM lactate (0.87–1.21) and 51.91 μM pyruvate (44.13–73.14). Basal levels were stable during the 2 h prior to $KCl_{120\ mM}$ perfusion (Fig. 1; $p>0.1$ for each compounds).

Perfusion with the $KCl_{120\ mM}$ ACSF resulted in an abrupt change in the levels of glucose ($p<0.01$), lactate ($p<0.01$), and pyruvate ($p<0.01$) and subsequently also the lactate/pyruvate ratio ($p<0.01$). Specifically, $KCl_{120\ mM}$ infusion resulted in a 22.89% decrease in glucose (Fig. 1a; maximal change at 135 min; $p<0.05$), a 29.69% decrease in pyruvate (Fig. 1c; maximal change at 150 min; $p<0.05$), and an 84.68% increase in lactate (Fig. 1b; maximal change at 135 min; $p<0.01$). Consequently, the ratio of lactate to pyruvate was also significantly increased (Fig. 1d; maximal change at 135 min; $p<0.02$). Ninety minutes post-$KCl_{120\ mM}$ infusion, all variables stabilized back to their original baseline control values (Fig. 1; $p>0.1$ for each compounds).

3. Discussion

Infusing $KCl_{120\ mM}$ into the striatum of freely behaving rats elicited decreases in glucose and pyruvate levels and a concurrent increase in lactate. Our data suggest that the increase in lactate evoked by high extracellular potassium (Bures et al., 1984; Mayevsky and Weiss, 1991; Rosenthal and Somjen, 1973; Scheller et al., 1992; Somjen, 2001; Taylor et al., 1997) is related to a net increase in the lactate to pyruvate ratio.

Microdialysate levels of glucose and its metabolites have been used to characterize changes in energy metabolism (Ungerstedt, 1997). However, the relationship between these two entities is complex and depends upon factors that can affect the extracellular compartment and/or the diffusion of a substance through the tissue and the microdialysis membrane (Amberg and Lindefors, 1989; Lindefors et al., 1989). Although these parameters are unlikely to change during most experimental paradigms, exposing brain tissue to a high extracellular potassium concentration would result in neuronal and glial swelling due to water accumulation (Dietzel et al., 1982). Extracellular space would subsequently decrease while extracellular tortuosity would increase (Mazel et al., 2002). While these changes would alter probe recovery, prior data reveal that during application of high levels of extracellular K^+ changes in microdialysate concentrations closely correlate with metabolic activity. For instance, studies using push–pull cannulae to administer a tracer for endogenous glutamate and aspartate (D-$_3$[H]aspartate), have shown that the levels of glutamate, aspartate and the amount of radioactivity increase concurrently in the hippocampus following infusion of high K^+ (Nielsen et al., 1989). Similar findings between metabolic pathways and high potassium infusion have also been noted in other structures (Fernandez-Galaz et al., 1993; Girault et al., 1986; Granata and Reis, 1983; Lantin le Boulch et al., 1991; Noto et al., 1986). Thus, even though high potassium can affect the recovery of microdialysis probes, the overall effect of this change appears to be relatively small compared to that associated with the metabolic change. Consequently, we posit that the change in the levels of glucose and its metabolites seen in the striatum following high K^+ infusion primarily reflects the metabolic response evoked by high extracellular K^+. In addition, recent works on the endogenous levels of glucose and its metabolites in the rat cortex corroborate the findings of the present study (Selman et al., 2004).

The extracellular lactate/pyruvate reflects the equilibrium of pyruvate to lactate conversion. This process is a cytosolic and mitochondrial reaction that is catalyzed by lactate dehydrogenase and requires coenzyme nicotinamide adenine dinucleotide in its reduced state ($NADH+H^+$). Lactate dehydrogenase works in near equilibrium with the concentration of pyruvate and the redox state of the tissue (NAD/NADH) regulating pyruvate to lactate conversion (Siesjo, 1978). Subsequently, changes in neuronal and/or glial redox states can affect the lactate/pyruvate ratio. For instance, potassium-induced spreading depression shifts the redox state of brain tissue to reduction (Hashimoto et al., 2000). Therefore, the increase in NADH occurring following potassium infusion is likely to contribute to an enhanced conversion of pyruvate to lactate. During conditions of increased activity, glial cells and neurons have different metabolic responses, and data suggest that lactate becomes a neuronal substrate preferentially (Bouzier et al., 2000; Magistretti and Pellerin, 1999). Specifically, a metabolic shuttle between astrocytes and neurons has been proposed in which astrocytes release lactate during periods of intense activity that is subsequently taken up and metabolized by neurons (Pellerin and Magistretti, 1994, 1996); the net flux of lactate from astrocytes to neurons can account for up to 85% of CMR_{glc} in the central nervous system (Dienel and Hertz, 2001). Therefore, the increase in the lactate to pyruvate ratio seen following $KCl_{120\ mM}$ infusion could reflect this synergistic glial–neuronal metabolic response with glial glycolysis increasing (their redox state shifted to reduction) in