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Glucose consumption of inflammatory cells masks metabolic deficits in the brain

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ABSTRACT

Inflammatory cells such as microglia need energy to exert their functions and to maintain their cellular integrity and membrane potential. Subsequent to cerebral ischemia, inflammatory cells infiltrate tissue with limited blood flow where neurons and astrocytes died due to insufficient supply with oxygen and glucose. Using dual tracer positron emission tomography (PET), we found that concomitant with the presence of inflammatory cells, transport and consumption of glucose increased up to normal levels but returned to pathological levels as soon as inflammatory cells disappeared. Thus, inflammatory cells established sufficient glucose supply to satisfy their energy demands even in regions with insufficient supply for neurons and astrocytes to survive. Our data suggest that neurons and astrocytes died from oxygen deficiency and inflammatory cells metabolized glucose non-oxidatively in regions with residual availability. As a consequence, glucose metabolism of inflammatory cells can mask metabolic deficits in neurodegenerative diseases. We further found that the PET tracer did not bind to inflammatory cells in severely hypoperfused regions and thus only a part of the inflammation was detected. We conclude that glucose consumption of inflammatory cells should be taken into account when analyzing disease-related alterations of local cerebral metabolism.

Introduction

Harmful or beneficial — disorders of the brain may induce neuroinflammation affecting the course of disease (Graeber and Streit, 2010; Graeber, 2010). After an ischemic injury, the peak of the inflammatory response is typically observed one week after the onset of ischemia (Hallenbeck et al., 1986). In ischemic regions, neurons and astrocytes die because of insufficient supply of nutrients and oxygen. Inflammatory cells, however, also rely on energy supply to exert cellular functions and to maintain their membrane potential. In a double tracer long-term follow-up positron emission tomography (PET) study in rats we analyzed the development of inflammation in relation to local glucose metabolism following permanent occlusion of the middle cerebral artery (MCAo). Inflammation was localized and quantified using [11C]PK11195, a PET tracer that binds to the translocator protein expressed by activated microglia and macrophages (Banati, 2002; Politis et al., 2012; Rojas et al., 2007; Thiel and Heiss, 2011). Since [11C]PK11195 does not allow for differentiation, we summarize here activated microglia and microglia- or monocyte-derived macrophages as *inflammatory cells*, keeping in mind that other types of inflammatory cells could be involved. Glucose metabolism was measured using [18F]-2-fluoro-2-deoxy-D-glucose ([18F]FDG) as PET tracer. Additionally, we measured blood flow 1 h after MCAo using [15O]H_2O-PET for localization of the primarily affected ischemic territory and performed structural magnetic resonance imaging (MRI) before each PET scan. In vivo data were compared with ex vivo immunostaining.

Materials and methods

Animals and Surgery

All animal procedures were performed in accordance with the German Laws for Animal Protection and were approved by the local animal care committee and local governmental authorities (LANUV NRW). Male Wistar rats (n = 5, Janvier, France; weight: 320 to 363 g; age ~ 10 weeks; pairwise housed in type-4 cages filled with Lignocel in an inverse 12 h day–night cycle with lights on at 8:30 pm in a temperature

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(22 ± 11 °C) and humidity (55 ± 5%) controlled room; experiments performed between 9 am and 4 pm) were anesthetized with 5% isoflurane and maintained with 2.5% isoflurane in 65%/35% nitrous oxide/oxygen. Throughout the surgical procedure and the MRI and PET imaging procedures, body temperature was maintained at 37.0 ± 0.5 °C using a thermostatically controlled heating pad (MEDRES, Cologne, Germany). Before acute PET imaging, animals were prepared for induction of ischemia using macrospheres (Gerriets et al., 2004). Briefly, the left common carotid artery, internal carotid artery, and external carotid artery were exposed through a midline incision of the neck and the external carotid artery and the pterygopalatine branch of the internal carotid artery were ligated. A PE-50 catheter was filled with saline and two TiO2 macrospheres (diameter of 0.315–0.355 mm; BRACE, Alzenau, Germany). This catheter was inserted into the common carotid artery, advanced to the origin of the pterygopalatine artery, and fixed in place. After placing the rats in the micro-PET scanner and running baseline regional cerebral blood flow (rCBF) measurements using [^{15}O]H_2O as tracer, macrospheres were injected through a saline-filled catheter placed in the internal carotid artery to occlude the middle cerebral artery. Following PET imaging, the catheter was removed and the animals were allowed to recover. Each animal was additionally imaged by T2-weighted MRI and PET using [^{11}C]PK11195 and [^{18}F]FDG at baseline (7 days before MCAo) as well as at days 2, 7, 14, 21 and 42 after MCAo. During the experiment, all animals received an intensified care with moistening of food pellets, subcutaneous saline injections (5 mL 0.9%NaCl/day for 8 days) and an intra- and postoperative analgesia with Carprofen (Rimadyl®; 5 mg/kg/day s.c. for 3 days).

PET

PET imaging was performed using a microPET Focus 220 scanner (Concorde Microsystems, Inc, Knoxville, TN; 63 image planes; 1.5-mm full width at half maximum). For each animal and each imaging session transmission data from a Co57 point source were acquired for attenuation correction. During induction of ischemia rats received an intravenous bolus injection of [^{15}O]H_2O (59–89 MBq/rat in 0.5 mL) before as well as 5, 30, and 60 min after MCAo without changing the position of the animals in the PET scanner. Emission data were acquired for 2 min. Thereafter, an intravenous bolus of [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) (52–74 MBq/rat in 0.5 mL) was injected into the tail vein at 75 min after embolization of macrospheres, and emission data were acquired for 60 min.

At baseline (7 days before MCAo) as well as 2, 7, 14, 21, and 42 days, rats received an intravenous bolus injection of [^{11}C]PK11195 (59–89 MBq/rat, specific activity 18.5–81.4 GBq/μmol) in the tail vein. Emission data were acquired for 30 min and the animals remained in the μPET-scanner without changing their position. 100 min after injection of [^{11}C]PK11195 (~5 half-lives of the [^{11}C]-labeled tracer) a bolus of [^{18}F]FDG (52–74 MBq/rat in 0.5 mL) was injected in the tail vein and emission data were acquired for 60 min.

[^{18}F]FDG and [^{11}C]PK11195 data were histogrammed in time frames of 6 × 30 s, 3 × 60 s, 3 × 120 s, and either 12 × 240 s for [^{18}F]FDG or 4 × 240 s for [^{11}C]PK11195, Fourier rebinned, and images (voxel size: 0.4 mm × 0.4 mm × 0.8 mm) were reconstructed using 2-dimensional filtered back projection. Binding potential of the radiotracer [^{11}C]PK11195 was calculated using the simplified reference tissue model (SRTM) using the contralateral hemisphere as reference region (Lammertsma and Hume, 1996). [^{15}O]H_2O data were Fourier rebinned and images were reconstructed using 2-dimensional filtered back projection. CBF was assessed as percentage injected dose of [^{15}O]H_2O averaged over 2 minute acquisition time. A 1.5 mm Gaussian Filter was applied to the CBF images.

An image derived input function was extracted from the [^{18}F]FDG-PET data and parametric images of the kinetic constants were determined by a voxel-by-voxel application of a two-tissue-compartment kinetic model (Backes et al., 2011). K_1 is the rate constant for [^{18}F]FDG transport from blood into the brain tissue and is related to regional cerebral blood flow (Walberer et al., 2012). The metabolic rate constant or net influx rate constant for [^{18}F]FDG is given by $K_i = K_1\ k_3 / (k_2 + k_3)$. The cerebral metabolic rate of glucose CMR_{glc} is given by: $CMR_{glc} = K_{glc}\ C_p$, where K_{glc} is the net influx rate constant of glucose ($K_{glc} = K_i / [0.38 + 1.22\ K_i/K_1]$) and C_p the plasma glucose level. Note, that K_{glc} takes into account changes of the lumped constant that result from changes in the relative contributions of glucose transport and hexokinase activity (Backes et al., 2011). The plasma glucose level was determined after each [^{18}F]FDG PET measurement from a tail vein blood sample (measured values: 75–233 mg/dL, mean = 128.5 ± 28.8 mg/dL).

Magnetic resonance imaging

At baseline (7 days before MCAo) as well as 2, 7, 14, 21 and 42 days after induction of ischemia, rats were (re-)anesthetized with isoflurane, and experiments were conducted on a 4.7 T BioSpec system (Bruker BioSpin, Ettlingen, Germany) with a 30 cm bore horizontal magnet, equipped with a self-shielded gradient system (max gradient: 100 mT/m; rise time: <250 μs). Radio frequency transmission was achieved with a Helmholtz coil (12 cm diameter), and the signal was detected with a 22 mm diameter surface coil. The animals were positioned prone in a dedicated cradle using a stereotactic head holder with the surface coil placed directly over the head. A multislice

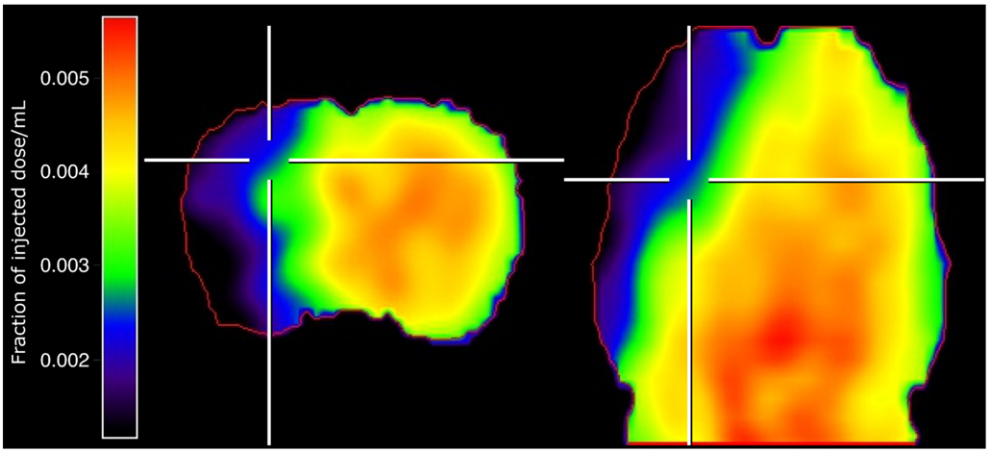

Fig. 1. Cerebral blood flow 1 h after permanent occlusion of the middle cerebral artery. The region supplied by the MCA was characterized by a severe reduction of CBF measured by [^{15}O]H_2O PET. Data is from the same rat and spatially co-registered to the data shown in Fig. 2. Left: coronal section, right: axial section. Locations of the sections are indicated by white markers. The red line indicates the boundary of the brain.