

Enhancement of antral contractions and vagal afferent signaling with synchronized electrical stimulation

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Peles, Shachar, Jaime Petersen, Ricardo Aviv, Shai Policker, Ossama Abu-Hatoum, Shlomo A. Ben-Haim, David D. Gutterman, and Jyoti N. Sengupta. Enhancement of antral contractions and vagal afferent signaling with synchronized electrical stimulation. *Am J Physiol Gastrointest Liver Physiol* 285: G577–G585, 2003. First published June 11, 2003; 10.1152/ajpgi.00109.2003.—Gastric filling activates vagal afferents involved in peripheral signaling to the central nervous system (CNS) for food intake. It is not known whether these afferents linearly encode increasing contractions of the antrum during antral distension (AD). The aim of this study was to investigate effects of AD and electrically enhanced antral contractions on responses of vagal afferents innervating the antrum. Single-fiber recordings were made from the vagal afferents in anesthetized male Long-Evans rats. Antral contractions were measured with a solid-state probe placed in the antrum. A nonexcitatory electrical stimulation (NES) inducing no smooth muscle contractions was applied during the ascending phase of antral contractions to enhance subsequent antral contractions. Fifty-six fibers identified during AD (1 ml for 30 s) were studied through different types of mechanical stimuli. Under normal conditions, one group of fibers exhibited rhythmic firing in phase with antral contractions. Another group of fibers had non-rhythmic spontaneous firing. Responses of 15 fibers were tested with NES during multiple-step distension (MSD). NES produced a mean increase in antral contraction amplitude ($177.1 \pm 35.3\%$) and vagal afferent firing ($21.6 \pm 2.6\%$). Results show that both passive distension and enhanced antral contractions activate distension-sensitive vagal afferents. Responses of these fibers increase linearly to enhanced antral contraction induced by NES or MSD up to a distending volume of 0.6 ml. However, responses reached a plateau at a distending volume >0.8 ml. We concluded that enhanced contraction of the antrum can activate vagal afferents signaling to the CNS.

antrum; gastric contraction; motility

FOOD INTAKE IS REGULATED BY chemical and mechanical factors acting in concert to produce sensations of hunger and satiety. The vagus plays a major role in peripheral signaling in satiety. Gastric distension is correlated with an increase in firing of vagal mechanosensitive afferent fibers (1, 8, 11, 16, 18, 23, 24, 34). However, the patterns of response of these fibers de-

pend on the part of the stomach they innervate. Electrophysiological studies in different species have shown that mechanosensitive afferent fibers located in the antrum muscle wall respond to changes in smooth muscle transmural and local tension with an increased firing rate (1, 11, 30). It has been suggested that various levels of sensitivity of stomach mechanoreceptors to distension may account for the generation of satiety signals long before distension is maximal (10, 27). Antral contractions first appear a few minutes after food intake and increase in amplitude as the stomach continues to distend throughout a meal (17). Impulses arriving to the brain stem nuclei (i.e., nucleus tractus solitarius, area postrema) via the vagus nerve contribute to the perception of fullness and satiety (21).

Morphological studies have revealed two types of afferent terminals innervating the stomach, intraganglionic laminar endings (IGLEs) and intramuscular array (IMA) fibers (3, 8, 35). The IGLEs, which act as mechanoreceptors, are located throughout the stomach with high density in the antrum (3, 8, 35). These are probably the mechanosensors associated with distension-mediated satiety signals to the central nervous system (CNS). IGLEs are expected to sense transmural pressure gradients and deformations initiated by gastric filling and during active smooth muscle contraction (36). In contrast, IMAs are strategically located in the lower esophageal sphincter, pyloric sphincter, longitudinal muscle of the greater curvature, and in the lesser curvature (24, 27, 35, 36). On the basis of its structure and location, it has been proposed that IMAs are stretch-sensitive afferent fibers responsible for detecting the change in length of the smooth muscle (28). Behavioral studies have demonstrated that both IGLEs and IMAs are actively involved in detection of food ingestion. The selective loss of IGLEs or IMAs greatly influences the meal pattern, but not the total food intake (13, 14, 15). For example, neurtrophin-4-deficient mice that lose 80–90% IGLEs in the small intestine exhibit a prolonged meal duration compared with the wild-type litter mate (13). It has been shown that interstitial cells of Cajal are important for the development and maintenance of IMAs (14, 15). There

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is a marked loss of IMAs in steel factor (a ligand for c-kit receptor) and c-kit mutant mice. A recent study (14) has also shown that c-kit mutant mice lacking IMAs exhibit decreased meal size and increased meal frequency. These observations suggest that IGLEs and IMAs play differential roles in feeding behavior.

It is known that when active contractions are superimposed on passive distension, the maximal discharge rate is greater than that resulting from distension or contraction alone (18). Andrews et al. (1) showed that antral mechanoreceptors are able to respond to active contraction as well as to passive distension. However, it is not known whether these afferent fibers respond in an intensity-dependent manner to increasing antral contractions. The use of nonexcitatory electrical stimulation (NES) in cardiac contractility modulation (6) gave rise to the idea of applying NES to modulate the contractility of gastric smooth muscle. Excitatory stimulation (i.e., pacing) is a well-known therapy for maintaining normal heart rate. It has been suggested that pacing of the stomach can ameliorate symptoms related to postpartial gastrectomy and gastroparesis (2, 20, 22). Moreover, unsynchronized pacing has been attempted in experimental animals and in morbid obesity in humans to reduce food intake (7, 9).

The present study was designed to evaluate the effect of nonexcitatory electrical stimulation (NES) for enhancement of antral contractions and modulation of responses of vagal afferent fibers innervating the antrum. Unlike pacing of gastric smooth muscle, NES is delivered to the antral smooth muscle synchronized to the ascending phase of the rhythmic antral contraction and thus does not change the native rhythm acting on already depolarized tissue. NES has been used successfully in heart failure patients for the improvement of left ventricular muscle contractions and cardiac performance (6, 12, 25).

The present study has three goals: 1) to assess changes in firing frequency of vagal afferent fibers associated with antral distension (AD)-induced antral contractions, 2) to study the effect of NES applied during the ascending phase of the antral contraction, and 3) to evaluate changes in firing frequency of fibers responding to enhanced antral contraction evoked by NES. Responses of antral mechanoreceptors to spontaneous and NES-enhanced contractions may contribute to understanding the role of antral contractility in vagal signaling. NES is a novel approach to modulate vagal signals via enhanced antral contractions.

MATERIALS AND METHODS

General Procedures

Experiments were performed on 42 male Long-Evans rats (Harlan, Indianapolis, IN) weighing 400–500 g. Rats were deprived of food but not water 16–18 h before the experiment. Of the 42 rats, 39 were anesthetized with pentobarbital sodium (50 mg/kg ip) and maintained with constant intravenous infusion of pentobarbital (5–10 mg·kg⁻¹·h⁻¹). The remaining three rats were anesthetized with an intraperitoneal injection of α -chloralose (50 mg/kg). The right femoral vein was cannulated for infusion of anesthetic and

the left carotid artery was cannulated for monitoring the blood pressure. The trachea was intubated for artificial ventilation with room air. The rats were paralyzed with an initial dose (10 mg/kg iv) of gallamine triethiodide (Flaxedil) and mechanically ventilated with room air (~60 strokes/min). Paralysis was maintained throughout the experiment with supplemental doses of gallamine triethiodide (5 mg·kg⁻¹·h⁻¹). After completion of the experiment, rats were euthanized by injecting Beuthanasia-D (390 mg pentobarbital, 50 mg phenytoin sodium, 2% benzyl alcohol; Schering-Plough Animal Health) at a dose of 2 ml/kg. All experimental protocols were approved by the Medical College of Wisconsin Animal Care Committee.

Abdominal Surgery

The abdomen was opened by a transverse incision (5–6 cm length) just below the diaphragm. The stomach was located, clamped at the fundus, and retracted to allow surgical procedures. A pair of stainless steel stimulating electrodes (type-A5631 wire; Cooner) was placed in the muscle layer at the antrum. The distance between the leads was ~0.4–0.8 cm. Another pair of electrodes was placed just below the fundus of the stomach (0.4- to 0.6-cm distance between the leads) and was used to record myoelectrical activity propagating distally toward the stimulating leads. A small hole was made in the apex of the fundus to insert a highly compliant latex balloon (1.5 cm length, 2–3 cm inflated diameter) connected to a fluid delivery catheter (0.965 mm outer diameter, 0.58 mm inner diameter). Through the delivery catheter, a solid-state pressure recording probe (Millar Instruments, Houston, TX) was passed and positioned at the center of the balloon. The balloon assembly was securely placed in the antrum without obstructing the pylorus. The probe provided voltage readings calibrated by a pressure transducer connected to the balloon assembly. Once the surgical procedure was complete, the abdomen was closed in layers.

Recording of Afferent Nerve Activity

A midline incision was made in the neck, and the sternocleidomastoid, sternohyoid, and omohyoid muscles were removed to expose the right cervical vagus nerve. Skin was retracted by using silk sutures tied to a stereotaxic frame creating a warm mineral oil pool (37° C). The sympathovagal trunk was dissected away from the carotid artery. The vagus nerve was separated from the sympathetic and aortic nerves and transected below the nodose ganglion. The nerve was gently placed on a black microbase plate and the perineural sheath was removed. The vagal trunk was separated into fine bundles and was further split into fine filaments to obtain a single-unit recording. Nerve action potentials were recorded by draping a nerve filament over one arm of the bipolar platinum recording electrode. An equally thin fiber of connective tissue was placed on the other arm of the electrode to allow differential recordings. Action potentials were monitored continuously by analog delay and displayed on an oscilloscope after amplification through a low-noise AC differential amplifier (model 3000; AM Systems). A window discriminator (model DDIS-1; BAK Electronics, Mount Airy, MD) was used to discriminate the action potentials, and the firing frequency of the nerve was recorded on line by using the Spike2/CED 1401 data acquisition system (Cambridge Electronic Design). The nerve action potentials, intra-antral pressure, myoelectric activity, delivery current and voltage, and blood pressure were recorded on line. Data were also recorded on an analog tape for offline analysis (31).

Experimental Protocol

AD. Vagal afferent fibers innervating the antrum were identified by observing an increase in firing of the fiber to 1 ml of AD. Nerves responding positively to the distension were subjected to a stimulus-response function (SRF) to a graded phasic distension and to multiple-step distension (MSD) at volumes of 0.2, 0.4, 0.6, 0.8, and 1.0 ml. For phasic AD, distending volume was maintained for 30 s at each volume and the interstimulus time interval was 2 min. For MSD, the volume was incremented in steps, and a minimal residual volume of 0.2 ml was maintained in the balloon throughout MSD protocols to generate detectable antral contractions. Each step of distension was maintained for 5 min. The pressure generated due to distension was measured at the plateau stage of each distension level, and the balloon distension was performed manually.

Spinalization. In a set of experiments ($n = 5$), responses of vagal afferent fibers and antral contractions were recorded on spinal-transected rats. A laminectomy was performed to expose the cervical (C_1 – C_2) spinal cord. The dura membrane was gently removed and 5–10 μ l of 4% xylocaine were applied to the surface of the exposed spinal cord. After 10 min, the spinal cord was completely transected by using a scalpel blade. The animals exhibited a vasodepressor response immediately after spinal transection. The vasodepressor response slowly recovered in 15–30 min. Experiments were performed after full recovery of the spinal shock.

Antral contraction enhancement by NES. Firing frequency of the nerves and antral contractions were tested for enhanced antral contractions induced by NES. The stimulation parameter was 80 Hz frequency, 1- to 2-s pulse width, and current ranging from 0.5–1.0 mA. These supraphysiological stimulation parameters work by enhancing the antral contractions that mechanically activate the afferents to further increases in firing frequency on top of the ongoing afferents firing from the distension-induced antral contractions. The frequency is not supraphysiological, because it is aimed at modulating smooth muscle activity as opposed to vagal afferents and, more specifically, the spike activity. In preliminary

studies, the strength of the current was set to elicit a sufficient response from the muscle (>1 mmHg) during a spontaneous contraction, but set to <1 mA to maintain the NES status. Electrical signals were generated by using a Spike3 digital-analog current (DAC) pulse, which triggered a signal generator. Current and voltage delivered to the muscle were continuously measured and recorded across the leads. NES was either synchronized to propagating myoelectrical activity of the smooth muscle by designing a threshold algorithm or was triggered manually at the ascending phase of the antral contractions. Application of NES was conducted on two protocols, tonic single distension and MSD. The single distension protocol involved a 5-min tonic distension at the volume of 0.2 or 0.4 ml. This protocol was conducted with the purpose of determining how NES would affect the early stages of stomach distension. In the second protocol, NES was recorded in MSD (0.2–1.0 ml) to examine the progressive increases in amplitude of antral contraction at different volumes of tonic AD (5 min). The MSD protocol was split into three sections at each distension volume. During the first 2 min, the firing rate of the fibers was recorded. In the following 2 min, NES was delivered, and response to enhanced antral contraction was recorded. Finally, 1 min was used to record post-NES nerve activity.

Data Analysis

Data are expressed as means \pm SE. To evaluate the effect of the NES on nerve firing rates and contraction amplitude in the single distension and MSD protocols, a baseline period was taken at the start of each protocol. The firing frequency of the nerve was measured by averaging the total count for the first 2 min in each step of distension. Increase in nerve response is displayed as a percentage, or an average of the increased firing activity above baseline. Antral contractions were measured from the baseline tone to the peak of the contraction. During application of NES, nerve response was measured from the time the antral contraction reached 30% in amplitude over the baseline, to

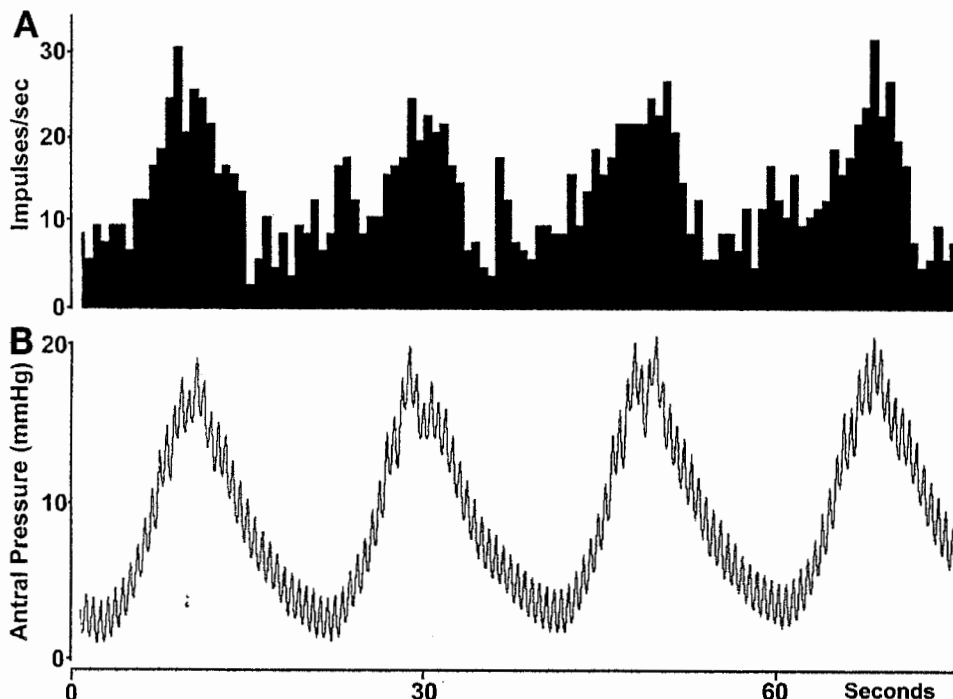


Fig. 1. Correlation between antral contractions and rhythmic vagal afferent fiber innervating the antrum. **A:** activity of the nerve represented as peristimulus time histogram (PSTH; 1-s binwidth). **B:** spontaneous antral contractions. Firing of the fiber started to increase during the ascending phase of the antral contraction and peaked at the peak of the contraction. Small oscillations over the antral pressure wave represent respiratory movement.

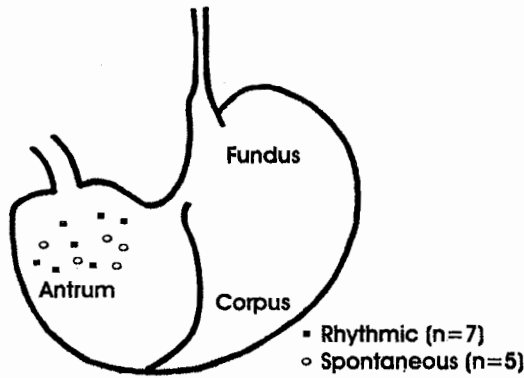


Fig. 2. Receptive fields of 12 distension-sensitive afferent fibers located on the posterior part of the antrum. The solid squares ($n = 7$) represent the receptive fields of rhythmic fibers that responded in phase with spontaneous antral contraction. Open circles ($n = 5$) represent the receptive fields of fibers that fired nonrhythmically to antral contractions. However, these fibers were excited to enhanced antral contractions.

the time the antral tone returned to baseline. This distinction was used to allow sufficient time to observe the effect of NES on antral contractions and the corresponding firing of the afferent fiber.

In the MSD protocol, the dynamic nerve firing of the antral contraction was measured by averaging the firing frequency of the afferents occurring at the 4- to 6-s peak of contraction. Percent increase in nerve firing frequency was calculated by dividing the average of the NES enhancement period by the control period at each level of step distension. All data from control and NES-enhanced antral contraction periods were compared by a Mann-Whitney U -test with Bonferroni correction for multiple comparisons.

RESULTS

Sample

A total of 56 vagal afferent fibers from 42 rats were identified that increased their firing rate by $\leq 50\%$ to 1 ml of AD. All fibers exhibited spontaneous resting activity with the balloon positioned in the antrum. The mean resting activity of the fibers was 7.47 ± 0.96 impulses/s (range: 1.54–12.54 impulses/s). The mean increase in activity of these fibers to 1 ml AD was $184.6 \pm 32.3\%$. The spontaneous nerve firing exhibited two types of behavior. In a group of fibers ($n = 23$), firing was in phase with the ascending phase of the antral contraction (Fig. 1). There was little or no response of these fibers during the descending phase of the antral contraction. The second group of fibers ($n = 33$) was nonrhythmic and exhibited no increase in firing during the ascending or the descending phase of the antral contraction.

Receptive Fields

Receptive fields of 12 fibers were mapped by probing the serosal surface with a fine-tip glass probe after the completion of the experimental protocol. Seven of the 12 fibers exhibited rhythmic correlations to antral contraction, and five exhibited spontaneous nonrhythmic activity. All 12 fibers were located on the posterior part of the antrum (Fig. 2).

Effects of Single Distension and MSD on Antral Pressure and Nerve Activity

In 11 fibers, SRFs were constructed to a short-duration (30 s) AD at volumes of 0.2, 0.4, 0.6, 0.8, and 1 ml.

Fig. 3. Response of a rhythmic vagal afferent fiber to short duration (30 s) antral distension (AD). A: firing frequency of the fiber represented as PSTH (1-s binwidth). B: antral pressure (AP). Arrows indicate the dynamic increases in firing during rapid distension; imp, impulses. A drop in firing frequency occurred during the fall in AP. Firing frequencies either increased or maintained a tonic state at the last phase of distension.

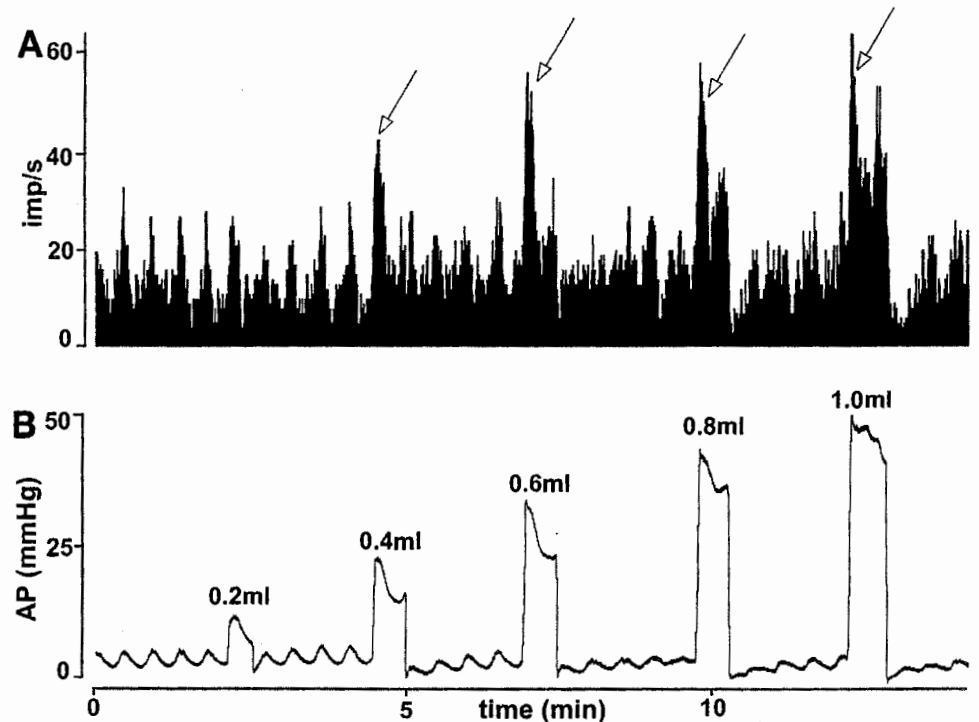


Figure 3 illustrates an SRF of a rhythmic fiber to different volumes of AD. A biphasic nerve firing response occurred at 0.4–1.0 ml distensions. The biphasic response involved a transient dynamic firing at the onset of distension followed by a tonic response (Fig. 3). There were no differences in thresholds for response and slope of the SRFs between rhythmic and nonrhythmic fibers to AD. Therefore, these two types of fibers were grouped together in subsequent analysis. All fibers showed a linear increase in firing with increasing volume of distension.

SRF was also constructed to MSD with an incrementing volume of 0.2 ml ($n = 21$). Each volume of distension was maintained for 2 min. Figure 4 illustrates response of a fiber to MSD. The amplitude of the antral contractions increased with each step of distension ≤ 0.6 ml, and was associated with an increase of vagal afferent firing frequency (Fig. 4). Unlike phasic AD, the longer duration of distension produced adaptation of the response and relatively smaller increase in overall nerve activity compared with that of short-

duration graded AD (Fig. 5). In MSD, the 0.2-, 0.4-, and 0.6-ml distensions produced increases in firing frequency of 25.6 ± 6.4 , 43.3 ± 9.5 , and $77.1 \pm 17.9\%$, respectively. Similar volumes of distensions in short-duration AD produced increases in firing of 35.5 ± 7.6 , 82.9 ± 17.7 , and $150.7 \pm 34.9\%$, respectively. However, mean response of fibers during MSD at 0.8 ml ($115.6 \pm 33.9\%$) and 1.0 ml ($120.6 \pm 15.3\%$) volumes was significantly ($P < 0.05$) less than that of 0.8 ml ($209.5 \pm 46.9\%$) and 1.0 ml ($286.1 \pm 57.9\%$) short-duration AD, indicating an adaptation of response of these fibers to tonic distensions. The change in antral steady-state pressure (i.e., maintained pressure after initial accommodation) to increasing balloon volume during tonic MSD was linear, indicating that the procedure did not reach the compliance of the antrum (Fig. 6).

Effect of Spinalization

MSD was carried out in five fibers from five spinally (C_1 – C_2) transected animals. Response patterns of the

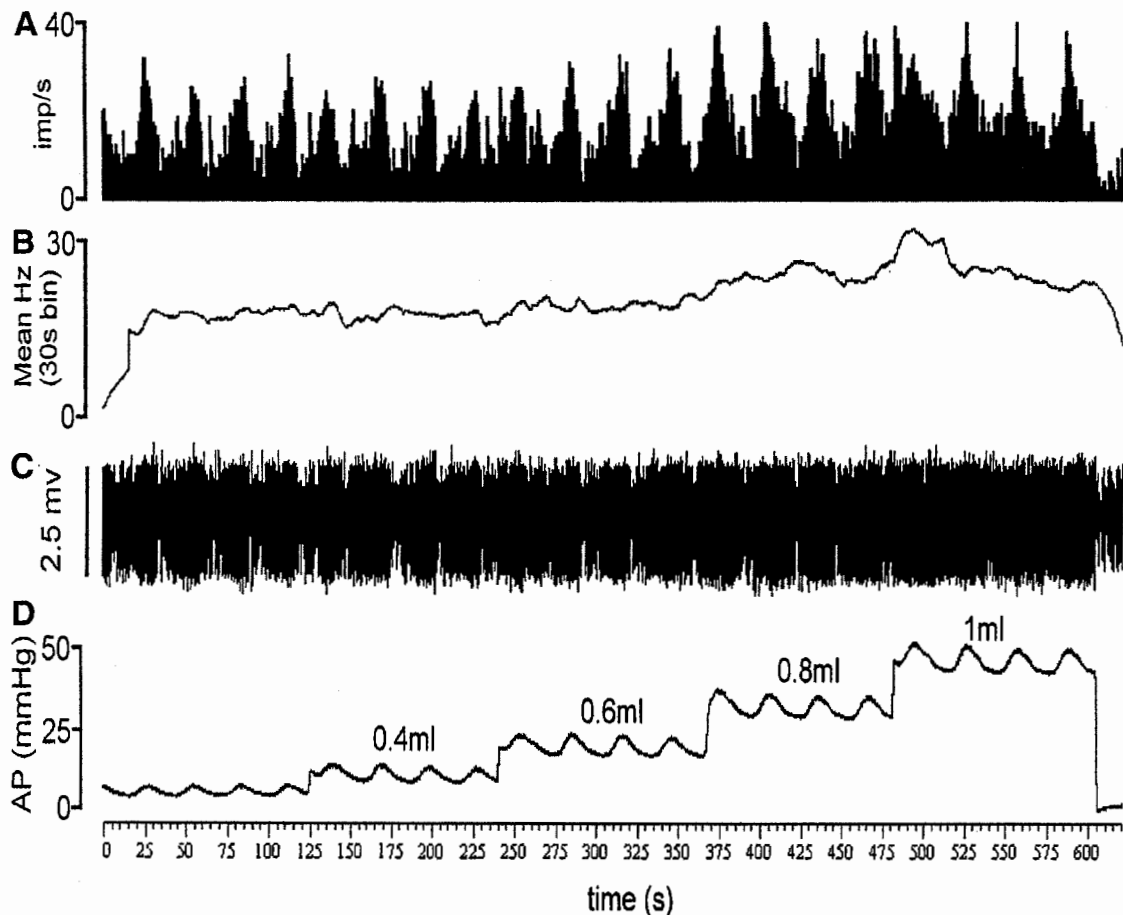


Fig. 4. Response of a rhythmic vagal afferent fiber to multiple-step distension (MSD). *A*: firing frequency of the fiber represented as PSTH (1-s binwidth). *B*: mean frequency integrated as total impulses per 30 s. *C*: nerve action potentials. *D*: antral pressure. A residual volume of 0.2 ml was necessary to elicit significant antral contractions in most experiments. This volume generated 10–12 mmHg of antral pressure. *D*: antral pressure linearly increased to subsequent distension and reached ≥ 50 mmHg at 1 ml of distension. There was tonic increase in firing during step distension. A dynamic increase in firing was also observed synchronized to spontaneous antral contractions ≤ 0.8 ml. No further increase was observed at 1 ml. Firing of the fiber abruptly decreased when fluid was withdrawn.

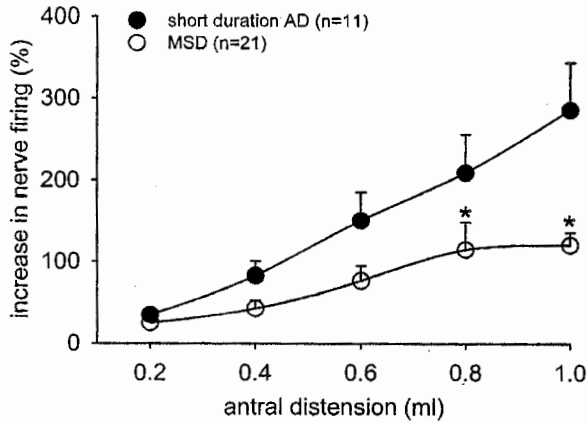


Fig. 5. Summary data of mean stimulus-response functions (SRFs) to short-duration AD and tonic MSD. Fibers exhibited a linear increasing response to short-duration AD, whereas the firing reached a plateau in MSD at a volume of >0.6 ml ($*P < 0.05$).

fibers to MSD in spinalized rats were similar to those of nonspinalized animals (Table 1). There was no difference in antral pressure change between spinalized and intact animals, suggesting that spinal motor neurons have no influence in the maintenance of antral tone.

Antral Contraction Modulation by NES

In initial experiments, 10 fibers were tested during tonic single distension. The volume of distension was either 0.2 ($n = 5$) or 0.4 ml ($n = 5$). Table 2 shows the summary data. Figure 7A illustrates response of a rhythmic fiber to enhanced antral contraction induced by NES. NES increased the firing rates of the fiber due to mechanical stretch by larger antral contractions and not direct stimulation of the nerve. The peak afferent response correlated to the peak of the antral contraction. To examine whether the amplitudes of antral contractions increase to NES at increasing antral pressure, a study was designed to deliver NES during MSD. Response patterns of 15 vagal afferents were tested with NES during MSD. As the distension volume was incremented, the amplitude of the NES-induced antral contractions increased (Fig. 7B). The dif-

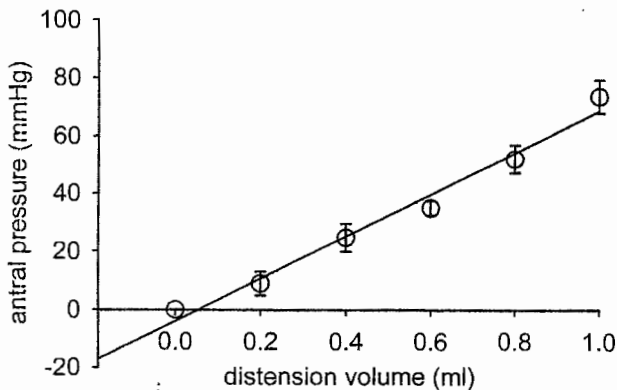


Fig. 6. Linear relationship of antral pressure and distension volume during MSD. The 0.2-ml increment in distension generated a constant increase of pressure (14.4 ± 3.2 mmHg) for each increased distension level ($y = 72.6x - 3.8$, $R = 0.99$, $P < 0.05$, $n = 15$).

Table 1. Effects of cervical (C_1 - C_2) spinalization on the membrane-spanning domain

Distension Volume, ml	Spinalized		Control	
	Antral pressure, mmHg	Nerve firing, %	Antral pressure, mmHg	Nerve firing, %
0.2	1.3 ± 1.4	54.6 ± 17.1	1.2 ± 1.3	25.6 ± 6.4
0.6	15.8 ± 1.6	73.8 ± 18.6	25.2 ± 2.0	77.1 ± 17.9
1.0	41.9 ± 0.8	186.5 ± 70.7	54.6 ± 3.1	120.6 ± 15.3

Values for spinalized ($n = 5$ rats) and control ($n = 21$ rats) are means \pm SE.

ference between the spontaneous contractile force exerted by the antrum during baseline and NES application was significant at distensions >0.4 ml (Fig. 8). In the presence of NES, contractile forces of the antrum were on average $177.1 \pm 35.3\%$ greater than control contractile forces ($n = 15$). The enhanced contractions increased in a linear fashion between distension volumes of 0.2–0.6 ml, but reached a plateau at distensions of 0.8 and 1.0 ml (Fig. 8).

During the MSD protocol, NES-enhanced contractions increased the nerve firing at ≤ 0.6 ml distension volume. At higher distension volumes (0.8–1.0 ml), the firing rate during enhanced and control contractions decreased. The nerve firing rate of both the control and NES-enhanced contractions followed a similar pattern as shown in Fig. 9. Paired *t*-test of control vs. test for each balloon volume showed statistical significance for all tested volumes ($P < 0.01$). The mean increase of firing between control and NES-enhanced contraction throughout the five distension volumes was $21.6 \pm 2.6\%$ ($n = 15$). To isolate the dynamic effect of NES-enhanced contractions on the firing frequency of vagal afferent fibers, the changes in nerve firing between the minimum and maximum contraction at each cycle were assessed. Thus dynamic nerve firing was sampled within the top 20% contraction levels. The electrical signals significantly augmented nerve firing ($P < 0.05$) in this setting as well. The increased rate of change in antral contraction amplitude during NES application was accompanied by a rapid dynamic nerve response (Figs. 7A and 9, bar graphs).

DISCUSSION

The present study has shown that it is possible to augment the amplitude of antral contractions and the

Table 2. Summary data of changes in amplitude of antral contractions and firing of the fiber during tonic single distension

Distension Volume (ml)	Contraction Amplitude (mmHg)	Nerve Firing (imp/s)
0.2	71.1 ± 10.3	51.6 ± 4.0
0.4	84.4 ± 5.7	44.8 ± 6.9

Values are mean \pm SE, expressed as %increase from baseline of 5 experiments. Contractility and nerve firing rate were significantly enhanced by nonexcitatory electrical stimulation compared to baseline ($P < 0.05$ vs. baseline). Imp, impulses.

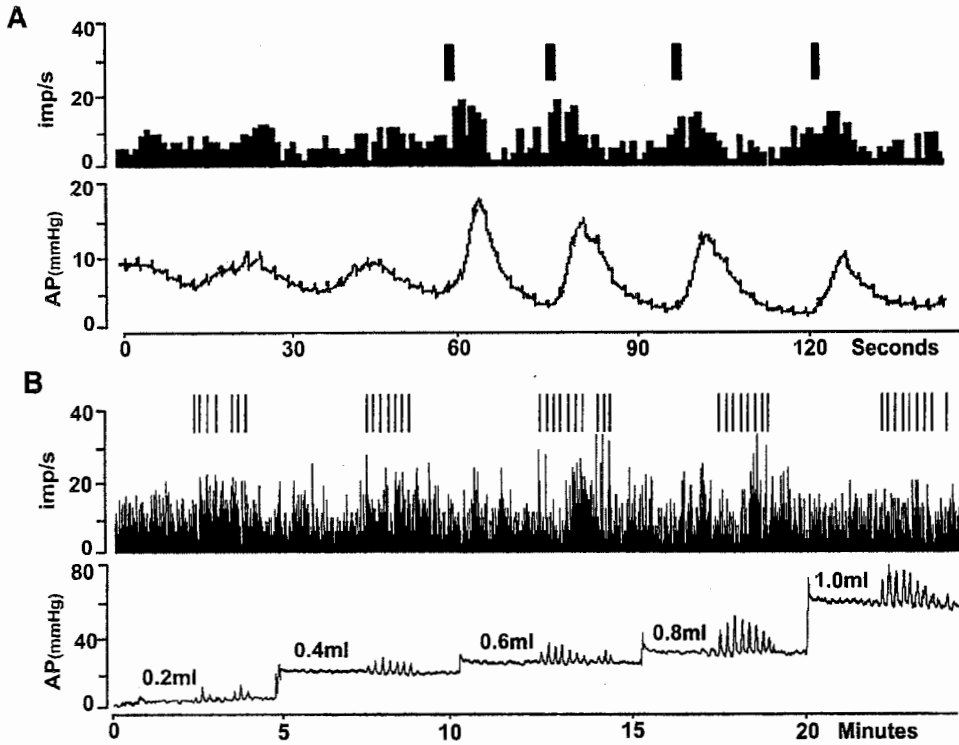


Fig. 7. Examples of nonexcitatory electrical stimulation (NES)-induced enhanced antral contractions and responses of vagal afferent fibers during tonic single distension and MSD. *A* and *B*: top traces represent firing frequency of fiber represented as PSTH (1-s bin-width) and the lower traces represent AP. Vertical bars on top of PSTH indicate the NES delivery (80 Hz, 0.5–1.0 mA, 1–2 s). *A*: NES application during tonic single distension (0.2 ml) produced an increase in response of the fiber and enhancement of amplitude of antral contraction. *B*: NES application during MSD increased antral contractions. Maximum firing of the neuron occurred at 0.4 ml. However, the fiber did not exhibit increasing response at greater volume of distensions (0.6–1 ml) despite enhanced antral contraction to NES.

frequency of firing of vagal afferent fibers by applying NES synchronized to the ascending phase of the antral contraction. The results indicate that the active contraction and intraluminal distension play a dominant role in modulating the firing rate of vagal distension-sensitive afferent fibers innervating the antrum. A short-duration AD produces a dynamic response of these fibers followed by slow decline in firing during the fall in antral pressure. On the other hand, tonic distension lasting >1 min produces a tonic increase in firing (Fig. 4). With regard to the spontaneous resting

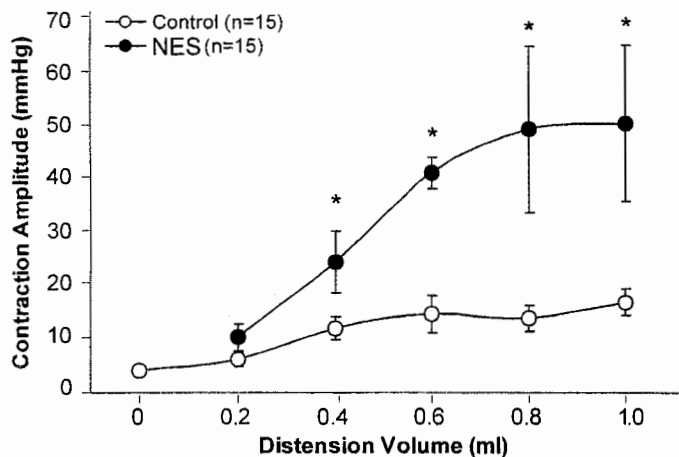


Fig. 8. Effect of distension on the amplitude of basal and NES-induced antral contractions. Open circles represent antral contraction induced by MSD ($n = 15$) and filled circles represent antral contraction induced by NES at each distension level ($n = 15$). Amplitudes of antral contractions were significantly greater during NES application ($*P < 0.05$).

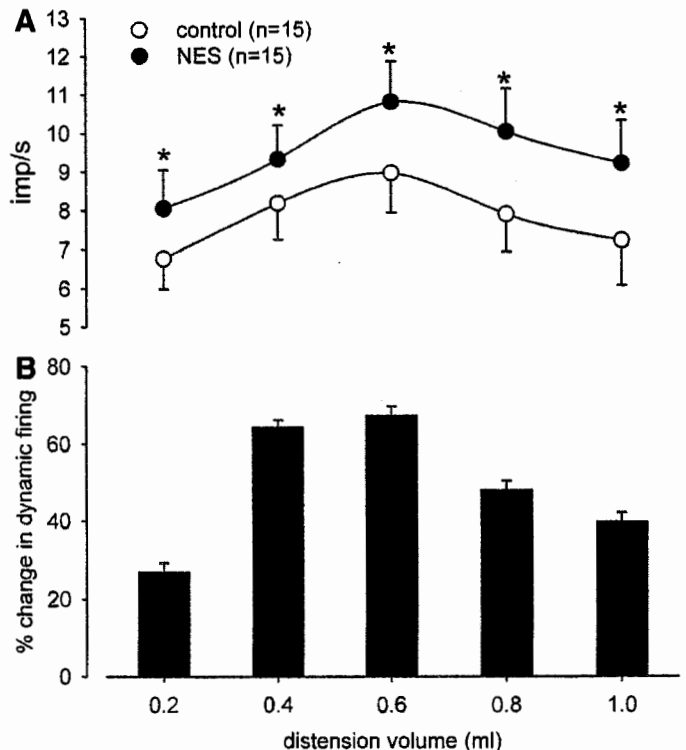


Fig. 9. Effect of NES-enhanced antral contraction on the firing frequency of the vagal afferent fibers ($n = 15$) during MSD. *A*: open circles indicate mean firing (impulses/s) of fibers during the first 2 min of each level of step distension. Solid circles indicate mean firing (impulses/s) of the fibers during a 2-min application of NES. *B*: mean increases (%) in dynamic firing associated with antral contraction between control and NES-induced enhanced antral contractions at various levels of distension ($*P < 0.05$ in control vs. NES-enhanced contractions).

activity under nondistended condition, a group of fibers exhibit rhythmic discharge synchronized to antral contraction. These fibers attain the peak firing at the peak of the antral contraction. The second group of fibers exhibits a nonrhythmic spontaneous firing and does not synchronize to antral contraction. The distinction between rhythmic and nonrhythmic fibers both located in the antrum (Fig. 2) is made to understand the response patterns of these fibers under normal physiological condition. A possible explanation for the non-rhythmic nature of the second group of fibers could be that the thresholds for responses of these fibers to antral contraction are greater compared with rhythmic fibers. These fibers are also tension sensitive because they respond to antral contractions enhanced by NES in a similar fashion to the rhythmic fibers. The nature of response of tension-sensitive mechanoreceptors largely depends on the structure of the muscle they innervate and their thresholds for response to active contractions (1, 4, 33, 34). For example, the mechanosensitive afferent fibers innervating the fundus and corpus generally do not exhibit any firing to normal gastric contraction. However, they respond when relatively strong contractions of the proximal stomach are induced by stimulating the distal end of the cervical vagus nerve (1, 4).

Vagal Afferent Firing During AD

SRFs to short-duration graded AD and tonic MSD were used to examine the intensity-dependent firing of vagal afferent fibers. The transient nature of the short-duration AD elicited greater increase in vagal mechanoreceptor activity compared with the MSD protocol (Fig. 5). The difference in neural response between the two protocols is related to the nature of the mechanical stimulus applied. The graded AD involved a passive distension (30-s balloon filling) applied in a graded manner. The initial dynamic increase in nerve firing was very much dependent on rapid development of tension in the smooth muscle (28). On the other hand, the MSD imposes a more complex stimulus composed of both transient and tonic mechanical stimulation of the smooth muscle. The responses of the afferents in the short-duration graded distension were larger than that of MSD protocol due to initial dynamic response to the transient balloon inflation (Fig. 3).

In the present study, cervical spinalization had no influence on antral tone and nerve activity, suggesting that spinal motorneurons do not contribute to antral contraction or to NES-induced enhancement of contractions.

Modulation of Antral Contractions

Use of the balloon at small volumes allowed us to accurately detect nerve firing with spontaneous contractions at minimal distension (0.2 ml). The balloon was inflated in steps of 0.2 ml/2–3 s, which represents small increments at a rate slightly higher than that recorded in a previous study (23). The linear increase in basal antral pressure to the increase in balloon

distension indicates that the distension range chosen does not change the compliance of the stomach (Fig. 6). It was found that contractions significantly increased in amplitude with increasing volume of ≤ 0.6 ml distension (Fig. 8). Nerve firing correlated to the increase of contraction amplitude ≤ 0.6 ml and thereafter decreased, whereas contraction was maintained at a higher level. This dynamic change in the firing rate at low distension volumes (0.2–0.6 ml) could represent an important vagal signaling to the CNS conveying enhanced activity of the antrum. It appears that maximum signaling takes place much earlier than the development of the maximum tone of antral smooth muscle. The present study also shows there is no one-to-one relationship between contractions and afferent firings (see Fig. 9). It is obvious that NES is not contributing to uncouple the nerve response from the antral contraction, because the decrease in firing at greater magnitude of contraction was also evident without NES (Fig. 9). A possible explanation for the decrease of nerve firing beyond 0.6 ml distension might involve the saturation of the nerve activity. The saturation of nerve firings at greater intensities is a typical phenomenon that has been observed in vagal afferents to passive distensions of the other parts of the gastrointestinal tract (5, 19, 23).

Possible Mechanism for NES-Enhanced Antral Contractions

The mechanism of action of NES has been envisaged in the cardiac muscle where NES applied during the refractory period increased Ca^{2+} transients and enhanced contractility (29, 32). However, structural and functional differences between cardiac and smooth muscle tissue require further research.

In conclusion, the findings of this study suggest that the antral mechanoreceptors respond in a phasic manner to transient stimuli, whereas these mechanoreceptors respond in both a phasic and tonic manner to sustained stimuli. It is possible to increase the amplitude of antral contractions by delivering NES synchronized to the ascending phase of the antral contraction. This novel approach allows modulation of the mechanical contraction of the antrum and corresponding sensory afferent signals to the CNS. Further behavioral studies are necessary to elucidate whether the phasic response of the enhanced contractions can influence food intake and satiety.

DISCLOSURES

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