Journal of Neuroimmunology 212 (2009) 102–105

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Increased rat serum corticosterone suggests immunomodulation by stimulation of the vagal nerve

V. De Herdt [a,*], L. Puimege [b], J. De Waele [c], R. Raedt [a], T. Wyckhuys [a], R. El Tahry [a], C. Libert [b], W. Wadman [a], P. Boon [a], K. Vonck [a]

[a] Laboratory for Clinical and Experimental Neurophysiology, Reference Center for Refractory Epilepsy, Department of Neurology 1K12IA, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium
[b] Department for Molecular Biomedical Research, VIB, Technologiepark 927, 9052 Ghent, Belgium
[c] Intensive Care Unit, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 11 January 2009
Received in revised form 21 April 2009
Accepted 23 April 2009

Keywords:
Vagal nerve
Vagal nerve stimulation
HPA-axis
Serum corticosterone
Immunomodulation

ABSTRACT

The role of the vagal nerve within the immune system has not been fully elucidated. Vagal afferents connect to several central nervous system structures, including the hypothalamus. We investigated the effect of vagal nerve stimulation (VNS) on serum corticosterone levels in rats. Corticosterone levels were measured following 1 h of high frequency (30 Hz) or low frequency (1 Hz) VNS in awake animals. There was a significant increase ($p<0.05$) in serum corticosterone levels following 30 Hz VNS compared to 1 Hz VNS or sham stimulation. These results suggest an immediate effect of VNS on the hypothalamic pituitary-adrenal (HPA) axis and support the role of the vagal nerve in immunomodulation.

1. Introduction

The vagal nerve is the tenth cranial nerve originating in the brain stem. Vagal nerve efferents are mainly parasympathetic motor fibers that innervate the visceral organs. Vagal afferents carry gustatory, visceral sensory and other peripheral information toward the central nervous system (CNS). Vagal afferents primarily project to the nucleus of the solitary tract (NTS) that is located in the brain stem. Ascending fibers from the NTS reach the hypothalamic nuclei, the amygdala, and the locus coeruleus, among others (Berthoud and Neuhuber, 2000).

Recent insights into the immune system have identified the vagal nerve as a neural pathway that monitors and adjusts the inflammatory response (Tracey, 2002). Pro-inflammatory cytokines activate sensory vagal afferents that signal the brain in case of peripheral inflammation (Watkins et al., 1995). Within the CNS, different pathways are activated to modulate a centrally mediated anti-inflammatory immune response (Tracey, 2002). One of these pathways presumably includes the hypothalamic–pituitary–adrenal (HPA) axis (Pavlov et al., 2003).

This study aims to investigate whether direct electrical stimulation of vagal afferents activates the HPA-axis, leading to an increase in serum corticosterone. Serum corticosterone levels were measured following VNS in awake and freely moving rats. High frequency (30 Hz) stimulation was compared to sham stimulation and low frequency (1 Hz) stimulation. High frequency VNS has proven to be efficient in activating brain structures in patients (Vonck et al., 2008). Low frequency VNS was used as an additional control, as human studies as well as animal experiments have shown that low frequency stimulation is not effective compared to high frequency stimulation (Osharina et al., 2006; Woodbury and Woodbury, 1990; Handforth et al., 1998).

2. Methods

2.1. Animals

Sixteen male Wistar rats (Harlan, The Netherlands) weighing 250–300 g, were treated according to the 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*.

2.2. Surgical procedure

The animals were implanted with a custom-made silicone spiral cuff-electrode with platinum contacts around the left vagal nerve. All

* Corresponding author. Tel.: +32 9 332 64 81; fax: +32 9 332 45 47.
E-mail address: Veerle.Deherdt@UGent.be (V. De Herdt).

0165-5728/$ – see front matter
doi:10.1016/j.jneuroim.2009.04.013

rats were anesthetized with a ketamine/xylazine (80 mg/kg and 7.5 mg/kg respectively, i.p.) mixture. To implant the stimulation cuff-electrode, an incision was made over the left anterior cervical region. The cuff-electrode was wound around the left vagal nerve and was positioned in a way to allow preferential afferent stimulation, with the anode located caudally. The electrode ends were tunnelled under the skin over the back of the neck towards the head, where they were fixed in a head cap using acrylic cement. The animals were allowed to recover from surgery for 2 weeks before the experiments were performed.

2.3. Blood sampling and corticosterone measurement

Blood sampling was performed in awake animals by means of an incision in the lateral tail vein. The time between the first handling of the rat and the sample collection was kept as short as possible and a blood sample was always obtained within a few minutes. The blood sample was centrifuged at room temperature and serum aliquots were frozen until further analysis. Serum corticosterone was measured using a radio-immuno assay (Diagnostic Products Corporation, Los Angeles, CA, USA).

2.4. Experimental set-up

The experiments were performed between 09.00 am and 01.00 pm. The animals were connected through a commutator to an external current stimulator (NCP, model 100; Cyberonics Inc. USA) for vagal or sham stimulation. Rats were freely moving in their cages. To ensure proper delivery of the stimulation intensity, the impedance of the electrode-to-vagal nerve interface was measured in every rat before every VNS session. Randomly, vagal and sham stimulation sessions were performed on the same animal and repeated several times on different days, with at least 3 days in between sessions. One experimental session consisted of 1 h of VNS (0.75 mA) or 1 h of sham stimulation, immediately followed by blood sampling. During the 1 h of sham stimulation, rats were handled in exactly the same way as during the VNS session, but the output current of the pulse generator was programmed to 0 mA.

In a first experiment ($n=16$), serum corticosterone levels following high frequency (30 Hz) VNS were compared to serum corticosterone levels following sham stimulation. In a second experiment performed in a subset of rats ($n=5$), serum corticosterone levels following high frequency (30 Hz) VNS were compared to serum corticosterone levels following low frequency (1 Hz) VNS or sham stimulation.

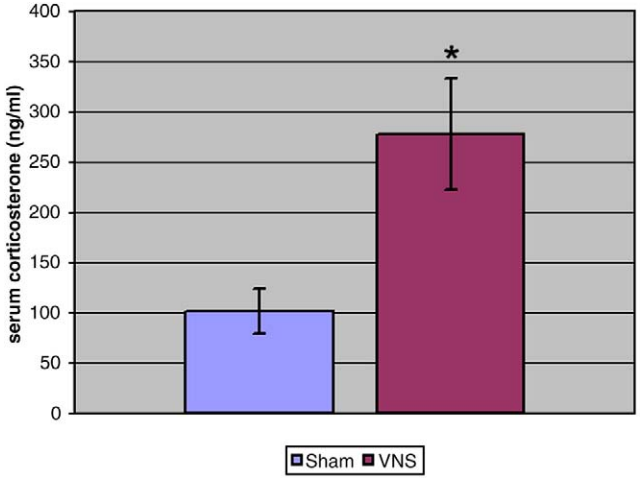

Fig. 1. Serum corticosterone after 1 h of sham ($n=16$) and after 1 h of VNS ($n=16$). * denotes a statistically significant difference of serum corticosterone after VNS compared to sham stimulation (paired Student's *t*-test; $p<0{,}05$).

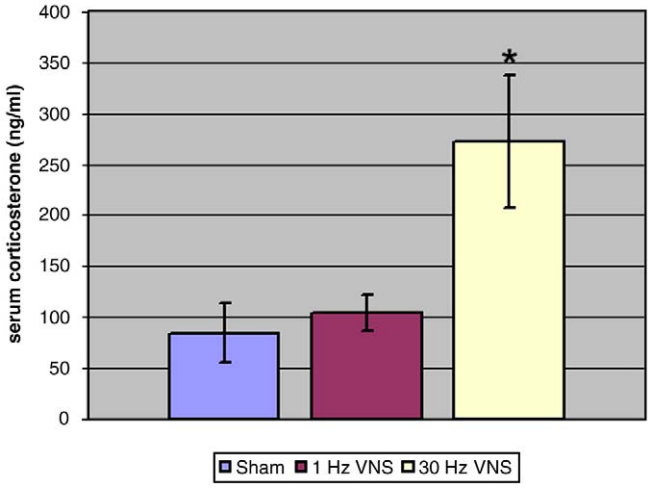

Fig. 2. Serum corticosterone after 1 h of sham ($n=5$), 1 h of low-frequency VNS ($n=5$) and 1 h of high frequency VNS ($n=5$). * denotes a statistically significant difference of serum corticosterone after high frequency stimulation compared to sham and low-frequency stimulation (Wilcoxon signed rank test; $p<0{,}05$).

2.5. Analysis of data

The results are presented as group's mean ± standard error of the mean. Statistical evaluation was performed by a paired Student's *t*-test for the first experiment ($n=16$). A Wilcoxon signed rank test was performed for the second experiment ($n=5$). Statistical significance was set to $p<0.05$. All calculations were performed using SPSS 15.0.

3. Results

All measured vagal nerve electrode impedance values were within the normal low range before each experimental session.

For the first experiment, several blood samples following sham and following high frequency VNS were gathered for each rat during different experimental sessions. In total 43 blood samples after sham stimulation and 42 blood samples after VNS were gathered for the 16 rats. Per rat, 1 mean serum corticosterone change following VNS compared to sham stimulation was calculated based on these different sessions. A significant increase ($p=0.014$) in serum corticosterone levels was found after high frequency VNS (mean: 278 ng/ml, SEM: 22, range: 30–776 ng/ml) compared to sham stimulation (mean: 101 ng/ml, SEM: 55, range: 22–355 ng/ml) (Fig. 1).

For the second experiment, several blood samples following sham stimulation, low frequency stimulation and high frequency stimulation were randomly gathered for each rat during different experimental sessions. In total, 8 blood samples after sham stimulation, 9 blood samples after high frequency VNS and 9 blood samples after low frequency VNS were collected for 5 rats.

Serum corticosterone significantly increased following high frequency stimulation (median: 294, range: 110–490 ng/ml) compared to sham stimulation (median: 56, range: 31–191 ng/ml, $p=0.043$) and compared to low frequency stimulation (median: 102, range: 58–153 ng/ml, $p=0.043$) (Fig. 2).

4. Discussion

In this study, 1 h of high frequency VNS significantly increased serum corticosterone compared to sham stimulation in awake and freely moving rats. We aimed to investigate the effects of electrical

stimulation of the vagal nerve on serum corticosterone levels under physiological conditions. This approach and set-up allowed separation of the implantation procedure and the experiments in time, and to perform biological repeats in the same animal. Our findings suggest that electrical stimulation of the vagal nerve activates the HPA-axis.

It has been known for several decades that the peripheral administration of cytokines such as IL-1 β activate the HPA axis and lead to an enhanced production of ACTH and corticosterone (Sapolsky et al., 1987; Besedovsky et al., 1986). Possible mechanisms by which cytokines signal the brain include blood-borne and neural routes (Banks et al., 1991; Van Dam et al., 1993; Saper and Breder, 1994; Tilders et al., 1994; John and Buckingham, 2003) or indirect pathophysiological changes such as hypotension or hypovolemia (Tkacs and Strack, 1995). Strong evidence exists that vagal afferents represent a functional link between peripheral cytokine release and HPA-axis activation, as subdiaphragmatic vagotomy blocks ACTH and corticosterone production when low doses of cytokines are administered intraperitoneally or intravenously (Gaykema et al., 1995; Fleshner et al., 1995, 1998). HPA-axis activation with higher doses of cytokines may involve additional neural and/or humoral pathways (Gaykema et al., 1995; Fleshner et al., 1998; Wieczorek and Dunn, 2006).

Activation of a nerve implies the presence of a depolarising electrical current so that an action potential is elicited. Nerve depolarisation can be obtained by chemical compounds or other molecules like cytokines, but also by applying direct electrical current to the nerve (Rowbottom and Susskind, 1984). Once a nerve fibre is activated i.e. a nerve action potential is elicited, either by chemicals or by electrical stimulation, nerve to nerve or nerve to brain tissue communication is established. The NTS, the main terminal of the vagal nerve afferents within the CNS, is known to have anatomical connections with the corticotropin releasing hormone cells in the paraventricular nucleus of the hypothalamus (Berthoud and Neuhuber, 2000; Cunningham and Sawchenko, 1988). Several imaging studies have shown activation of the hypothalamus upon electrical stimulation of the vagal nerve (Vonck et al., 2008; Henry et al., 1998). Hosoi et al. showed elevation of serum corticosterone and ACTH when the vagal nerve was electrically stimulated in anaesthetized rats (Hosoi et al., 2000). These arguments support the idea that indeed electrical stimulation of the vagal nerve under experimental conditions activates brain structures that are part of the HPA axis.

Previously, it has been shown that electrical stimulation of the vagal afferents enables modulation of the HPA-axis under specific conditions. A study by O'Keane et al. found that stimulation of the vagal nerve in a group of patients treated with VNS for chronic depression normalized the increased adrenocorticotropic hormone (ACTH) response to a corticotropin-releasing hormone (CRH) challenge test (O'keane et al., 2005). Hosoi et al. demonstrated that direct electrical stimulation of the proximal end of the vagal nerve in vagotomized and anaesthetized rats resulted in an increase in the expression of corticotropin releasing factor mRNA in the hypothalamus after 2 h of VNS. Plasma levels of ACTH and corticosterone were also increased by this stimulation (Hosoi et al., 2000). Our experiments support these findings in awake and freely moving animals.

Because vagotomy was not performed in this study, efferent vagal nerve activation cannot be fully excluded. Due to the specific design and implantation of the electrode an anodal block is achieved eliminating most efferent stimulation. An anodal block is never complete and therefore we cannot fully exclude that other mechanisms also contributed to corticosterone increases. However, to our knowledge, no experimental evidence exists showing an increase of serum corticosterone mediated by vagal efferents. In contrast clear evidence exists that vagal afferents activate the HPA axis (Gaykema et al., 1995; Fleshner et al., 1998).

In our study, serum corticosterone levels significantly increased after 1 h of high frequency stimulation in contrast to low frequency stimulation. Low frequency stimulation is considered not effective in activating vagal nerve fibers and has therefore no effect on brain activity (Woodbury and Woodbury, 1990; Osharina et al., 2006; Handforth et al., 1998). This finding suggests the superiority of high frequency stimulation to increase serum corticosterone.

Current clinical applications of electrical stimulation of the vagal afferents include treatments for refractory epilepsy and depression. The precise mechanism of action of these treatments is still not understood (Ben-Menachem, 2002). It is possible that activation of the HPA-axis by stimulating the vagal afferents affects the brain in these pathological conditions. The latter is in agreement with considerable evidence showing that patients with major depression suffer from a dysfunctional HPA-axis (Holsboer, 2003; O'keane et al., 2005). Evidence also exists that immune mechanisms are involved in the pathophysiology of epilepsy (Vezzani et al., 2008; Ransohoff, 2009). Moreover, ACTH and corticoids are used to treat certain severe childhood epilepsies (Verhelst et al., 2005). Thus, it is not unlikely that immunomodulatory changes triggered by VNS would lead to an altered immune response in the CNS and thereby modify neuropathological processes in the brain. If this is the case, future clinical applications using electrical stimulation of the vagal nerve could aid the treatment of numerous acute and chronic inflammatory diseases. For patients with life-threatening sepsis or chronic inflammatory diseases, modulation of the immune response by electrical stimulation of the vagal nerve could become a treatment option. With this in mind, transcutaneous VNS is a treatment modality worthwhile to further investigate (Huston et al., 2007).

References

Banks, W.A., Ortiz, L., Plotkin, S.R., Kastin, A.J., 1991. Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. J. Pharmacol. Exp. Ther. 259, 988–996.

Ben-Menachem, E., 2002. Vagus–nerve stimulation for the treatment of epilepsy. Lancet Neurol. 1, 477–482.

Berthoud, H.R., Neuhuber, W.L., 2000. Functional and chemical anatomy of the afferent vagal system. Auton. Neurosci. 85, 1–17.

Besedovsky, H., Del Rey, A., Sorkin, E., Dinarello, C.A., 1986. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science 233, 652–654.

Cunningham Jr., E.T., Sawchenko, P.E., 1988. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. J. Comp. Neurol. 274, 60–76.

Fleshner, M., Goehler, L.E., Hermann, J., Relton, J.K., Maier, S.F., Watkins, L.R., 1995. Interleukin-1 beta induced corticosterone elevation and hypothalamic NE depletion is vagally mediated. Brain Res. Bull. 37, 605–610.

Fleshner, M., Goehler, L.E., Schwartz, B.A., Mcgorry, M., Martin, D., Maier, S.F., Watkins, L.R., 1998. Thermogenic and corticosterone responses to intravenous cytokines (IL-1beta and TNF-alpha) are attenuated by subdiaphragmatic vagotomy. J. Neuroimmunol. 86, 134–141.

Gaykema, R.P., Dijkstra, I., Tilders, F.J., 1995. Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. Endocrinology 136, 4717–4720.

Handforth, A., Degiorgio, C.M., Schachter, S.C., Uthman, B.M., Naritoku, D.K., Tecoma, E.S., Henry, T.R., Collins, S.D., Vaughn, B.V., Gilmartin, R.C., Labar, D.R., Morris III, G.L., Salinsky, M.C., Osorio, I., Ristanovic, R.K., Labiner, D.M., Jones, J.C., Murphy, J.V., Ney, G.C., Wheless, J.W., 1998. Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial. Neurology 51, 48–55.

Henry, T.R., Bakay, R.A., Votaw, J.R., Pennell, P.B., Epstein, C.M., Faber, T.L., Grafton, S.T., Hoffman, J.M., 1998. Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: I. Acute effects at high and low levels of stimulation. Epilepsia 39, 983–990.

Holsboer, F., 2003. High-quality antidepressant discovery by understanding stress hormone physiology. Ann. N.Y. Acad. Sci. 1007, 394–404.

Hosoi, T., Okuma, Y., Nomura, Y., 2000. Electrical stimulation of afferent vagus nerve induces IL-1beta expression in the brain and activates HPA axis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R141–R147.

Huston, J.M., Gallowitsch-Puerta, M., Ochani, M., Ochani, K., Yuan, R., Rosas-Ballina, M., Ashok, M., Goldstein, R.S., Chavan, S., Pavlov, V.A., Metz, C.N., Yang, H., Czura, C.J., Wang, H., Tracey, K.J., 2007. Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. Crit. Care Med. 35, 2762–2768.

John, C.D., Buckingham, J.C., 2003. Cytokines: regulation of the hypothalamo–pituitary–adrenocortical axis. Curr. Opin. Pharmacol. 3, 78–84.

O'keane, V., Dinan, T.G., Scott, L., Corcoran, C., 2005. Changes in hypothalamic–pituitary–adrenal axis measures after vagus nerve stimulation therapy in chronic depression. Biol. Psychiatry 58, 963–968.

Osharina, V., Bagaev, V., Wallois, F., Larnicol, N., 2006. Autonomic response and Fos expression in the NTS following intermittent vagal stimulation: importance of pulse frequency. Auton. Neurosci. 126–127, 72–80.

Pavlov, V.A., Wang, H., Czura, C.J., Friedman, S.G., Tracey, K.J., 2003. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol. Med. 9, 125–134.
Ransohoff, R.M., 2009. Immunology: barrier to electrical storms. Nature 457, 155–156.
Rowbottom, M., Susskind, C., 1984. Electricity and Medicine, History of Their Interaction. San Francisco Press, San Francisco.
Saper, C.B., Breder, C.D., 1994. The neurologic basis of fever. N. Engl. J. Med. 330, 1880–1886.
Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P., Vale, W., 1987. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238, 522–524.
Tilders, F.J., Derijk, R.H., Van Dam, A.M., Vincent, V.A., Schotanus, K., Persoons, J.H., 1994. Activation of the hypothalamus–pituitary–adrenal axis by bacterial endotoxins: routes and intermediate signals. Psychoneuroendocrinology 19, 209–232.
Tkacs, N.C., Strack, A.M., 1995. Systemic endotoxin induces Fos-like immunoreactivity in rat spinal sympathetic regions. J. Auton. Nerv. Syst. 51, 1–7.
Tracey, K.J., 2002. The inflammatory reflex. Nature 420, 853–859.
Van Dam, A.M., Brouns, M., Man, A.H.W., Berkenbosch, F., 1993. Immunocytochemical detection of prostaglandin E2 in microvasculature and in neurons of rat brain after administration of bacterial endotoxin. Brain Res. 613, 331–336.
Verhelst, H., Boon, P., Buyse, G., Ceulemans, B., D'hooghe, M., Meirleir, L.D., Hasaerts, D., Jansen, A., Lagae, L., Meurs, A., Coster, R.V., Vonck, K., 2005. Steroids in intractable childhood epilepsy: clinical experience and review of the literature. Seizure 14, 412–421.
Vezzani, A., Balosso, S., Ravizza, T., 2008. The role of cytokines in the pathophysiology of epilepsy. Brain Behav. Immun. 22, 797–803.
Vonck, K., De Herdt, V., Bosman, T., Dedeurwaerdere, S., Van Laere, K., Boon, P., 2008. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. Seizure 17 (8), 699–706.
Watkins, L.R., Maier, S.F., Goehler, L.E., 1995. Cytokine-to-brain communication: a review & analysis of alternative mechanisms. Life Sci. 57, 1011–1026.
Wieczorek, M., Dunn, A.J., 2006. Effect of subdiaphragmatic vagotomy on the noradrenergic and HPA axis activation induced by intraperitoneal interleukin-1 administration in rats. Brain Res. 1101, 73–84.
Woodbury, D.M., Woodbury, J.W., 1990. Effects of vagal stimulation on experimentally induced seizures in rats. Epilepsia 31 (Suppl 2), S7–19.