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Role of Nitric Oxide in Esophageal Peristalsis in the Opossum

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To explore the involvement of NO in normal peristalsis, the effects of inhibitors of NO synthase, including N^{ω}-nitro-L-arginine (L-NNA) and N^{ω}-nitro-L-arginine methyl ester (L-NAME), on esophageal peristaltic contractions induced by diverse stimuli that may involve different neuronal circuits were studied. Studies were performed in opossums. Experimental conditions in vivo included primary peristalsis (P) induced by pharyngeal stroking, short-train (1 second) electrical stimulation of the vagus nerve which caused peristaltic (S) contractions, and long-train (10 second) electrical stimulation of the vagus nerves which caused contractions at the onset of (A contractions) and after (B contractions) the stimulation period. In vitro experiments were performed on strips of esophageal circular muscle using electrical field stimulation which caused contractions at the onset of (on contractions) and after (off contractions) the stimulation period. The administration of L-NAME significantly decreased the latency period and reduced the latency gradient for P contractions, thereby increasing the velocity of peristalsis. Concomitant administration of atropine prolonged the latency period but did not restore the latency gradient. L-NAME abolished B contractions in a dose-dependent fashion. In vitro, L-NAME caused dose-dependent inhibition of off contractions and augmentation of on contractions. These studies support the hypothesis that NO may be involved in (a) both the latency period and the latency gradient, as well as in the contraction amplitude of esophageal peristalsis; and (b) esophageal B and off contractions.

There is a latency period between the onset of a swallow and contraction of esophageal circular smooth muscle.[1,2] The duration of the latency period increases progressively from the proximal to the distal esophagus, and this latency gradient is the basis for esophageal peristalsis.[1-4] Early in the latency period, intramural neurons release a nonadrenergic, noncholinergic neurotransmitter that causes initial hyperpolarization and inhibition of esophageal smooth muscle.[5,6] The nature of the inhibitory neurotransmitter and its role in the latency period and in peristaltic contraction amplitude have not been established (see references 1 and 2). Recently, a nitric oxide–related compound has been proposed as an inhibitory neurotransmitter or an intermediary in the action of an unknown neurotransmitter in the gut.[7-12] NO is produced by the metabolism of L-arginine through the action of NO synthase (NOS), an enzyme present in myenteric neurons.[13] Certain analogues of L-arginine, including N^{ω}-nitro-L-arginine methyl ester (L-NAME) and N^{ω}-nitro-L-arginine (L-NNA), reversibly inhibit NOS and thereby block the production of NO.[14] These agents are useful tools for exploring the role of endogenous NO in physiological processes.[10-14] Recently, Murray et al.[11] reported that L-NNA decreases the latency and amplitude of off contraction in the esophageal circular muscle in vitro. However, Tottrup et al. failed to find any effect of L-NNA on vagal stimulated esophageal peristalsis.[12] The effect of inhibitors of NOS on swallow-induced peristalsis is not known.

We have used L-NAME and L-NNA to investigate the role of NO in swallow-induced esophageal peristalsis and esophageal contraction induced by diverse stimuli involving different neuromuscular mechanisms.[1,2,15-19] Our studies suggest that an NO-related substance functions as an inhibitory neurotransmitter or is involved in the action of an inhibitory neurotransmitter in the esophagus and may play a role in producing both the latency period and the aborally progressive latency gradient. NO is also the effector of esophageal off contractions, which may contribute to the amplitude of primary peristaltic contraction associated with swallow-induced peristalsis. NO or a related compound, along with acetylcholine and a yet undefined noncholinergic excitatory transmitter, appears to be involved in swallow-induced peristaltic contractions.

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Materials and Methods

Studies were performed in anesthetized opossums (*Didelphis virginiana*) weighing 2.8–3.5 kg. After an overnight fast, animals were initially anesthetized with sodium pentobarbital [40 mg/kg intraperitoneally (IP)]. Subsequently, α-chloralose [30–50 mg intravenously (IV)] was administrated slowly as needed to maintain anesthesia.

Anesthetized animals were strapped supine on an animal board and maintained at 37°C with a heating pad. The brachial artery was cannulated to monitor blood pressure, and the brachial vein was cannulated for administration of test agents as needed.

Intraluminal esophageal pressures were measured with a catheter assembly consisting of six individual polyvinyl catheters. Each catheter had a side hole; the five proximal holes were situated 2 cm apart, and the sixth, most distal, hole was 1 cm away from the fifth. The outside diameter of the assembly was 5 mm. Each catheter was continuously perfused with bubble-free distilled water using a low-compliance pneumohydraulic system as described earlier.[15,18]

Swallowing was induced by stroking the pharynx with a cotton swab. The onset of swallow-induced peristalsis was determined in relation to the onset of spike bursts in the mylohyoid muscle. Mylohyoid electromyography was recorded using a conventional bipolar electrode as described previously.[17,20]

In some animals the vagi were isolated in the neck and severed, and the peripheral end of one decentralized vagus was used for electrical stimulation with a bipolar electrode. Vagal stimulations (VS) of short (1 second) and long (10 seconds) trains were applied with square waves pulses of 60 V and 0.5-millisecond pulse duration at 5, 10, and 20 Hz using a Grass stimulator (Model S11; Quincy, MA).

In all animals, control responses were obtained first, followed by responses after L-NAME administration. L-NAME was dissolved in 3 mL of saline and administered over 30 seconds as an IV bolus. L-NAME was used in in vivo studies because it is water soluble.[14] L-NAME causes a dose-dependent increase in blood pressure.[14] We monitored blood pressure as an indicator of L-NAME effect. L-NAME caused a maximal increase in blood pressure in a dose of 20 mg/kg IV. However, we used a fivefold higher dose of 100 mg/kg IV as a supramaximal dose. This dose of L-NAME increased mean arterial blood pressure from 101 ± 5 mm Hg to 134 ± 6 mm Hg ($n = 10$; $P < 0.01$). In some animals, atropine sulfate (20–30 μg/kg) was added to produce muscarinic receptor antagonism.[16]

For in vitro studies, animals were anesthetized with sodium pentobarbital (40 mg/kg IP) and after exposure the esophagus and part of the stomach were removed by thoracotomy and laparotomy.[18] Circular muscle strips of the esophagus (3 cm above gastroesophageal junction) were isolated and suspended in an organ bath containing 2 mL of Krebs' solution. The solution contained (mmol/L) NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 0.57; $NaHPO_4$, 1.0; $NaHCO_3$, 25.0; and glucose, 11.1. The Krebs' solution was equilibrated with 95% O_2 and 5% CO_2 and maintained at 37°C. It had a pH of 7.42. One end of the esophageal circular muscle strip was attached to a force-displacement transducer to monitor isometric tension. Muscle strips were equilibrated at an applied tension of 1.5g for 1 hour. Electrical field stimulation (EFS) was applied via a coaxial electrode delivered from a stimulator (Grass S11). Stimulus parameters used were square wave pulses of 0.5 milliseconds, 2–20 Hz, 10-second train, and 60 V.

Control responses were obtained first. Tissues were then exposed to various concentrations of L-NAME dissolved in saline and L-NNA dissolved in 0.25N HCl. Tissue responses to different concentrations of L-NAME and L-NNA were studied.

Quantitative data are expressed as mean ± SE. Animals served as their own controls. Statistics of effects of various treatments were determined in each animal separately and also cumulatively in all animals. Statistical analyses were performed using paired or unpaired *t* tests and analysis of variance (ANOVA) for multiple comparisons.

L-Arginine, L-NNA, and L-NAME were all purchased from Sigma Chemical Company, St. Louis, MO. Atropine sulfate was purchased from American Regent Laboratories Inc., Shirley, NY.

Results

Influence of L-NAME on Primary Peristalsis (P Contractions)

Esophageal primary peristalsis is produced in response to a swallow.[1] As shown in Figure 1, swallows induced by pharyngeal stimulation caused contractions that were peristaltic, i.e., the latency gradient increased distally along the esophagus.

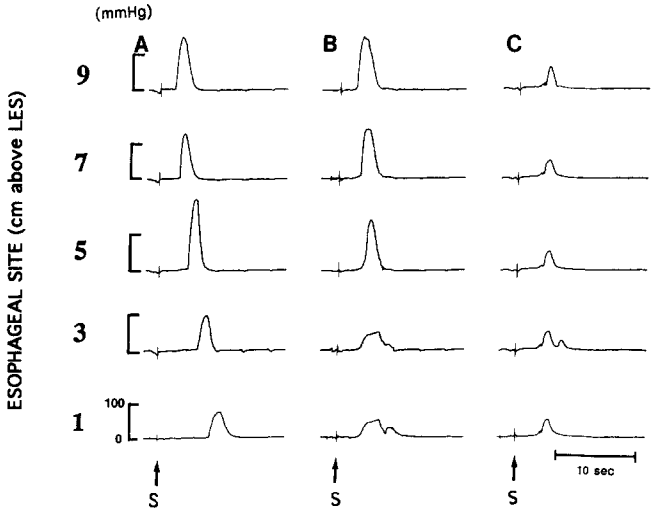

Figure 1. Manometric tracing of the effects of L-NAME and atropine on primary peristalsis in vivo in the smooth muscle portion of the opossum esophagus. (*A*) Normal peristaltic contraction with an increasing latency gradient along the esophagus in response to a swallow. The *arrows* labeled S mark the onset of swallowing. (*B*) Effect of L-NAME. Note that L-NAME reduced the latencies and the latency gradient. Also note the decrease in amplitude of contractions in the distal esophageal sites. (*C*) The addition of atropine (30 μg/kg) increased latencies but did not restore the latency gradient. Atropine also caused a reduction in the amplitude of contractions.

Treatment with L-NAME reduced latencies throughout the esophagus, particularly at the distal esophageal sites. This reduced the latency gradient so that the contractions seemed almost simultaneous. The treatment also slightly reduced the amplitude of contractions, particularly at distal esophageal sites. These effects of L-NAME treatment disappeared within 2 hours. L-Arginine administration (up to 100 mg IV) by itself had no effect on the latencies, amplitude, or duration of swallow-induced esophageal contractions. L-Arginine (20 mg/kg) restored the latency gradient reduced by L-NAME (20 mg/kg). Addition of atropine to L-NAME treatment caused a reduction in the amplitude of contractions, particularly at the proximal site, and some prolongation of the latencies of contraction.

Cumulative data from six animals (five to seven observations each) showed that the latency of contractions caused by swallows varied from 2.0 ± 0.1 seconds to 5.2 ± 0.2 seconds depending on the esophageal site (Figure 2). ANOVA showed that the gradient of latencies increased significantly distally along the esophagus ($P < 0.01$). The mean latency of contractions was 3.2 seconds longer at the 1-cm site than at the 9-cm site, resulting in a conduction velocity of 2.3 ± 0.1 cm/s.

The latency gradient persisted after L-NAME treatment ($P < 0.01$), although L-NAME treatment decreased latencies at all esophageal sites but particularly at the distal sites (Figure 2). After L-NAME treatment, the latency of contractions was 1.7 ± 0.2 seconds at the 9-cm site and 2.8 ± 0.1 seconds at the 1-cm site, resulting in a calculated conduction velocity of 8.8 ± 1.5 cm/s. The change in contraction velocity with L-NAME treatment vs. control was significant ($P < 0.01$). Addition of atropine prolonged the latencies of contractions, particularly at the proximal sites. ANOVA showed no significant proximal-to-distal latency gradient after administration of L-NAME plus atropine ($P > 0.05$).

L-NAME did not significantly modify the amplitude of contractions at any site (Figure 2). However, the addition of atropine caused a significant reduction in the amplitudes of contractions at all esophageal sites in all animals individually as well as cumulatively (Figure 2).

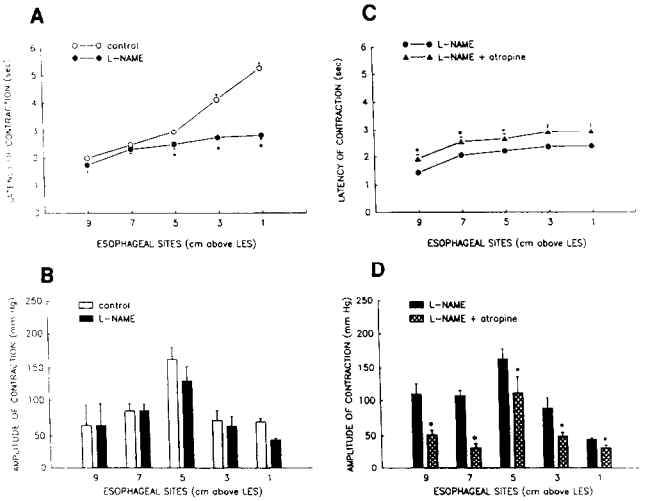

Figure 2. Effects of L-NAME and atropine on primary peristalsis. (*A* and *B*) Effects of L-NAME on latencies and latency gradient (*A*) and amplitudes (*B*) of contractions in the smooth muscle portion of the opossum esophagus (n = 33 observations in six animals). Note that L-NAME treatment reduced the latencies and the latency gradient. The mean amplitudes of P contractions were not changed. (*C* and *D*) Effect of adding atropine (30 μg/kg; n = 10 observations in two animals). Atropine treatment increased the latencies of contraction from those during L-NAME treatment but did not change the latency gradient. The amplitude of P contractions was significantly reduced by atropine at most esophageal sites. Values are mean ± SE. *Statistically significant ($P < 0.05$) difference by paired *t* test.

Influence of L-NAME on Esophageal Contractions Caused by Vagal Stimulation

Vagal stimulation produces contractions in the esophageal body due to peripheral neuromuscular mechanisms and excludes the central deglutitive reflex pathways.[1,2] Short trains of vagal stimulation produce esophageal peristaltic contractions whose latencies, peristaltic velocities, amplitudes, and neurotransmitter mediators vary with the parameters of electrical stimulation.[21] These contractions have been called S contractions.[11]

Long trains of vagal stimulation (10 seconds) cause contractions soon after the onset of the stimulus (A contraction) and after cessation of the stimulus (B contraction).[11,15,21] The prevalence and quantitative features of A and B contractions also depend on the parameters of electrical stimulation and show considerable interspecies variations.[10,11,15,22] Moreover, the neural mediators of these two responses are also different; A waves are largely cholinergic because they are sensitive to atropine, whereas B waves are noncholinergic and nonadrenergic because they are resistant to antimuscarinic as well as to antiadrenergic agents.[10,11,15,16] Because the S, A, and B waves caused by vagal efferent stimulation may involve different neurotransmitters, we examined the effect of L-NAME on each of these contractions.

Influence on S Contractions

Short-train vagal efferent stimulation produced contractions at all esophageal sites. Figure 3 illustrates the effect of L-NAME and L-NAME plus atropine on S contractions, and Figure 4 shows the cumulative data on 10 observations in four animals. With the stimulus parameters of 60 V, 0.5 millisec-

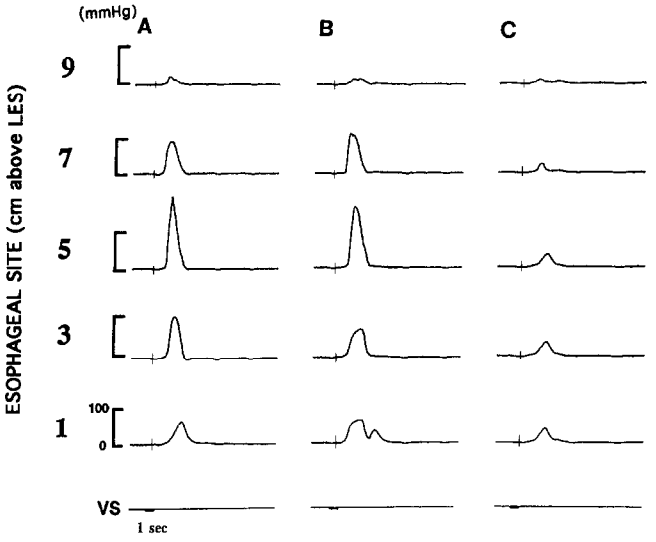

Figure 3. Effect of L-NAME and atropine on S contractions due to short-train vagal efferent stimulation in one animal. (*A*) Esophageal contraction during the control period. (*B*) L-NAME treatment reduced the latencies and the latency gradient. (*C*) L-NAME plus atropine treatment reduced the amplitude of contractions, particularly at the upper sites, and prolonged the latencies. Vagal efferent stimulation parameters: 60 V, 0.5-milliseconds square wave pulse at 20 Hz for 1-second train.

onds, 1-second train, and 20 Hz, the latencies tended to increase distally along the esophagus. However, ANOVA shows that the latency gradient was not statistically significant ($P > 0.05$).

L-NAME treatment reduced the latencies, particularly at the distal sites. Addition of atropine (30 μg/kg) increased the latencies of contractions at all esophageal sites by approximately 0.5–1 second (Figure 4).

The mean amplitude of contractions varied from 25 ± 5 mm Hg to 150 ± 27 mm Hg depending on the esophageal site. L-NAME treatment caused a small and insignificant decrease in the amplitude of contractions. Addition of atropine caused a significant ($P < 0.05$) reduction in amplitudes of contractions at all sites except the 9-cm site (Figure 4).

Influence on A Contractions

Figure 5 shows an example of the effect of L-NAME and L-NAME plus atropine on A and B contractions in one animal, and Figure 6 shows the cumulative data in four animals. A contractions with 10-second vagal efferent stimulation (0.5 milliseconds, 60V, and 20 Hz) had mean latencies of contraction that varied from 1.2 to 1.9 seconds and amplitudes of contraction varying from 15 ± 3 mm Hg to 145 ± 27 mm Hg depending on the esophageal site in different animals (Figure 6). Although the latencies of A contractions were greater in the distal than in the proximal esophageal sites, ANOVA showed no significant differences ($P > 0.05$). L-NAME treatment reduced the latencies of contractions, particularly at distal esophageal sites.

Addition of atropine increased the latencies of contractions, prolonging them even beyond control levels. After L-NAME and atropine treatment, the mean latencies were 1.7–2.5 seconds at different esophageal sites (Figure 6).

The amplitude of A contractions was not significantly modified by L-NAME treatment. Moreover, in some animals intrastimulus intermediate contractions appeared after L-NAME treatment. Addition of atropine markedly inhibited the amplitude of A contractions.

Influence on B Contractions

As shown in Figure 5, L-NAME treatment completely abolished B contractions at all esophageal sites. Atropine treatment alone has been reported not to modify the amplitude of B contractions.[16] B contractions occurred at short latencies after the end of the stimulus. The mean amplitude of B contractions during the control period varied from 30 to 90 mm Hg at different esophageal sites. The latency of contractions was 1.0–1.5 seconds, consistent with

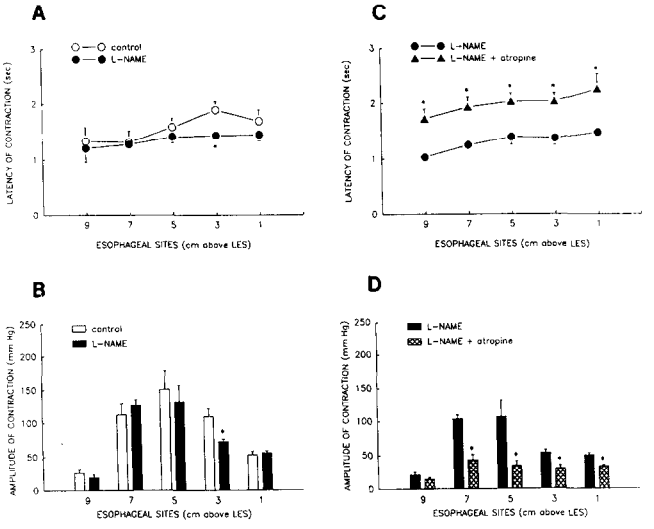

Figure 4. Effects of L-NAME and atropine on esophageal S contractions. (*A*) Modest effect of L-NAME in reducing the latencies of contraction. Note that the control latencies of S contractions are much smaller than those of P contractions. Also note that L-NAME reduced the latency gradient. (*B*) L-NAME did not significantly influence the amplitude of S contractions except at the 3-cm level (n = 10 observations in four animals). (*C* and *D*) Atropine increased the latencies and reduced the amplitudes of contraction at almost all levels (n = 6 observations in two animals). Values are mean ± SE. *Statistically significant ($P < 0.05$) difference by paired *t* test. Vagal efferent stimulus parameters: 60 V, 0.5-millisecond pulse duration, at 20 Hz for 1 second.

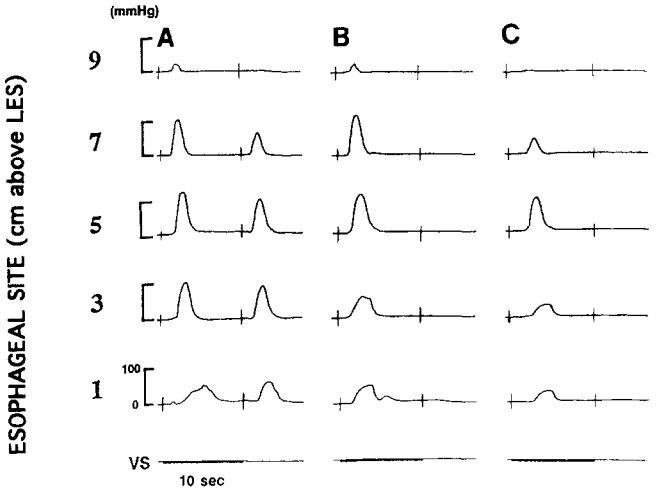

Figure 5. Influence of L-NAME on A and B contractions in response to long-train vagal efferent stimulation in one animal. (*A*) Both A and B contractions. Note that A contractions have a prominent latency gradient. (*B*) L-NAME reduced the latencies and the latency gradient of A contractions and abolished B contractions. (*C*) Addition of atropine (30 μg/kg) prolonged the latencies and decreased the amplitudes of A contractions. Vagal efferent stimulation parameters: 60 V, 0.5-millisecond square wave pulses, at 20 Hz for 10 seconds.

very fast conduction velocities for B contractions. L-NAME treatment completely abolished B contractions at all the esophageal sites in all observations in all four animals tested.

Influence of L-NNA and L-NAME on Circular Muscle Off Contraction In Vitro

The effect of the NOS inhibitor L-NAME with VS could be exerted at one or more levels in the neural pathway or at the neuromuscular sites. Therefore we examined the effects of NOS inhibitors on circular muscle strips in vitro. EFS with long trains causes on and off contractions of the esophageal body circular muscle.[2,18] The prevalence and amplitude of these different contractions vary with different stimulus parameters and esophageal sites and among animal species.[18,23] In this study using circular muscle strips from 3 cm above the lower esophageal sphincters of opossums, off contractions were most prominent and on contractions were inconspicuous.

As shown in Figure 7, L-NAME treatment caused a concentration-dependent decrease in off contractions and evoked on contractions, particularly at high frequencies of stimulation. The inhibitory effects of L-NAME were reversed by L-arginine (10^{-3} mol/L).

The inhibitory effects of L-NAME on off contractions and its excitatory effects on on contractions were concentration dependent and varied with different frequencies of stimulation. Higher concentrations of L-NAME abolished off contractions. Off contractions occurring before the end of stimulus (negative latencies) were not seen. Although on contractions occurred at high frequencies of stimulation

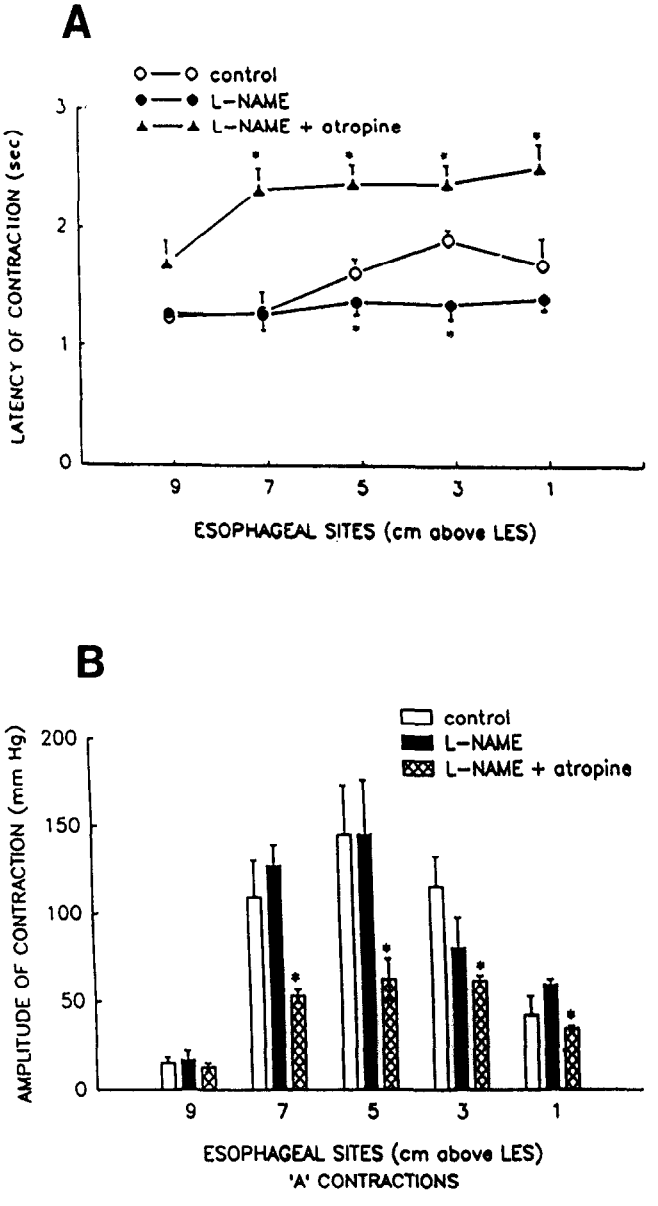

Figure 6. Influence of L-NAME and atropine on A and B esophageal contractions in response to long-train vagal efferent stimulation. (*A*) L-NAME reduced the latencies of A contractions at the distal esophageal sites and reduced the latency gradient (n = 12 observations in four animals). Addition of atropine lengthened the latencies of A contractions (n = 9 observations in three animals). (*B*) L-NAME did not modify the amplitude of A contractions, whereas the addition of atropine caused a significant reduction in amplitude. (*C*) L-NAME (10^{-3} mol/L) and L-NAME plus atropine (10^{-5} mol/L) completely abolished the B contractions (n = 12 observations in four animals). Values are mean ± SE. *Statistically significant ($P < 0.05$) difference by paired *t* test. Vagal effect stimulus parameters: 60 V, 0.5-millisecond pulse duration, 20 Hz for 10 seconds.

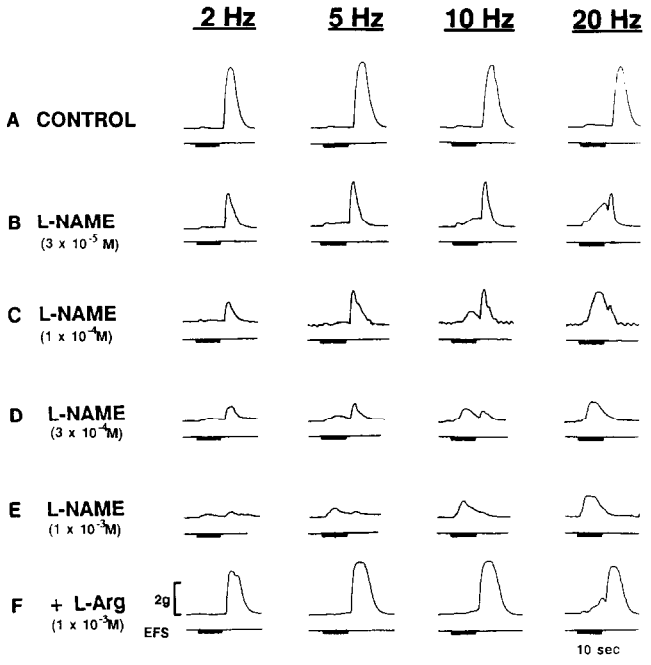

Figure 7. Effect of L-NAME on on and off contractions of esophageal circular muscle strips induced by EFS in vitro from one animal. (*A*) EFS caused prominent off contractions and a small on contraction at frequencies of stimulation of 2–20 Hz. Increasing concentrations of L-NAME caused a progressive decrease in the latencies and amplitudes of off contraction (*B–E*). (*F*) L-Arginine reversed the action of L-NAME. Similar effects were seen at higher frequencies of stimulation. Electrical field stimulation parameters: 60 V, 0.5-millisecond pulse duration, 2–20 Hz for 10 seconds.

after L-NAME treatment, they could be distinguished readily from off contractions.

The latencies of off contractions increased with increasing frequencies of stimulation. The latencies of off contractions were 1.0 ± 0.2, 1.1 ± 0.2, 1.5 ± 0.2, and 1.8 ± 0.2 seconds at 2, 5, 10, and 20 Hz, respectively (n = 5). L-NAME treatment caused reduction in latencies as well as reduction in the amplitude of off contractions. At 10 Hz, the latencies and amplitude of off contractions were 1.5 ± 0.2 seconds and 3.8 ± 0.7 g in control and 0.7 ± 0.1 seconds and 2.6 ± 0.9 g after L-NAME treatment (10^{-4} mol/L), with no off contractions observed after L-NAME treatment (10^{-3} mol/L) L-NNA produced similar effects.

Figure 8 shows cumulative information on the inhibitory effects of L-NAME and L-NNA on off contractions at different stimulus frequencies. Based on EC_{50} values, L-NNA was five times more potent than L-NAME as an inhibitor of off contractions.

L-Arginine (10^{-3} mol/L) itself had no effect on esophageal circular muscle, nor did it modify responses to EFS (2–20 Hz; n = 3). Atropine treatment (10^{-5} mol/L) had no effect on off contractions but moderately inhibited on contractions (n = 3).

Discussion

These studies show that inhibition of L-arginine–NO metabolic pathway with L-NAME shortens the latency period (from stimulus to contraction) throughout the esophagus. This effect is most marked in the distal esophagus, where the latency period ordinarily is the longest.[3,4] Consequently, L-NAME reduces the aborally progressive latency gradient to the point that esophageal contractions become nearly simultaneous. This decrease in latency period and latency gradient is observed for peristaltic contractions evoked by swallowing (P contractions), in which the latency gradient is most marked. Given in sufficient doses, L-NAME abolishes B contractions in vivo. L-NNA also reduces the latencies and amplitudes of off contractions of esophageal muscle strips induced by EFS in vitro. These findings suggest that endogenous NO or a related compound mediates both the latency period and the latency gradient responsible for esophageal peristalsis and is the effector of both B contractions and off contractions.

An L-NAME–associated decrease in the latency period and latency gradient for primary peristaltic contractions in vivo has not been described before, but reduction in the latency period and a loss of latency gradient for off contractions in vitro with L-NNA has recently been reported.[11,24] Tottrup et al. did not find an inhibitory effect of L-NNA on latencies or amplitudes of S contractions.[12] The latencies and the latency gradients of S and A contractions are small and therefore may not show the effects of L-NAME. La-

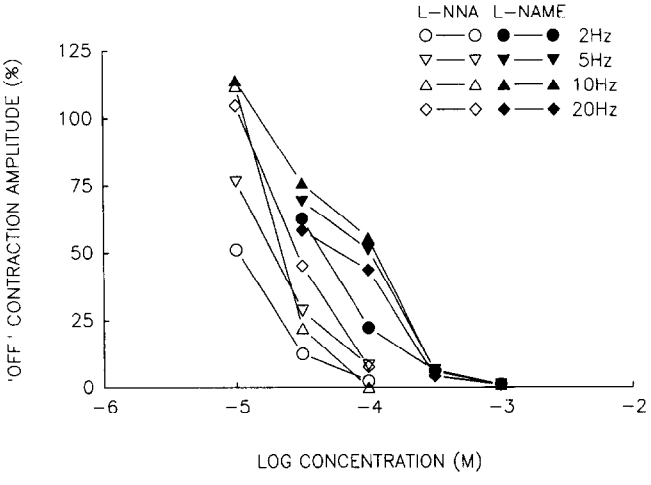

Figure 8. Effects of L-NNA and L-NAME on the amplitude of off contractions. Note that both L-NNA and L-NAME cause concentration-dependent inhibition of off contractions at all frequencies of EFS examined (n = 7–8 observations in three animals). L-NNA was approximately five times more potent than L-NAME based on comparison of EC_{50} values. Contraction amplitudes are represented as a percent of control amplitudes taken as 100%.

tency of peristaltic waves appears to be a function of inhibitory neurotransmission, and therefore our observations suggest that L-NAME acts by eliminating the action of an inhibitory neurotransmitter. Whereas L-NAME inhibits NO synthesis, these findings support an involvement of NO or a related substance in noncholinergic, nonadrenergic inhibitory neurotransmission. NO has been shown to cause hyperpolarization of smooth muscle, an effect that may be the electrophysiological basis for the inhibition of esophageal muscle contraction.[24]

In addition to its effects on latency, L-NAME inhibited, and in sufficient concentration abolished, B contractions in vivo and off contractions in vitro. These findings suggest that NO or a related compound mediates both B contractions in vivo and off contractions in vitro. Antagonism of the off contractions in vitro was accompanied by a decrease in its latency period and by the appearance of on contractions.

The mechanism whereby NO mediates off contractions is not clear. One explanation is that NO-induced hyperpolarization and inhibition of esophageal smooth muscle is followed by rebound excitation.[25,26] This would explain the total disappearance of off contractions with sufficient concentrations of L-NAME.

In contrast to its dramatic effect on off contractions, L-NAME had little apparent effect on the amplitude of peristaltic waves induced by swallowing. These observations suggest that primary peristaltic contractions do not represent B or off contractions. However, the contribution of B or off contractions to swallow-induced peristalsis remains uncertain. Although at first glance these observations might suggest that the NO-mediated off contractions (rebound excitation) do not contribute to normal peristalsis, this may not be the case. L-NAME presumably eliminates the inhibitory influence of NO, and therefore the actions of the excitatory neurotransmitters on esophageal smooth muscle should be unopposed during L-NAME administration. If NO causes only inhibition without rebound excitation, then L-NAME should increase the amplitude of peristaltic contractions by eliminating NO production. The observation that contraction amplitude does not change appreciably with L-NAME administration does not therefore exclude a contribution of the NO-mediated off contractions to the amplitude of primary peristaltic contractions. Further studies are needed to define the relationship of the experimentally produced off and B contractions to primary peristalsis.

The combination of L-NAME and atropine reduced the amplitude of peristaltic contractions by 20%–80%. This finding suggests that an important cholinergic element of these contractions is antagonized by atropine. These observations are consistent with the model of esophageal peristalsis that incorporates a role for the regional gradient of cholinergic influence in peristalsis.[18,27–29] The atropine-resistant component of the contraction may be the result of the release of an unknown noncholinergic, excitatory neurotransmitter. Further studies are needed to characterize this substance and to define the involvement of vasoactive intestinal polypeptide and calcitonin gene-related peptide in esophageal peristalsis.[30–33]

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