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BRAIN RESEARCH

Research Report

Caudal hindbrain lactate infusion alters glucokinase, SUR1, and neuronal substrate fuel transporter gene expression in the dorsal vagal complex, lateral hypothalamic area, and ventromedial nucleus hypothalamus of hypoglycemic male rats

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ABSTRACT

While *in vitro* studies show that the oxidizable energy substrate, lactate, is a preferred fuel for CNS neurons during states of energy crisis, and that lactate may regulate neuronal glucose uptake under those conditions, its role in neuronal function *in vivo* remains controversial. Glucose-excited neurons in hindbrain dorsal vagal complex (DVC) monitor both glucose and lactate, and express both the glucose sensor, glucokinase (GK), and the SUR1 subunit of the plasma membrane energy transducer, K_{ATP}. Fourth ventricular lactate infusion exacerbates insulin-induced hypoglycemia (IIH) and IIH-associated patterns of DVC neuronal activation. We investigated the hypothesis that during glucoprivation, lactate regulates neuronal monocarboxylate and glucose transporter gene transcription in the DVC, and adjustments in these gene profiles are correlated with altered GK and SUR1 mRNA expression. We also examined whether caudal hindbrain lactate repletion alters the impact of hypoglycemia on substrate fuel uptake and metabolic sensing functions in other characterized metabolic monitoring sites, e.g., the ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic area (LHA). qPCR was used to measure MCT2, GLUT3, GLUT4, GK, and SUR1 transcripts in the microdissected DVC, VMH, and LHA from groups of male rats treated by continuous infusion of aCSF or lactate into the caudal fourth ventricle (CV4), initiated prior to injection of Humulin R or saline. Blood glucose was decreased in response to insulin, a response that was significantly augmented by CV4 lactate infusion. IIH alone did not alter mean DVC MCT2, GLUT3, GLUT4, GK, or SUR1 mRNA levels, but these transcripts were increased in the lactate plus insulin group, relative to both euglycemic and aCSF-infused hypoglycemic rats. IIH decreased *MCT2*, *GLUT3*, and *SUR1* gene profiles in the VMH; CV4 lactate infusion during IIH further diminished these transcripts, and suppressed GLUT4 and GK mRNA levels in this site. In LHA, IIH increased *GLUT3* and *SUR1* gene expression to an equal extent, with or without lactate, while GLUT4, MCT2, and GK mRNA levels were elevated only in response to lactate plus insulin. These studies show that caudal hindbrain-targeted delivery of exogenous lactate during IIH upregulates neuronal monocarboxylate and glucose transporter, *GK*, and *SUR1* gene profiles in the DVC, and results in increased or decreased GLUT4 and GK mRNA in LHA and VMH, respectively. These data suggest that lactate and

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glucose utilization by DVC neurons may be enhanced in response to local lactate surfeit, alone or relative to glucose deficiency, and that increases in intracellular glucose and net energy yield may be correlated with elevated *GK* and *SUR1* gene transcription, respectively, in local glucose sensing neurons. The results also imply that GLUT4- and GK-mediated glucose uptake and glucose sensing functions in the VMH and LHA may be reactive to DVC signaling of relative lactate abundance within the caudal hindbrain, and/or to physiological sequelae of this fuel augmentation, including amplified hypoglycemia.

1. Introduction

In vitro studies support the concept of compartmentalization of glucose metabolism in the brain, and astrocytic trafficking of the oxidizable fuel, lactate, to support neuronal aerobic respiration. Neuronal reliance upon this energy source is bolstered by evidence that lactate preserves synaptic activity in the absence of glucose (Izumi et al., 1997), serves as the primary oxidative substrate when both fuels are present (Bouzier-Sore et al., 2003), and sustains neuronal function during states of energy crisis (Schurr et al., 1997a,b). Reports that exogenous lactate reverses glucoprivic stupor/coma and coincident reductions in CNS levels of tricarboxylic acid cycle intermediates (Thurston et al., 1983) suggest that this molecule is utilized *in vivo* to derive energy. However, it should be noted that *in vivo* evidence for the astrocyte-neuron lactate shuttle hypothesis and for neuronal lactate utilization in the adult remains controversial (Chih et al., 2000, 2003). Lactate is mainly incorporated into neurons by action of the low K_m monocarboxylate transporter (MCT) variant, MCT2 (Miki et al., 2001), while glucose internalization is achieved by facilitative and insulin-dependent uptake mechanisms, involving GLUT3 and GLUT4 transporters, respectively (Devaskar and Mueckler, 1991; Vannucci et al., 1997). Recent *in vitro* studies reveal that preference of hippocampal neurons for lactate during energy deficiency reflects, in part, inhibition of glucose uptake by lactate under such conditions (Bliss and Sapolsky, 2001).

Neural signals of cellular metabolic imbalance originate within discrete brain sites, including the hindbrain dorsal vagal complex (DVC), structures that contain neurons that exhibit rapid electrophysiological reactivity to small adjustments in substrate fuel levels (Adachi et al., 1995; Mizuno and Oomura, 1984; Oomura et al., 1969; Silver and Erecinska, 1998; Yeffefti et al., 1995, 1997). The sensory component of the DVC, the nucleus of the solitary tract, is the principal CNS visceral sensory structure in the brain and the projection sites of vagal visceral afferent fibers, whereas the motor element, the dorsal vagal motor nucleus, contains parasympathetic preganglionic visceral efferent neurons. Glucose-excited neurons in the DVC display increased synaptic firing *in vitro* in response to microiontophoretically applied glucose and lactate (Himmi et al., 2001), and are characterized by the expression of both the substrate sensor, glucokinase (GK), and the inwardly rectifying, ATP-dependent potassium channel, K_{ATP} (Balfour et al., 2005). K_{ATP} mediates the impact of energy availability on plasma membrane voltage of hypothalamic neurons (Ashford et al., 1990; Miki et al., 2001) and is critical for metabolic signaling by DVC glucose-excited neurons (Dallaporta et al., 2000). Lactate infusion into the caudal fourth ventricle can exacerbate insulin-induced hypoglycemia and modify IIH-associated patterns of DVC neuronal activation (Patil and Briski, 2005), findings that suggest that this substrate fuel, alone or against a background of glucose scarcity, can attenuate local metabolic deficit signaling. The present studies investigated the hypothesis that DVC patterns of expression of the sulfonylurea receptor subunit of K_{ATP}, e.g., SUR1, and/or GK are modified due to hypoglycemia, and that repletion of the caudal hindbrain with lactate may reverse the former response. We also examined the premise that this lactate treatment paradigm may alter MCT2 and neuronal glucose transporter gene expression in the DVC of hypoglycemic animals.

The neuroanatomical connectivity of the DVC with other characterized metabolic monitoring sites in the brain, e.g., the lateral hypothalamic area (LHA) and ventromedial hypothalamic nucleus (VMH), raises the possibility that information on local substrate availability may be conveyed to these structures, and thereby influence substrate uptake, glucose

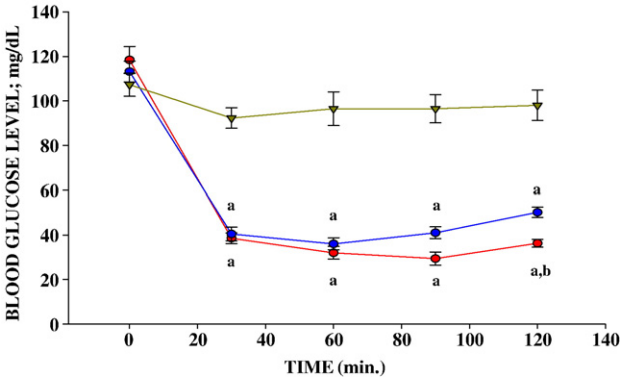

Fig. 1 – Effects of continuous caudal fourth ventricular (CV4) lactate infusion on blood glucose levels following insulin injection of intact adult male rats. Groups of saline- and insulin (Humulin R; 10 U/kg, s.c.)-injected rats [injections were administered at time zero] were infused with artificial cerebrospinal fluid (aCSF) or L-lactate (50 μM/2.0 μl/h) into the CV4 [infusions were carried out from −10 to +120 min]. Treatment combinations are depicted as follows: *green triangles/green lines*: aCSF infusion plus s.c. saline injection; *blue circles/blue lines*: aCSF infusion plus s.c. insulin injection; *red circles/red lines*: lactate infusion plus s.c. insulin injection. Data points depict mean blood glucose values ± SEM for $n = 6$ rats per group. [a] $p < 0.05$, compared to saline-injected controls; [b] $p < 0.05$, compared to the aCSF plus insulin-treated group.