Brain Stimulation 8 (2015) 702–707

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Original Article

Acute Vagal Nerve Stimulation Lowers α2 Adrenoceptor Availability: Possible Mechanism of Therapeutic Action

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ARTICLE INFO

Article history:
Received 16 October 2014
Received in revised form
23 January 2015
Accepted 7 February 2015
Available online 7 March 2015

Keywords:
Brain stimulation
Minipig
Noradrenaline
Positron emission tomography
Vagal nerve stimulation
Yohimbine

ABSTRACT

Background: Vagal nerve stimulation (VNS) emerged as an anti-epileptic therapy, and more recently as a potential antidepressant intervention.
Objective/hypothesis: We hypothesized that salutary effects of VNS are mediated, at least in part, by augmentation of the inhibitory effects of cortical monoaminergic neurotransmission at appropriate receptors, specifically adrenoceptors. Our objective was to measure the effect of acute VNS on α2 adrenoceptor binding.
Methods: Using positron emission tomography (PET), we measured changes in noradrenaline receptor binding associated with acute VNS stimulation in six anesthetized Göttingen minipigs. We used the selective α2 adrenoceptor antagonist [^{11}C]yohimbine, previously shown to be sensitive to competition from the receptor's endogenous ligands, as a surrogate marker of monoamine release. PET records were acquired 4–6 weeks after the implant of a VNS electrode in minipigs before and within 30 min of the initiation of 1 mA stimulation. Kinetic analysis with the Logan graphical linearization generated tracer volumes of distribution for each condition. We used an averaged value of the distribution volume of non-displaceable ligand (V_{ND}), to calculate binding potentials for selected brain regions of each animal.
Results: VNS treatment markedly reduced the binding potential of yohimbine in limbic, thalamic and cortical brain regions, in inverse correlation with the baseline binding potential.
Conclusion: The result is consistent with release of noradrenaline by antidepressant therapy, implying a possible explanation for the antidepressant effect of VNS.

Introduction

Vagal Nerve Stimulation (VNS) is a form of stimulation of the brain which has anticonvulsant effects in animal models of epilepsy [1,2] and in humans with seizures [3]. As such, it has been approved as an adjunct treatment for partial complex seizures since 1994 in Europe and since 1997 in the USA.

Follow-up of VNS-treated epileptic patients revealed improvements in mood in the majority of patients, including patients with no apparent changes of seizure frequency or intensity. These observations prompted further studies of the possible antidepressant effect of VNS. Since then, VNS has been shown to exert some antidepressant effects in a model of depression in rats and in

Part of this work has been presented at international scientific meetings: A.M. Landau, S. Dyve, A.K.O, Alstrup, S. Jakobsen, A. Gjedde, and D.J. Doudet. Acute vagal nerve stimulation promotes noradrenaline release in minipig brain. International Union of Basic and Clinical Pharmacology (IUPHAR). Oral Presentation, Copenhagen, Denmark, July 2010; and D.J. Doudet, S. Dyve, A.K.O Alstrup, S. Jakobsen, M. Simonsen, A. Møller, P. Videbech, G. Wegener, A. Gjedde, and A.M. Landau. Noradrenaline release: potential antidepressant mechanism of brain stimulation? 8th International Symposium on Functional Neuroreceptor Mapping of the Living Brain. Oral Presentation, Glasgow, Scotland, July 2010.

This work was supported by a Lundbeck Foundation grant (grant number 2008-2607 to DJD).

The authors declare no conflict of interest.

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http://dx.doi.org/10.1016/j.brs.2015.02.003
1935-861X/

depressed humans [4–7]. As a result, the Food and Drug Administration approved VNS in 2005 for adjunctive therapy in treatment-resistant depression. The exact therapeutic mechanisms of VNS in epilepsy and mood disorders are unknown. However, evidence from the abundant literature on specific antidepressant and anti-epileptic therapies, and from the known anatomical connections of the vagus nerve in brainstem and pons, makes it likely that effects of VNS are mediated by monoaminergic neurotransmission. Through the nucleus tractus solitarius, the ascending pathways from the vagus nerve connect indirectly to the locus coeruleus (LC), the main origin of noradrenergic innervation of the cortex [8] and the hippocampus [9]. While it is unknown whether the vagal influences on the LC are excitatory or inhibitory, lesions of the LC confirm a role of this nucleus in seizures [10]. Observations of effects of pharmacological antidepressant therapy suggest that both serotonergic and noradrenergic (NA) neurotransmissions are affected in mood disorders. It is possible that modulation of noradrenergic neurotransmission plays a role in at least some of the therapeutic effects of VNS.

We recently developed [^{11}C]yohimbine, an antagonist primarily of the α2 adrenoreceptors, as a tracer of noradrenergic neurotransmission by means of PET, and validated its use in pigs [11]. In pharmacological doses, yohimbine is not exclusively selective for α2 receptors, as it has been reported to bind with moderate or weak affinity to other receptors *in vitro*, such as D_2, α1 and $5HT_{1A}$, albeit with 5–10-fold lower affinity [12–14]. However, in tracer concentrations, yohimbine is highly selective for α2 sites *in vivo* [11]. Central α2 NA receptors are expressed presynaptically on noradrenergic neurons in the LC, where they control the release of the neurotransmitter, and postsynaptically in the widely distributed projection areas of the NA neurons throughout the cortex where they modulate signaling pathways by limiting signal dissipation in dendritic spines [15]. At rest, the distribution of labeled yohimbine in the living pig brain is consistent with the known *in vitro* distribution of α2 receptors, with density in cortex and thalamus greater than density in mesencephalon, which in turn has greater density than cerebellum, pons, and medulla [16]. Furthermore, we recently demonstrated displacement of yohimbine binding in response to amphetamine challenge in pigs and rats, suggesting competition with the endogenous ligand [17,18]. These observations, together with reports that stimulation of the vagal nerve in rodents induces NA release [19], prompted the current test *in vivo* of the hypothesis that the acute effects of a human VNS device are consistent with the release of NA or other monoamines, or both, in a healthy brain.

Göttingen minipigs are large animals in which the device designed for use in humans easily is implanted, and stimulation is executed with similar parameters, facilitating the translation of these observations for clinical application. To test the hypothesis, we implanted VNS electrodes on the left vagus nerve of minipigs and imaged the brain with [^{11}C]yohimbine before and shortly after stimulation to allow us to determine the effect of VNS on the binding of the tracer.

Material and methods

Animals

Six 14-month old normal female Göttingen minipigs weighing 25–32 kg (Ellegaard Minipigs ApS, Dalmose, Denmark) were used in the VNS study (labeled VNS1-VNS6), in accordance with a protocol approved by the Danish Animal Experimentation Inspectorate. The brain size (about 80 g) is adequate for imaging, and the existence of an MRI-based atlas allows co-registration of PET data for accurate analysis of regional distribution of the tracer. The minipigs were fed a restricted pellet diet (DIA plus FI, DLG, Aarhus, Denmark). They were fasted overnight, with free access to tap water, prior to the VNS surgery and the PET scans. Environmental conditions in the animal facility were 20 °C and 50–55% relative humidity, and the air was changed 8 times every hour. Pigs were single housed in a 4.6 m^2 enclosure with fence-line contact with congeners.

VNS surgery and stimulation

The minipigs were pre-medicated with a mixture of 1.25 mg/kg midazolam and 6.25 mg/kg s-ketamine intramuscularly (IM). After placing of an ear vein catheter (21G Venflon), anesthesia was induced with a mixture of 1.25 mg/kg midazolam and 3.13 mg/kg s-ketamine intravenously (IV). The minipigs were intubated, and anesthesia was maintained on 3.7 mg/kg/h to 4.0 mg/kg/h propofol IV. The minipigs were mechanically ventilated with approximately 8 ml/kg/min of a mixture containing 1 O_2 and 2.2 medical air. Pulse, arterial oxygen (SaO_2) and body temperature were monitored during the whole procedure and a saline drip prevented dehydratation. Analgesics such as Flunixin and Temgesic were administered for up to 5 days post-surgery to minimize pain and discomfort. The antibiotic Penovet was administered at least 30 min before the start of the procedure and then daily for up to 5 days post-surgery. The animal was placed supine and the head was immobilized and taped towards the right to expose the left neck area. The implant of the vagal nerve stimulator (Cyberonics Inc) was performed by an experienced neurosurgeon (SD) under sterile conditions using a modified human protocol. As in human subjects, the implant was placed on the left vagus in order to avoid the cardiac branches of the vagal nerve. The nerve was carefully dissected and exposed and the stimulating coils were wrapped gently around it. A second incision was placed on the left side of the back of the neck to create a pocket in which to slide the stimulating device. During the surgery, we tested the stimulator efficacy and connection with the control device by turning on the VNS for a 1-min period at 1 mA. The device was turned off again and remained off until the time of the PET studies. None of the animals developed side effects or infection from the surgical procedure.

The PET studies were performed 4–6 weeks after stimulator implant to allow the animal ample time for recovery from surgery and to ensure the lack of infection and adverse reactions. Baseline and experimental tomography occurred on the same day. This design ensured that the possible changes in the VNS ON condition could not be attributed to possible damage, trauma or inflammation of the vagus nerve due to surgery compared to baseline. It also reduced the variability due to positioning in the scanner and other physiological differences. The baseline study was performed first (baseline OFF condition) and the challenge study approximately 30 min after turning the stimulator on (ACUTE ON). The choice of stimulation intensity was based on the data by Roosevelt [19] reporting a significant increase in NA concentrations in both the cortex and hippocampus of rats at 1 mA stimulation intensity of the vagus nerve. Furthermore, this intensity is used during the surgical procedure for the testing of the stimulator and leads.

Tomography

The anesthesia regimen and preparation of the pigs for scanning was described in detail elsewhere [20] and was similar to the one described above for the VNS implant. The minipigs were positioned in a state of the art High-Resolution Research Tomograph (HRRT, CTI/Siemens) in a dorsal recumbent position, with the head immobilized with a custom device, and covered with a heating blanket set to maintain a stable body temperature. The HRRT permits the acquisition of 207 slices, 1.2 mm apart center to center and has a reconstructed resolution of about 2 mm FWHM. After a brief