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Vagus Nerve Stimulation in Refractory Epilepsy: Effects on Pro- and Anti-Inflammatory Cytokines in Peripheral Blood

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Key Words
Inflammation · Anticholinergic reflex · Tryptophan · Serotonin · Kynurenin · Neurostimulation

Abstract
Objective: The vagus nerve has important immunological functions that may be relevant for its anticonvulsive action. We postulate that this anticonvulsive action is activated by a shift in the immune system resulting in a reduction of neurotoxic and an increase of neuroprotective tryptophan metabolites. ***Methods:*** Eleven patients with refractory epilepsy and 11 controls matched for age and gender were included in this study. The primary outcome measure was a 50% seizure reduction. Other variables were pro-inflammatory cytokines IL-6 and TNF-α, anti-inflammatory cytokine IL-10, cortisol, and the tryptophan metabolites 3-hydroxykynurenine (3-OH-KYN), kynurenic acid (KYNA), kynurenine, serotonin (5-HT) and 5-hydroxyindol acetic acid (5-HIAA). Blood samples were scheduled during baseline, and in week 28 of add-on treatment. ***Results:*** IL-6 levels were higher in the responders than in the control group, and decreased after vagus nerve stimulation (VNS), whereas IL-10 was low and increased after VNS. In nonresponders, VNS resulted in an increase of IL-6 plasma levels and in a decrease of IL-10. Cortisol concentrations are higher in the epilepsy group than in the control group. After VNS, these concentrations decreased. The concentrations of the tryptophan metabolites were lower in the epilepsy group than in the control group. The KYNA ratios are defined as the ratio of neuroprotective KYNA versus neurotoxic 3-OH-KYN and KYNA versus neurotoxic kynurenine: these ratios were lower in epilepsy patients than in controls, and they both moderately increased after VNS. ***Conclusion:*** The outcome of this preliminary study indicates that VNS causes a rebalancing of the immune system. This results in: (1) a reduction of neurotoxic and an increase of neuroprotective kynurenine metabolites and (2) in the normalization of cortisol levels.

Introduction

Vagus nerve stimulation (VNS) is an alternative treatment for intractable epilepsy. The mode of action is assumed to be the result of modulation of deep brain structures by neuroactive substances. The severity of epileptic seizures is causally related to central inflammatory and neurotransmitter functions [1–5].

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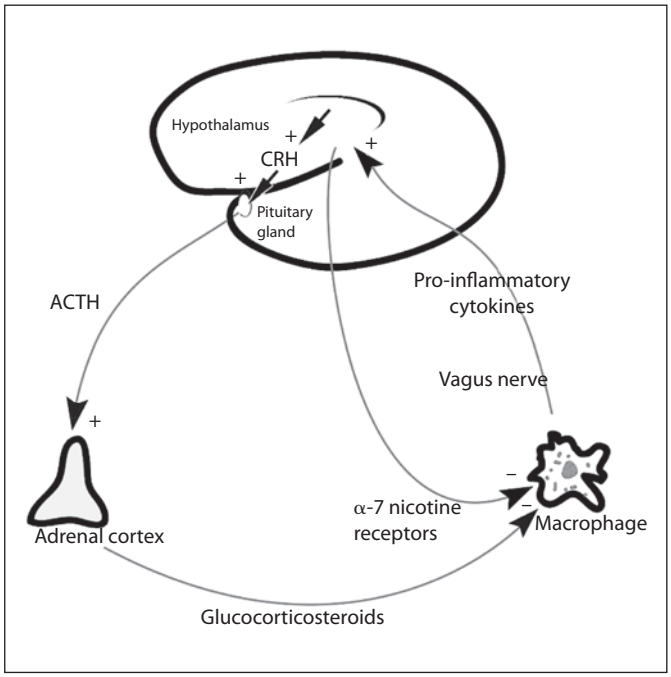

Fig. 1. The anticholinergic reflex loop.

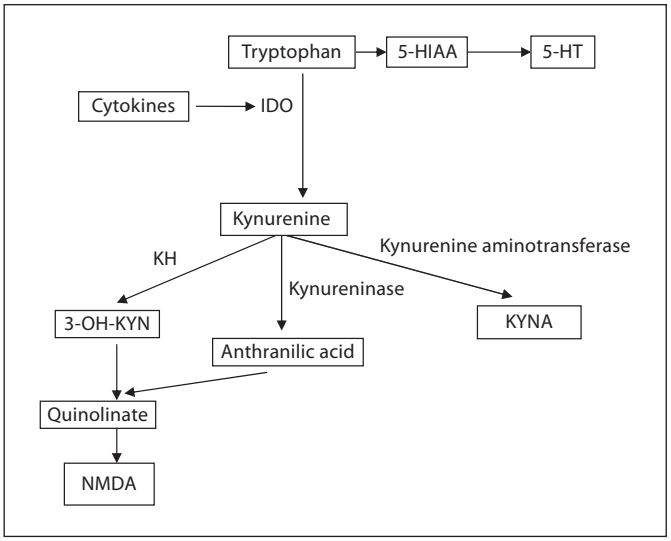

Fig. 2. 5-HT = Serotonin; 3-OH-KYN = 3-hydroxykynurenine; KYNA = kynurenic acid; IDO = indoleamine (2,3)-dioxygenase; KH = kynurenine hydroxylase.

Tryptophan metabolites are involved in the stimulation of glutaminergic N-methyl-D-aspartate (NMDA) receptors. Overstimulation of NMDA receptors has been implicated in the generation of seizures [6, 7]. The vagus nerve has a major impact in attenuating inflammatory responses via the so-called anticholinergic reflex loop [8] (fig. 1). The pro-inflammatory cytokines influence the serotonin (5-HT) and kynurenine metabolism [7–9]. VNS also results in changes of tryptophan metabolite concentrations in the brain [10, 11] (fig. 2). However, the exact mechanism behind seizure frequency reduction and improvement of well-being in VNS is still unknown.

We postulate that the beneficial effect of VNS is activated by a shift in the immune system, resulting in a reduction of neurotoxic and an increase of neuroprotective tryptophan metabolites.

In the presented pilot study we aimed at: (1) evaluating the effectiveness of VNS, (2) evaluating the effects of VNS on the immune system and changes in the tryptophan metabolic pathway, and (3) linking the therapeutic effect of VNS to changes in the tryptophan metabolic pathway.

Subjects and Methods

We studied 11 patients with intractable epilepsy and 11 controls matched for age and gender. Patients and controls with known immune compromising diseases, infectious diseases or pregnancy were excluded. No changes in medication were allowed during the study. The study was approved by the medical ethics committee.

Seizure frequency per month at 6 months of VNS was compared with seizure frequency per month at baseline. The primary endpoint was a seizure frequency reduction of 50% or more. The main variables were: demographics, epilepsy-specific data, and biochemical and neuro-immunological variables in peripheral blood. Blood sampling was scheduled during baseline and in week 28 of add-on treatment. The time interval between the last seizure and blood sampling was 24 h or more.

IL-6 and TNF-α (pro-inflammatory cytokines), IL-10 (anti-inflammatory cytokine), 5-HT, kynurenine, kynurenic acid (KYNA), 3-hydroxykynurenine (3-OH-KYN), and 5-hydroxyindol acetic acid (5-HIAA) were assessed. The 'neuroprotective ratio' was determined to evaluate whether the effect of VNS is neuroprotective or neurotoxic. As we were unable to determine the concentration of quinolinic acid (neurotoxin), the KYNA/kynurenine and KYNA/3-OH-KYN ratios were determined to assess the balance between the neuroprotective and neurotoxic arm of the kynurenine metabolism. EDTA blood was collected and centrifuged. It was stored at –70°C until analysis was performed. For determination, 7 mm of plasma were used.

The cytokine assessments were performed with an ELISA technique (R&D Quantikine HS). In some samples, the cytokine concentrations were below the detection level. The level of detection of IL-6 was 0.4 pg/ml, 1 pg/ml for TNF-α and 1.5 pg/ml for IL-10. While results below the level of detection cannot be regard-

Table 1. Peripheral blood samples

	Control	n	Baseline	n	After	n	p^1	p^2
IL-6, pg/ml	0.68 (0.2–1.57)	8	0.50 (0.2–1.45)	7	0.61 (0.2–1.42)	6	0.75	0.37
TNF-α, pg/ml	0.5 (0.5–1.89)	4	1.03 (0.5–2.39)	6	0.5 (0.5–2.56)	2	0.65	0.74
IL-10, pg/ml	1.68 (0.75–34.12)	7	2.45 (0.75–21.59)	8	2.13 (0.75–15.18)	10	0.9	0.15
Tryptophan, μg/ml	11.3 (8–14.6)	11	10.8 (4.6–15.3)	11	9.8 (4.2–352.8)	11	0.52	0.66
5-HIAA, ng/ml	7.8 (2.7–41.5)	11	3.6 (2.6–9.5)	11	6.0 (2.3–7)	11	0.08	0.29
KYNA, ng/ml	6.6 (5.1–14.9)	11	4.8 (3.7–17.6)	9	6.4 (3.3–10)	8	0.24	0.75
3-OH-KYN, ng/ml	9.2 (7.5–11.2)	11	9.1 (6.3–12)	11	9.8 (8.2–12.4)	11	0.9	0.92
Kynurenine, ng/ml	503.5 (402.9–873.4)	11	399.5 (277–742.7)	11	317.4 (14.1–593)	11	0.07	0.18
KYNA/3-OH-KYN	0.72 (0.52–1.99)	11	0.64 (0.36–1.71)	9	0.65 (0.5–2.67)	8	0.4	0.92
KYNA•1,000/kynurenine	13 (7.1–37)	11	12.8 (5.79–47.86)	9	16.1 (11.92–156.13)	8	0.9	0.46

Values are medians (min.–max.). n = Number of samples.
[1] Mann-Whitney test (control – baseline). [2] Wilcoxon (baseline – after VNS).

ed as missing, they cannot be regarded as true values. We therefore assumed IL-6 values <0.4 pg/ml to be 0.2 pg/ml, TNF-α <1 pg/ml to be 0.5 pg/ml and IL-10 <1.5 pg/ml to be 0.75 pg/ml.

Tryptophan, 5-HT and kynurenine metabolites were assessed by use of HPLC.

Non-parametric statistics were used to analyze the data. The Mann-Whitney test was used to compare the controls with the baseline group. The Wilcoxon test was used to compare the results at baseline with the results after 28 weeks of treatment.

Results

The control group consisted of 6 males and 5 females with ages ranging from 10–64 years (median age: 28). The epilepsy group also consisted of 6 males and 5 females. The ages in the epilepsy group ranged from 12–58 years (median age: 30).

Six patients had cryptogenic localization-related epilepsy, four had symptomatic epilepsy and one had generalized epilepsy. The median duration of the epilepsy was 19 years. The patients had used 5–15 drugs before. One patient had been operated on and one had been on a ketogenic diet.

The reduction of the mean seizure frequency was 21%. The responder rate was 36%.

VNS resulted in an increase of the IL-6 (pro-inflammatory) plasma levels and in a decrease in IL-10 (anti-inflammatory) plasma levels, thus converging to the values of the control group. TNF-α concentrations were above the detection level in only 33% of the samples, which makes it difficult to interpret the results. For IL-10 and IL-6, 76 and 64% of the measured concentrations were above the detection level.

Most of the responders had detectable cytokine levels. In this subgroup of responders, we measured a decrease in the pro-inflammatory cytokine IL-6 in all responders. An increase of anti-inflammatory cytokine IL-10 after VNS was seen in two out of four responders. The responders who already showed high levels of IL-10 (n = 2) did not show a further increase after VNS. None of the differences reached a level of statistical significance.

Epilepsy patients had a higher cortisol concentration than controls (346 vs. 326 nmol/l). After VNS, the levels decreased (286 nmol/l).

In the subgroup of responders, three out of four patients showed a decrease of cortisol concentrations after VNS. The one patient whose cortisol level did not change after VNS already had a low cortisol level at baseline.

Most tryptophan metabolites were lower in epilepsy patients than in healthy individuals. After treatment, kynurenine and tryptophan decreased. We noticed an increase in the serotonin metabolite 5-HIAA and kynurenine metabolites KYNA and 3-OH-KYN. None of the differences reached a level of statistical significance (table 1). Only the KYNA assessment had missing values (2 samples at baseline and 3 samples after VNS).

Epilepsy patients showed lower KYNA ratios than controls. These ratios moderately increased after VNS (table 1). Unfortunately, three of five missing values occurred in the subgroup of responders (n = 4), leaving us with only one responder in whom we were able to assess the baseline and post-VNS KYNA/3-OH-KYN ratio. In

this patient, the neuroprotective ratio was increased after VNS because the increase in KYNA was larger than the increase in 3-OH-KYN.

Discussion

A responder rate of 36% is in line with the results known from observational and controlled studies on VNS in epilepsy [12, 13].

Based on the literature, one would expect a pro-inflammatory status in patients with epilepsy [3, 14, 15]. Our preliminary data did not confirm the previous findings. Again, based on the literature one would expect VNS to provoke an anti-inflammatory reaction [3, 4, 16–19]. Our preliminary data did not confirm this observation either. An increased pro-inflammatory reaction after VNS was also described by Corcoran et al. [20], who studied cytokine reactions in 10 patients with therapy-resistant depression. Nevertheless, marked increases in pro-inflammatory cytokines are presumably non-therapeutic. Our findings are in line with our expectations only if we analyze the responders as a subgroup: most responders showed a pro-inflammatory status before VNS treatment and an anti-inflammatory reaction after VNS. Those responders who already had high anti-inflammatory IL-10 levels at baseline did not show a further increase after VNS.

The decrease of 5-HIAA concentration in intractable epilepsy patients that we observed can be explained by the following hypothesis, which is also explained in figure 2. According to this neurodegeneration hypothesis, increased levels of pro-inflammatory cytokines influence the tryptophan metabolism, resulting in decreased metabolism of tryptophan to 5-HT and increased metabolism of tryptophan to kynurenine. This subsequently leads to an imbalance between the neuroprotective NMDA receptor antagonist KYNA and the neurotoxic 3-OH-KYN [7–9]. The reduced KYNA/kynurenine and KYNA/3-OH-KYN ratios in epilepsy patients are in line with this neurodegeneration hypothesis. These results are indicative of an imbalance between neuroprotective and neurotoxic metabolites in epilepsy patients, and with previous reports showing significantly lower levels of neuroprotective KYNA in children with intractable epilepsy than in healthy controls [21–23].

Hypothalamic pituitary adrenal axis dysfunction or a state of hypercortisolemia can be correlated with cognitive deficits specific to medial temporal lobe memory, depression and epilepsy [24, 25]. The high cortisol levels at baseline and the decrease after VNS are in line with these studies and with our anti-inflammatory concept.

The purpose of this preliminary study was to determine which parameters were of value in assessing the anti-inflammatory action of VNS as a possible explanation of its therapeutic action. The interpretation of our results is only tentative due to the small number of patients, the rapid changes in cytokine concentrations, and the involvement of several central and peripheral networks. However, it appears that epilepsy patients who respond to VNS show a trend towards rebalancing the immune system as well as the 5-HT and kynurenine metabolism.

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