Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Real-time effects of insulin-induced hypoglycaemia on hippocampal glucose and oxygen

*John Kealy**, *Rachel Bennett*, *John P. Lowry*

Maynooth University Department of Chemistry, Maynooth University, Maynooth, Co. Kildare, Ireland

ARTICLE INFO

Article history:
Accepted 5 December 2014
Available online 13 December 2014

Keywords:
Insulin
Hypoglycemia
Fasting
Oxygen
Glucose

ABSTRACT

The hippocampus plays a vital role in learning and memory and is susceptible to damage following hypoglycaemic shock. The effect of an acute administration of insulin on hippocampal function has been described in terms of behavioural deficits but its effect on hippocampal oxygen and glucose is unclear. Glucose oxidase biosensors (detecting glucose) and carbon paste electrodes (detecting oxygen) were implanted into the hippocampus of Sprague Dawley rats. Animals were allowed to recover and real-time recordings were made in order to determine the effects of fasting, insulin administration (15 U/kg; i.p.) and reintroduction of food on hippocampal oxygen and glucose. Fasting caused a significant decrease in hippocampal glucose over the course of 24 h. Insulin administration produced a significant decrease in hippocampal glucose along with a significant increase in hippocampal oxygen. Finally, the reintroduction of food resulted in glucose levels significantly increasing along with a transient but significant increase in oxygen levels. The findings presented here suggest that even a single acute period of hypoglycaemia may substantially disrupt hippocampal oxygen and glucose and therefore affect hippocampal function.

1. Introduction

While there are many known effects of insulin-induced hypoglycaemia in the periphery, its effect on the brain is only beginning to become evident and even so, a large proportion of research has focussed on the chronic effects of hypoglycaemia due to the growing body of evidence that chronic hypoglycaemia is associated with pathological processes in the brain (McCrimmon, 2012; Witsch et al., 2012). There is evidence to suggest that along with long-term effects on brain function, short-term periods of hypoglycaemia may affect different regions of the brain. For example, the hippocampus plays a pivotal role in declarative memory (Scoville and Milner, 1957; Cohen et al., 1999; Eacott and Easton, 2010), spatial navigation (O'Keefe and Nadel, 1978; Morris et al., 1982; D'Hooge and De Deyn, 2001) and it is linked to various neurodegenerative/psychiatric disorders including Alzheimer's disease (Heckers and Konradi, 2010; Marlatt and Lucassen, 2010; Bast, 2011; Dhikav and Anand, 2011; Bonilha et al., 2012). The hippocampus is susceptible to damage following hypoglycaemic shock (Auer and Siesjö, 1988) and hippocampal damage is associated with impairments on hippocampus-dependent tasks (Whishaw et al.,

Abbreviations: BOLD, blood–oxygen-level dependent; CPA, constant potential amperometry; CPE, carbon paste electrode; GLUT, glucose transporter; Pt/PPD/GOx, platinum/poly(o-phenylenediamine)/glucose oxidase

*Correspondence to: Maynooth University Department of Chemistry, Science Building, Maynooth University, Co. Kildare, Ireland. Fax: +353 1 708 3815.
E-mail address: John.Kealy@nuim.ie (J. Kealy).

http://dx.doi.org/10.1016/j.brainres.2014.12.015
0006-8993/

1994). It has been previously shown that long-term insulin-induced hypoglycaemia in neonatal rats is associated with neuronal damage in many parts of the brain, including the dentate gyrus of the hippocampus, with subsequent impairment of spatial learning (Zhou et al., 2012).

Normally, glucose is transported into the brain across the blood–brain barrier via the GLUT-1 transporter (Oldendorf, 1971; Banks et al., 2012) and, as uptake of glucose within the brain is largely insulin-independent with insulin-insensitive variations of GLUT dominating over GLUT-4 (McEwan and Reagan, 2004; Grillo et al., 2009), any insulin-induced decreases in cerebral glucose are most likely due to decreased availability of glucose from the periphery (Boutelle et al., 1986). It has been shown that insulin-induced hypoglycaemia rapidly reduces the amounts of glucose available for the brain to use (Lowry et al., 1994; Lowry and O'Neill, 1994) and hypoglycaemic conditions (both insulin- and non-insulin-induced) have a direct effect on neurotransmission and behaviour (Gazit et al., 2003; Auer, 2004a; 2004b). Specifically, insulin-induced hypoglycaemia leads to decreases in cholinergic (Sherin et al., 2011) and GABAergic neurotransmission (Antony et al., 2010) and to increases in glutamatergic neurotransmission (Joseph et al., 2008; Anu et al., 2010). Together, these changes can cause disruption of normal brain function and, in the case of glutamate, neuronal damage in the form of excitotoxicity (Hynd et al., 2004). Use of a competitive NMDA glutamate receptor blocker protects against insulin-induced hypoglycaemic damage in a dose-dependent manner (Papagapiou and Auer, 1990).

The uptake of peripherally-administered insulin directly into the brain (Banks et al., 1997a; 1997b) causes further complications due to insulin's role as a transmitter in the nervous system, acting both as a regulatory peptide for satiation (Banks, 2004) and as a modulator of learning and memory (Park, 2001). Furthermore, decreased transport of insulin from the periphery is associated with Alzheimer's disease (Craft et al., 1998) whereas increased levels of insulin in the nervous system are associated with obesity (Israel et al., 1993; Kaiyala et al., 2000). In terms of learning and memory, it is unsurprising that the hippocampus expresses the insulin-sensitive glucose transporters GLUT-4 (Messari et al., 1998) and GLUT-8 (Reagan et al., 2001) along with insulin receptors (Heidenreich et al., 1983; Doré et al., 1997). Trafficking of GLUT-4 (McEwen and Reagan, 2004) and GLUT-8 (Piroli et al., 2002) within the hippocampus seems to be regulated by insulin levels and disruption of insulin signalling in diabetes may affect glucose transport within the hippocampus leading to cognitive deficits associated with diabetes (Reagan, 2005).

Compounding the direct effects of insulin on the brain are the associated effects of glucose availability in the brain with increased cognitive demand associated with a decrease in hippocampal glucose measured using microdialysis (McNay et al., 2000). Using glucose biosensors combined with CPA recording in real-time, increased neuronal activation has been associated with changes in tissue concentrations of glucose in the striatum (Lowry and Fillenz, 1997) and the hippocampus (Kealy et al., 2013). In the case of the hippocampus, these changes in glucose are also associated with changes in oxygen availability measured using a CPE (Kealy et al., 2013) with such changes in oxygen being associated with neuronal activity (Li et al., 2011; Russell et al., 2012; Kealy et al., 2013) akin to the BOLD signal from functional magnetic resonance imaging (Lowry et al., 2010, Francois et al., 2012). In the striatum, decreases in glucose concentration have been reported following a peripheral administration of insulin (Lowry and O'Neill, 1994) suggesting that insulin-induced hypoglycaemia directly affects brain metabolism. Additionally, it has also been shown using amperometry that insulin- and glucagon-induced changes in striatal glucose are directly correlated to changes in plasma glucose concentrations (Boutelle et al., 1986).

In this study, we implant a Pt/PPD/GOx biosensor, which has been shown to be highly selective and sensitive to glucose (Lowry and O'Neill, 1994; Lowry et al., 1998; Dixon et al., 2002) and stable for up to two weeks following implantation (Lowry et al., 1994). Along with the Pt/PPD/GOx biosensor, we co-implanted a CPE that has been shown to be highly selective and sensitive towards oxygen (Bolger et al., 2011a, 2011b). CPEs show excellent stability over several months (O'Neill and Fillenz, 1985; Bolger et al., 2011b) and can be used as an indirect measure of regional cerebral blood flow (Lowry et al., 1997; Bolger and Lowry, 2005). Real-time changes in hippocampal glucose and oxygen levels measured by these sensors are then monitored over the course of fasting, acute insulin-induced hypoglycaemic conditions and reintroduction of food in order to determine the effects of peripheral hypoglycaemia on hippocampal oxygen and glucose in freely-moving rats.

2. Results

2.1. *Effect of fasting on hippocampal glucose and oxygen*

For recordings made during the fasting period ($n=6$), the real-time data was divided into 15 min time bins for analysis. All data was normalised to baseline levels of hippocampal glucose or oxygen (2 h prior to removal of food). One-way ANOVAs revealed that there was an overall significant effect for time on glucose when animals were fasting for 24 h ($F=3.428$; $df=103$; 515; $p<0.001$; Fig. 1A) but there was no overall significant effect for oxygen ($F=1.134$; $df=103$; 515; $p>0.05$; Fig. 1B).

For hippocampal glucose, a one-way ANOVA also showed that there was a significant difference between time intervals during the fasting period ($F=68.23$; $df=3$, 28; $p<0.001$; Fig. 1C). Following Tukey *post-hoc* analysis, it was revealed that hippocampal glucose levels during the early fasting period (time 0–2 h following the removal of food) were significantly higher than the levels observed during the baseline ($p<0.01$), middle fasting (12–14 h following removal of food; $p<0.001$) and late fasting periods (22–24 h following removal of food; $p<0.001$). However, there was a significant decrease in hippocampal glucose with increased time, Tukey *post-hoc* analysis showed that compared to baseline, there were significantly lower levels of hippocampal glucose in the middle ($p<0.001$) and late fasting periods ($p<0.001$). There were no significant differences between the middle and late fasting periods ($p>0.05$). When comparing fasting to the control period over 24 h, area under the curve (AUC) analysis confirmed that there were significant lower levels of hippocampal glucose in the fasting group ($n=6$; $t=3.018$; $df=5$; $p<0.05$; Fig. 1E).