Stimulation of the paraventricular nucleus modulates the activity of gut-sensitive neurons in the vagal complex

XUEGUO ZHANG, RONALD FOGEL, AND WILLIAM E. RENEHAN
Division of Gastroenterology, Henry Ford Health System, Detroit, Michigan 48202

Zhang, Xueguo, Ronald Fogel, and William E. Renehan. Stimulation of the paraventricular nucleus modulates the activity of gut-sensitive neurons in the vagal complex. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G79-G90, 1999.—There is good evidence that stimulation of the lateral hypothalamus excites neurons in the dorsal vagal complex (DVC), but the data regarding the role of the paraventricular nucleus (PVN) in vagal function are less clear. The purpose of this study was to clarify the effect of PVN stimulation on the activity of neurons in the DVC. We utilized extracellular and intracellular neuronal recordings with intracellular injections of a neuronal tracer to label individual, physiologically characterized neurons in the DVC of rats anesthetized with pentobarbital sodium. Most (80%) of the gut-sensitive dorsal motor nucleus of the vagus (DMNV) neurons characterized in this study exhibited a change in activity during electrical stimulation of the PVN. Stimulation of the PVN caused an increase in the spontaneous activity of 59% of the PVN-sensitive DMNV neurons, and the PVN was capable of modulating the response of a small subset of DMNV neurons to gastrointestinal stimuli. This study also demonstrated that the PVN was capable of influencing the activity of neurons in the nucleus of the solitary tract (NST). Electrical stimulation of the PVN decreased the basal activity of 66% of the NST cells that we characterized and altered the gastrointestinal response of a very small subset of NST neurons. It is likely that these interactions play a role in the modulation of a number of gut-related homeostatic processes. Increased or decreased activity in the descending pathway from the PVN to the DVC has the potential to alter ascending satiety signals, modulate vago-vagal reflexes and the cephalic phase of feeding, and affect the absorption of nutrients from the gastrointestinal tract.

vagus; gastrointestinal neurophysiology; dorsal motor nucleus of the vagus; nucleus of the solitary tract

IT IS WELL KNOWN that the hypothalamus modulates endocrine and autonomic function. The hypothalamus influences many aspects of autonomic function via its interactions with the pituitary, but there is also evidence that the hypothalamus may affect certain aspects of autonomic activity via direct descending projections to the dorsal vagal complex (DVC). Retrograde (3, 22, 27) and anterograde (8, 13) tracing studies have shown that there is a substantial projection from the paraventricular nucleus (PVN) and lateral hypothalamus (LH) to the DVC, with most descending fibers terminating in the ipsilateral brain stem. Electrophysiological data provide additional evidence that the hypothalamus plays an important role in the regulation of the vagal complex. The information regarding the role of the LH in the modulation of vagal activity is particularly clear. It has been demonstrated that stimulation of the LH excites neurons in the dorsal motor nucleus of the vagus (DMNV; Ref. 12) and increases the activity of axons in the hepatic branch of the vagus (32). It has also been shown that electrolytic lesions of the LH result in a rapid and strong reduction in vagal nerve activity (32, 33). It is reasonable to expect that modulation of vagal neuronal activity by the LH would have a number of effects on autonomic function. Indeed, this has been shown to be true, with numerous studies demonstrating that stimulation or ablation of the LH alters activities such as gastric acid secretion, glucose utilization, and gastrointestinal motility (see Ref. 6 for review).

Although the data from the LH physiology experiments are fairly consistent, studies that have examined the role of the PVN in vagal function have produced results that are more difficult to reconcile. Some investigators have reported data that are compatible with the hypothesis that the neurons in the DMNV are excited by activation of the PVN (9, 19, 29), but others have shown that the influence of the PVN may be more complex. For example, Banks and Harris (2) have shown that some DMNV neurons are excited by PVN stimulation (consistent with the LH effect), whereas others exhibit a decrease in activity. Furthermore, another group (33) has presented data indicating that vagal activity is increased following lesions of the PVN (suggesting that the PVN exerts a tonic inhibitory influence on the DMNV that is eliminated by the destruction of the subnucleus). Thus, although there is reason to believe that the PVN is capable of altering the activity of vagal neurons, the precise nature of this interaction is not clear. There are a number of factors that may be contributing to this discrepancy. One potential explanation for the conflicting results obtained in the PVN experiments is the possibility that different subgroups of DMNV and nucleus of the solitary tract (NST) neurons have different responses to stimulation of this region of the hypothalamus. Thus one subset of DMNV neurons may be excited by PVN stimulation, whereas another is inhibited by this input. If true, this scenario might indicate that the PVN has the capability to modulate the activity of discrete subsets of vagal neurons and may be able to regulate the function of specific regions of the gastrointestinal tract. This would have important implications for our understanding of the PVN's modulation of gastrointestinal function. It is also possible that investigators who have used standard extracellular recording techniques may have had difficulty distinguishing the responses that were associated with DMNV neurons from those
that were obtained from NST cells. This predicament is due, at least in part, to the substantial intermingling of NST and DMNV neurons at the border between these two nuclei. The situation is complicated further by the fact that a significant proportion of DMNV neurons send their dendrites into the overlying NST (5, 11, 16, 25, 34, 35). As a result, many of the hypothalamic axons that terminate in the NST may actually synapse on the dendrites of DMNV neurons. Although one might expect that the use of collision tests and latency data would permit the investigator to distinguish DMNV from NST neurons, our own experience has taught us that these methodologies are unreliable in cases in which one is relying on results obtained with stimulation of the subdiaphragmatic vagus nerve (see Ref. 35).

Answers to the questions posed above require that we employ a methodology that permits us to differentiate the response of NST and DMNV neurons with confidence. We have addressed this issue by utilizing an experimental paradigm that combines extracellular and intracellular neuronal recordings with intracellular injections of a neuronal tracer to label individual, physiologically characterized neurons in the DVC. This strategy has allowed us to determine the precise location of every neuron we have recorded in the vagal complex. The data we have obtained confirm that the neurons in the DVC are sensitive to stimulation of the PVN and provide some important insights into the role of the PVN in the regulation of NST and DMNV function.

MATERIALS AND METHODS

Animal preparation. Adult male Sprague-Dawley rats weighing 270–350 g were anesthetized with pentobarbital sodium (50 mg/kg ip), and supplemental doses of pentobarbital sodium were administered as needed to maintain a deep level of surgical anesthesia and muscle relaxation. A tracheotomy was performed, and a tube was inserted into the trachea for artificial ventilation with room air (100 strokes/min, 2.0–2.4 cm3 tidal volume). A midline abdominal incision was made to expose the abdominal vagus, stomach, and duodenum. Teflon-coated pure gold wire stimulating electrodes (76 µm outside diameter) were placed around the anterior and posterior branches of the subdiaphragmatic vagal nerve, ~1–2 cm above the gastroesophageal junction and immediately above the accessory and celiac branches of the vagus nerve. The stimulating electrodes were loosely sutured to the esophagus to limit displacement. An incision was made in the gastric corpus after the stimulating electrodes were placed, and a gastric efflux catheter was inserted into this opening and fixed to the greater curvature of the gastric corpus. An incision was then made immediately proximal to the pylorus, and two tubes were inserted into the gut, one oriented toward the stomach and the other oriented toward the duodenum. The tube oriented toward the stomach served as the gastric efflux catheter, whereas the duodenal tube served as the duodenal influx catheter. Finally, the gut was transected 10 cm distal to the ligament of Triitz. The resulting caudal open end was closed with silk sutures, and the proximal end was cannulated with a tube that served as the duodenal efflux catheter.

Stimulus presentation and neuronal labeling. The animals were placed in a Kopf small animal stereotaxic frame, and body temperature was maintained by a thermostatically controlled heating table that also warmed all perfusion fluids to body temperature. The brain stem was exposed by removing the atlanto-occipital membrane and a portion of the occipital bone. Beveled glass micropipettes (A-M Systems, Everett, WA; tip diameter of 0.08–0.1 µm, resistance of 50–70 MΩ), filled with 2.0% Neurobiotin (Vector Laboratories, Burlingame, CA) in 1 M KCl, were lowered into the vagal complex between 100 µm rostral and 400 µm caudal to the obex. Biphasic electrical pulses (0.5 ms duration, 0.5–3 mA, 1 Hz) delivered to the abdominal vagus were used as search stimuli. The recording micropipettes were advanced until a unit that was driven by the vagal stimulating electrode was encountered. All units driven by the stimulating electrode were tested for a response to duodenal or gastric distension as described previously (34–36). The distension was accomplished by raising the efflux catheter to a level that produced a 13-mmHg increase in intraluminal pressure (a nonpainful stimulus). The distension was maintained for 60 s. Unit discharges were amplified by an A-M Systems high-input impedance preamplifier and displayed and stored on an IBM-compatible Pentium computer with the use of Axotape software (Axon Instruments, Foster City, CA).

Neurons responding to either of the gastrointestinal stimuli were tested for a response to stimulation of the PVN. Stimulation was accomplished using a concentric bipolar electrode (Kopf, SNES-100) that was placed 2.0 mm caudal to bregma, 0.5 mm from the midline, and 8.5 mm from the skull surface. The PVN was stimulated with a 20- to 40-µA current (0.5 ms duration) at 15 Hz for 1 min (preliminary studies using frequencies between 5–40 Hz demonstrated that a 15-Hz PVN stimulation was the most effective stimulus for eliciting changes in DMNV or NST activity). The cell’s response to PVN plus gastrointestinal stimulation was tested if the neuron appeared stable (steady membrane potential, consistent action potential amplitude, and so forth) following PVN stimulation alone.

The recording micropipette was advanced after the response characterization until the neuron was impaled (passing small positive current pulses from the recording electrode facilitated this process). Penetration of the cell membrane was accompanied by a 20- to 40-mV drop in the voltage measured at the electrode tip, an increase in the amplitude of the action potential, and a shift from a bipolar to a monopolar action potential (see Ref. 15, for a depiction of this process). When this confirmation was completed, the cells were labeled with Neurobiotin (used to demonstrate the location of the neuron in the DVC) by passing 2- to 4-nA, 250-ms positive current pulses at 2–7 Hz for 1–6 h after the first injection. The rinse solution was followed by 500 ml of 0.9% saline containing 2,000 U/l heparin in 0.1 M sodium phosphate buffer (pH 7.3, room temperature) 1–6 h after the first injection. The rinse solution was followed by 500 ml of a fixative solution containing 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (4°C, pH 7.4). The brain was stored overnight in 0.1 M phosphate buffer containing 20% sucrose. Fifty-micrometer coronal sections through the brain stem were incubated in 0.4% Triton X-100 for 1.5 h at room temperature and then placed in avidin D-horseradish peroxidase (Vector Laboratories) for 2 h. The sections were incubated in a solution containing 100 mg dianinobenzidine, 5.0 ml of 1.0% cobalt chloride, and 4.0 ml of 1.0% nickel ammonium sulfate in 200 ml phosphate buffer (pH 7.4) for

Ammonium sulfate in 200 ml phosphate buffer (pH 7.4) for 5.0 ml of 1.0% cobalt chloride, and 4.0 ml of 1.0% nickel ammonium sulfate in 200 ml phosphate buffer (pH 7.4) for
Period 2

Prestimulus level of 1.73 electrical stimulation of the PVN caused an increase in response obtained during gastrointestinal distension, rons is illustrated in Fig. 1.

Period 3

Driven by the PVN-stimulating electrode at frequencies PVN. None of the PVN-sensitive neurons could be investigated responded to electrical stimulation of the eight of the 85 DMNV neurons characterized in this investigation.

Period 4

This 120-s trace was divided into four periods (30 s each) to test the effect of each stimulus. Period 1 represented basal spontaneous activity. Period 2 included the immediate response to the stimulus, whereas period 3 represented the late response. Period 4 contained the first 30 s after the stimulus was discontinued and therefore included any delayed response or changes induced by removal of the stimulus. We determined whether a neuron's response to a given stimulus was statistically significant by comparing the mean activity during periods 2–3 with the mean activity during the pre- and poststimulus periods (periods 1 and 4, respectively) using ANOVA and the Bonferroni test for multiple comparisons.

RESULTS

DMNV neurons: response to gastrointestinal stimuli.

We characterized and labeled a total of 85 DMNV neurons in this study. Most (84/85) of these neurons were inhibited by one or both of the gastrointestinal distension stimuli. Fifty-four of the 85 DMNV neurons were inhibited by both stimuli, whereas 30 neurons were inhibited by one stimulus and either excited by or unresponsive to the other. Intestinal distension reduced the mean activity of the total sample of DMNV cells from a prestimulus (period 1) level of 1.83 ± 0.16 (SE) Hz to 1.21 ± 0.24 Hz in period 2 (the first 30 s after stimulus onset) and 0.72 ± 0.17 Hz in period 3 (the second 30 s after stimulus onset). This decrease in activity in response to intestinal distension was statistically significant [P < 0.01 (ANOVA F = 7.3) and P < 0.05 (Bonferroni) for periods 2 and 3 compared with period 1]. Similarly, gastric distension reduced the mean activity from a prestimulus level of 1.64 ± 0.14 to 0.62 ± 0.12 Hz in period 2 and 1.08 ± 0.19 Hz in period 3. This distension-induced decrement in neural activity was also statistically significant [P < 0.01 (ANOVA F = 9.7) and P < 0.05 (Bonferroni) for both comparisons].

DMNV neurons: PVN effect on basal activity.

Sixty-eight of the 85 DMNV neurons characterized in this investigation responded to electrical stimulation of the PVN. None of the PVN-sensitive neurons could be driven by the PVN-stimulating electrode at frequencies >1.0 Hz. The average response profile for these neurons is illustrated in Fig. 1A. In contrast to the response obtained during gastrointestinal distension, electrical stimulation of the PVN caused an increase in the mean activity of these DMNV neurons. Stimulation of the PVN increased the average activity from a prestimulus level of 1.73 ± 0.26 to 2.60 ± 0.39 Hz in period 2 and 2.57 ± 0.54 Hz in period 3. This increase in activity was statistically significant [P < 0.01 (F = 10.6) for periods 2 and 3 compared with period 1 and P < 0.05 (Bonferroni)]. It is important to note, however, that PVN stimulation did not increase the spontaneous activity of all of the PVN-sensitive DMNV neurons. Although the majority (48/68) of the PVN-sensitive cells did exhibit an increase in activity, a substantial subset (20/68) responded to PVN stimulation with a decrease in activity. The mean response profile for the group that was excited by PVN stimulation is illustrated in Fig. 1B. Figure 1B indicates that electrical stimulation of the PVN had a dramatic effect on the basal activity of these neurons, increasing the mean activity from a prestimulus level of 1.57 ± 0.22 to 4.02 ± 0.51 Hz in period 2 and 3.78 ± 0.44 Hz in period 3 [P < 0.01 (ANOVA F = 12.1) and P < 0.05 (Bonferroni) for both comparisons]. The response profile of the subset that was inhibited by the PVN is illustrated in Fig. 1C. The activity of these neurons was decreased from a prestimulus level of 1.98 ± 0.24 to 0.91 ± 0.17 Hz in period 2 and 0.87 ± 0.18 Hz in period 3 [P < 0.01 (ANOVA F = 8.2) and P < 0.05 (Bonferroni) for both comparisons].

Examples of the responses exhibited by individual neurons in these two subgroups are presented in Figs. 2 and 3. Figure 2 illustrates the response profile of a DMNV neuron that was excited by electrical stimulation of the PVN. This neuron was completely inhibited by both intestinal (Fig. 2A) and gastric (Fig. 2B) distension. Conversely, electrical stimulation of the PVN increased the neuron's basal activity from 1.23 Hz in period 1 to 4.53 Hz in period 2 and 6.00 Hz in period 3 (Fig. 2C). The response profile illustrated in Fig. 3 shows the opposite response to PVN stimulation. In this instance, the DMNV neuron was almost completely inhibited by intestinal distension (Fig. 3A), moderately excited by distension of the stomach (1.2 Hz in period 1, 1.9 Hz in period 2, and 2.7 Hz in period 3; Fig. 3B; note that contrasting responses to intestinal and gastric distension are not uncommon in this system, see Ref. 5), and completely inhibited by stimulation of the PVN (Fig. 3C).

Effect of PVN stimulation on the DMNV response to gastrointestinal stimuli.

Thirty-two of the 68 PVN-sensitive cells that we recorded were sufficiently stable to test the combined effect of PVN and gastrointestinal stimulation. We found that the PVN stimulation parameters utilized in this investigation affected the gut-related response properties of six DMNV neurons. Figure 4 shows the electrophysiological properties of a DMNV neuron whose response to one of the two gastrointestinal stimuli was counteracted by PVN stimulation. Figure 4A demonstrates the response to intestinal distension. This stimulus caused a reduction in neuronal activity from a basal level of 0.36 to 0.03 Hz in periods 2 and 3. Distension of the stomach (Fig. 4B) caused a reduction in activity from a baseline level of 0.83 to 0.0 Hz in periods 2 and 3 (a complete inhibition). Stimulation of the PVN alone produced a substantial increase in activity, from 0.7 Hz in period 1 to 2.43 Hz in
period 2 and 1.30 Hz in period 3 (Fig. 4C). The effect of simultaneous presentation of PVN stimulation and intestinal distension is illustrated in Fig. 4D. The simultaneous administration of these two stimuli caused the neuron’s activity to increase from 0.60 Hz in period 1 to 1.73 Hz in period 2 and 1.60 Hz in period 3. Interestingly, the influence of the PVN on the activity of the DMNV neuron was very stimulus specific. The simultaneous presentation of the PVN and gastric stimuli resulted in a response that was virtually identical to that seen when the gastric stimulus was presented alone (compare Fig. 4E with Fig. 4B; note, however, that the poststimulus activity was greater following PVN + gastric distension).

NST neurons: response to gastrointestinal stimuli. A total of 31 neurons were recorded and labeled in the NST. The majority (29/31) of these cells were excited by at least one of the gastrointestinal stimuli, with 10 of the 31 cells excited by both gastric and duodenal distension [the remaining 19 neurons were excited by one gastrointestinal stimulus and inhibited (or had no response) to the other]. Intestinal distension increased the mean activity of the total sample of NST cells from a prestimulus level of $1.59 \pm 0.36$ to $3.13 \pm 0.58$ Hz in period 2 and $2.92 \pm 0.52$ Hz in period 3. This increase in activity in response to intestinal distension was statistically significant [$P < 0.05$ (ANOVA $F = 3.9$) and $P < 0.05$ (Bonferroni) for both comparisons]. Gastric distension also demonstrated a tendency to increase the mean neural activity of the NST neurons (period 1 = $1.75 \pm 0.40$ Hz, period 2 = $2.67 \pm 0.8$ Hz, and period 3 = $2.55 \pm 0.61$ Hz), but this apparent increase was not
Fig. 2. Response profile of a DMNV neuron that was excited by electrical stimulation of the PVN. Solid arrows indicate stimulus onset, and open arrows indicate stimulus removal. This neuron was completely inhibited by both intestinal (A) and gastric (B) distension. Conversely, electrical stimulation of the PVN increased the neuron’s basal activity (C).

Fig. 3. Response properties of a DMNV neuron that was almost completely inhibited by intestinal distension (A), moderately excited by distension of the stomach (B), and completely inhibited by electrical stimulation of the PVN (C). Solid arrows indicate stimulus onset, and open arrows indicate stimulus removal.
statistically significant when the entire sample of NST neurons was considered. It is important to note, however, that the majority (18/22) of the NST neurons that responded to gastric distension did exhibit a significant increase in activity during the presentation of this stimulus (the failure to obtain a significant effect when the entire sample was examined reflects the fact that the 4 NST neurons that were inhibited by the gastric stimulus exhibited a very dramatic decrement in activity, thus producing a large standard deviation when the response average was calculated).

NST neurons: PVN effect on basal activity. Electrical stimulation of the PVN altered the basal activity of 18 of the 31 NST neurons in our sample. One of the 18 PVN-sensitive NST neurons could be driven at frequencies \(1 \text{ Hz}\) (this neuron was capable of following 150 Hz, suggesting that it projected to the PVN).

Stimulation of the PVN at 15 Hz resulted in a significant [\(P < 0.01\) (\(F = 25.07\)] and \(P < 0.05\) (Bonferroni) for periods 2 and 3 compared with period 1] decrease in mean NST activity (Fig. 5A). Similar to the results obtained in the DMNV, the sample of NST neurons was composed of two distinct subsets. One group contained the majority (12) of the 18 NST neurons and was inhibited by the PVN (Fig. 5B). Electrical stimulation of the PVN decreased the mean activity of this group of neurons from a baseline level of 3.87 ± 0.09 to 1.37 ± 0.2 Hz in period 2 and 0.97 ± 0.16 Hz in period 3 (this decrease was statistically significant; \(P < 0.01\) (\(F = 52.3\)) and \(P < 0.05\) (Bonferroni) for both comparisons). An example of a neuron exhibiting this response profile is shown in Fig. 6. This cell was excited by both intestinal (Fig. 6A) and gastric (Fig. 6B) distension. In contrast to the excitatory response to
gastrointestinal stimuli, electrical stimulation of the PVN (Fig. 6C) reduced the neuron’s activity from a basal level of 5.93 Hz (in period 1) to 1.67 Hz in period 2 and 0.47 Hz in period 3 [P < 0.05 (Bonferroni) for both comparisons].

Six of the 18 PVN-sensitive NST neurons were excited by electrical stimulation of the PVN. The response of one such NST neuron is illustrated in Fig. 7. This particular neuron was not sensitive to distension of the intestine (Fig. 7A) but did exhibit a brief increase in activity following gastric distension (Fig. 7B). Although the neuron’s response to gastrointestinal stimulation was modest, it did show a robust response to PVN stimulation. Electrical stimulation of the PVN increased the neuron’s activity from a basal level of 0.5 to 3.76 Hz in period 2 and 2.96 Hz in period 3 [P < 0.05 (Bonferroni) for both comparisons].

Effect of PVN stimulation on the NST response to gastrointestinal stimuli. Only 2 of the 18 PVN-sensitive NST neurons studied in this investigation exhibited an altered response to gastrointestinal distension during PVN stimulation. The response profile of one of these neurons is presented in Fig. 8. This NST neuron responded to intestinal distension with an increase in activity from a baseline level of 1.8 to 2.4 Hz (in period 2; Fig. 8A). Gastric distension was also an effective excitatory stimulus, increasing the neuron’s activity from 1.7 to 2.8 Hz (Fig. 8B). Electrical stimulation of the PVN proved to be an inhibitory stimulus, however, reducing the cell’s activity from 2.5 to 1.4 Hz (Fig. 8C). Interestingly, the inhibitory influence of the PVN blocked the neuron’s response to gastric and intestinal distension (Fig. 8, D and E). In each instance, the cell failed to exhibit a valid response to distension during the simultaneous administration of the gastrointestinal and PVN stimulation [there was a trend that indicated that the response to gastric distension was reduced during period 3; there was also a valid increase in activity (an apparent “rebound” effect) following the cessation of the intestinal and PVN stimuli, Fig. 8D].

DISCUSSION

NST and DMNV response to PVN stimulation. Approximately 80% of the DMNV neurons and 58% of the NST neurons we characterized responded to electrical stimulation of the PVN. In each nucleus, the predominant effect of the PVN stimulation was contrary to the predominant effect of the gastrointestinal stimuli. For example, whereas most of the DMNV neurons we
Fig. 6. NST neuron that was excited by both intestinal (A) and gastric (B) distension but inhibited by electrical stimulation of the PVN (C). Solid arrows indicate stimulus onset, and open arrows indicate stimulus removal.

Fig. 7. Response properties exhibited by one of the 6 PVN-sensitive NST neurons that were excited by electrical stimulation of the PVN. Solid arrows indicate stimulus onset, and open arrows indicate stimulus removal. This particular neuron was not sensitive to distension of the intestine (A) but did exhibit a brief increase in activity after gastric distension (B). Electrical stimulation of the PVN resulted in a robust increase in the neuron's activity (C).
studied exhibited a decrease in activity during gastric and/or intestinal distension, the majority (59%) of the neurons that responded to electrical stimulation of the PVN were excited by this hypothalamic stimulus. Similarly, although 94% of the NST cells in our study were excited by gastric and/or intestinal distension, 67% of the PVN-sensitive NST neurons were inhibited by electrical stimulation of the hypothalamus.

Although it is tempting to speculate on the potential role(s) of the PVN-vagal interaction based on the major effect of the PVN in the NST and DMNV, we would remind the reader that each of the vagal nuclei contained two subsets of neurons: one excited and one inhibited by the PVN (see Fig. 9). It is quite possible that this diversity of responses provides an explanation for the apparent discrepancies in the results obtained in prior investigations of the PVN influence on vagal activity. It is understandable that many laboratories (e.g., Refs. 9, 19, 29) have concluded that the PVN excites neurons in the DMNV, given that this appears to be the dominant response. It is also clear that our results support the data presented by Banks and Harris (2), who have argued that the DMNV contains two neuronal subsets, with some cells excited by the PVN and others that are inhibited.

Given the nature of the interaction between NST and DMNV neurons, we must consider the possibility that the influence of the PVN on DMNV activity is actually an indirect result of its effect on neurons in the NST. There is a growing body of evidence to support the contention that many (if not most) of the gut-sensitive neurons in the NST exert an inhibitory influence on the DMNV. Accordingly, any input that acted to decrease the activity of NST neurons (such as increased activity in the PVN-NST pathway) would tend to increase the activity of a substantial number of the neurons in the

Fig. 8. Neurophysiological properties of a NST neuron that responded to intestinal (in period 2; A) and gastric (B) distension with an increase in activity. Electrical stimulation of the PVN resulted in a decrease in the cell's activity (C). Inhibitory influence of the PVN blocked the neuron's response to gastric and intestinal distension (D and E). In each instance, the cell failed to exhibit a valid response to distension during simultaneous administration of gastrointestinal and PVN stimulation (there was a trend that indicated that the response to gastric distension was reduced during period 3; there was also a valid increase in activity (an apparent "rebound" effect) following the cessation of the intestinal and PVN stimuli, D). Solid arrows indicate stimulus onset, and open arrows indicate stimulus removal.
DMNV (as we found when we recorded from neurons in this portion of the vagal complex). It is not clear, therefore, whether the responses we recorded in the DMNV during stimulation of the PVN reflected the influence of a direct or indirect pathway. The anatomic evidence would indicate that both scenarios are possible. A number of anterograde (8, 24, 30) and retrograde (27) labeling studies have shown that axons from the PVN terminate in both the NST and the DMNV. It would appear that an answer to this important question would depend on electrophysiological recording studies that examine the response of the DMNV to PVN stimulation in a model that eliminates any potential contribution from the NST. It is possible that this goal could be accomplished by inhibiting the activity of NST neurons via local injection of a reversible anesthetic such as lidocaine while stimulating the PVN and recording the activity of neurons in the DMNV.

The potential role of the descending PVN-vagal pathway. The multiple effects of the descending PVN pathway on vagal response properties provide a substrate for both excitatory and inhibitory influences on gastrointestinal function. As noted above in the discussion of the influence of the PVN on vagal neuron activity, this potential for diverse effects may explain the disparate results that have been obtained following electrical and/or chemical stimulation of the PVN (see Refs. 6 and 10 for reviews). For example, there is evidence that stimulation of the PVN evokes large increases in gastric acid secretion (20), a transient increase in gastric motility (21), and a general increase in parasympathetic activity (28), yet other laboratories have reported that stimulation of the PVN results in a decrease in gastric acid secretion (e.g., Refs. 26, 31). There are a number of possible explanations for these disparate results, including the activation of vagal vs. spinal pathways as suggested by Yoneda and Taché (31) and/or the selective excitation of cholinergic and noncholinergic pathways as proposed by Rogers and collaborators (21, 23). It is reasonable to suggest that our understanding of these interactions will improve as we obtain additional data regarding the neurochemistry and targets of the DMNV neurons that are excited or inhibited by the PVN. One issue that remains to be resolved is the importance of the small number of DVC neurons that exhibited an altered response to gastrointestinal stimuli in the presence of PVN stimulation. It is not clear, for example, whether the PVN can exert a meaningful influence on gastrointestinal activity if it only alters the distension-induced response of 19% of the gut-sensitive DMNV neurons and 11% of the gut-sensitive NST neurons. Despite this caveat, we would point out that PVN stimulation did alter the basal activity of a substantial number of DVC neurons. This influence, plus potential subthreshold changes in DVC electrophysiology (not examined in this study), may serve as an important regulatory function.

Finally, we would point out that the descending modulation of vagal activity by the PVN may play a role in other processes as well, such as feeding behaviors and the genesis or promulgation of feeding disorders. A descending input that alters the activity of DMNV neurons, for example, could lead to changes in the activity of the vagal afferents that would in turn influence the activity of NST neurons that participate in circuits related to appetite, satiety, and feeding behaviors (see Refs. 17 and 18).

Response to gastrointestinal stimuli. Almost all (84/85) of the DMNV neurons studied in this investigation exhibited a decrease in activity in response to gastric and/or duodenal distension. This finding replicates the results we (5, 34) and others (1, 4, 7, 14, 23) have obtained in similar studies of the vagal efferent response to gastrointestinal stimulation. In addition, we found that the majority of the neurons in the NST were excited by distension of the stomach and/or duodenum. This result is also consistent with data we have obtained in previous investigations (35, 37).

Technical limitations. There are a number of technical issues that should be considered when evaluating the results obtained in this investigation. First, we must consider the possibility that our PVN-stimulating electrode has stimulated fibers from other portions of the forebrain that pass through this region. We have examined this potential confound by employing a dual stimulating electrode in a limited (n = 3) number of control animals (unpublished results). The electrode had the ability to administer electrical and chemical stimuli and was used to inject the PVN with a small volume of 0.01 M glutamate. We found that this protocol resulted in a decrease in the activity of most of the NST neurons and an increase in the activity of most of the DMNV neurons that we recorded, a result that replicates the data presented in the present paper. We would propose that this finding supports the reliability of the data obtained using the electrical stimulating electrode. Of course, it is still possible that the electrical stimulation activated areas (e.g., the LH) adjacent to the PVN. Furthermore, it is likely that the stimulat-
ing electrode activated multiple neuronal subsets in the PVN. Unfortunately, this is an inherent difficulty in any study that employs electrical stimulating electrodes in the central nervous system and is difficult to address. The extant literature provides relatively little guidance in this regard, although a respected study by Rogers and Hermann [21] did use a similar PVN stimulation paradigm. These investigators stimulated the PVN with a tungsten electrode that delivered a 25-µA current (approximately the same magnitude used in our investigation) for 0.3 ms (similar to our 0.5 ms duration) at 10 Hz (similar to our 15 Hz frequency). We can state, therefore, that our stimulation parameters were consistent with a protocol used by another group that has investigated the role of the PVN in gastrointestinal function.

We were able to test the response of a relatively small (~50% of the total data set) number of neurons to the simultaneous presentation of gastrointestinal and PVN stimulation. This was due to the fact that we were able to maintain a stable recording for a limited amount of time, and we were often forced to skip the final portion of the stimulus presentation protocol so that we could inject the neuron with the label. Despite this limitation, the results we obtained do verify that the PVN has the ability to modulate the gastrointestinal response of a small number of NST and DMNV neurons. Furthermore, it is clear that the nature of this modulation can be quite variable. One factor that may have contributed to the fact that the PVN modulated the gastrointestinal response of a small number of vagal neurons may be the magnitude of the gastrointestinal stimuli. We have shown previously that the distension protocol employed in this study increases the intraluminal pressure in the stomach and duodenum to ~12–13 mmHg (see Ref. 5). This pressure is not considered to be noxious (15), but it is greater than the resting intraluminal pressure. We do not know whether the magnitude of the distension is substantially greater than the distension that occurs during normal feeding, but, if it is, it may have caused us to underestimate the number of vagal neurons whose response to gastrointestinal stimuli could be modulated by the PVN. It is possible that the distension pressure that we employed may have resulted in a greater response to the gastrointestinal stimuli than would occur during normal feeding, and this may have masked the response of some vagal neurons to stimulation of the PVN. It would be helpful to expand on the present study by utilizing a stimulating protocol that exposed the stomach and duodenum to a range of distension pressures (indeed, a similar “dose-response” strategy could also be employed to test the effect of various stimulating current amplitudes in the PVN).

In conclusion, the data obtained in this investigation demonstrate that descending inputs from the PVN have the ability to 1) alter the baseline activity of gut-sensitive NST and DMNV neurons and 2) modulate the response of a small number of vagal neurons to gastrointestinal stimuli. These interactions may play an important role in a number of gut-related homeostatic processes. Increased or decreased activity in the descending pathway from the PVN to the DVC has the potential to alter ascending satiety signals, modulate the cephalic phase of feeding, and affect the absorption of nutrients from the gastrointestinal tract. The study provides a foundation for future investigations that will examine the neuropharmacology of this and related forebrain-brainstem interactions.

We thank Drs. R. Alberto Travagl, Richard Rogers, and Kirsteen Browning for comments on the manuscript.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-53159.

Present address and address for reprint requests and other correspondence: W. E. Renehan, Neurogastroenterology Research, Henry Ford Health System, One Ford Place-2D, 6071 Second Ave., Detroit, MI 48202 (e-mail: wrenenha1@hfhs.org).

Received 2 September 1998; accepted in final form 31 March 1999.

REFERENCES


