Recent animal studies have suggested that nitric oxide (NO) plays an important role in the regulation of esophageal motility, being partly responsible for latency period and latency gradient between the onset of a swallow and contractions of esophageal circular smooth muscles. The aim of this study was to evaluate whether endogenous NO is responsible for physiological timing of forthcoming contractions in the human esophageal body after swallowing.

Eight male volunteers (age 21—25 years, weight 67—82 kg) were involved in this placebo controlled study on the effects of increasing doses of the NO synthase blocker, N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA 1.0—4.0 μmol/min i.v.), and/or L-arginine (L-arg) (30 μmol/kg-min i.v.) on the peristalsis of esophageal body in response to wet swallows (5 ml of water) and lower esophageal sphincter (LES) resting pressure. The esophageal motor activity was determined manometrically using 3-channel electronic catheter. Additionally, during all examinations arterial blood pressure (BP) was measured every 5 min.

L-NMMA resulted in a significant and dose dependent reduction in the latency period between swallows and the onset of contractions which was most pronounced in the distal esophagus (control: 7.07 ± 0.74 s vs. L-NMMA 4.0 μmol/min: 5.87 ± 0.57 s), and this effect was partially reversed after addition of L-arg to the L-NMMA infusion (6.91 ± 0.62 s). L-NMMA infusion significantly reduced the duration of contractions and increased the velocity of onset propagation but did not change the amplitude of contractions and again, these effects were reversed during simultaneous infusion of L-arg. The resting tone of LES increased significantly during infusion of L-NMMA and these effects were reversed by addition of L-arg. The mean BP significantly increased during infusion of L-NMMA (control 97.0 ± 5.7 vs. L-NMMA 4.0 μmol/min: 116.4 ± 3.1 mm Hg) and this was also reversed by L-arg.

We conclude that in humans endogenous NO is involved, at least in part, in the physiological regulation of motility patterns of the distal esophageal body and LES.

**Key words:** nitric oxide, esophageal motility, NO synthase blocker.

**INTRODUCTION**

The primary function of the esophagus is to propel the food from pharynx into the stomach. This is achieved through a sequence of swallowing followed by peristaltic contractions in concert with appropriately timed relaxation.
creating aborally migrating pressure waves. The existence of neurally mediated latency period between the onset of swallow and peristaltic activity of the esophageal muscles has previously been established in animal experiments (1) and observed in humans (2), but the nature of transmitters involved remained the subject of controversy for many years. It has been postulated that for these inhibitory effects the nonadrenergic, noncholinergic (NANC) system is responsible. Recently is has been shown that nitric oxide (NO) previously identified as endothelium-derived relaxing factor (3) plays an important role in the latency gradient which is the basis for esophageal peristalsis (4). Intramural neurons release NO which induces inhibitory junction potentials consisting of an initial deep and short lasting hyperpolarization followed by a plateau phase responsible for inhibition of smooth muscles within the esophagus (5) as well as in the stomach (6). Adding considerable support to this hypothesis, the enzyme responsible for NO generation, NO synthase, has immunohistochemically been localized in intramural neurons (7). Lately Murray et al. (8) reported that the blocker of NO synthase $N^\omega$-nitro-L-arginine (L-NNA) decreases the latency and amplitude of contractions of circular muscles in vitro which, however, was not confirmed in in vivo experiments (9).

We have used L-NMMA ($N^G$-monomethyl-L-arginine) to investigate the role of endogenous NO in swallow induced esophageal peristalsis and in maintaining lower esophageal sphincter (LES) pressure in humans.

MATERIAL AND METHODS

Eight healthy male volunteers (age 21—25 years, weight 67—82 kg) were involved in this study after acceptance by the local Ethical Committee. Informed consent was signed by each subject. All of them were nonsmokers with no history of gastrointestinal complaints. L-NMMA 1.0—4.0 mol/min i.v. (Clinalfa, Lauflingen, Switzerland) and/or L-arginine (L-arg; Braun Melsungen AG, Germany) 10—30 mol/kg-min i.v. or saline were infused in random fashion. Each dose of L-NMMA or L-arg or saline was infused for a 10 min period and then doubled. On different occasion, after 10 min infusion of L-NMMA 4.0 mol/min, L-arg was added to the solution at a dose of 30 mol/kg-min and infused together for the next 10 min period. Simultaneously with infusions peristalsis of esophageal body in response to wet swallows (5 ml of tap water, at least 10 swallows for each dose tested at intervals of 30 sec or longer) and resting lower esophageal sphincter (LES) pressure were measured using 4 channel microtransducer Koenigsberg catheter (Koenigsberg, Pasadena, USA) and 2MB Microdigitrapper (Synectics, Stockholm, Sweden). The catheter was localized with first transducer within LES as measured by stationary pull through. Force and duration of contractions were measured at 5, 10 and 15 cm above LES. Each measurement was a mean of 10 wet swallows. Values were expressed as means±SEM and statistical differences of the means were determined by applying the paired Student’s “t” test. Motility patterns and statistics were analysed with specially developed software (Gastrosoft, Irvine, USA). During studies blood pressure and ECG were continuously monitored for sake of security and, additionally, as an antidote to L-NMMA effects, i.v. solution of glycercyl trinitrate (GTN) was kept ready to infuse.
RESULTS

Latency period and esophageal contractions

Intravenous L-NMMA infusion resulted in a significant and dose dependent reduction in latency period between the onset of swallowing and esophageal contractions and this effect was most pronounced in the distal esophagus. The control latency as determined in the lowest part of esophagus was 7.07 ± 0.74 sec and 5.87 ± 0.57 sec after L-NMMA being infused at the highest dose (Fig. 1). Administration of L-arg in increasing doses had by itself no effects on latencies, amplitude or duration of water induced esophageal contractions. However, the reduced latency gradient that occurred following NO synthase inhibitor, was partially restored by L-arg infusion which caused the latency gradient to increase to 6.91 ± 0.62 sec. (Fig. 2). Duration of contractions and the velocity of onset propagation during i.v. saline infusion were 4.07 ± 0.15 sec and 3.93 ± 0.82 cm/sec, respectively. L-NMMA infusion significantly reduced the duration of contractions to 3.63 ± 0.21 sec and increased the velocity of onset propagation to 4.82 ± 0.6 cm/sec (Fig. 3) but did not changed the amplitude of contractions (Fig. 4). These effects as brought about by the NO synthase inhibitor were reversed by subsequent L-arg infusion (Fig. 5).

![Graph showing latency periods in the lower part of esophagus 5, 10 and 15 cm above upper LES border before and after L-NMMA and/or L-arg infusions. S = swallow.](image)
**Fig 2.** Latency periods at the level of 5 cm above upper LES border before and after L-NMMA and/or L-arg infusions.

**Fig 3.** Duration of contractions and onset propagation at the level of 5 cm above the upper border of LES before and after L-NMMA and/or L-arg infusions.
Fig 4. Amplitude of contractions at the level of 5 cm above the upper LES border before and after L-NMMA and/or L-arg infusions.

Fig 5. Manometric tracing of the effect of L-NMMA and L-arg on water induced primary peristalsis at the level of 5 cm above LES. Normal peristaltic contraction, effect of L-NMMA at highest dose and/or L-NMMA and L-arg infusions in response to swallow. The arrow labelled with S mark the onset of swallowing. Note that L-NMMA reduced the latencies.
Lower esophageal sphincter pressure

The resting maximal pressure of LES increased significantly during L-NMMA infusion from $27.6 \pm 8.3$ to $42.2 \pm 11.3$ mm Hg at the highest dose used. Infusion of L-arg reversed effects of L-NMMA on LES by decreasing the resting pressure to $33.2 \pm 9.7$ mm Hg (Fig. 6).

![Graph showing LES resting pressure](image)

*Fig 6. Resting LES pressure before and after L-NMMA and/or L-arg infusions.*

Blood pressure

L-NMMA infusion at maximum dosage increased the mean blood pressure (MBP) from $97.0 \pm 11.5$ to $115 \pm 17.4$ mm Hg ($p < 0.05$). This effect was also reversed towards normal by co-administration of L-arg (Fig. 7).

![Graph showing mean arterial pressure](image)

*Fig 7. Mean arterial pressure before and after L-NMMA and/or L-arg infusions.*
DISCUSSION

Recently it has become increasingly apparent that primary esophageal peristalsis is a biphasic process consisting of an inhibition followed by contraction as shown by Gidda & Goyal (2). The preceding inhibition of contraction is called deglutitive inhibition or initial inhibition. The duration inhibition increases distally along the esophagus and corresponds to the latency of esophageal contractions. It has been shown by Rattan et al. (10) that initial inhibition is associated with hyperpolarization of esophageal circular muscles. It is well known that swallow induced primary peristalsis of the smooth muscle portion of the esophagus partially depends on vagal pathways which is evidenced by the fact that bilateral cervical vagotomy or vagal cooling abolishes primary peristalsis (11—12). The body of the esophagus is a hollow organ and therefore shows no basal tone and, consequently, its inhibition is not demonstrable in manometric recordings. It becomes evident, however, when two swallows are taken within few seconds. Moreover, the amplitudes of contractions are variables presumably due to the phenomenon of deglutitive inhibition (13). This neurally mediated inhibition increases progressively from proximal to distal esophagus creating a latency gradient which is the basis for the propulsive force of esophageal peristalsis. The nature of the inhibitory neurotransmitter and its role in the progression of the latency period and in the development of peristaltic contractions have not been established yet (14). Weisbrodt & Christensen (15) pointed out that there is a gradient of cholinergic and noncholinergic neurons being involved in peristalsis along the esophagus. Cholinergic nerves dominate proximally whereas noncholinergic nerves do so distally. Thus, under physiological conditions esophageal peristalsis involves both cholinergic and noncholinergic contractions. As has been shown by Christensen & Fang (16), esophageal myenteric plexus contains NO synthase together with other inhibitory substances. Nonadrenergic-noncholinergic, nitrinergic neurons conduct inhibitory signals that induce esophageal muscles to relax. In our study, blockade of NO synthase by L-NMMA resulted in reduction in both duration of contraction and onset propagation thus proving that in motor activity of the esophagus NO inhibition is involved not only in the initiation but also in the maintenance of contractions. Our results support the study of Chakder et al. (17) who showed that NO scavenger hemoglobin caused impairment of relaxation and almost simultaneous contractions of esophageal smooth muscles. Gidda & Goyal (2) found that there are two types of preganglionic efferent fibers; short latency fibers which correlate with initial inhibition and long latency fibers which correlate with peristaltic contractions. This suggests that NO is the mediator of both fiber types and, therefore, it can modulate speed, amplitude and duration of peristaltic waves. However, NO is not the only inhibitory transmitter of NANC nerves in esophageal muscles.
Correspondingly, it has been proposed that vasoactive intestinal polypeptide (VIP) or similar inhibitory peptides like calcitonin gene-related peptide (CGRP) and galanin might mediate at least some of the relaxation processes which were shown to be resistant to antagonists of NO synthase (18). We used L-NMMA to investigate the role of endogenous NO in swallow induced esophageal peristalsis. Our results suggest that inhibition of the NO metabolic pathway decreased the latency period between swallow onset and contraction which was most pronounced in the distal esophagus where latency period normally is the longest one. It suggests that NO and related substances function as inhibitory neurotransmitters mostly in the distal esophagus and may play a role in producing both latency period and the aborally progressive latency gradient. Similar results were obtained by Yamato et al. (19) on opossum muscle strips where he found that L-NAME reduces the latencies as well as amplitudes of the off contraction. Therefore he suggests that NO is an effector of esophageal off contractions, which may contribute to the amplitude of primary peristaltic contractions associated with swallow induced peristalsis. In our data we did not observe any significant effects of L-NMMA on the amplitude of contractions in the distal part of the esophagus despite of the fact that mean arterial pressure increased. Discrepancies resulted probably mainly from different preparation, doses and species used. Vagal nerves are generally held to exert inhibitory actions and their stimulation is thought to induce LES relaxation (10, 20). Whether tonic excitatory vagal discharge plays a role in the maintenance of LES tone is controversial. In our study the blockade of NO synthase by L-LMMA resulted in an increase of the mean LES pressure suggesting that nitrinergic fibers are partially involved in the maintenance of LES pressure counteracting intrinsic myogenic activity. Our study demonstrates for the first time in vivo the involvement of endogenous arginine/NO pathway in the physiological regulation of (3) peristalsis in the smooth muscle part of esophageal body and (7) maintance of LES pressure in humans.

REFERENCES


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