Neurons in the vagal complex of the rat respond to mechanical and chemical stimulation of the GI tract

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Zhang, Xueguo, William E. Renehan, and Ronald Fogel. Neurons in the vagal complex of the rat respond to mechanical and chemical stimulation of the GI tract. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G331–G341, 1998.—Perfusing the duodenum with acid solutions dramatically reduces gastric motility and acid secretion. We propose that the presence of acid in the proximal small intestine initiates a vagovagal reflex that excites inhibitory neurons in the nucleus of the solitary tract (NST) and reduces the activity of the neurons in the dorsal motor nucleus of the vagus nerve (DMNV). However, results from several investigations suggest that the relevant circuit may not be as simple as we had believed. The present study was designed to address this dilemma by employing intracellular and extracellular recording and intracellular labeling techniques to provide direct information on the activity of neurons in the NST and DMNV during and after intestinal exposure to acid solutions. The results obtained prove that NST and DMNV neurons respond to HCl in the duodenum. In some instances, these neurons were very stimulus specific, although the majority of the cells in our sample (47% of NST neurons and 86% of DMNV neurons) also responded to distension of the stomach and/or duodenum. It is important to note, however, that many of the more broadly responsive neurons in the dorsal vagal complex were able to distinguish between mechanical and chemical stimulation of the gastrointestinal (GI) tract. Most of the NST neurons that responded to duodenal perfusion with HCl were excited by this stimulus. Conversely, activity of most of the DMNV neurons decreased after the onset of the HCl stimulus. These findings verify the existence of a vagovagal reflex pathway initiated by duodenal perfusion with acid. Presumably, this reflex would decrease gastric motility and acid secretion, reducing the amount of acid that enters the duodenum and ultimately protecting the intestinal mucosa.

nucleus of the solitary tract; dorsal motor nucleus of the vagus nerve; intracellular labeling; electrophysiology; mechanoreceptor; chemoreceptor

SENSORY INFORMATION FROM gastrointestinal receptors, particularly the data transmitted by vagal primary afferents, has been a focus of many investigations due to the recognized importance of this information in the regulation of satiety and gastrointestinal function (12, 16–19). Much of this work has concentrated on gastrointestinal mechanosensation, but there is substantial evidence that vagal primary afferent neurons sensitive to chemical stimuli also play an important role in gastrointestinal activity. It is now quite clear, for example, that perfusing the duodenum with acid solutions causes a dramatic reduction in gastric motility (13, 20, 24) and gastric acid secretion (21, 23). Given that at least some vagal primary afferents are sensitive to acid (4, 7, 10) and decreased vagal afferent activity causes a reduction in gastric acid secretion and gastric motility (see Ref. 18 for review), we propose that the presence of acid in the proximal small intestine initiates a vagovagal reflex that results in a reduction in the activity of the neurons in the dorsal motor nucleus of the vagus nerve (DMNV; the cells that give rise to the efferent fibers in the vagus nerve). Furthermore, because most vagovagal reflexes appear to involve inhibitory neurons in the nucleus of the solitary tract (NST) (19, 27, 28), we can also postulate that the acid-induced inhibition of DMNV activity is mediated by inhibitory neurons in the NST. This argument would appear to provide a relatively straightforward and logical explanation for the gastric secretion and motility data, but the results obtained in several other experiments suggest that the relevant circuit may not be as simple as we have proposed. Molan and Roman (11), for example, have shown that some vagal efferent fibers may exhibit a decrease in activity in the presence of intestinal HCl (as we would expect), but other efferents appear to be excited by this stimulus. The presence of vagal efferent fibers that are excited by the presence of HCl in the proximal intestine has also been noted by Blackshaw and colleagues (3). An immunohistochemical study by Zittel et al. (29) appears to cast additional doubt on the validity of the proposed circuit. This group has shown that perfusion of the intestine of awake rats with HCl had no effect on the number of c-fos-positive neurons in the NST (suggesting that NST neuronal activity is not increased by the presence of HCl in the small intestine).

It is clear that the extant literature contains a number of observations that are difficult to reconcile with our current understanding of the role of the dorsal vagal complex in the regulation of gastrointestinal function. The present study was designed to address this dilemma by providing direct information regarding the activity of neurons in the NST and DMNV during and after intestinal exposure to acid solutions. Intracellular and extracellular recording techniques were combined with an intracellular labeling protocol to characterize and label individual gut-sensitive neurons in the presence of intestinal and gastric distension and perfusion of the intestine with HCl. We believe that the resultant data 1) provide a clear demonstration of the NST and DMNV response to acids, 2) help to explain the seemingly contradictory evidence obtained in prior studies, and 3) make a significant contribution to our understanding of the central nervous system control of gastrointestinal function.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Henry Ford Health Sciences Center. Sprague-Dawley male rats were anesthetized with an intraperitoneal injection of pentobarbital so...
duodenum (45 mg/kg), and the trachea was cannulated for ventilator-assisted respiration (Harvard rodent ventilator, South Natick, MA). A midline abdominal incision exposed the abdominal vagus nerve, the stomach, and the duodenum. Teflon-insulated pure gold wire stimulating electrodes (76 µm OD) were placed around the anterior and posterior branches of the subdiaphragmatic vagus nerve, −1–2 cm above the gastroesophageal junction and immediately above the accessory and celiac branches. These stimulating electrodes were fixed to the esophagus and stomach to prevent displacement.

Each animal was exposed to gastric and duodenal distension as well as perfusion of the duodenum with an acid solution. Gastric distension was accomplished by using an influx catheter (4.0 mm ID) that was placed in the most dependent portion of the greater curvature of the stomach (care was taken not to damage the extrinsic nerves innervating the stomach and duodenum) and an efflux catheter that was inserted through a small incision in the pylorus. The duodenum was distended and perfused with HCl via an influx catheter in the duodenal bulb and an efflux catheter that was placed 10 cm distal to the ligament of Treitz.

The abdominal incision was closed, and each rat was placed in a Kopf small animal stereotaxic frame after placement of the gastric and duodenal catheters. While in the stereotaxic frame, body temperature was maintained by a heating platform that sustained the temperature of the animal and the perfusion fluids at 37°C. The brain stem was exposed by removing the atlanto-occipital membrane and a portion of the occipital bone. Beveled glass micropipettes (tip diameter, 0.08–0.1 µm; resistance = 50–70 MΩ) filled with 2.0% Neurobiotin (Vector Laboratories) in 1 M KCl were lowered into the NST or DMNV between 100 µm rostral and 400 µm caudal to the obex. Search stimuli consisted of biphasic pulses (0.5-ms duration, 3 mA, 1 Hz) delivered to the abdominal vagus nerve. Recording micropipettes were advanced until a unit that responded to stimulation of the abdominal vagus nerve was encountered. The response of the neuron to perfusion of the stomach and the duodenum with 0.9% saline was determined while the gastric or duodenal efflux catheter was in the plane of the animal and again when the efflux catheter was elevated 20 cm above the plane of the animal. We have shown previously (5) that this protocol elevates intragastric and intraduodenal pressure by −12–13 mm Hg. The presentation of the gastric and duodenal distension stimuli was followed by the delivery of the acid stimulus with the efflux catheter in the plane of the animal. Acid concentrations used in the study are discussed in Determination of Optimal Acid Stimulus Concentration.

The change in firing rate in response to distension and perfusion with HCl was tested at least three times. If the response was equivocal, the stimulus was presented up to five times. Unit discharges were amplified by a high-input impedance microelectrode amplifier (A-M Systems, Everett, WA) and displayed and stored on an IBM-compatible computer with the use of Axotape software (Axon Instruments, Foster City, CA) at an acquisition rate of 2.5 kHz. After response characterization, the micropipette was advanced until the NST or DMNV neuron was impaled (this process was facilitated by passing small positive current pulses from the recording electrode). Penetration of the cell membrane was accompanied by a 20–40 mV drop in the voltage potential, an increase in the amplitude of the action potential, and a shift from a bipolar to a monopolar action potential (see Ref. 28 for an illustration of this process). Receptive fields were checked before Neurobiotin injection to confirm that the impaled cell was the cell that had been characterized. We have learned that we can only allow the electrode tip to be in the intracellular compartment for ~6 min before damaging the neuron and causing a degradation of labeling, so most of the electrophysiological characterization was performed before penetrating the cell membrane. After confirming that the response properties were the same, cells were labeled with Neurobiotin by passing 2- to 4-NA, 250-ms positive current pulses at 2 Hz for ~2–7 min. The injection was stopped if at any time the resting potential returned to prepenetration levels. A maximum of two injections was attempted on each side.

Rats were administered a lethal (100 mg/kg ip) dose of pentobarbital sodium and perfused through the heart with 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 fixative containing 1% paraformaldehyde and 2.5% glutaraldehyde, in 0.1 M phosphate buffer (4°C, pH 7.4). The brain stem was stored overnight in 0.1 M phosphate buffer containing 20% sucrose. Tissue was developed with avidin-D-horseradish peroxidase (Vector Laboratories) solution containing cobalt and nickel to enhance visualization of Neurobiotin-labeled neurons. Sections were mounted on gelatin-coated slides, and some sections were counterstained with neutral red to facilitate identification of NST and DMNV borders.

Neurophysiological Analysis

Neuronal response properties were examined using the Datapac software system (Run Technologies, Laguna Niguel, CA). Peristimulus time histograms were constructed for the period beginning 30 s before and ending 90 s after initiation of distension or presentation of HCl (the stimuli were maintained for 60 s). This 120-s trace was divided into four periods (30 s each) to test the effect of gastric and duodenal stimuli. Period 1 represented basal spontaneous activity. Period 2 included the immediate response to the stimulus, and period 3 represented the late response. Period 4 was the first 30 s after the stimulus was discontinued and therefore included any delayed response or changes induced by removal of the stimulus. To determine whether a given response was significantly more or less than the baseline, the mean activity during periods 2–3 was compared with the mean activity during the pre- and poststimulus periods (periods 1 and 4, respectively) using analysis of variance, with the Tukey B-test employed for post hoc comparisons. Only corrected P values <0.05 were considered statistically significant.

Morphometric Analysis

Three-dimensional reconstructions of individual Neurobiotin-labeled neurons, digitized at a magnification of ×400 (×40 dry objective), were made using the Eutectic neuron tracing system (Eutectic Electronics, Raleigh, NC). The integrity of each reconstruction was verified using the "mathematical correctness" subroutine of the Eutectic neuron tracing system. This subroutine tests the mathematical validity of the reconstructed neuronal processes using several measures [such as number of origins per process (correct value is 1) and the number of branch endings per process (correct value is 1 more than the number of branch points)] and allows the investigator to identify many of the more common data entry errors. Optical and physical compression of the material did occur in the plane of focus, but a compression factor was obtained for each individual section and the neuronal components in each section were rescaled to 50 µm (original thickness at the time of sectioning).
Sixteen morphological features were assessed: 1) total dendritic length, 2) total membrane surface area (includes soma), 3) total internal volume (includes soma), 4) soma form factor (FF; a measure of circularity for which a value of 1.0 indicates a perfect circle and 0 indicates a line; \( FF = 4\pi A / P^2 \), where \( A \) equals the somatic cross-sectional area in the coronal plane and \( P \) equals the perimeter of the soma in the coronal plane), 5) somatic cross-sectional area, 6) total number of dendritic branches, 7) highest dendritic branch order, 8) number of dendritic spines, 9) spine density, 10) number of fiber swellings, 11) swelling density, 12–14) mediolateral, rostrocaudal, and dorsoventral extent of the dendritic arbor, 15) soma-dendritic receptive area (the area of a convex two-dimensional (coronal plane) polygon that circumscribes the neuron, touching the outermost endings of the dendritic arbor), and 16) average length of dendritic branch segments. These features were chosen based on their utility in prior studies of structure-function relationships in the NST and DMNV (22, 27, 28).

Determination of Optimal Acid Stimulus Concentration

We characterized the response of NST and DMNV neurons to intestinal perfusion with 0.01, 0.1 (an acid concentration used in several prior studies; see Ref. 13), and 0.15 N HCl in a series of preliminary experiments. We found that the 0.01 N HCl stimulus failed to produce a reliable response, whereas there was no significant difference between the responses to 0.1 and 0.15 N HCl. Most of the cells reported in this manuscript were characterized using 0.15 N HCl, but the cells characterized with 0.1 N HCl in this preliminary phase of the study were pooled with the 0.15 N HCl data set to arrive at our final sample size.

RESULTS

Neurons in the NST

Response to mechanical and chemical stimuli. A total of 72 NST neurons were characterized and labeled (only 66 neurons were filled completely and subjected to computer reconstruction, but all contained enough label to permit the accurate assessment of the location of each cell within the vagal complex). It was possible to place each cell into one of seven major groups based on the response to perfusion of the duodenum with HCl, intestinal distension, or gastric distension (Table 1). Table 1 shows a number of important characteristics of the NST response to the acid and chemical stimuli. It is clear, for example, that most (86%) NST neurons were excited by the effective stimulus or stimuli. It is also apparent that many (58%) of the neurons in the NST responded to more than one stimulus. In fact, 19 of the 72 neurons responded to all stimuli. These neurons were labeled “CONV” to indicate the fact that they received convergent mechanical and chemical input. Eight of these neurons were excited by all three stimuli, six were excited by both duodenal stimuli but inhibited by gastric distension, and one neuron was inhibited by all three stimuli. The remaining four cells had different patterns of response to the three stimuli.

Twenty-two neurons responded to only two of the three stimuli. Eighteen of these 22 neurons were excited by both stimuli and four were inhibited by both stimuli.

Figure 1 depicts a neuron that belongs to the convergence subset. The soma of the neuron was located in the left medial NST (mNST). The dendrites of the cell were oriented primarily dorsomedial and ventrolateral to the soma. The dorsomedial dendrites proceeded through the subpostrema NST (a region of the NST that exhibits intense endogenous peroxidase activity after prolonged stimulation of the subdiaphragmatic vagal nerve and is largely coexistent with the commissural region of the NST, see Ref. 28) and terminated in the area postrema. The cell emitted dendrites that terminated in the mNST as well, with one branch entering the subjacent DMNV. The axon left a lateral primary dendrite and terminated in the lateral mNST. Figure 1, B–D, shows the response of this neuron to intestinal distension, gastric distension, and perfusion of the duodenum with 0.15 N HCl. Each of these stimuli elicited a significant increase in the activity of the NST neuron. Distending the intestine (Fig. 1B) increased the activity from a baseline (period 1) level of 0.6 to 1.3 Hz in the first 30 s after stimulus onset (period 2) and 1.7 Hz in the second 30-s period after stimulus onset (period 3). Distending the stomach (Fig. 1C) increased the activity from 0.5 Hz in period 1 to 1.3 Hz in period 2 and 1.9 Hz in period 3. Finally, perfusing the intestine with HCl (Fig. 1D) produced a change from a baseline of 0.5 to 1.5 Hz in period 2 and 4.3 Hz in period 3. In this instance and in several others, we observed that the increase in neuronal firing rate continued after the saline flush that removed the acid from the lumen. This change in activity is not due to a mechanical response to the saline flush, as none of the neurons that were included in this sample responded to saline flush alone.

Some NST neurons responded to one or both of the mechanical stimuli in a manner that differed from their

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NST, nucleus of solitary tract; GD, gastric distension; ID, intestinal distension. CONV, neurons that received convergent mechanical and chemical input. n = no. of neurons.
response to HCl. The cell shown in Fig. 2 provides an example of this response profile. This neuron exhibited a statistically significant response to all stimuli. However, whereas the neuron exhibited a moderate but significant increase in activity during intestinal distension (stimulus onset is indicated by the closed arrow and stimulus removal is indicated by the open arrow), C and D: the response of the neuron to gastric distension and perfusion of the duodenum with 0.15 N HCl. AP, area postrema; NST, nucleus of solitary tract; sNST, subpostremal region of the NST; mNST, medial subnucleus of the NST; DMNV, dorsal motor nucleus of the vagus nerve; CC, central canal.

Fig. 1. Morphology and physiology of an acid-sensitive neuron in the NST. A: the neuron as it would be seen in a coronal/transverse section through the caudal brain stem. B: the response of the cell to intestinal distension (stimulus onset is indicated by the closed arrow and stimulus removal is indicated by the open arrow). C and D: the response of the neuron to gastric distension and perfusion of the duodenum with 0.15 N HCl. AP, area postrema; NST, nucleus of solitary tract; sNST, subpostremal region of the NST; mNST, medial subnucleus of the NST; DMNV, dorsal motor nucleus of the vagus nerve; CC, central canal.

The differential sensitivity to mechanical and chemical stimuli is perhaps most apparent when one examines the HCl group. The six neurons in this category were only sensitive to the HCl stimulus. An example of one such neuron is presented in Fig. 3. This small but complex neuron was located in the dorsal rostral NST. Figure 3, B and C, depicts the failure of the cell to respond to either of the distension stimuli. In contrast, perfusion of the duodenum with 0.1 N HCl elicited a significant response (period 1 = 0.2 Hz, period 2 = 0.7 Hz, and period 3 = 0.7 Hz) that was maintained for the duration of the stimulus presentation.

Relationships between neuronal structure and function. Our ability to label and reconstruct the majority of the neurons recorded in the NST allowed us to examine potential relationships between neuronal morphology and physiology. A number of structure-function relationships were identified when we focused on those NST neurons that responded to HCl (these neurons could belong to the CONV, HCl and intestinal distension, HCl and gastric distension, or HCl groups). We found that the neurons that were inhibited by the stimulus (n = 6) had longer dendritic branch segments (117.8 ± 11.3 vs. 84.8 ± 6.6 µm) than the neurons that were excited by duodenal HCl (n = 31; P = 0.04). The neurons that were inhibited by HCl also possessed larger somata (237.0 ± 42.2 vs. 62.2 ± 11.1 µm² in the transverse plane) than the cells that exhibited an increase in activity during acid perfusion (P < 0.01).
Neurons in the DMNV

Response to mechanical and chemical stimuli. Data were gathered on the DMNV response to gastric and duodenal distension and perfusion of the duodenum with HCl from a total of 61 neurons (57 of the 61 neurons were judged to be labeled in their entirety). A summary of the response properties of these DMNV neurons is shown in Table 2. From the data in this table, we can make two important general observations. First, it is evident that the majority (80%) of the DMNV neurons responded to all three stimuli (the CONV group). Second, it is clear that most DMNV neurons were inhibited by the mechanical and/or chemical gastrointestinal stimuli. Only 16% of the responses to mechanoreceptor stimuli and 17% of the responses to duodenal acid were excitatory.

Figure 4 portrays the morphology and physiology of one of the neurons in the CONV group. The soma of this neuron was located in the ventral aspect of the middle region of the right DMNV. The soma emitted dendrites that arborized in the medial and lateral DMNV as well as in the medial NST (most of the DMNV neurons labeled in this study possessed dendrites that entered the NST) and dorsal hypoglossal nucleus. It was possible to trace the axon to the ventrolateral border of the medulla, where it joined the vagus nerve. Figure 4, B–D, shows the effects of intestinal distension, gastric distension, and perfusion of the duodenum with 0.15 N HCl. In each case, the gastrointestinal stimulus produced a total inhibition of neuronal activity. Interestingly, the response of the cell to mechanical and chemical stimulation of the duodenum was maintained (with the exception of 1 spike after the release of the intestinal distension stimulus) for a long period beyond the cessation of the stimuli. In fact, the inhibition lasted for over 2 min after the stimulus was removed. Figure 4E shows the response of the neuron to a train of stimuli (150 Hz) delivered to the subdiaphragmatic vagus nerve. The neuron was able to follow this frequency and exhibited a latency of ~90 ms, which is slightly less...
than the average response latency of 104.8 ± 1.9 ms. The reduced amplitude of the second and third responses to the stimulus train is consistent with other studies of DMNV electrophysiology (e.g., Refs. 5, 8, 26) and is believed to be the result of an initial segment-somatodendritic block. The subdiaphragmatic vagus nerve stimulating electrodes were ~10.0 cm from the recording site, resulting in an average conduction velocity of ~1 m/s, in the range typically associated with small-diameter unmyelinated axons.

In most cases, the response of a given DMNV neuron to HCl replicated its response to gastric and/or intestinal distension, although the relative magnitude of the responses may have differed. This was not true of all DMNV neurons, however. Eighteen neurons showed an ability to differentiate among the stimuli. In one in-

![Table 2. DMNV response to mechanical and chemical stimuli](Fig. 3. Structure and function of an NST neuron that only responded to perfusion of the duodenum with 0.1 N HCl. See Fig. 1 legend for conventions and abbreviations for A–D. E: neuronal response to a single stimulation of the subdiaphragmatic vagus nerve.

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DMNV, dorsal motor nucleus of the vagus nerve.
stance, a DMNV neuron was excited by HCl but failed to respond to either gastric or intestinal distension (HCl group in Table 2). In other instances, DMNV neurons responded to HCl and at least one of the two mechanical stimuli, but the nature of the response to intestinal perfusion with HCl differed from the response to distension. An example of this response profile is presented in Fig. 5. Note that the spontaneous activity of the cell was dramatically inhibited by intestinal (Fig. 5B; period 1 = 1.0 Hz, period 2 = 0.0 Hz, period 3 = 0.16 Hz) and gastric (Fig. 5C; period 1 = 0.7 Hz, period 2 = 0.0 Hz, period 3 = 0.0 Hz) distension, but perfusion of the intestine with HCl (Fig. 5D) elicited a substantial increase (period 1 = 0.7 Hz, period 2 = 3.3 Hz, period 3 = 4.9 Hz) in the firing rate.

Relationships between neuronal structure and function. There was a difference between the neurons that were excited by HCl and the neurons that were inhibited by this stimulus. We found that the cells that were excited by the acid stimulus (8 neurons reconstructed) had longer dendrites (5,034.4 ± 687.7 µm; P = 0.03) than the cells that were inhibited (n = 42). The cells that were excited by HCl also exhibited larger somata (464.9 ± 58.6 µm² vs. 333.2 ± 17.3 µm² in the transverse plane; P < 0.01).

Location of neurons in the vagal complex. The NST neurons characterized in this study were located in the subpostremal region (71%), medial subnucleus (23%), and the gelatinous subnucleus (16%). Ninety-five percent of the cells were located between 100 µm rostral and 400 µm caudal to the obex, a region that receives sensory information from the stomach and duodenum (28). All DMNV neurons were located in the medial two-thirds of the nucleus, with the same rostrocaudal extent as the NST neurons. There was no geographic segregation of the neurons by physiological subtype.

DISCUSSION

The results obtained in this study prove that neurons in the NST and DMNV respond to the presence of HCl in the duodenum. In some instances, these neurons
were very stimulus specific, such as the six NST neurons that only responded to HCl. More commonly, the cells in our sample (47% of the neurons in the NST and 86% of the neurons in the DMNV) also responded to distension of the stomach and/or duodenum. It is important to note, however, that a large number of the more broadly responsive neurons in the dorsal vagal complex were also able to distinguish between mechanical and chemical stimulation of the gastrointestinal tract. These neurons would typically exhibit one response to the chemical stimulus (e.g., an increase in activity) and another (e.g., a decrease in activity) to one or both of the mechanical stimuli.

Most of the NST neurons that responded to perfusion of the proximal small intestine with HCl were excited by this stimulus. This feature is common to most NST neurons that respond to gastrointestinal stimuli (see Ref. 28). Conversely, the majority of the DMNV neurons exhibited a decrease in activity after the onset of the HCl stimulus, typical of the DMNV response to gastric or intestinal mechanical stimulation (see Ref. 5).

This study also uncovered a number of relationships between the structure and response properties of neurons in the NST and DMNV that are affected by duodenal acid perfusion. We found, for example, that the NST neurons that were inhibited by HCl had longer dendritic branch segments and larger somata than the neurons that were excited by the presence of acid in the duodenum. When we examined the morphology of acid-sensitive neurons in the DMNV, we found that the converse was true, with the cells that were inhibited by HCl exhibiting shorter dendrites and smaller somata than the neurons that responded with an increase in activity.

**NST and DMNV Response to HCl**

There is general agreement that some (perhaps many) vagal primary afferents are excited by the presence of acid in the duodenum (see Refs. 10, 12 for reviews). Because most vagal afferents terminate on neurons in the NST, it is reasonable to suggest that this excitatory input would result in an increase in the activity of NST neurons. Furthermore, given the large body of circumstantial evidence indicating that many of the NST neurons that project to the DMNV are inhibi-

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**Fig. 5.** DMNV neuron inhibited by intestinal (B) and gastric (C) distension but excited by perfusion of the duodenum with HCl (D). E: neuronal response to a single stimulation of the subdiaphragmatic vagus nerve.
tor (5, 27, 28), we might expect that the typical DMNV neuron would respond to the presence of acid in the duodenum with a decrease in activity. Indeed, this is the result obtained in the present investigation. As we noted earlier, however, one laboratory has provided evidence that NST neurons may not be excited by intestinal acid (29) and at least two laboratories (3, 11) have shown that many vagal efferents may be excited by this stimulus. Although the results of these prior studies may seem to contradict our data, we believe that some of these apparent contradictions can be placed in the proper perspective after a closer examination of the methods that were employed. Miolan and Roman (11), for example, did not measure vagal efferent activity directly, but instead used a crossed-reinnervation paradigm that measured the activity of motor units in the diaphragm to indicate the activity of vagal efferents. Although this electromyographic technique offered the advantage of examining the vagal response to intestinal stimulation in awake animals, it obviously provides an indirect measure of vagal efferent activity and is complicated by the many difficulties that one encounters when a nerve is transected and the proximal stump of one nerve is sutured to the distal end of another (in this instance the transected vagus nerve was connected to the cut phrenic nerve).

Our results also differ from those of Blackshaw et al. (2, 3), who reported that chemical stimulation had a "clear-cut" effect on the activity of 29% of vagal efferents during perfusion of the antrum with 150 mM HCl and on 19% of vagal efferents during perfusion of the duodenum and jejunum with acid. In both experiments the predominant response was an increase in activity. There are several possible explanations for the differences between our results and those of Blackshaw et al. (2, 3). One possible explanation may concern species differences. The present study used a rat model, whereas Blackshaw and co-workers (2, 3) studied the ferret. It is also possible that Blackshaw and colleagues (2, 3) may have recorded a secondary motor response to their chemical stimulus, since the presentation of the HCl often elicited a retching/emetic response (this phenomenon appears to have been more common after perfusion of the stomach with HCl). A third possible reason for the discrepant results concerns differences in the experimental technique. The teased-fiber studies conducted by Blackshaw et al. (2, 3) may be subject to interpretive difficulties. Unfortunately, the investigators do not state how they distinguished afferent units from efferent units in their studies. Therefore, we must consider the possibility that at least some of the efferents that exhibited an increase in activity in response to gastric or intestinal HCl may in fact have been primary afferents. Fortunately, this is an area where our results may prove particularly useful. Our data confirm that some DMNV (i.e., efferent) units are excited by the presence of acid in the intestine, but most (83%) are inhibited. We know that these neurons were in fact in the DMNV because they were labeled with Neurobiotin. Thus there would appear to be agreement regarding the fact that HCl excites some vagal efferents, but we contend that this population is very much outnumbered by the efferents that exhibit a decrease in activity.

The apparent contradiction between our NST data and the results obtained in the c-fos study conducted by Zittel et al. (29) is more difficult to reconcile. Zittel and co-workers (29) found that perfusion of the intestine of awake rats had no effect on the number of fos-positive neurons in the NST (implying that there was no increase in the activity of these cells). One could maintain that the c-fos technique provides an indirect measure of neuronal activity and therefore may be misleading, but the fact that the investigators did demonstrate fos-like immunoreactivity in the NST when the intestine was perfused with lipid or glucose weakens this argument. It is possible that the number of fos-positive cells and/or the intensity of the fos labeling after exposure to the lipid or glucose may be dependent on the fact that the concentrations of the nutrient stimuli were quite high (as noted in Zittel et al. (29)). The 0.1 N HCl stimulus may not have induced a level of activity in the NST that was high enough to be detected by the c-fos technique.

Relationships Between Neuronal Structure and Function

Morphological features have been used by several investigators to classify neurons in the central nervous system (see Ref. 22 for review). Typically, these investigators have been interested in identifying potential correlations between specific morphological features and the physiological response properties of a given neuron. As one might expect, these efforts to determine relationships between neuronal structure and function have been most fruitful in cases in which the investigator has been able to label neurons with known response properties (14, 15, 28). In the present study, we have demonstrated that NST neurons that are excited by the presence of acid in the duodenum can be distinguished from those neurons that are inhibited by this stimulus on the basis of certain structural features. Specifically, we found that neurons that exhibited a decrease in activity during perfusion of the duodenum with HCl had longer dendrites and larger somata than the cells that were excited during acid perfusion. These findings are virtually identical to those reported for NST neurons that respond to duodenal distension (28), raising the possibility that the longer dendrites and larger somata are permitting the receipt of more inhibitory inputs. There is certainly abundant evidence to support the contention that inhibitory interactions modulate the response properties of NST neurons. Wang and Bradley (25) and Liu et al. (9) have demonstrated clearly that most (if not all) neurons in the rostral NST are highly sensitive to inhibitory neurotransmitters. If the neurons in the caudal NST exhibit a similar sensitivity, the longer dendrites may be associated with an inhibitory response due to the fact that these neurons receive more inhibitory synapses. It would be extremely helpful to know whether the synapses on the dendrites of these NST neurons were predominantly...
excitatory or inhibitory (such data could be obtained using combined immunocytochemical and ultrastructural techniques). It would also be very useful to know more about the synaptic inputs to DMNV neurons, given that the DMNV neurons that were inhibited by HCl had shorter dendrites and smaller somata than the neurons that were excited by this stimulus (in this case we would predict that the DMNV neurons that were excited by HCl received more excitatory inputs). It is likely that this issue will not be resolved until this information is available.

The Potential Role of the NST and DMNV Response to HCl

Perfusion of the duodenum with acid decreases proximal gastric motility (1, 12, 20) and gastric acid secretion (21) and may initiate bicarbonate production by the pancreas and duodenal mucosa (6). It has been suggested that each of these effects is aimed at limiting and/or counteracting the influx of additional acid into the duodenum (6, 21). The response properties of the NST and DMNV neurons studied in the present investigation would appear to illustrate physiological and anatomic substrates for this process. We have shown that neuronal subsets in each nucleus are capable of distinguishing acid stimuli from mechanical stimuli. Most acid-sensitive NST neurons were excited and most acid-sensitive DMNV neurons were inhibited by this stimulus (these response profiles are consistent with the postulate that the NST projection to the DMNV is primarily inhibitory; see Refs. 19, 27, 28). One can therefore envision a pathway that includes acid-sensitive vagal primary afferents that terminate on and excite inhibitory neurons in the NST. These NST neurons would inhibit the activity of a subset of DMNV neurons and would thereby produce a decrease in gastric motility and gastric acid secretion. Presumably, the result of this reflex would be a decrease in the amount of acid that enters the duodenum, ultimately protecting the intestinal mucosa.

What is the functional role of the DMNV neurons that are excited by visceral stimuli? Although our experimental paradigm did not allow us to determine the target for the physiologically characterized DMNV neurons, we do know that the axons of these neurons exit the brain stem. These neurons could innervate structures other than the stomach or duodenum. A second possibility is that these neurons synapse on nonadrenergic, noncholinergic neurons (e.g., nitric oxide- or vasoactive intestinal polypeptide-positive neurons) in the myenteric plexus and mediate the inhibition of gastric motility by the excitation of these enteric neurons.

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