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Research Report

Acute seizure-suppressing effect of vagus nerve stimulation in the amygdala kindled rat

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ABSTRACT

Purpose: Vagus nerve stimulation (VNS) is a moderately effective anti-epileptic treatment. Clinically relevant animal models that are suitable to study the mechanism of action of VNS are not available. The aim of the current study was to develop a clinically relevant animal model for VNS-treated epilepsy that can be used to study the mechanism of action of VNS. *Methods*: The anticonvulsive effect of VNS was studied in fully kindled rats by measuring behavioral and electrophysiological parameters. Afferent vagus nerve activation was confirmed by quantifying nNOS immunoreactive cells in the nucleus of the solitary tract (NTS). *Results*: VNS rats had more nNOS immunoreactive cells/mm^2 in the NTS than shams. VNS induced a >25% decrease in stage 5 duration (S5D) in 32% of rats. Prior to VNS this type of responders suffered from seizures with a longer total seizure duration (TSD) than non-responders. In 21% of rats VNS resulted in a >25% decrease in TSD. This type of responders had a shorter TSD prior to VNS than non-responders. In 29% of rats VNS resulted in >200% increase in stage 5 latency (S5L). This type of responders had higher kindling rates than non-responders. *Conclusion*: The VNS-treated kindled rat is a clinically relevant animal model because it is a chronic epilepsy model that responds to VNS with effects that are comparable to the effects of VNS in epilepsy patients. In addition, this study demonstrates that VNS-treated kindled rats can be used to study the mode of action of VNS using immunohistochemical techniques.

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Abbreviations: ADT, afterdischarge threshold; AK, amygdala kindling; EEG, electroencephalography; ICC, intraclass correlation coefficient; ir, immunoreactivity; nNOS, neuronal nitric oxide synthetase; NO, nitric oxide; NTS, nucleus of the solitary tract; ΔADT, difference between pre-kindling afterdischarge threshold and post-kindling afterdischarge threshold; pre-KADT, pre-kindling afterdischarge threshold; post-KADT, post-kindling afterdischarge threshold; S5D, stage five duration; S5L, stage five latency; sVNS, sham vagus nerve stimulation; TSD, total seizure duration; VNS, vagus nerve stimulation

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1. Introduction

Vagus nerve stimulation (VNS) is a type of neuromodulation therapy that has been used to treat refractory epilepsy patients since 1988 (Penry and Dean, 1990). Average seizure frequency reduction is 12.8%, while 11.7% of patients have >50% seizure frequency reduction (The Vagus Nerve Stimulation Study Group, 1995; Handforth et al., 1998; Landy et al., 1993). Besides this chronic effect on seizure frequency reduction observed after 3–6 months of VNS treatment (Shahwan et al., 2009; Vonck et al., 1999), VNS has an acute seizure-suppressing effect as well. Acute activation of the pulse generator can abort 20% of seizures in patients who experience auras or simple partial seizures, while placebo-activation aborts approximately 10% (Ben-Menachem et al., 1994; Boon et al., 2001; Morris, 2003).

Even though VNS has been applied in a number of animal models (McLachlan, 1993; Naritoku et al., 1995; Sunderam et al., 2001; Woodbury and Woodbury, 1990; Zabara, 1992; Zagon and Kemeny, 2000; Zanchetti et al., 1952; Zhang et al., 2008a,b; Dedeurwaerdere et al., 2006; Fernandez-Guardiola et al., 1999; Krahl et al., 1998; Krahl et al., 2001; Krahl et al., 2003; Lockard et al., 1990; McLachlan, 1993; Munana et al., 2002; Naritoku et al., 1995; Sunderam et al., 2001; Takaya et al., 1996; Woodbury and Woodbury, 1990; Zabara, 1992; Zagon and Kemeny, 2000; Zanchetti et al., 1952; Zhang et al., 2008a,b; Dedeurwaerdere et al., 2004), including three studies in freely moving, awake animals suffering from experimental epilepsy (Dedeurwaerdere et al., 2006; Fernandez-Guardiola et al., 1999; Lockard et al., 1990), a study using a clinically relevant animal model and experimental design has not been conducted so far.

The aims of the current study were to develop a clinically relevant animal model for VNS-treated epilepsy that can be used to study the mechanism of action of VNS, and to characterize rats that responded to VNS.

Therefore we applied VNS in fully amygdala kindled (AK) rats, and evaluated its acute seizure-suppressing effect by measuring behavioral and electrophysiological parameters. The AK rat has been developed as an epilepsy model several years ago and has been used to study the effectiveness of several anti-epileptic drugs (Racine, 1972; Loscher et al., 2000). After establishing the effectiveness of VNS, responders were further analyzed regarding epilepsy-related parameters before VNS. To confirm afferent vagus nerve activation, we quantified neuronal NO synthetase (nNOS) immunoreactivity (ir) in the nucleus of the solitary tract (NTS), the primary projection site of the vagal afferents.

2. Results

Forty-eight rats were operated on. Seventeen rats did not complete the experiments because of anesthesia-related death ($n=2$), severe post-operative dyspnea due to glottisedema ($n=2$), loss of AK electrode ($n=9$), defective AK electrode ($n=2$), or not reaching the fully kindled state ($n=2$). We observed left sided Horner's syndrome immediately after surgical placement of the vagus nerve electrode in 20 of 33 rats (Aalbers et al., 2009). Thirty-one (12 AK, 8 VNS and 11 sVNS) completed the experiments and were used for further analysis.

In all VNS rats, the VNS electrode was located around the vagus nerve and carotid artery during post-mortem examination, while in 3 sVNS rats, the electrode was found detached from artery and nerve but still in close proximity to these structures.

Kindling rates did not significantly differ between VNS rats (19±4 stimuli), sVNS rats (16±4) and AK rats (16±5 stimuli).

2.1. *VNS effects*

2.1.1. *Seizure severity*

The amount of measurement error for the two observers concerning seizure severity was low (κ_ω 0.96). Seizure severity during VNS treatment (stage 5) did not differ from the average seizure severity of the 5 seizure preceding VNS (stage 5).

2.1.2. *Stage 5 duration*

Determination of S5D by two observers resulted in a low amount of measurement error (ICC 0.918). We therefore averaged the S5Ds of the two observations and used this number for analysis. The S5D of the five seizures preceding VNS or sham treatment did not significantly differ between VNS (25±6), sVNS (25±6) and AK rats (23±9 s). Animals with >25% reduced S5D upon VNS treatment were considered responders (S5D-responder), and represented 50% of VNS, 18% of sVNS and 9% of AK rats (Fig. 1A). We attributed the S5D reductions in sVNS and AK rats as normal fluctuations, and the corrected responder rate in the VNS group was therefore 32%. Further analysis of the S5D-responders in the VNS group showed that their average TSD prior to VNS was significantly longer than the TSD of non-responders (Table 1).

2.1.3. *Seizure duration*

The mean TSD prior to VNS or sham treatment did not significantly differ between VNS rats (184±80 s), sVNS rats (246±66 s) and AK rats (181±76 s). Animals with a >25% reduced TSD upon VNS treatment were considered responders (TSD-responder), and represented 57% of VNS, 36% of sVNS, and 9% of AK rats Fig. 1B). The TSD reductions in sVNS rats and AK rats were considered normal fluctuations in TSD. Therefore the corrected responder rate based on TSD reduction is 21%. Further analysis of the TSD-responders in the VNS group showed that their average TSD prior to VNS was significantly shorter than that of non-responders (Table 2).

2.1.4. *Stage 5 latency*

The mean S5L of the five seizures preceding VNS or sham treatment did not significantly differ between VNS (6±6 s), sVNS (2±2 s) and AK rats (4±3 s). After the last kindling stimulus, those rats that were treated with VNS had a longer S5L (9±8 s) than sVNS (1±1 s) and AK rats (3±3 s). There was a trend towards statistical significance between VNS-treated rats and sVNS-treated rats ($p=0.056$).

Animals in which S5L increased with >200% upon VNS treatment, were considered responders (S5L-responder), and represented 38% of VNS, none of sVNS and 8% of AK rats (Fig. 1C). The corrected responder rate based on S5L increase was therefore 30%. Further analysis of S5L-responders in the VNS group showed that their kindling rate was significantly slower than that of non-responders (Table 3).