Vagal-Evoked Activity in the Parafascicular Nucleus of the Primate Thalamus

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INTRODUCTION

There are almost no prior physiological studies of the effects of vagal afferent activity in the primate thalamus. The few findings obtained in other species include numerous inconsistencies. The actions of vagal afferent activity in the primate thalamus need to be identified because left vagus nerve stimulation (VNS) is now used clinically for treatment-resistant epilepsy and depression, these actions need to be identified. We used a roving microelectrode to record vagal-evoked potentials in the thalamus of the macaque monkey. In addition to the anticipated activation in the gustatory/visceral thalamic relay nucleus, we found an unexpectedly larger and earlier response focus with multi-unit discharges in the adjacent parafascicular nucleus. These data reveal a potent vagal input to this intralaminar nucleus, which is normally considered to be involved in motor control. This finding indicates that a role for this vagal activation site in the anti-epileptic effects of VNS needs to be considered.

METHODS

The experiments were performed in six cynomolgus monkeys (Macaca fascicularis) using procedures that conform with the animal-welfare guidelines of the National Institutes of Health and the American Physiological Society and were approved by the local institutional review board. The animals were anesthetized with intravenous pentobarbital (bolus: 40 mg/kg and \(-10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) through an angiocath in the saphenous vein after induction with intramuscular ketamine (10 mg/kg). These were also survival experiments for tracing anatomical projections, and so sterile precautions were observed, and prophylactic antibiotics and dexamethasone (10 mg) were administered. Blood pressure, heart rate, rectal temperature, and tissue oxygenation were monitored and stabilized.

Vagus nerve stimulation

The common cervical vagus nerves on both left and right sides were isolated as they course below the neck muscles together with the common carotid artery and the jugular vein. Each nerve was isolated rostrally as far as its dorsomedial traverse just caudal to the branch point of the superior laryngeal nerve, and caudally as far as the clavicle, over a distance of \(\sim 2.5\) cm. The few tiny branches along this part of the nerve were severed.

A custom-made electrode was placed on each nerve that consisted of polyethylene tubing (2.5 cm long) with a spiral slit cut along its entire length and two pairs of flattened platinum wire attached to its inner face, one at each end. The electrode was gently placed around each vagus nerve by winding the nerve along the spiral slit. The electrodes were insulated (with Parafilm, American National Can, Menasha, WI) and stabilized with sutures to the nearby muscles. They were removed at the completion of the recordings.

The threshold current strength for eliciting dyspnea (an immediate pause in ongoing respiration) or alimentary contraction (retching) was determined using a long (2 s) train of 0.4-ms pulses at 50 Hz. This intensity was usually \(\sim 0.3\) mA.

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Microelectrode recording

The animal was placed in a stereotaxic holder. A hole in the right calvarium allowed repeated vertical penetrations with a platinum-plated tungsten-in-glass microelectrode (tip: ~20 μm, impedance: ~250 KΩ). Microelectrode signals were amplified, band-pass filtered (10–5,000 Hz for VEP recordings; 500–15,000 Hz for unit recordings), monitored with an oscilloscope and a speaker, recorded on audio tape, and stored on a laptop PC using a CED Micro1401 and the program Spike2 (Cambridge Electronic Design, Cambridge, UK). Single- and multunit responses to innocuous and noxious mechanical and thermal stimulation of the body were used to locate the somatosensory ventral posterior (VP) thalamus and the spinothalamic-reciprocant posterior ventromedial nucleus (Vp). Based on that map, the initial microelectrode track for recording VEPs was selected near the estimated location of the posterior aspect of the basal part of the ventral medial nucleus (Vm).

VEP mapping

In each animal, a series of microelectrode tracks was made at different mediolateral (ML) and anteroposterior (AP) levels, proceeding from posterior and lateral to anterior and medial, generally in 0.5-mm steps. The locations of mapping penetrations were restricted in some cases by the surface vasculature. The VEP was recorded at preselected depths in each track, usually at 0.5-mm intervals. The locations and depths were chosen with the intent of spanning the extent of Vm in the initial three experiments, but in the later three experiments, they were directed at Pf. At each depth, a set of 12 stimulus events was delivered once every 4 s over a period of 45 s. Each event consisted of two pulses of 0.4 ms width at 300 Hz with an intensity of 5 times threshold or ~1.5 mA. These pulse parameters are comparable to those found useful for VNS clinically (Schachter and Schmidt 2003); a double pulse was used to enhance synaptic transfer. The stimuli produced no visible movements. The evoked response was averaged across all 12 events at each recording site. Lesions were made at selected recording locations by passing 7- to 20-μA cathodal current for 10–30 s.

Histology

After a survival period appropriate for the anatomical tracers that were injected in each brain (to be reported later), the animals were killed with an overdose of pentobarbital (60 mg/kg ip) and perfused transcardially with 1 L warm phosphate-buffered saline (0.1 M, pH 7.4, 38°C, 200 mmHg), followed by fixation with 1 L cold 4% paraformaldehyde and 0.2% picric acid in phosphate buffer (0.1 M, pH 7.4, 4°C, 2 min), and then 1.5 L 2% paraformaldehyde, 0.5% glutaraldehyde and 10% sucrose in phosphate buffer (0.1 M, pH 7.4, 4°C, 60 min). The brains were removed and cryoprotected by immersion in buffered 30% sucrose for 3 days. The thalamus was sectioned coronally at 50 μm on a horizontal freezing microtome, and every other section was mounted, stained with thionin, dehydrated, and coverslipped. Alternate sections were processed for tracer detection. Immunohistochemical staining for calbindin 28Kd was performed in some cases as described in Craig (2004).

Reconstruction

A three-dimensional VEP map was made in each case by arranging all averaged VEP recordings according to stereotaxic location in a single overview using Adobe Photoshop. The electrode tracks were identified individually in the thionin-stained sections by closely examining the pattern of gliotic penetrations and the locations of marking lesions and tracer injections. Corrections for tissue shrinkage (usually ~15–20%) were based on estimates from lesions and parallel penetrations. Cytoarchitectonic drawings of the thalamic sections were made with the camera lucida on a dissecting microscope (at 14×) depicting the trajectories of all electrode tracks and the locations of all recording sites. Cytoarchitectonic delineations were made as described by Craig (2004). Digital photomicrographs that were obtained using a Leaf Micromublisher scanner (3,800 × 2,253 pixels) or a Hamamatsu Orca-HR CCD camera (4,000 × 2,624 pixels) mounted on a Nikon Epiphot microscope or a Nikon Multiphot macrophotographic system were sharpened and contrast-enhanced using Photoshop.
a more rostral and lateral region of the ventral posterior thalamus. Furthermore, a distinct latency shift (−2 ms) was observed by shifting from the proximal to the distal vagus nerve electrode, consistent with a peripheral conduction velocity of ∼10 m/s.

Figure 3 illustrates the three-dimensional VEP map obtained in one of the later three cases (M142) in which more thorough mapping directed at the neighborhood of Pf was performed. This map shows with greater clarity that the VEP in lateral Pf is indeed focal, that is, that it diminishes in size in all spatial directions. (In this and more recent cases, there were no indications of vagal evoked potentials in medial thalamic regions dorsal to Pf.) This map also differentiates the large, short-latency VEP in Pf (peak: ∼35 ms; centered at AP 6.9, ML 1.8, depth: 24.0; marked with *) from a smaller, longer-latency VEP in VMb (peak: ∼55 ms; centered at AP 6.5, ML 2.8, depth: 24.0, and marked with †; to be described in detail in a separate report). The cytoarchitectonic reconstructions shown on the right in Fig. 3 demonstrate that the VEP focus in this case was located in lateral Pf between the habenulo-interpeduncular tract and the medial tip of VMb, as in the earlier cases. Nevertheless, this reconstruction also reveals that VEPs were recorded throughout Pf, some of which had multiple peaks. We interpret this finding to indicate that considerable further analysis, including identification of the stimulus-response characteristics of differentiable components and comparisons of activation by the asymmetric left and right vagus nerves in left and right thalamus, are now compelled in order to elucidate the detail and the extent of the VEP field in Pf.

FIG. 1. Results from an early case (M122), showing the histological localization of the large early vagal-evoked potential (VEP) in the monkey thalamus to parafascicular nucleus (Pf). Average VEPs (left) at different mediolateral (ML) locations and different vertical depths are shown as an array at each of 4 anteroposterior (AP) levels. The large dots in the photomicrographs (right) of coronal thionin-stained sections indicate the reconstructed sites of the corresponding recordings. The encircled star demarcates the VEP focus. Time-locked evoked multi-unit discharges (bottom inset) and heart rate-related ongoing discharges (top inset) were recorded at this VEP focus in this and other cases. Note that recording tracks could not be made at some medial locations because of surface vasculature. CM, center median; hyp, hypothalamus; VMb, basal part of the ventral medial n.; VP, ventral posterior n. Positive up (left) and dorsal up, medial left (right). Scale bars: left: 100 ms and 5 mV, bottom inset: 50 ms and 8 mV, upper inset: 600 ms (separate scale); right: 1 mm.
DISCUSSION

The vagus nerves innervate the viscera as well as the pharynx, larynx, inner ear, branchiomeric striate muscles, and the female genitalia, and together with the glossopharyngeal, facial and pelvic nerves, provide the parasympathetic efferents of the autonomic nervous system (Berthoud and Neuhuber 2000; Tracey 2002; Zagon 2001). The densest terminations occur in the middle, caudal, and commissural parts of medial NTS, which project strongly to the PB in the pons and to other homeostatic regions of the brain stem (e.g., ventrolateral medulla, n. ambiguus, locus coeruleus). In rats, but apparently not in monkeys, NTS also projects to numerous forebrain sites, including infralimbic and olfactory cortices, amygdala, hypothalamus, and the thalamic paraventricular nucleus (Ruggiero et al. 1998). Conversely, there is a direct projection from NTS to VMb in thalamus in the primate (Beckstead et al. 1980), but not in the rat (Ruggiero et al. 1998). Further, the PB projects to VMb, amygdala, and hypothalamus in both rodent and primate, but in the rat, PB also projects to various cortical regions and to portions of intralaminar and midline thalamus, including the Pf (Krouth and Loewy 2000; Rinaman and Schwartz 2004), whereas in the monkey, such ancillary projections were reported as weak or nonexistent (Pritchard et al. 2000). These stark neuroanatomical distinctions in NTS and PB projections are consonant with the phylogenetic differences observed in ascending homeostatic afferent activity from spinal and trigeminal levels (Craig 2002), and together these findings indicate that the evolutionary encephalization in primates is reflected in profound differences in afferent autonomic integration between rat and monkey. In primates, vagal afferent activity can be conveyed to the thalamus by major projections from both NTS and PB, and a focus of activation in the VMb should be expected.

Anatomy of central vagal projections

Studies in monkeys, cats and rats indicate that vagal afferents terminate mainly in the NTS and weakly in the superficial layers of the trigeminal dorsal horn, the area postrema, and the adjacent medullary reticular formation (Gwyn et al. 1985; Hamilton et al. 1987). Vagal afferent activity must be significant for neural control of the physiological condition of the body.

Prior physiological studies of central vagal afferent activation

Vagal activation of NTS and PB neurons is well documented (Loewy and Spyer 1990), but the physiological evidence at more rostral sites is incomplete. Vagal activation was observed in the cingulate, insular, and lateral sensorimotor cortices in rat and cat (for references, see Ito 2002; Ito and Craig 2003). This tripartite projection parallels the forebrain projections of the gustatory system (Benjamin and Burton 1968; Ogawa et al. 1985) and the lamina I homeostatic (sympathetic) afferent system in primates (Craig 2002) and is proposed as an archetypical pattern of parallel homeostatic afferent activation of limbic motor (cingulate), limbic sensory (insula), and viscerosomatic (lateral sensorimotor) cortical control regions (Craig 2002, 2004). In monkey, short-latency vagal activation was demonstrated in cingulate (Bachman et al. 1977) and lateral sensorimotor cortices (O’Brien et al. 1971), yet the tripartite pattern is apparent in functional imaging studies of visceral sensory activation in human cortex (Aziz et al. 2000; Critchley et al. 2000; Strigo et al. 2003). With regard to thalamus, an early report stated that vagal activation was recorded (with concentric bipolar electrodes) throughout medial thalamus and hypothalamus of the cat (Dell and Olson 1951); yet, no potentials were shown, most “primary” sites were near the nucleus submedius, and the reported
latencies (5–6 ms) were remarkably short. Two single-unit studies in monkey reported a variety of excitatory and inhibitory responses throughout medial thalamus, hippocampus, entorhinal cortex, and putamen at considerably longer latencies (Hallowitz and MacLean 1977; Radna and MacLean 1981). Activation was not reported in VMb in any of these reports, in contrast to the anatomical literature. In rat, vagal-activated units were reported in the region equivalent to VMb (“VPpc”), but almost no responses were reported in the adjacent Pf (Rogers et al. 1979; Saleh and Cechetto 1993; Zhang and Oppenheimer 2000), in contrast to the anatomical evidence in that species. Except for one report of microstimulation-evoked visceral sensation from the region of VMb in human patients (Lenz et al. 1997), there seem to be no other pertinent physiological findings. Possible reasons for this deficiency are that thalamic VEPs are not clearly visible in single trials or with single shocks, perhaps due to anesthesia (Ito 1994).

Thus the present identification of a VEP in Pf that is much larger and earlier than the VEP in VMb was unexpected. Our results provide a new insight into thalamic activation by vagal afferents and indicate that our understanding of afferent autonomic processing in the primate forebrain is very incomplete. Physiological and anatomical re-examinations of NTS and PB projections that convey vagal afferent activity in the primate are warranted.

**Functional significance**

The Pf nucleus is regarded as an evolutionarily old component of the intralaminar thalamus (Royce et al. 1991; Sadikot et al. 1992; Smith et al. 2004). The Pf projects to the basal ganglia, the subthalamic nucleus, and the substantia nigra, and it receives pallidal, cerebellar, tectal, and motor cortical inputs. Accordingly, it is regarded as an integral component of the striatal network controlling movement. Therefore vagal activation of Pf would be expected to affect motor control, and so a role for this VEP focus in the anti-seizure effects of VNS needs to be considered (see following text).

Our findings indicate a role for Pf in autonomic processing, and this is consistent with other interconnections of Pf with limbic forebrain regions, e.g., hypothalamus, substantia innominata, and n. accumbens. Neurons in Pf were labeled from anterior cingulate cortex in monkey (Vogt et al. 1987), and retrograde labeling in PB from Pf was reported in rat and cat (Krout and Loewy 2000; Royce et al. 1991). We are not aware of any prior physiological evidence relating Pf to autonomic processing, yet one can speculate that the robust heartbeat-evoked potential identified in electroencephalographic recordings (Pollatos and Schandry 2004), which might modulate emotional and attentional networks, could originate in the VEP foci in Pf and VMb.
Potential role of Pf in the anti-epileptic effects of VNS

VNS is recognized as an effective treatment for drug-resistant epilepsy in humans (Henry 2002; Schachter and Schmidt 2003). Trains of sub-millisecond pulses having low to moderate current intensities (which probably activate only myelinated afferent fibers) at 30–50 Hz produce abortive and prophylactic effects. The mechanisms of VNS action are unknown, but all reviewers assert that the thalamus must be an important component. Prior analyses of VNS actions in the rat suggested that noradrenergic modulation of thalamic activity is altered by vagal effects on the locus coeruleus (LC) (Krahl et al. 1998); yet, the lidocaine injections in LC underlying this suggestion unavoidably would have interrupted vagal input to the thalamus (including Pf) by way of PB, which lies immediately ventrolateral to LC. The hypothesis that the potent vagal input to Pf demonstrated by the present observations may be significant for the anti-epileptic actions of VNS is consistent with other observations. Clinical reports indicate that stimulation in the region of Pf or its main projection target, the basal ganglia, has anti-epileptic effects (Chkhhenkeli and Chkhhenkeli 1997; Velasco et al. 2001). Functional imaging studies indicate that VNS produces strong activation of the basal ganglia (e.g., Narayanam et al. 2002), which have been suggested to play a crucial role in epileptic seizures (Deransart and Depaulis 2002). The Pf may be essential for the propagation of theta waves associated with arousal (Marini et al. 1998; see also Juhasz et al. 1985), which is consistent with this hypothesis. Thus our findings strongly recommend that the role of Pf in the anti-epileptic actions of VNS be tested directly.

Notably, the VEP in VMb could also be important because the insular cortical region that it probably activates lies adjacent to, and may overlap with, the epileptogenic "area tempestas" described in rat and monkey (Gunderson et al. 1999; M. Dubach and V. Gunderson, personal communications). Yet, the VMB and insular cortex may be particularly relevant to the anti-depressive effects of VNS (see Craig 2002; Mayberg et al. 1999). Finally, other medial thalamic areas (Cassidy and Gale 1998; Zhang and Bertram 2002) may also be important for epilepsy.

N O T E A D D E D I N P R O O F : A new report by Nail-Boucherie et al (2005) has added direct evidence supporting the hypothesis that Pf has a critical role in the control of epilepsy.

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R E F E R E N C E S


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