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Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures

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Objective – The precise mechanism of action of vagus nerve stimulation (VNS) in suppressing epileptic seizures remains to be elucidated. This study investigates whether VNS modulates cortical excitability by determining the threshold for provoking focal motor seizures by cortical electrical stimulation before and after VNS. *Material and methods* – Male Wistar rats ($n = 8$) were implanted with a cuff-electrode around the left vagus nerve and with stimulation electrodes placed bilaterally on the rat motor cortex. Motor seizure threshold (MST) was assessed for each rat before and immediately after 1 h of VNS with standard stimulation parameters, during two to three sessions on different days. *Results* – An overall significant increase of the MST was observed following 1 h of VNS compared to the baseline value (1420 μA and 1072 μA, respectively; $P < 0.01$). The effect was reproducible over time with an increase in MST in each experimental session. *Conclusions* – VNS significantly increases the MST in a cortical stimulation model for motor seizures. These data indicate that VNS is capable of modulating cortical excitability.

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Introduction

Vagus nerve stimulation (VNS) is indicated in patients with refractory epilepsy who are unsuitable candidates for epilepsy surgery. By means of a helical electrode that is wound around the left vagus nerve, electrical stimuli are administered through an implantable and programmable pulse generator. The precise mechanism of action (MOA) by which VNS suppresses epileptic seizures remains to be elucidated. It has been demonstrated that VNS has both an acute effect on seizures, is able to interrupt ongoing seizure activity, as well as having a more chronic seizure preventative effect following long-term treatment (1). The acute and chronic effects are likely to be based on a distinct MOA that involves different neurochemical and neuromodulatory changes affecting cortical excitability. Further investigation of these distinct effects may clarify the underlying MOA of VNS, which may ultimately improve responder rates in patients, as currently, VNS treatment is unsuccessful in about one third of the treated patients (2).

In 1989, Voskuyl et al. described an animal model that allows to investigate cortical excitability (3). In this cortical stimulation model, the threshold for evoking convulsions is determined by electrical stimulation of the motor cortex in unanaesthetized rats. It is a reliable acute seizure model that allows to repeatedly test changes in seizure threshold and it has previously been used to evaluate anticonvulsant drug activity (3–7). We hypothesized that modulation of cortical excitability could be the MOA through which VNS exerts its acute anti-seizure effect.

Material and methods

Animals

Eight male Wistar rats (Harlan, the Netherlands) weighing 250–300 g, were treated according to guidelines approved by the European Ethics Committee (decree 86/609/EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University Hospital (ECP 05/17). All animals were kept under environmentally controlled conditions (12 h light/dark cycles, 20–23°C and 50% relative humidity) with food and water intake *ad libitum*.

Surgical procedures

The animals were implanted with six epidural registration/stimulation electrodes and a custom made silicone spiral cuff-electrode with platinum contacts around the left vagus nerve. All rats were anaesthetized with a ketamine/xylazine (80 and 7.5 mg/kg respectively, i.p.) mixture. For implantation of the vagus nerve cuff-electrode an incision was made over the left anterior cervical region. The cuff-electrode was wound around the left vagus nerve and the ends were tunnelled under the skin over the back of the neck towards the head, where they were fixated in a head cap using acrylic cement. For stimulation of the motor cortex, two epidural stainless steel screw electrodes were positioned over the motor area of the left and right frontal cortex (3.0 mm left and right of the midline, 1.7 mm anterior to bregma). Four epidural stainless steel screw electrodes were implanted bilaterally on the parietal cortex; three of them were used for electroencephalogram (EEG) recording, the fourth was used as the reference electrode. The leads of the epidural electrodes were fixed together with the leads of the vagus nerve cuff-electrode in the head cap on the skull of the rat using acrylic cement. The animals were allowed to recover from surgery for 2 weeks before the experiments were performed.

Cortical stimulation, vagus nerve stimulation and video-EEG monitoring

The experiments were performed between 9.00 am and 01.00 pm and repeated several times for each animal on different days. In total, two to three sessions were performed for each animal, with at least 3 days in between sessions. During the experiments, the animals were connected through a multifunctional commutator: (i) to allow seizure observation and EEG recording using a digital video-EEG monitoring system, (ii) to perform cortical stimulation using a custom made external current stimulator and (iii) to perform VNS using an external current stimulator (NCP, model 100; Cyberonics Inc., Houston, TX, USA). Rats were freely moving in their cages.

At the beginning of each experiment, the impedance of the electrode-to-vagus nerve interface was measured.

One experimental session started with a baseline cortical stimulation session (baseline condition). Immediately following 1 h of VNS (0.75 mA, 30 Hz, 250 μs, 30 s on/1.8 min off), a second cortical stimulation session was performed (VNS-condition). Stimulation of the motor cortex was performed using a ramp-shape pulse train of biphasic rectangular pulses (600 μs, 50 Hz, 0–2000 μA). The maximal duration of the cortical stimulation train was 15 s. The stimulation was interrupted when a focal seizure was noticed by visual inspection. During one cortical stimulation session, at least three consecutive stimulation trains were given with a minimum interval of 3 min.

To determine the motor seizure threshold (MST), a *post hoc* analysis of the video images was performed by an experienced observer, without prior knowledge of the treatment (baseline or VNS condition). The MST is the current intensity corresponding to the first clinical symptoms of a focal seizure. A focal seizure is characterized by forelimb clonus, a tonic backward movement of the body or an axial myoclonic seizure. In the recorded video image a timer with a precision of 1/100 s was used to determine the exact beginning of the focal seizure. This time moment was then converted to the stimulation intensity (μA) of the ramp-shape pulse train at that exact moment. The MST during the baseline and VNS condition in each rat were calculated as the mean of at least three consecutive stimulation trains.

As an additional control to ensure that MST values did not increase over time within one experimental session, several sessions of SHAM stimulation were performed in rats, that showed the greatest increase in MST during the VNS condition. A SHAM stimulation session comprised a baseline MST determination followed by a second MST determination after 1 h without VNS.

Statistical analysis

Differences in MST were calculated parametrically using the paired Student's *t*-test ($P < 0.05$). Delta values were defined as the difference in MST during the baseline and VNS condition.

Results

In total, 21 experimental sessions were performed in eight rats (two or three sessions per rat). The impedance of the electrode-to-vagus nerve interface showed normal values in all rats during all experiments. The mean MST per rat during the baseline and VNS condition per session are shown in Table 1.

For each rat, one mean baseline MST and one mean VNS MST was calculated based on the different sessions (Fig. 1). Of the eight rats tested, seven showed an increase in MST with a delta value ranging between 258 and 784 μA. One rat did not respond to the treatment.

During the VNS condition, the mean overall MST was significantly increased (1420 μA, SEM 94 μA) compared with the mean overall MST during the baseline condition (1072 μA, SEM 54 μA) ($P < 0.01$) (Fig. 2).

Also, during each individual cortical stimulation session a statistically significant increase in mean MST was found ($P < 0.05$, paired t-test) when baseline and VNS condition were compared. No significant differences were found between cortical stimulation sessions within the conditions. An overview of the data per session is shown in Table 2.

Table 1 Motor seizure threshold (MST) values during baseline and vagus nerve stimulation (VNS) conditions per rat per session

	Session	Mean MST baseline condition (SEM)	Mean MST VNS condition (SEM)
Rat 1	1	983 (189)	1524 (145)
	2	1461 (147)	1788 (59)
	3	1755 (51)	1829 (0)
Rat 2	1	1093 (91)	1687 (104)
	2	1227 (16)	1217 (27)
Rat 3	1	989 (132)	1999 (0)
	2	1001 (139)	1559 (124)
Rat 4	1	937 (32)	1413 (356)
	2	1413 (93)	1364 (97)
	3	968 (43)	1824 (121)
Rat 5	1	673 (25)	1037 (43)
	2	1084 (28)	1271 (28)
	3	1089 (63)	1597 (135)
Rat 6	1	908 (4)	957 (79)
	2	1323 (57)	1563 (57)
	3	876 (52)	1633 (96)
Rat 7	1	916 (55)	869 (44)
	2	983 (133)	1040 (53)
Rat 8	1	1112 (177)	1319 (92)
	2	824 (51)	1156 (31)
	3	1015 (15)	1252 (61)

Mean MST values during baseline and VNS conditions per rat are shown for each session. The baseline condition and VNS condition MST values were calculated as the mean of at least three consecutive stimulation trains. All values are presented in μA. SEM: standard error of the mean.

In total, five sessions of SHAM stimulation were performed in two rats (rat 3 and rat 4). The mean MST before SHAM stimulation (684 μA) showed no difference from the mean MST after SHAM stimulation (694 μA) ($P > 0.05$, paired Student's t-test).

EEG recording was used to confirm the focality of the induced epileptic seizures. During a focal seizure, no epileptiform discharges were observed

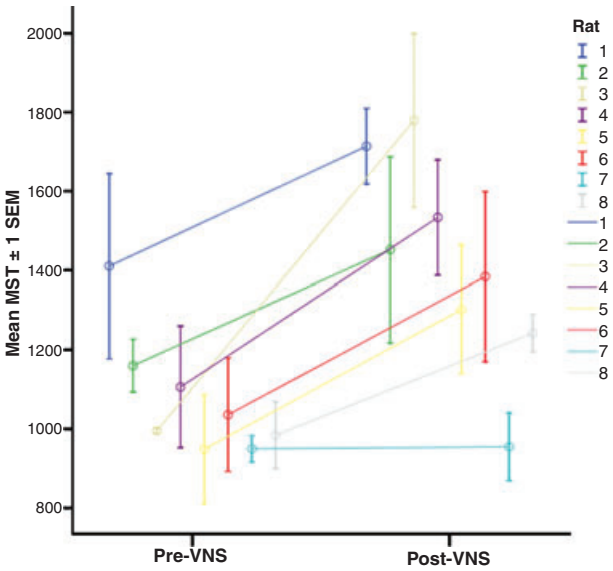

Figure 1. Motor seizure threshold (MST) values during baseline and vagus nerve stimulation (VNS) condition per rat. Mean MST values ± 1 SEM during baseline and VNS condition per rat are shown. Values were calculated based on the different experimental sessions. Rat 2, 3 and 7 underwent two experimental sessions, the other five rats underwent three experimental sessions.

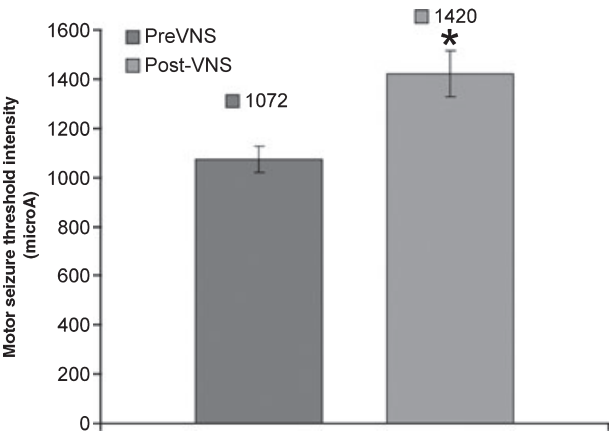

Figure 2. Overall motor seizure threshold (MST) value during baseline and vagus nerve stimulation (VNS) condition. The mean MST value for all rats during baseline ($n = 8$) and VNS ($n = 8$) condition is shown. *Statistically significant difference compared to the mean baseline MST value (paired Student's t-test; $P < 0.05$).

Table 2 Motor seizure threshold (MST) values during baseline and vagus nerve stimulation (VNS) conditions over consecutive experimental sessions

	Mean MST baseline condition (SEM)	Mean MST VNS condition (SEM)
Session 1 ($n = 8$)	951 (48)	1351 (137)*
Session 2 ($n = 8$)	1165 (80)	1370 (88)*
Session 3 ($n = 5$)	1141 (124)	1627 (83)*

Mean MST values during baseline and VNS conditions are shown for the number of rats tested in each session. *All sessions showed statistical significant increase in mean MST ($P < 0.05$) based on Student's t-test. All values are presented in μA. SEM: standard error of the mean.

in the parietal locations posterior from the location of cortical stimulation, pointing to isolated epileptiform activity in the motor cortex, in agreement with the results of Krupp and Loscher (7). When stimulation was interrupted, the clinical seizure symptoms immediately stopped in all rats and no afterdischarges were identified on the EEG, confirming localized seizure activity restricted to a pure motor seizure.

Discussion

The data obtained in the present study demonstrate that VNS increases the threshold for focal motor seizures in the cortical stimulation rat model. It supports the hypothesis that VNS is able to modulate cortical excitability. These findings are in agreement with the reported acute anti-seizure effect of VNS, that has been demonstrated in animal experiments and observed in clinical practice e.g. in patients who use the magnet feature of the device (8–12).

Our findings are in agreement with several other studies that have indicated a direct or indirect effect on cortical excitability of VNS. EEG studies in humans have shown an acute decrease in interictal epileptiform discharges after VNS, indicating a change in cortical neuronal activity (13, 14). Naritoku et al. found an increased latency in thalamocortical somatosensory evoked potentials after 1 month of VNS, suggesting modulation of the thalamocortical pathway by VNS (15). The nucleus of the solitary tract is the main terminal for vagal afferents in the brainstem and has direct and indirect projections to the locus coeruleus, the raphe nuclei, the reticular formation, the thalamus and ultimately the cortical neurons (1, 16). The anti-seizure effects of VNS could therefore be mediated through modulation of synaptic activity in the thalamus and the thalamocortical projection pathways. Several studies have shown VNS-induced changes in brain activation and cerebral blood flow in particular in the thalamus (17–20). Moreover, a positive correlation between thalamic activation and a favourable clinical outcome has previously been reported (17).

Based on *in vivo* intracellular cortical recordings, Zagon et al. proposed that slow hyperpolarization may be one of the mechanisms underlying the seizure-reducing effect of VNS, by means of reducing the excitability of neurons involved in seizure propagation (21). The acute effect of VNS on cortical excitability has also been investigated using transcranial magnetic stimulation (TMS). VNS was responsible for a pronounced increase in the inhibitory response produced by paired-pulse TMS but did not affect the excitatory response by single-pulse TMS. This observation specifically points to a $GABA_A$ intracortical inhibitory mechanism explaining the modulatory effect of VNS on cortical excitability (22). The latter is in agreement with other studies demonstrating that GABA plays a major role in the MOA of VNS. Cerebrospinal fluid studies have shown an increase in GABA levels during VNS (23). A SPECT study in humans before and after 1 year of VNS treatment resulted into normalization of $GABA_A$ receptor density in patients with a clear therapeutic response (24). More recently, Neese et al. found that VNS following experimental brain injury in rats protects cortical GABAergic cells from cell death (25). Apart from the GABA hypotheses, there is experimental evidence indicating that also noradrenalin plays a role in the anti-seizure effect of VNS. Already in 1998, Krahl et al. demonstrated that lesioning the locus coeruleus, i.e. the major source of noradrenalin in the brain, abolished the seizure-attenuating effect of VNS (26). A few years later, Groves et al. found a significant increase in the discharge rate of locus coeruleus neurons following short term VNS in the anaesthetized rat (27). Other experimental work confirmed this potential involvement of noradrenalin in the mechanism of action of VNS (28, 29). A pilot trial in our laboratory has shown responder-correlated increases in noradrenalin in the pilocarpine model using microdialysis (30).

This study shows efficacy of VNS in the cortical stimulation rat model using standard VNS stimulation parameters. These standard stimulation parameters are currently used in clinical practice, but are not evidence-based (2, 31, 32). This animal model may be a useful tool for future evaluation of optimized stimulation parameters as it may also be combined with microdialysis in freely moving animals. These studies may lead to improved clinical efficacy in patients treated with VNS.

In the present study, one rat did not show a significant increase in MST after VNS. We could

not identify any external factor causing the non-responsiveness of this rat. However, a minor defect of the VNS electrode can not be excluded, even with normal impedance values for the electrode-to-vagus nerve interface. On the other hand, it is plausible that this finding reflects the non-responders also observed in clinical practice.

In this study VNS significantly increased the threshold for focal motor seizures in a cortical stimulation model. The obtained data indicate that VNS is capable of modulating electrically induced cortical excitability. Further research is needed to elucidate the precise mechanism of action of these VNS effects.

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