Effect of Vagus Nerve Stimulation on Serotonergic and Noradrenergic Transmission

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Received March 14, 2006; accepted May 9, 2006

ABSTRACT

Vagus nerve stimulation (VNS) is an antiepileptic treatment, which has recently shown promise as an antidepressant. Yet, its antidepressant mechanisms of action are unknown. Serotonergic [5-hydroxytryptamine (5-HT, serotonin)] and noradrenergic [norepinephrine (NE)] systems are involved in the pathophysiology of depression and in the mechanisms of action of antidepressants. The present study analyzes 5-HT and NE neuronal firing rates in their brainstem nuclei: the dorsal raphe nucleus (DRN) and locus coeruleus (LC), respectively. The basal firing rates in the DRN and LC were significantly increased after long-term treatments with VNS. After short-term VNS treatments, firing rates were significantly higher for LC (at 1 h and 3 days). As changes in their firing rate may have been due to altered autoreceptor sensitivities, the responses of autoreceptors to the acute administration of their respective agonists were assessed. However, no significant difference was seen in the DRN. No significant differences in dose response curves for 5-HT1A somatodendritic and α2-adrenergic autoreceptors were noticed between long-term VNS and controls. VNS appears to have a novel mechanism of antidepressant action, enabling its effectiveness in treatment-resistant depression. LC firing rates significantly increased earlier than the DRN basal firing. As the LC has an excitatory influence on DRN, it is possible that the increased DRN firing rate is secondary to an initial increased LC firing rate from VNS.

The vagus nerve (cranial nerve 10) is generally thought of as a group of efferent parasympathetic fibers regulating autonomic functions. However, this nerve consists of 80% afferent fibers (Foley and DuBois, 1937) from the head, neck, and body, leading up toward the cerebrum. These afferent fibers (Foley and DuBois, 1937) from the head, neck, and body, leading up toward the cerebrum. These afferent fibers were targeted for therapeutic use by stimulation in medially and surgically intractable epilepsy, with several patients reporting a large decrease in seizure frequency (reviewed in Groves and Brown, 2005).

In addition to decreasing seizure frequency, an improvement in mood was witnessed in patients with vagus nerve stimulation (VNS), even in those with little or no change in seizure frequency (Harden et al., 2000). Clinical studies with VNS in treatment-resistant depression have generated positive results, with 3-month treatments inducing a 31% response rate and a 15% remission rate, based on Hamilton Rating Scales for Depression scores. Response and remission rates increased further with longer VNS treatments (Nahas et al., 2005). Recently, Krahl et al. (2004) showed that VNS significantly reduced immobility in rats with the forced-swim test, an animal model used to predict antidepressant treatments efficacy. The immobility levels of VNS-treated animals were similar to those of animals treated with desipramine and electroconvulsive therapy (ECT), two antidepressant treatments. VNS therapy is now approved for treatment-resistant depression in the European Union, the United States, and Canada, but its mechanisms of action in depression are unknown.

This work was supported by the Canadian Institute in Health Research and Cyberonics Inc.

The data presented in this manuscript were part of a thesis accepted at McGill University in April 2005, entitled The Effect of Vagus Nerve Stimulation on the Efficacy of Serotonergic and Noradrenergic Transmission: an Electrophysiological Study in the Rat.


Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.106.104166.

ABBREVIATIONS: VNS, vagus nerve stimulations; ECT, electroconvulsive therapy; 5-HT, 5-hydroxytryptamine, serotonin; DRN, dorsal raphe nucleus; MAOI, monoamine oxidase inhibitor; SSRI, selective serotonin reuptake inhibitor; NE, norepinephrine; LC, locus coeruleus; YM992, [N-(2-fluoro-4-indanyl)xoxy[methyl]morpholine monohydrochloride; LSD, lysergic acid diethylamide; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; WAY-100635, [2-(4-methoxyphenyl)-1-piperaziny]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride; ANOVA, analysis of variance.
Serotonin and norepinephrine are involved in the pathophysiology of depression and mechanisms of action of antidepressant treatments (reviewed in Millan, 2004). The major brainstem cell body nucleus for 5-HT is the dorsal raphe nucleus (DRN). Acute MAOI and SSRIs treatments, both of which increase extracellular 5-HT levels, show an initial decrease in DRN 5-HT firing rate in rats, whereas long-term decreases in firing rate are due to negative feedback from inhibitory 5-HT_{1A} somatodendritic autoreceptors, and these receptors become desensitized during long-term exposure to endogenous or exogenous agonists (reviewed in Blier, 2003).

NE reuptake inhibitors show beneficial effects with depression treatment (Massana et al., 1999; Montgomery et al., 2003), as do dual 5-HT and NE reuptake inhibitors (Stahl et al., 2002) and α_{2}-receptor antagonists (Nutt and Pinder, 1996). The locus coeruleus (LC) is the major NE brainstem nucleus that sends projections to many brain areas, including limbic structures. It receives innervation from the nucleus of the solitary tract (Van Bockstaele et al., 1999), a brainstem nucleus for vagus nerve afferents (Barraco, 1994). In addition, the DRN innervates the LC (Cedarbaum and Aghajanian, 1978; Leger and Descarries, 1978), and, conversely, the LC gives NE inputs to the DRN (Baraban and Aghajanian, 1980), creating ample opportunity for cross-modulation. Antidepressant treatments affecting one system, either NE or 5-HT, can indirectly affect the other on the basis of this cross-modulation. For instance, NE acts on 5-HT DRN neurons to induce tonic activation mediated by excitatory α_{2}-adrenoceptors (Baraban and Aghajanian, 1980), whereas SSRIs treatments produce a net inhibition of LC firing rate after 2 weeks (Szabo et al., 2000; Grant and Weiss, 2001). NE neurons have inhibitory α_{2}-adrenergic autoreceptors on the soma and terminals that decrease the NE firing rate or NE terminal release, respectively, in the presence of excess endogenous NE or α_{2}-agonists such as clonidine. After long-term treatment with the dual SSRI and 5-HT_{3A} antagonist YM992, NE neurons showed an increase in firing rate induced by a decrease in sensitivity of the α_{2}-adrenergic autoreceptor (Szabo and Blier, 2002). Interestingly, lesioning the LC in animal studies blocks the antiepileptic action of VNS, suggesting that the LC is involved in its effective circuitry (Krahnl et al., 1998). Recent electrophysiological studies showing an increased discharge rate of LC NE neurons in response to acute VNS within a very short time frame of seconds to minutes poststimulation has come to light (Groves et al., 2005). It is interesting to note, however, that no significant change in cerebrosplinal fluid metabolites of NE and 5-HT was seen in VNS patients treated for 3 months compared with pretreatment levels (Carpen ter et al., 2004).

The present study examines the effect of VNS on 5-HT and NE systems. In vivo extracellular unitary recordings were obtained in anesthetized rats to assess the basal firing rates of 5-HT and NE in the DRN and LC, respectively. The sensitivity of the 5-HT_{1A} somatodendritic autoreceptor in the DRN and the α_{2} somatodendritic autoreceptor in the LC was assessed by administration of their agonists, LSD and clonidine, respectively.

Materials and Methods

Animals. All experiments were carried out on Sprague-Dawley rats (Charles River Canada, St. Constant, QC, Canada). Rats weighed a minimum of 275 g for surgery and electrophysiological experiments and were housed under standard laboratory conditions (12:12 light/dark cycle with access to food and water ad libitum). Body temperature was kept at 37°C during the surgery and electrophysiological experiments. The electrophysiological experiments were carried out by standard mounting of anesthetized rats in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and inserting a catheter into the lateral tail vein to for systemic i.v. administration of drugs. Rats were kept hydrated during the experiments by i.v. injection of 0.1 ml of saline every 30 min.

Surgery. Using sterile techniques, male Sprague-Dawley rats were operated on under equithesine anesthetic, 1 ml i.p./300-g rat (4.26% chloral hydrate and 0.96% sodium pentobarbital). Supplemental doses of equithesine were given i.p., 0.1 ml at a time, when a nociceptive response to a hind paw pinch was noticed, to ensure that the rat was fully anesthetized. A horizontal incision was made, slightly above the clavicle. The left omohyoid and sternomastoid muscles were carefully separated, to allow a clear view of the left vagus nerve. In the rat, the vagus nerve lies lateral to the carotid artery. Bipolar leads were wrapped around the left carotid artery and vagus nerve, allowing close contact between the vagus nerve and the electrodes. This method of wrap of the lead to the control arm of the carotid artery and the electrode was performed before, with success (Handforth and Krahl, 2001). The electrodes were sutured to the underlying muscle to keep the leads in place. The leads were tunneled s.c., within and around the neck toward a horizontal dorsal incision made in the back. For stimulator placement, a pocket was made under the skin of the back and wiped with iodine. The tunneled leads were then connected to the stimulator, the stimulator was placed in the dorsal pocket, and antibiotics and fluid replacements were given to ease recovery. A sham control group underwent the same surgical procedure with leads and a dummy 102 pulse stimulator in place. The lead impedance was checked to assure a tight connection between the nerve and coils, using the device diagnostic setting on the NCP handheld computer and programming wand. A recovery period of 2 days was allowed before the stimulator was turned on in treated rats. The stimulator was programmed with outputs similar to those used in humans (30 s on, 5 min off; continuous cycle; 20 Hz, pulse width of 500 μs, 0.25 mA) (Sackei et al., 2001). One-hour, 24-h, and 3-day stimulator groups were used for short-term VNS treatment. Two-week, 3-week, and 3-month treatment groups were used for long-term treatment groups. Sham treatments ranged from 3 days post-surgery to 3 months. No significant difference was noticed between different sham groups, allowing all sham treatments to be grouped together into one sham group (data not shown).

Electrophysiological Experiments: Dorsal Raphe Nucleus. Experiments were carried out with the VNS device in place; however, the device was inactivated for the duration of the experiment because of electrical interference. Rats were anesthetized with chloral hydrate i.p at 400 mg/kg. Extracellular unitary in vivo recordings were conducted with a pulled single-barrel glass micropipette. The electrode was filled with 2% pontamine sky blue solution, 0.5 M, with physiologic saline and programming wand. A recovery period of 2 days was allowed before the stimulator was turned on in treated rats. The stimulator was programmed with outputs similar to those used in humans (30 s on, 5 min off; continuous cycle; 20 Hz, pulse width of 500 μs, 0.25 mA) (Sackei et al., 2001). One-hour, 24-h, and 3-day stimulator groups were used for short-term VNS treatment. Two-week, 3-week, and 3-month treatment groups were used for long-term treatment groups. Sham treatments ranged from 3 days post-surgery to 3 months. No significant difference was noticed between different sham groups, allowing all sham treatments to be grouped together into one sham group (data not shown).
10-s histogram (as obtained by the Spike2 program during recording) and dividing by length of time recorded in seconds. All neuronal firing rates for all rats in one group were added together and divided by the number of neurons recorded per group. Lysergic acid diethylamide (LSD), an agonist for 5-HT₁₄ receptors, was administered i.v. with the last 5-HT neuron, after at least 60 s of recording for baseline firing rate determination, to test the sensitivity of the 5-HT₁₄ somatodendritic autoreceptor. LSD was used instead of 8-OH-DPAT, as the latter 5-HT₁₄ receptor agonist can also inhibit 5-HT firing through a long feedback loop from the frontal cortex (Romero et al., 1994). The 5-HT₁₄ receptor antagonist WAY-100635 was administered i.v., after at least 60 s of LSD inhibition, to bring the firing rate back to basal levels, confirming that the inhibition was indeed due to the agonistic action at the somatodendritic 5-HT₁₄ receptor. Percentage inhibition for each neuron was calculated by first determining the basal firing rate and then the firing rate after agonist administration, expressing the firing rate due to the agonist as a percentage of the basal firing rate. The percentage inhibition was 100 minus this percentage.

Electrophysiological Experiments: Locus Coeruleus. Similarly to the DRN experiments, LC experiments were done with the inactivated stimulator in place. Rats were anesthetized with chloral hydrate i.p at 400 mg/kg. Extracellular unitary in vivo recordings were conducted with a pulled single-barrel glass micropipette. The electrode was filled with 2% pontamine sky blue solution, 0.5 M, with an impedance range of 2 to 4 MΩ. A burr hole was drilled 1.1 mm posterior to lambda and 1.1 mm lateral to the midline. The electrode was lowered at 0.7 mm interaural and 1.1 to 1.4 mm lateral (Paxinos and Watson 1986).

Spontaneously active NE cells were identified by their regular 1-to 5-Hz firing rate, a positive action potential of long duration (0.8–1.2 ms), and their burst discharge due to nociceptive pinch of the contralateral hind paw (Aghajanian, 1978). For averaging the firing rate for each group, at least five descents were made per rat with the electrode at 100 μm from previous descents. Each neuron was recorded for at least 60 s. Neuronal firing rates were calculated by adding each discharge per 10-s histogram (as obtained by the Spike2 program during recording) and dividing by length of time recorded in seconds. All neuronal firing rates for all rats in one group were added together and divided by number of neurons recorded per group. Clonidine, an agonist for α₂-adrenergic receptors, was administered i.v. with the last NE neuron, after at least 60 s of recording for baseline firing rate determination, to test the sensitivity of LC α₂ autoreceptors. The α₂-adrenergic receptor antagonist idazoxan was administered i.v., after clonidine inhibition of at least 60 s, to bring LC firing rates back to basal levels, confirming that the inhibition was indeed due to the agonistic action at the α₂-adrenergic autoreceptor. Percentage inhibition for each neuron was calculated by first determining the basal firing rate and then the firing rate after agonist administration, expressing the firing rate due to the agonist as a percentage of the basal firing rate. The percentage inhibition was 100 minus this percentage.

Drugs and Materials. The following drugs were used: LSD (Sandoz, Boucherville, QC, Canada), WAY-100635 (Sigma Chemical, Oakville, ON, Canada), clonidine (Sigma), and idazoxan (Sigma). All drugs were dissolved in distilled water. Concentration ranges were chosen on the basis of previous successful experiments in our laboratories. Cyberonics (Houston, TX) provided the leads, 102 pulse stimulators, and dummy stimulators.

Statistical Comparisons. Basal firing rates are expressed as mean ± S.E.M. Nonparametric tests were used as firing rates were not normally distributed. Average firing rates for control and sham groups were compared using Mann-Whitney rank sum tests. Average firing rates for control and treated groups were compared using the Kruskal-Wallis one-way analysis of variance on ranks test. Post hoc tests were performed using Dunn’s multiple comparisons test to assess the difference between control and treated groups. The numbers of neurons encountered per track were analyzed using the Kruskal-Wallis one-way analysis of variance on ranks test. Regression analysis was used to assess any difference between dose-response curves for control and treated groups and to find the effective dose for inhibiting neuronal activity by 50% (ED₅₀).

Results

Effect of Sham VNS Surgery on Basal DRN 5-HT and LC NE Firing Rates. The sham data from all time lengths of VNS treatments were pooled as no significant difference in firing rates were noticed between different sham VNS groups (data not shown). Comparing control and sham basal 5-HT firing rates in anesthetized rats revealed no significant difference [Mann-Whitney rank sum test, T(174) = 6670.51, p = 0.340] (Fig. 1A). Comparing control and sham basal NE firing rates in anesthetized rats also showed no significant difference [Mann-Whitney rank sum test, T(139) = 4158.50, p = 0.111] (Fig. 1B). Therefore, the surgery itself, following a sufficient recovery time of 2 days, had no effect on DRN 5-HT or LC NE neuronal basal firing rates.

Effect of Short-Term and Long-Term VNS Treatment on Basal 5-HT Firing Rates. To determine the basal 5-HT firing rate changes due to short- and long-term VNS, in vivo extracellular neuronal activity was sampled from five to six rats per treatment group, with at least 10 neurons per rat. No significant difference for short-term VNS (1-h, 1-day, and 3-day treatments) was found when compared with control.
5-HT firing rates [Kruskal-Wallis one-way ANOVA on ranks, \( H(3) = 6.86; p = 0.076 \)] (Fig. 2A). However, there was a clear significant group effect for long-term VNS (14-, 21-, and 90-day treatments) compared with control 5-HT firing rates [Kruskal-Wallis one-way ANOVA on ranks, \( H(3) = 41.87, p < 0.001 \)]. Post hoc analysis of long-term VNS groups revealed a statistically significant increase in firing rate for 14-, 21-, and 90-day treatments versus control [Kruskal-Wallis one-way ANOVA on ranks, \( H(3) = 11005, H(11005) = 6.86; p = 0.076 \)] (Fig. 2A). However, there was a clear significant group effect for long-term VNS (14-, 21-, and 90-day treatments) compared with control 5-HT firing rates [Kruskal-Wallis one-way ANOVA on ranks, \( H(3) = 41.87, p < 0.001 \)]. Post hoc analysis of long-term VNS groups revealed a statistically significant increase in firing rate for 14-, 21-, and 90-day treatments versus control [Dunn’s method, \( p < 0.05 \)] (Fig. 2B). Ninety-day treated rats had a mean 5-HT firing rate that was 2-fold higher compared with control (2.22 ± 0.42 Hz for 90-day VNS versus 1.11 ± 0.18 Hz for control), indicating a substantial increase in 5-HT firing due to long-term VNS. In addition, mean firing rates showed a trend to increase as length of stimulation increases (slope = 0.18) (Fig. 2C).

Assessment of 5HT1A Somatodendritic Autoreceptor Activity. LSD dose-response curves for control and long-term VNS were constructed to compare the sensitivity of the 5-HT1A somatodendritic autoreceptor. The sensitivity of the 5-HT1A receptor was not assessed after short-term VNS treatment, as there was no significant difference in 5-HT firing rate for short-term VNS versus control. After recording a 5-HT neuron for 60 s (Fig. 3A), various doses of LSD (ranging from 1 mg/kg to 60 \( \mu \)g/kg) were administered i.v. The ED50 value for control was 11.22 \( \mu \)g/kg whereas the ED50 for long-term VNS was 10.47 \( \mu \)g/kg (Fig. 3B). There is no significant difference between the dose-response curves for control and long-term VNS (\( r^2_{\text{control}} = 0.94, r^2_{\text{VNS}} = 0.95; F(12,2) = 3.85, p = 0.688 \)) (Fig. 3C). In all cases, the suppression of the firing activity induced by LSD was reversed by the subsequent administration of WAY 100635 (Fig. 3A).

Effect of Short-Term and Long-Term VNS Treatment on Basal NE Firing Rates. To determine the basal NE firing rate changes due to short- and long-term VNS, in vivo extracellular neuronal activity was sampled in the LC from five to six rats per treatment group, with at least 10 neurons per rat. After a short-term treatment with VNS, a significant main group effect was observed between VNS and controls [Kruskal-Wallis one-way ANOVA on ranks, \( H(3) = 9.60, P = 0.022 \)] (Fig. 4A). Dunn’s method for post hoc analysis revealed a significant difference in the direction of a higher NE firing rate comparing acute (\( p < 0.05 \)) and 3-day (\( p < 0.05 \)) treatments with control; however, 1-day (24 h) treatment showed no significant difference.

Long-term VNS treatment showed a highly significant difference in firing rates compared with controls [\( H(3) = 57.20, p < 0.001 \)]. Dunn’s method for post hoc analysis revealed significant differences for 14-, 21-, and 90-day versus control (\( p < 0.05 \)) (Fig. 4B). NE firing rate proved to be 2-fold higher for 90-day treatment compared with control (4.75 ± 0.13 Hz for 90-day VNS versus 2.23 ± 0.36 Hz for control), indicating a large facilitatory effect on NE firing due to VNS. These data show a trend for a time-dependent increase in firing rate (slope = 0.34) (Fig. 4C).
Assessment of $\alpha_2$-Adrenergic Autoreceptor Activity on NE Soma. Clonidine dose-response curves were compared between control and long-term VNS treatments to assess the sensitivity of the $\alpha_2$-adrenergic autoreceptors on the LC NE cell bodies. After recording a LC neuron for 60 s (Fig. 5A), various doses of clonidine (ranging from 1 to 60 $\mu$g/kg) were administered i.v. The ED$_{50}$ values were 7.50 and 8.13 $\mu$g/kg for control and long-term VNS, respectively (Fig. 5B). Regression analysis revealed no significant difference between control and VNS dose-response curves ($r^2$$_{\text{control}}$ =...
suggesting that there is no desensitization of the α₂-adrenergic autoreceptors.

Effect of VNS on Number of Neurons Per Track. The number of neurons encountered per descent into the brain was analyzed for both short-term and long-term VNS in the DRN and LC. Indeed if more neurons are encountered per track, it is possible that a previously silent population of neurons have been activated during VNS, thus affecting the average firing rates and our comparisons with control groups. For the DRN, a Kruskal-Wallis one-way ANOVA on ranks revealed no significant difference versus control for all VNS groups \( \text{H}(6) = 5.03; p = 0.541 \) (Fig. 6A). Similarly for the LC, the Kruskal-Wallis one-way ANOVA on ranks revealed no significant difference versus control for all VNS groups \( \text{H}(6) = 3.03; p = 0.805 \) (Fig. 6B). It therefore appears that VNS does not activate a previously silent population of neurons in either brainstem nuclei of interest in this study.

Discussion

The main results of our studies are that VNS treatments induce large time-dependent increases in basal neuronal firing in the brainstem nuclei for serotonin and norepinephrine: the dorsal raphe nucleus and locus coeruleus, respectively. All classes of antidepressant treatments, including norepinephrine reuptake inhibitors, ECT, and neurokinin-1/substance P antagonists, act at least in part, by increasing 5-HT neurotransmission (reviewed in Haddjeri et al., 1995; Hadjæjeri and Blier, 2000; Santarelli et al., 2001); however, NE probably also plays an important role in antidepressant effects, and NE is thought to be involved in the pathophysiology of depression (Delgado and Moreno, 2000). Long term, SSRIs increase 5-HT neurotransmission, while decreasing spontaneous NE activity (Szabo et al., 2000; Grant and Weiss, 2001). Conversely, norepinephrine reuptake inhibitors are efficient antidepressant treatments and seem to affect 5-HT neurotransmission (Massana et al., 1999; Montgomery et al., 2003) as do dual 5-HT and NE reuptake inhibitors (Stahl et al., 2002). However, to our knowledge VNS represents the first reported antidepressant treatment able to induce increased firing activity of both serotonergic and noradrenergic neurons.

Indeed, endogenous or local applications of exogenous 5-HT in the DRN decrease the 5-HT firing rate through activation of 5-HT₁A somatodendritic autoreceptors, as do the 5-HT₁A agonists LSD and 8-OH-DPAT (Aghajanian et al., 1968). Prolonged exposure to increased 5-HT levels or 5-HT agonists progressively desensitizes these receptors, leading to recovery of the 5-HT firing rate. Antidepressant treatments that increase 5-HT concentrations in the vicinity of 5-HT cell bodies, such as SSRIs and MAOIs, initially decrease the firing activities of DRN 5-HT neurons, whereas long-term treatments return the firing rate back to the baseline level due to 5-HT₁A autoreceptor desensitization while keeping the high synaptic availability of 5-HT (reviewed in Blier, 2003). These treatments increase the efficacy of 5-HT neurotransmission via a change in the amount of 5-HT released and altered sensitivity of several subtypes of 5-HT.
was reversed by the selective WAY-100635 (Fig. 3A) suggests that this effect was indeed mediated by the 5HT1A receptor. Despite the significantly increased DRN 5-HT firing rate with long-term VNS (Fig. 2B), no change in 5-HT1A receptor sensitivity was observed (Fig. 3, B and C). In contrast with results for SSRIs published so far, despite an increase in the firing activity of DRN 5-HT neurons, the 5HT1A somatodendritic autoreceptors were fully functional after long-term VNS. The increase in firing rate was therefore not due to a desensitization of these autoreceptors but rather to a distinct mechanism. One possible explanation for the absence of 5-HT1A receptor desensitization could be that VNS increases the release of 5-HT in the terminal regions such as the hippocampus or the medial prefrontal cortex but not in the vicinity of DRN 5-HT cell bodies.

The next step was to examine the basal firing rate of LC NE neurons. We found a progressive and statistically significant increase in NE firing over long-term VNS treatments. This is in keeping with previous data showing that an intact NE system is necessary for effective VNS treatments, at least for epilepsy (Krah et al., 1998) and that increased NE levels were present in LC terminal areas in VNS-treated animals (Hassett et al., 2004). LC firing activity is normally controlled by the tonic activation of somatodendritic α2-autoreceptors (Mongeau et al., 1997). Similarly to the 5-HT1A receptors, a desensitization of α2-autoreceptors has been shown to appear progressively, after prolonged exposure to increased NE concentrations. However, if most studies agree on a desensitization of terminal α2-autoreceptors, there is still some controversy regarding the existence of such desensitization of LC somatodendritic receptors (for a review, see Invernizzi and Garattini, 2004).

Thus, the sensitivity of NE α2-adrenergic receptors on LC NE neurons was also investigated. Our findings do not indicate any desensitization of the α2-adrenergic autoreceptors on the NE cell bodies and thus an increased firing rate must result through alternative methods. These results are in keeping with those obtained with antidepressants acting selectively on the noradrenergic system (Invernizzi and Garattini, 2004). However, they differ from those obtained with compounds acting on both serotonergic and noradrenergic neurotransmissions (Szabo and Blier, 2002). Because VNS is acting by modifying both neurotransmissions, one might expect to find a desensitization of α2-autoreceptors. Again, similarly to what has been proposed for 5-HT1A autoreceptors, the lack of α2 desensitization observed after treatment with VNS (up to 90 days), even in the presence of important increases in the firing activity of 5-HT and NE neurons, could suggest that VNS increases NE release in the terminal region but not in the LC. For both neurotransmitters, this hypothesis remains to be verified in microdialysis studies. A modification of terminal autoreceptors sensitivity (5-HT1B for 5-HT neurons and α2 for NE neurons) is unlikely to be involved in the observed firing activity changes as these autoreceptors regulate the amount of neurotransmitter released; however, our present experiments cannot rule out the possibility of such a desensitization.

One possibility for explaining the increased firing rate of 5-HT and NE neurons after VNS treatments might have been the appearance of fast firing neurons, which would have been silent in control conditions and become activated only after the VNS treatments. However, the number of neurons

receptors, but they never increase the firing activity of 5-HT neurons above their baseline activity.

Our results indicate a large increase in 5-HT firing rate, which is normally associated with increased endogenous 5-HT release, which should activate the somatodendritic autoreceptor. Activation of 5-HT1A somatodendritic autoreceptors would hyperpolarize the cell through Gi/o proteins and decrease the 5-HT firing rate (Innis and Aghajanian, 1987). We therefore tested the sensitivity of 5HT1A somatodendritic autoreceptors after long-term VNS treatments. We chose LSD, as the 5HT1A agonist 8-OH-DPAT seems to have a stronger effect on postsynaptic 5HT1A receptors (Romero et al., 1994). The postsynaptic 5-HT1A receptors are involved in a long-loop inhibitory feedback mechanism (Artigas et al., 1996; Hajos et al., 1998); therefore, using 8-OH-DPAT may cause DRN 5-HT inhibition that is unrelated to 5HT1A somatodendritic autoreceptor inhibition. LSD cannot be considered as highly selective for the 5-HT1A receptor. However, the fact that its suppression of the firing activity of 5-HT neurons...
found per track was identical in all treatment conditions, suggesting that we were always recording from the same neuronal population.

The DRN and LC have wide projections within the cortex, acting on areas involved in mood, such as limbic structures (Levitt et al., 1984). In addition, the DRN and LC are highly interconnected, affecting each other's overall activity (Cedarbaum and Aghajanian, 1978; Leger and Descarries, 1978; Baraban and Aghajanian, 1980; Szabo and Blier, 2001), thereby playing a role in each other's downstream target areas. The LC, but not the DRN, receives direct inputs from the NTS (Van Bockstaele et al., 1999) which itself receives afferences from the vagus nerve. As we observed a more rapid increase in the LC NE firing rate than in the DRN 5-HT firing rate (3 days instead of 14 days), it can be postulated that VNS may act initially and/or predominantly on the LC, and indirectly with the DRN via afferents from the LC. Indeed, the DRN is tonically activated by the LC by way of excitatory α2-receptors (Baraban and Aghajanian, 1980). The activity of the 5-HT neurons may therefore be increased by enhanced noradrenergic tone on α1 adrenergic receptors. Testing the sensitivity of these receptors on 5-HT DRN neurons in long-term VNS rats would be interesting, as the degree of activation of rrons in long-term VNS rats would be interesting, as the

References


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