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Understanding of cerebral energy metabolism by dynamic living brain slice imaging system with [^{18}F]FDG

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Abstract

Recently, lactate has been receiving great attention as an energy substrate in the brain. In this study, the role of lactate was evaluated by ''bioradiography'' system with 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), which is a positron emitting radiotracer for glucose uptake quantification. ''Bioradiography'' is the dynamic living tissue slice imaging system for positron-emitter labeled compounds. We investigated the brain energy metabolism under resting state and neural activated conditions induced by KCl addition. The monocarboxylate transporter inhibitor, α-cyano-4-hydroxycinnamate (4-CIN), had no effect on [^{18}F]FDG uptake rate in rat brain slices before KCl addition. On the other hand, addition of 4-CIN induced larger [^{18}F]FDG uptake rates under the activated condition in comparison with the control condition. Because neurons cannot utilize lactate under the 4-CIN loaded conditions, this indicates that activated neurons consume lactate as an energy substrate. The lactate concentration in the incubation medium was increased with KCl treatment in both groups and the extent was slightly greater in 4-CIN group. These results suggested that: (1) the brain mainly uses glucose, not lactate, as an energy substrate in resting state; (2) when neuron is stimulated, excess amounts of lactate might be produced in astrocytes and the lactate is mobilized as an energy substrate.

Keywords: Lactate; Glucose; Rat brain slice; 2-Deoxy-2-[^{18}F]fluoro-D-glucose; Autoradiography; Cerebral energy metabolism

1. Introduction

The neuronal metabolic events that occur during functional activation are still partially unclear. Glucose has been thought as the only metabolic substrate of the brain for a long time (McIlwain and Bachelard, 1985). However, many researchers have recently been interested in lactate as an energy substrate in the brain, especially in neurons. Pellerin and Magistretti (1994) proposed an astrocyte–neuron lactate shuttle hypothesis (ANLSH), which claims the metabolic role of lactate in the brain. This model hypothesizes that neuronal activation stimulates the anaerobic production of lactate in neighbouring astrocytes. The produced lactate in astrocytes is then transferred to the neuron, which then uses the lactate as an energy substrate aerobically. In vivo studies have shown that the brain can consume lactate as a substrate (Ide and Secher, 2000; Smith et al., 2003). Magnetic resonance spectroscopy studies also show the increase of lactate production during cerebral activation (Frahm et al., 1997; Sappey-Marinier et al., 1992). Furthermore, in vitro studies have shown that lactate is utilized as an energy substrate in ischemic (Kitano et al., 2002) or hypoglycemic conditions (Izumi et al., 1997). On the other hand, Chih and Roberts (2003) support the conventional hypothesis that asserts that glucose is the primary substrate for both neurons and astrocytes during neural activated conditions. They claim that the experimental evidence of ANLSH is weak and that the existing evidence supports the conventional hypothesis. Therefore, the role of lactate in neuronal energy

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metabolism is not clearly elucidated, which has sparked interest in many researchers.

Recently, Tanaka et al. (2004) investigated the role of lactate in brain energy metabolism using the ''bioradiography'' system. ''bioradiography'' is the dynamic living brain slice imaging system for positron–emitter labeled compounds. They investigated the uptake of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), which is a positron emitting radiotracer for glucose metabolism measurement, to rat brain slices. The slices were incubated in lactate or a monocarboxylate transporter (MCT) inhibitor, α-cyano-4-hydroxycinnamate (4-CIN), added Krebs–Ringer solution. [^{18}F]FDG uptake was decreased by the lactate application and increased by the 4-CIN application. These results suggested that lactate is used as an energy substrate in the brain in resting state. However, the contribution of lactate as an energy substrate during neural activated conditions has not been clarified.

Therefore, in this study, we investigated energy metabolism under neural activated conditions in the brain using the ''bioradiography'' system with [^{18}F]FDG and compared under resting state conditions.

2. Materials and methods

2.1. Materials

All chemicals used were reagent grade. α-Cyano-4-hydroxycinnamate (4-CIN) was purchased from Sigma (Milwaukee, WI, USA). Wistar rats were supplied by Japan SLC Co. Ltd. (Sizuoka, Japan). The present animal study was approved by the Animal Care and Use Committee of the Hamamatsu University School of Medicine.

2.2. Preparation of rat brain slices

After the decapitation of male Wistar rats (230–250 g), the brain was quickly removed and placed in an ice-cold oxygenated (95% O_2, 5% CO_2) Krebs–Ringer solution (124 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.2 mM KH_2PO_4, 26 mM $NaHCO_3$, 5 mM glucose) for 3 min. Coronal slices (300 μm) were prepared using a microslicer (D.S.K. Super Microslicer, Dosaka EM, Kyoto, Japan) and transferred into the ice-cold Krebs–Ringer solution.

2.3. [^{18}F]FDG uptake study using the dynamic imaging system

[^{18}F]F^- was produced by a $^{18}O(p, n)^{18}F$ nuclear reaction using a Sumitomo CYPRIS HM18 cyclotron (Sumitomo Heavy Industries Ltd., Tokyo, Japan). Then [^{18}F]FDG was prepared from [^{18}F]F^- by the method of Hamacher et al. (1986) using an automated synthesis system (Cupid, Sumitomo Heavy Industries Ltd., Tokyo, Japan).

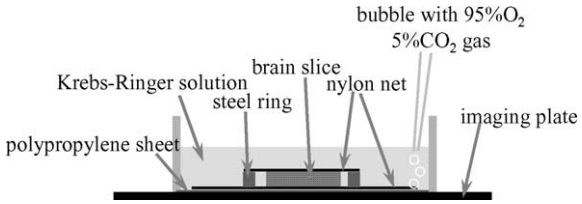

Fig. 1. Schematic side view of the incubation system for ''bioradiography''. Krebs–Ringer solution containing 1.5 MBq/mL of [^{18}F]FDG. Images were obtained in a dark environment at 35 °C.

The incubation was performed using the double polystyrene system as described by Murata et al. (1998). The system is described in Fig. 1. The Krebs–Ringer solution was filled into the outer chamber and the inner chamber was immersed in it. The bottom of the inner chamber was made with a nylon net and the bottom of the outer chamber was a 10 μm thick polyvinylidene chloride film that was penetrable to beta and gamma ray of ^{18}F. The prepared slices were placed in the inner chamber and were covered by a 300 μm stainless steel ring, as the upper side was braced by nylon net. After preincubation of the slices for 1 h at 35 °C, the inner chamber was taken out and immersed into another chamber that contained 1.5 MBq/mL of [^{18}F]FDG. The incubation volume was 100 mL for each chamber. During the incubation, Krebs–Ringer solution was bubbled with 95% O_2 and 5% CO_2 mixed gas. Ten brain slices were placed in each inner chamber.

For the 4-CIN added study, 4-CIN was added to the incubating solution and the pH was adjusted to 7.4 with NaOH (4-CIN added condition). The final concentration of 4-CIN was 0.5 mM. Control study was performed without 4-CIN (control condition). To take the images of the slices, the chamber was placed on the imaging plate (BAS-MS2040, Fuji Photo Film Co., Tokyo, Japan) and the plate was changed every 10 min. Then, the imaging plate was scanned with a computer-associated image analyzer (Fujix Bio-Imaging Analyzer, BAS-2000, Fuji Photo Film Co., Tokyo, Japan). To measure the background activity from the [^{18}F]FDG including solution over the brain slice, 300 μm nylon sheet was placed beside the brain slices. The incubation was carried out for 150 min. After 60 min incubation with [^{18}F]FDG, 50 mM of KCl with was added to each chamber. To adjust the osmolarity, KCl was dissolved in NaCl reduced Krebs–Ringer solution. For determination of lactate concentration of the incubation solution, 100 μL of the incubation solution was sampled every 20 min. The lactate concentration was then measured with an enzymatic bio-assay kit for L-lactate (R-Biopharm AG, Darmstadt, Germany).

2.4. Automatic registration of images

Sequential images from the imaging plate were automatically registered to the last image. To do this, each image was binarized by certain image threshold and then two-dimensional template matching technique was utilized for