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Small animal positron emission tomography during vagus nerve stimulation in rats: A pilot study

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Abstract

Vagus nerve stimulation (VNS) is an effective neurophysiological treatment for patients with refractory epilepsy, however, the mechanism of action remains unclear. Small animal positron emission tomography (PET) permits the monitoring of biochemical processes during multiple scans in the same animal. The aim of this pilot study was to explore the potential of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG)-PET to investigate the effect of acute and chronic VNS on glucose metabolism in the rat brain.

One week after EEG and VNS electrode implantation, a baseline FDG-PET scan was acquired during which animals were not stimulated. Secondly, scans were taken after first activation of the VNS electrode (acute VNS) and after one week of continuous VNS (chronic VNS). On the same time points, images were obtained in a control group. After acquisition, PET images were manually fused with MRI data. Normalized brain activities and left/right activity ratios of different brain structures were compared between control measurements and VNS group. During acute VNS, glucose metabolism was significantly decreased in the left hippocampus ($P < 0.05$). Significant increases were found in both olfactory bulbs ($P < 0.05$). During chronic VNS, a significant decrease in left/right ratio in the striatum ($P < 0.05$) was found.

Acute and chronic VNS induced changes in glucose metabolism in regions important for seizure control (hippocampus and striatum). Our results promote further brain research on VNS using small animal PET in rats.

Keywords: Small animal positron emission tomography (PET); Vagus nerve stimulation (VNS); Micro-PET; Mechanism of action; Rat; Epilepsy

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1. Introduction

Vagus nerve stimulation (VNS) is used to reduce the frequency and severity of epileptic seizures in refractory patients, although the precise mechanism of action (MOA) still remains to be elucidated (Vonck et al., 2001). Understanding the MOA is an important aspect of clinical VNS, as identification of responder groups and optimization of stimulation parameters is of great interest.

It is generally assumed that VNS exerts its effect by inducing action potentials in the afferents of the left vagus nerve, which has diffuse projections in the central nervous system. Several functional imaging studies have been conducted to investigate the activation or inhibition of brain structures by VNS. Our previous imaging studies in humans reported a different effect of acute and chronic VNS on cerebral blood flow (CBF), which is ascribed to a combination of an anti-seizure and anti-epileptic effect (Vonck et al., 2000; Van Laere et al., 2002). Generally, changes were found on both sides of the brain by unilateral left VNS, and a key role was pointed out for the thalamus and medial temporal lobe structures in the MOA of VNS (Vonck et al., 2001; Chae et al., 2003). However, there is no consensus on other activated structures neither on the type of the changes (inhibition or excitation). This discrepancy can be attributed to a number of confounding factors such as imaging techniques used (PET, SPECT and fMRI), tracer and contrast agents, scanning protocols, stimulation parameters, medication regimes, course of the illness and treatment response. Heterogeneity of relatively small patient samples is difficult to avoid, and, in addition, data gathering from healthy subjects are impossible for ethical reasons due to the invasiveness of VNS.

Small animal positron emission tomography (PET) is a technique used for in vivo, quantitative and high-resolution spatial determination of positron emitting isotopes in small animal tissues. It permits the monitoring of biochemical processes over time during a dynamic scan period but also longitudinally across multiple scans of the same subject. This technique is of increasing importance as fewer animals have to be used in comparison with post mortem techniques like autoradiographic methods (Phelps, 2000). Small animal PET is widely used in all fields of pathophysiological research such as experimental oncology, gene expression and CNS research, and is presently also emerging in animal research on epilepsy (Kornblum et al., 2000). Small animal PET could give the unique possibility to assess the effect of VNS on brain metabolism in healthy subjects, as two dynamical processes are going to interfere in epileptic animals (those due to epilepsy itself and those due to VNS). In addition, we will avoid many of the disease-related confounding factors listed above. The aim of this study was to explore whether it is feasible to investigate the acute and chronic effect of VNS on relative brain glucose metabolism in healthy rats using small animal PET.

2. Materials and methods

2.1. Animal preparation

Eight male Wistar rats, weighing 300–350 g, were used in this feasibility study. The animals were housed under environmentally controlled conditions (12-h light:12-h dark cycles, 20–22 °C) in the animal facility of the Ghent University Hospital with food and water ad libitum. All animals were treated according to guidelines approved by the European Ethics Committee (decree 86/609/EEC). The study protocol was approved by the Animal Ethical Committee of Ghent University Hospital (ECP 01/26).

All animals ($n = 8$) were implanted with five epidural EEG electrodes and a stimulation cuff-electrode around the left vagus nerve under deep ketamine/xylazine anesthesia (80 mg/kg and 7.5 mg/kg, respectively, i.p.) as previously described (Dedeurwaerdere et al., 2004). During surgery, additional ketamine (5 mg/kg) was given when sensorial pain prickles, by squeezing the foot pad, were felt. Following surgery, xylocaine (0.2%) was applied at the incision wound and animals were placed under a heating lamp in their home cages to maintain normal body temperature until behaviorally active. The experimental period started at least one week after surgery, and consisted of EEG recordings and repetitive scans.

2.2. EEG recordings

EEG was recorded during 3 h at the beginning and at the end of the experimental period to ensure that no spontaneous epileptic activity was present.