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Short communication

MicroPET detection of enhanced ^{18}F-FDG utilization by PKA inhibitor in awake rat brain

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Abstract

To obtain PET imaging of glucose metabolism in the brains of conscious rats, a method of rat head fixation was developed. PET measurement with microPET was performed for 60 min after ^{18}F-FDG injection. Significant enhancement of glucose utilization in the right striatum was observed with infusion of Rp-adenosine-3,5-cyclic phosphorothioate triethylamine (Rp-cAMPS). FDG uptake increments were also seen in the ipsilateral frontal cortex and thalamus. As initial FDG uptake in the brain was not significantly altered by Rp-cAMPS, increased glucose metabolism might be due to an increase in the phosphorylation rate by hexokinase rather than the delivery process from plasma to the brain. In contrast to awake rats, the effect of Rp-cAMPS was abolished by anesthesia using chloral hydrate, indicating that neuronal activity has an important role in short term regulation of hexokinase activity through the cAMP/PKA system in the brain. These results strongly demonstrated the value of measuring glucose utilization in the brains of conscious rats.

Theme: Other systems of the CNS
Topic: Brain metabolism and blood flow

Keywords: MicroPET; Conscious rats; Glucose metabolism; cAMP/PKA; Brain

Positron emission tomography (PET) is a highly sensitive, quantitative, and noninvasive detection method that provides three-dimensional molecular information within the living animal or human brain. The tracer ^{18}F-fluorodeoxyglucose (FDG) has been used as a marker of metabolic activity, in a manner analogous to the use of ^{14}C-DG in animals. Although FDG-PET has enormous value in clinical medicine, unfortunately, experimental application of FDG-PET for monitoring glucose metabolism has been restricted to large laboratory animals because of the limited resolution of the PET scanner relative to brain size.

Recently, the development of a microPET scanner has allowed application of this technology to small animal experiments, e.g. with mice and rats [4,8]. Although microPET has been useful in small animal studies, most of these studies were carried out in anesthetized animals. However, a variety of physiological responses are assumed to be significantly affected by anesthesia. Therefore, it would be of a great value to establish a method of measuring glucose metabolism in the rat brain, while conscious, using microPET. To perform the PET study in conscious rats, we recently developed a fixation system using a plastic plate [7].

In a previous study, we found that micro-infusion of Rp-adenosine-3,5-cyclic phosphorothioate triethylamine (Rp-cAMPS), a PKA inhibitor, resulted in a significant although transient increase in ^{14}C-DG uptake in the rat striatum [3]. These changes were only detected in the intact brain. Herein, we used this model and established an appropriate methodology for measuring brain metabolism in awake rats

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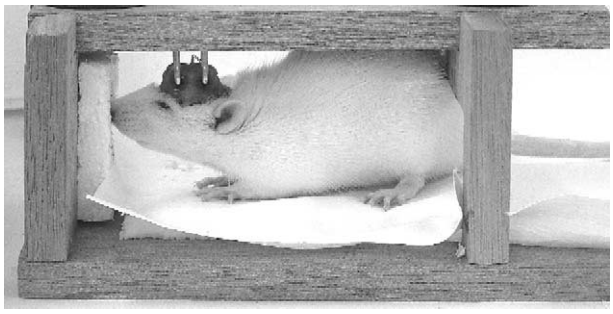

Fig. 1. Photograph of the rat setup in the wood frame for head fixation.

with microPET. We also determined whether chloral hydrate anesthesia alters the stimulant effect of Rp-cAMPS.

All experiments on the rats were performed with the permission of the Institutional Animal Care and Use Committee, School of Allied Health Sciences, Osaka University. The rats (male Wistar, 7–8 weeks old, Japan SLC, Shizuoka, Japan) were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus. Bilateral 26-gauge stainless steel guide cannulas fitted with 33-gauge stainless steel obturators were implanted in the striatum, according to the atlas of Paxinos and Watson [10]; 0.2 mm anterior to the bregma, 3.2 mm lateral to the midline, and 2.0 mm below the cortical surface. The guide cannulas were then fixed to the skull using stainless steel screws and acrylic cement. At the same time, two plastic tubes were fastened to the skull. One day after surgery, we began conditioning the rats to tolerate fastening of a right-angled hook of a wooden frame fixed with two plastic tubes (Fig. 1). The conditioning was carried out for 2 h per day over a period of 2 weeks to accustom the rats to the apparatus before the PET experiments.

Rp-cAMPS (300 nmol/μl, Sigma-Aldrich, St. Louis, MO, USA) was infused through a cannula (33-gauge, 3.5 mm longer than the guide cannula) into the left striatum of each rat, while awake. The infusion was performed for 4 min at a flow rate of 0.25 μl/min, and the infusion cannulas were left in place for an additional 3 min to reduce the reflux of infused drugs along the cannula track. At the same time, saline solution (1 μl) was infused into the right striatum.

^{18}F was produced by the ^{18}O(p, n)^{18}F nuclear reaction in a cyclotron at Osaka City University Hospital, and ^{18}F-FDG was synthesized by the method of Hamacher et al. [2], with an automated ^{18}F-FDG synthesis system (Nihonkokan, Tokyo, Japan).

Data were collected with a high-resolution small-animal PET, microPET Primate 4 (Concorde Microsystems, Knoxville, USA). The system parameters were described by Mastsumura et al. [6]. After the Rp-cAMPS infusion, animals were fixed to the wood frame and positioned in the microPET scanner. At 30 min after intrastriatal drug infusion, the animals were given an intravenous bolus injection of ^{18}F-FDG (37–74 MBq/rat) dissolved in 0.5 ml of saline. Data acquisition began at the same time and continued for 60 min. The image data acquired from microPET were displayed and analyzed by IDL ver. 5.5 (RESEARCH SYSTEMS, Colorado, USA) and ASIPro ver. 3.2 (Concorde Microsystems Inc, Knoxville, USA) software. The radioactivity concentrations in regions of interest (ROIs) were expressed as percent injected dose per tissue volume (% dose/cm^3).

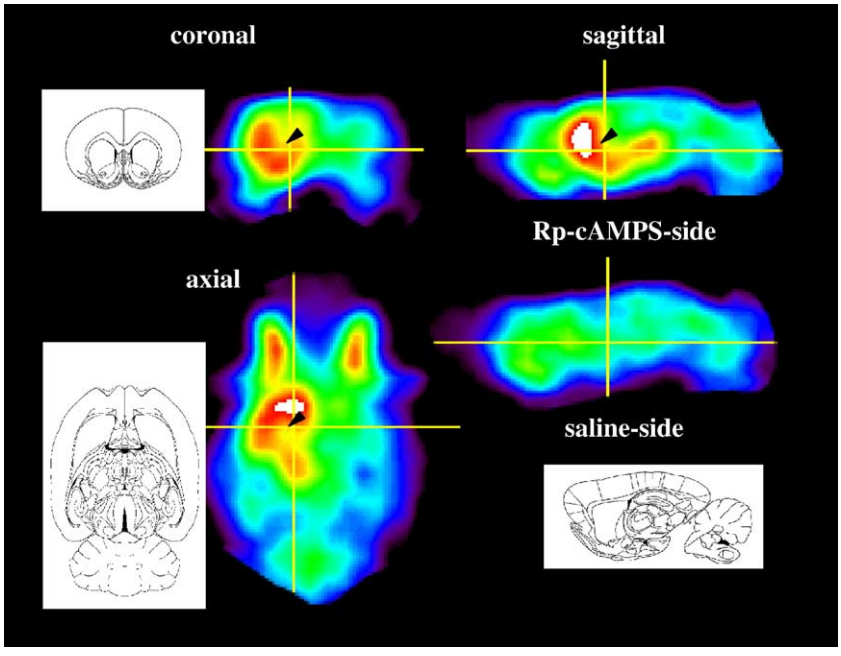

Fig. 2. Typical microPET images of ^{18}F-FDG in the brain of the Rp-cAMPS infused conscious rat. PET images were generated by summation of image data from 30 to 60 min after ^{18}F-FDG injection. Rp-cAMPS was infused into the left striatum (arrow) of the image, saline into the contralateral striatum.