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Long-term oral galactose treatment prevents cognitive deficits in male Wistar rats treated intracerebroventricularly with streptozotocin

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ABSTRACT

Basic and clinical research has demonstrated that dementia of sporadic Alzheimer's disease (sAD) type is associated with dysfunction of the insulin-receptor (IR) system followed by decreased glucose transport via glucose transporter GLUT4 and decreased glucose metabolism in brain cells. An alternative source of energy is D-galactose (the C-4-epimer of D-glucose) which is transported into the brain by insulin-independent GLUT3 transporter where it might be metabolized to glucose via the Leloir pathway. Exclusively parenteral daily injections of galactose induce memory deterioration in rodents and are used to generate animal aging model, but the effects of oral galactose treatment on cognitive functions have never been tested. We have investigated the effects of continuous daily oral galactose (200 mg/kg/day) treatment on cognitive deficits in streptozotocin-induced (STZ-icv) rat model of sAD, tested by Morris Water Maze and Passive Avoidance test, respectively. One month of oral galactose treatment initiated immediately after the STZ-icv administration, successfully prevented development of the STZ-icv-induced cognitive deficits. Beneficial effect of oral galactose was independent of the rat age and of the galactose dose ranging from 100 to 300 mg/kg/day. Additionally, oral galactose administration led to the appearance of galactose in the blood. The increase of galactose concentration in the cerebrospinal fluid was several times lower after oral than after parenteral administration of the same galactose dose. Oral galactose exposure might have beneficial effects on learning and memory ability and could be worth investigating for improvement of cognitive deficits associated with glucose hypometabolism in AD.

1. Introduction

Alzheimer's disease (AD) is a human neurodegenerative disorder associated with a progressive loss of cognition and memory. Among other pathophysiological features, AD is associated with decreased glucose utilization distributed all over the cerebral cortex, and particularly in organs with both high glucose demands and high insulin sensitivity like in the brain (Henneberg and Hoyer, 1995). Neuronal glucose metabolism is under the regulation of neuronal insulin receptor (IR), and defects in the brain glucose metabolism found in Alzheimer's disease have been suggested to be induced at the level of the insulin signal transduction system (Hoyer, 2002, 2004; Craft, 2005; de la Monte and Wands, 2005, 2008; de la Monte, 2012). Recent data indicate that insulin resistant brain state may play an important role in the ethiopathogenesis of the prevailing sporadic form of AD (sAD), instead of being its consequence (Salkovic-Petrisic et al., 2009; Baker et al., 2011; Bosco et al., 2011). However, specific nature and course of sAD do not allow such ethiopathogenetic research in humans, and no single experimental model has been found to be truly representative of the sAD, unrelated to the genetic manipulations or inheritance.

Streptozotocin (STZ) intracerebroventricularly (icv) treated rat has been proposed as a non-transgenic sAD animal model which seems to resemble this human disease at different levels (Lannert and Hoyer, 1998; Prickaerts et al., 1999; Sharma and Gupta, 2001; Salkovic-Petrisic et al., 2006, 2011; Lester-Coll et al., 2006; Agrawal et al., 2011). Central STZ administration in low doses (1–3 mg/kg, icv) does not produce diabetes mellitus type II (as it does

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after the peripheral administration (Blondel and Portha, 1989)), but produces cognitive impairment (Mayer et al., 1990), cholinergic deficits (Hellweg et al., 1992), oxidative stress (Sharma and Gupta, 2001) and morphological astroglyosis, neuronal loss (Shoham et al., 2003, 2006), amyloid angiopathy (Salkovic-Petrisic et al., 2006, 2011), and other Alzheimer-like alterations in the brain (reviewed by Salkovic-Petrisic and Hoyer, 2007; Correia et al., 2011). Additionally, brain glucose metabolism has been found to be markedly perturbed in the STZ-icv treated rats, demonstrated as decreased glucose and ATP contents in the cerebral cortex (Nitsch and Hoyer, 1991), decreased glucose utilization (30%–44%) (Pathan et al., 2006; Duelli et al., 1994) and decreased activities of glycolytic key enzymes (Plaschke and Hoyer, 1993) resulting in diminished concentrations of the energy-rich substrates ATP and creatine phosphate, respectively (Lannert and Hoyer, 1998; Nitsch and Hoyer, 1991; Hoyer and Lannert, 2008). STZ-icv treated rats consistently demonstrate deficits in learning and memory. Long-term cognitive deficits have been observed as early as 2 weeks after STZ-icv administration and are maintained at least 12 weeks post treatment (Lannert and Hoyer, 1998; Salkovic-Petrisic et al., 2006; Shoham et al., 2006; Grünblatt et al., 2007). Cognitive deficits are found 1 month post STZ-icv injection regardless of age in both 3-month and 1–2-years old rats, and regardless of a single or multiple 1 mg/kg STZ-icv doses (Lannert and Hoyer, 1998; Salkovic-Petrisic et al., 2006; Mayer et al., 1990; Shoham et al., 2006; Pathan et al., 2006; Grünblatt et al., 2007; Weinstock and Shoham, 2004; Mehla et al., 2013). Selective toxicity for insulin producing/secreting cells is related to the selective uptake of STZ via the glucose transporter GLUT2, which is predominantly located on the pancreatic β cell membrane (Szkudelski, 2001) but, in a lower extent, in the brain as well (Arluison et al., 2004a, 2004b). *In vitro* studies have demonstrated that STZ also reduces GLUT2 protein expression (Gai et al., 2004) and that within the cell it causes alkylation of β-cell DNA which triggers activation of poly ADP-ribosylation, leading to depletion of cellular NAD+ and ATP (Szkudelski, 2001; Lenzen, 2008). Additionally, low to moderate STZ doses have been shown to affect IR signaling by decreasing its autophosphorylation (Kadowaki et al., 1984; Giorgino et al., 1992). The exact mechanism of central STZ action and its target cells/molecules in the brain have not been elucidated yet, but given the presence of both GLUT2- and IR-expressing cells, similarity to the mechanism of the peripheral STZ-induced toxicity is assumed.

In line with different mechanisms proposed to explain the induction of cognitive deficits in STZ-icv rats, their prevention has been investigated by exploiting a number of drugs acting by different mechanism of action (as reviewed by Weinstock and Shoham, 2004; Salkovic-Petrisic et al., 2013) including those supposed to generate alternative energy sources such as acetyl-L-carnitine (Prickaerts et al., 1995) or preventing reduction of cerebral ATP (Lannert and Hoyer, 1998). Evidence accumulated from basic and clinical research has demonstrated that brain insulin and IR are involved in the brain cognitive functions, including learning and memory (Zhao et al., 2004). Therefore, it could be worth to investigate the treatments which may prevent or improve the decreased glucose concentration caused by dysfunction of IR signaling leading to a decrement of brain energy metabolism.

Glucose is the main source of energy in the brain and its entry into cells throughout the brain is facilitated by glucose transporter (GLUTs) proteins (Maher et al., 1994; Mueckler, 1994). The majority of glucose utilization in the central nervous system appears to be mediated through insulin-independent GLUT1 (responsible for glucose transport from blood into the extracellular space of the brain) and GLUT3 (responsible for glucose transport from the extracellular space into the neurons) (Duelli and Kuschinsky, 2001). Low intensity of GLUT2-immunoreactive signal has been found in the nerve cell bodies in the dentate gyrus granular layer and the periphery of nerve cells in the cerebral cortex, participating in the regulation of neurotransmitter release (Arluison et al., 2004a, 2004b) and in the hypothalamus, participating in cerebral glucose sensing (Pénicaud et al., 2002). GLUT4 is an intracellular, insulin-dependent glucose transporter which in the brain plays a role in rapidly providing additional glucose to neurons under conditions of high-energy demand (Vannucci et al., 1998; Apelt et al., 1999; McEwen and Reagan, 2004; Grillo et al., 2009). The pathophysiology of GLUT isoforms in sAD is not well characterized with the only consistent finding of reduced GLUT1 and GLUT3 density in the brain, respectively (Kalaria and Harik, 1989; Harik, 1992; Simpson and Davies, 1994; Patil et al., 2007; Liu et al., 2008;), which correlated well to the decrease in 0-GlcNAcylation and hyperphosphorylation of tau protein (Liu et al., 2008). No change in GLUT4 expression and a significant increase in GLUT2 protein levels, proposed to be caused by astrocyte activation, were found in the brain of AD patients by Bigl et al. (2003) while both decreased and unaltered expression of GLUT2 mRNA was found in basal forebrain neurons in the late stage of AD (Counts et al., 2009). Unaltered GLUT4 (Lee et al., 2013) and decreased GLUT1 and GLUT3 protein expression was found also in transgenic mice models of AD (Kouznetsova et al., 2006; Hoojimans et al., 2007; Um et al., 2008; Lee et al., 2013) while no such data have been reported in STZ-icv sAD model. It is logical to assume that impaired insulin receptor signaling in the brain such as that observed in human sAD and its animal counterpart, STZ-icv rat model, is accompanied by decreased insulin-dependent glucose transport in the brain. Thus, alternative sources of energy and/or their insulin-independent transport might play an important role. In line with this hypothesis, one should consider the C-4 epimer of D-glucose, D-galactose (refers to "galactose" further on in the text). The transport of galactose into the brain cells is mediated by the brain-specific glucose transporter GLUT3 (Gould et al., 1991; Seatter et al., 1997) which, unlike GLUT4, acts in an insulin-independent manner (Bell et al., 1990). The accumulation rate (not an absolute transport rate) of deoxyglucose and galactose by human GLUT3 *in vitro* demonstrated values of 3.39 and 0.81 pmol/(min•oocyte), respectively (Gould et al., 1991). After its uptake into cells expressing galactose-carring transporters (class 1 and 3 of GLUT family, respectively, Cura and Carruthers, 2012) in a concentration-dependent manner, galactose is quickly and quantitatively metabolized to glucose via the Leloir pathway occurring in all cells although most effectively in the liver cells (Cohn and Segal, 1973). Recently, experiments in rats have shown that galactose is taken up by brain cells to a similar extent as by liver cells (Roser et al., 2009). Additionally, it should be kept in mind that oral ingestion of galactose induces the intestinal release of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like-peptide-1 (GLP-1) which both promote insulin secretion (Phillips and Prins, 2011) but might (GLP-1) have extrapancreatic effect in improving learning and memory in the STZ-icv rat sAD model (Li et al., 2012) and in the transgenic APP/PS1 mice AD model (McClean et al., 2011). We have investigated whether STZ-icv induced cognitive deficits in rats might be improved or normalized by daily peroral galactose treatment and whether there has been an administration route-dependent difference in blood and cerebrospinal fluid (CSF) galactose concentration between the oral and parenteral galactose treatment.

2. Material and methods

2.1. Animals

Adult male Wistar rats weighing 280–470 g (University of Zagreb, School of Medicine, Department of Pharmacology) were used throughout the study. All animals were kept on standardized food pellets and water *ad libitum*.