Cerebral activation by fasting induces lactate accumulation in the hypothalamus


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Introduction

The hypothalamus is the primary site for appetite regulation and energy homeostasis. Several studies have previously addressed the role of neuropeptide signalling in the control of hypothalamic functions, including the control of feeding fasting cycles (1). Much less information is available, however, on the action of the amino acid neurotransmitters, glutamate and GABA (2), and how these could modulate the neuroglial coupling mechanisms underlying neurotransmission events during appetite regulation. On these grounds, methods providing further insight into hypothalamic metabolism and its disturbances entail considerable interest to improve our understanding, prognosis and therapy of the disorders in energy homeostasis and food intake. 13C MRS is an outstanding tool to investigate cerebral metabolism and neuroglial interactions during cerebral activation. Nonetheless, previous in vivo and in vitro 13C MRS studies required either, very large voxel sizes or extracts from large biopsies, precluding accurate cerebral regionalization. Here, we used 13C HR-MAS, an approach requiring very small tissue samples (ca. 10 mg), to study the hypothalamic activation and neuroglial-coupling during a feeding-fasting paradigm and under ghrelin (an orexigenic peptide) administration (3) to mice receiving (1-13C) glucose.

Methods

C57BL/6 male mice (6-8 weeks) were distributed in three groups (n=6 per group). Groups 1 (control) and 3 (ghrelin-treated) received water and food ad libitum. Group 2 (fasted) were fasted overnight before the experiment. All mice were anesthetized with isoflurane (1%). Groups 1 and 2 received an i.p. injection of (1-13C) glucose (20 nmol/g) while group 3 were administered an i.p. injection of 75 μM ghrelin (0.3 nmol/g) and fifteen minutes later an i.p. injection of (1-13C) glucose as in group 1. Fifteen minutes after glucose injection, cerebral metabolism was arrested using a high-power (5 kW) microwave fixation system and the brains dissected in two regions; hypothalamus and the “remaining brain”. Samples were analyzed in an 11.7 T Bruker AVANCE 500WB NMR spectrometer (4 kHz spinning, 4 ºC).

Results

13C HR-MAS spectra as obtained from small biopsies of brain tissue depicted good quality (Figure 1), allowing the study of metabolism in small brain areas. We investigated the incorporation of (1-13C) glucose into lactate C3, glutamate C4, glutamine C4 and GABA C2 resonances, relative to the unchanged natural abundance of myo-inositol C1, C3 resonance. No significant changes were observed in the glutamate and glutamine resonances. However, we detected significantly increased lactate C3 and GABA C2 (58% and 17%, respectively), only in the hypothalamus of fasting animals (Figure 2). In contrast, no significant changes in these resonances could be observed in control and in ghrelin-treated animals.

Conclusions

Increased hypothalamic content of lactate C3 and GABA C2 revealed increased astrocyte-to-neuron lactate shuttle activity and either augmented GABA synthesis, and/or decreased GABA degradation. Present results suggest that fasting reduces GABA degradation, since no parallel changes were observed in the glutamate and glutamine precursors. Administration of the main orexigenic peptide ghrelin is not able to mimic all the metabolic consequences of hypothalamic activation by fasting, suggesting the operation of additional factors. Finally, our study illustrates well the use of 13C HR-MAS to investigate metabolism in small brain areas. The approach can be easily extended to other paradigms and regions of cerebral activation.

References


Figure 1. Representative 13C HR-MAS spectrum (125.13 MHz, 4 ºC, 4 kHz) of the hypothalamus of a representative mouse fasted overnight following 15 min i.p. (1-13C) glucose administration (20 μmol/g body weight). Assignments: Ala, alanine; Cr, creatine; GABA, γ-aminobutyric acid; Glu, glutamate; Gln, glutamine; GPC, glycerophosphoryl choline; Lac, lactate; ml, myo-inositol; NAA, N-acetyl-aspartate; Tau, taurine; ppm, parts per million. (onde estao os assignments???)

Figure 2. 13C content of relevant metabolite carbons as normalized to the myo-inositol C1, C3 (73.2 ppm) resonance in the hypothalamus and “rest of the brain” of Groups 1 (control), 2 (fasted) and 3 (ghrelin). Lac, lactate; Gln, glutamine; Glu, glutamate; GABA, γ-aminobutyric acid. *p < 0.05, ***p < 0.001. Data are expressed as mean ± SEM.