Reflux Mechanisms in Gerd : Analysis of the role of transient lower esophageal sphincter relaxations

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EFFECT OF L-ARGININE ON LOWER ESOPHAGEAL SPHINCTER MOTILITY IN MAN

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ABSTRACT

Background: Inhibitory responses of the lower esophageal sphincter are mediated via an L-arginine/nitric oxide pathway. L-arginine is known as the precursor of NO. We have studied the effect of intravenous L-arginine on lower esophageal sphincter (LES) characteristics in man.

Design: Twelve healthy subjects participated in a double blind, placebo controlled randomized study.

Methods: We investigated the effect of continuous infusion of L-arginine (500mg/kg body weight/120 min) in six subjects under fasting conditions. Six other subjects were studied under postprandial conditions. LES pressure (LESP), swallow induced LES relaxations and transient lower esophageal sphincter relaxations (TLESR), were measured with sleeve manometry combined with pH metry. The meal consisted of a carbohydrate-high fat meal. Blood samples were taken before and after administration of L-arginine or saline to determine plasma levels of amino acids, cholecystokinin and gastrin.

Results: Plasma levels of arginine and citrulline significantly ($p<0.05$) increased during L-arginine infusion. L-arginine did not affect plasma hormone levels. Under fasting conditions LESP and TLESR were not influenced by L-arginine. Ingestion of the carbohydrate-high fat meal significantly decreased LESP. L-arginine did not significantly influence TLESR frequency, neither under fasting conditions, nor postprandially.

Conclusions: These results suggest that in humans under fasting or postprandial conditions intravenous infusion of L-arginine does not influence LES motility.

INTRODUCTION

Nonadrenergic noncholinergic (NANC) nerves mediate inhibitory responses in the gastrointestinal tract and regulate important physiological reflexes such as relaxation of the lower esophageal sphincter (LES) after swallowing and receptive relaxation of the proximal stomach [1-3]. Nitric oxide (NO) has been recognized as inhibitory neurotransmitter of NANC nerves in gastrointestinal smooth muscles [4]. NO is synthesized from the amino acid L-arginine by nitric oxide synthases. In the metabolic route L-arginine is the precursor of NO whereas citrulline is byproduct of the reaction. Recent studies have described the involvement of the L-arginine - nitric oxide pathway in esophageal motility. Studies in dog [5] and opossum [6,7] have shown that NO plays an important role in NANC mediated responses in the esophageal body and LES. The antagonistic action of nitric oxide synthase inhibitors is reversed by L-arginine but not by D-arginine. In vitro examination of circular smooth muscle of the human esophageal body and LES have shown similar effects [8,9].

Transient lower esophageal sphincter relaxations (TLESR) are prolonged relaxations of the LES not associated with swallowing [10]. TLESR are thought to be a venting mechanism allowing release of air from the stomach. However, during TLESR gastroesophageal acid reflux may occur [11]. In fact, in humans TLESR are the most common mechanism permitting gastroesophageal reflux. Gastric distension or ingestion of a meal are potent triggers for TLESR [12]. NO may be involved in the occurrence of TLESRs as suggested by Boulant *et al* [13] but the exact mechanism of triggering TLESR is not known.

If the availability of L-arginine is considered as a rate-limiting factor for NO production, administration of the precursor L-arginine may be expected to result in increased NO formation. The influence of intravenous L-arginine on the LES and TLESR, is unknown. We have investigated in healthy subjects the effect of intravenous administration of L-arginine on LES characteristics including LESP, TLESR and acid reflux, both under fasting and postprandial conditions.

METHODS

Subjects

In a double blind, randomized, placebo controlled study we have evaluated the effect of intravenous L-arginine on LES and esophageal motility. Twelve healthy subjects were studied twice in random order during iv infusion of saline or L-arginine. Six subjects were studied during fasting conditions (3 females, 3 males; age 18 - 26 years). Six other subjects (5 females, 1 male; age 19 - 31 years) were studied under postprandial conditions. None of the subjects had a history of gastro-intestinal disease or surgery or other illness or was on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

The manometry catheter consisted of a multilumen silicone tube (outer diameter 5.0 mm) with seven side holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greendale, Wisconsin, U.S.A.) at a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Synectics Medical, Stockholm, Sweden) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Synectics Medical, Stockholm, Sweden).

The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

Each test was performed on a separate day in random order with an interval of at least seven days. The experiments were started at 08.30 h. after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced into the esophagus and positioned as described above. Two intravenous cannulas were inserted into the antecubital vein of each arm, one for intravenous

infusion, the other for blood sampling. L-arginine (L-arginine.HCL 10%) was given intravenously starting with a bolus of 0.625 ml/kg followed by continuous infusion of 2.1875 ml/kg.h for 120 min (total 500 mg/kg bodyweight). In the control experiments saline was administered intravenously.
At regular intervals (-60, 0, 30, 60, 120, 180 min) three wet swallows with 5 ml of water were given to determine swallow induced LES relaxations. Blood samples for determination of plasma hormone levels were taken from -60 min to 180 min at regular intervals (-60, -30, 0, 15, 30, 60, 90, 120, 180 min). Plasma cholecystokinin (CCK) and gastrin concentration were determined by radioimmunoassay [14,15]. Blood samples to determine plasma levels of amino acids arginine en citrulline were drown at regular intervals: fasting (0 min), after start of infusion (30 min), at the end of infusion (120 min) and one hour after end of infusion (180 min). Plasma levels of amino acids arginine en citrulline were measured as described previously [16].

Fasting protocol

Two tests were performed in random order under fasting conditions [control vs L-arginine (ARG)]. Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by two hours (time 0 to 120 min) during infusion of L-arginine or saline (control). After the end of the infusion esophageal pH and motility were registered for one more hour (time 120 to 180 min).

Fed protocol

Two tests were performed in random order after ingestion of a meal (control-meal vs ARG-meal). The carbohydrate rich - high fat meal consisted of 200 g bananas blended with 125 ml cream and 25 ml Roosvicee (Koninklijke De Ruiter, Baarn, The Netherlands); (5 g protein, 44 g fat and 59 g carbohydrates, 2722 kJ). Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by two hours (time 0 to 120 min) during infusion of L-arginine or saline (control). The meal was given 15 min after start of the infusion. Subjects were asked to consume the meal within 10 min. After the end of the infusion esophageal pH and motility were registered for one more hour (time 120 to 180 min).

Data analysis

Lower esophageal sphincter

Lower esophageal sphincter tracings were analyzed for LES resting pressure (LESP) and LES relaxations (LESR). LESP was defined as mean end-expiratory LESP relative above intragastric pressure over a 2 min period. LESR are divided in swallow induced LESR and spontaneous LESR. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Residual LESP after wet swallows was defined as end-expiratory nadir LESP above intragastric pressure. Transient LES relaxation (TLESR) are defined as spontaneous decreases in LESP of $\geq$ 5 mmHg with a rate of $\geq$ 1 mmHg/sec, within 10 sec reaching a pressure of $\leq$ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related LESR are defined TLESR, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec [10].

pH analysis

Gastroesophageal reflux episodes are defined as a sudden fall of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted.
The mechanisms of each reflux episode were scored using the following criteria. Gastroesophageal reflux occurred during:
(1) TLESR (spontaneous LESR meeting the earlier mentioned criteria; swallow related LESR with the duration of LESR $\geq$ 10 sec)
(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR $\leq$ 10 sec or multiple swallowing).
(3) LES pressure drift (a gradual loss of basal LES pressure).
(4) Absent LES pressure (LESP less than 2 mmHg above intragastric pressure).
(5) Abdominal strain (an increase in abdominal pressure).

Statistical analysis

Data are expressed as mean values ± SEM. Data were analyzed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A p value of <0.05 was considered significant for all analyses.

RESULTS

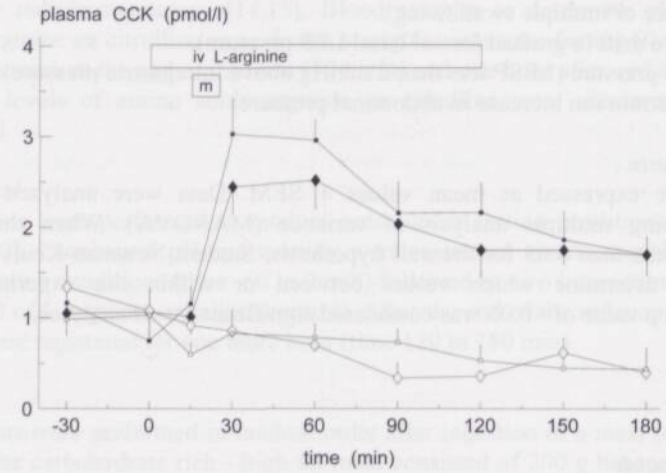
Plasma amino acids

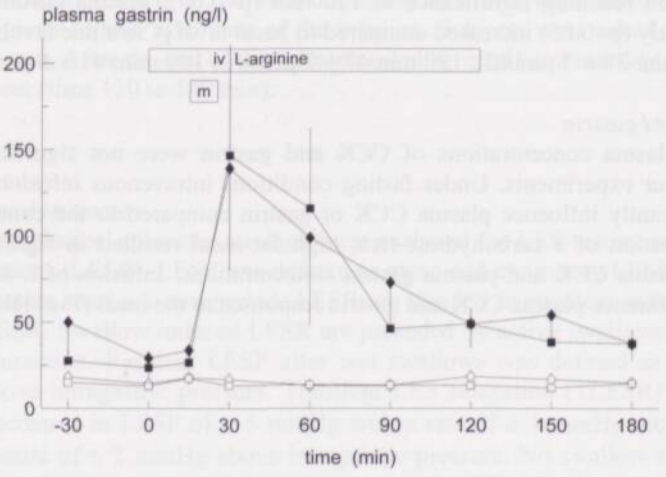
Intravenous infusion of L-arginine significantly ($p<0.01$) increased plasma arginine levels over basal (L-arginine levels at basal: 45 ± 9 µmol/L; 30 min: 1106 ± 132 µmol/L; 120 min 1758 ± 228 µmol/L; 180 min: 568 ± 108 µmol/L). Plasma citrulline increased during L-arginine infusion reaching significance at 120 min ($p<0.05$). Plasma citrulline increments were significantly ($p<0.05$) increased compared to basal level (Citrulline levels at basal: 31 ± 5 µmol/L; 30 min: 39 ± 5 µmol/L; 120 min 47 ± 7 µmol/L; 180 min: 41 ± 5 µmol/L).

Plasma CCK and gastrin

Basal plasma concentrations of CCK and gastrin were not significantly different between the four experiments. Under fasting conditions intravenous infusion of L-arginine did not significantly influence plasma CCK or gastrin compared to the control experiment (Fig. 1A). Ingestion of a carbohydrate-rich, high fat meal resulted in significant ($p<0.01$) increases in plasma CCK and plasma gastrin concentrations. Infusion of L-arginine did not significantly influence plasma CCK and gastrin responses to the meal (Fig. 1B).

Figure 1A-B. *Plasma levels (mean ± SEM) of [A] cholecystokinin (CCK) and [B] gastrin during control (open squares) or continuous infusion of L-arginine (open diamonds). L-arginine was given intravenously in a dosage of 500 mg/kg in 120 min. Six healthy volunteers were studies under fasting conditions. Six other healthy volunteers were studied after ingestion of a carbohydrate high fat meal (M) (closed squares and closed diamonds).*





Esophageal body motility

Esophageal body contractions and peristalsis were not significantly influenced by intravenous L-arginine neither under fasting conditions nor under postprandial conditions. For the distal esophageal body contractions, the amplitude was 69 ± 8 mmHg versus 67 ± 4 mmHg (arginine vs control), the duration was 3.2 ± 0.1 versus 3.3 ± 0.2 s. and the velocity was 4.7 ± 0.3 versus 4.0 ± 0.2 cm/s.

Swallow induced LES relaxation

Swallow induced LES relaxations were complete in all experiments. Residual LESP after 5 ml wet swallows was similar during ARG (≤ 1 mmHg) to that during control (≤ 1 mmHg). Residual LESP after ingestion of a meal was similar during ARG-meal (≤ 1 mmHg) and during control-meal (≤ 1 mmHg). The duration of LES relaxations during ARG and control were not significantly different (4.0 ± 0.3 sec vs 4.3 ± 0.2 sec). The duration of LES relaxation after ingestion of a meal was not influenced during ARG-meal (4.7 ± 0.4 sec) compared to control-meal (4.7 ± 0.3 sec).

Lower esophageal sphincter pressure

LESP in the basal period (-60 to 0 min) was not significantly different between the control and ARG experiment. During two hours of intravenous infusion of L-arginine there were no significant changes in LESP compared to the control experiment (Fig. 2A). LESP in the basal period was not significantly different between the control-meal and ARG-meal experiment. After ingestion of the fat-rich meal LESP decreased significantly ($p<0.01$) compared to basal level from t=20 to t=180 min. Intravenous infusion of l-arginine had no significant effect on LESP compared to the control-meal experiment (Fig. 2B).

Transient lower esophageal sphincter relaxation

The frequency of TLESR was not significantly different between the basal periods of the four experiments (Table 1). During the control experiment no significant changes in TLESR frequencies were observed. Intravenous infusion of L-arginine had no significant effect on the frequency of TLESR compared to the control experiment. After ingestion of the meal the frequency of TLESR significantly ($p<0.05$) increased in the first postprandial hour (4.5 ± 0.8 TLESR/h). During L-arginine infusion the postprandial increase of TLESR (5.3 ± 1.3 TLESR/h) was not significantly different compared to meal-control experiment. The duration of TLESRs was not significantly different between the four experiments (control: 15.3 ± 1.1 sec; ARG: 17.3 ± 1.9 sec; control-meal: 17.6 ± 1.7 sec; ARG-meal: 16.7 ± 0.3 sec).

Figure 2A-B. *Lower esophageal sphincter pressure (LESP) (mean ± SEM) during control (open squares) or continuous intravenous infusion of L-arginine (open diamonds) in six healthy volunteers under [A] fasting and [B] postprandial conditions. L-arginine was given intravenously in a dosage of 500 mg/kg in 120 min. The 400 ml carbohydrate high fat meal (M) consisted of 5 g protein, 44 g fat and 59 g carbohydrates (closed squares and closed diamonds).*

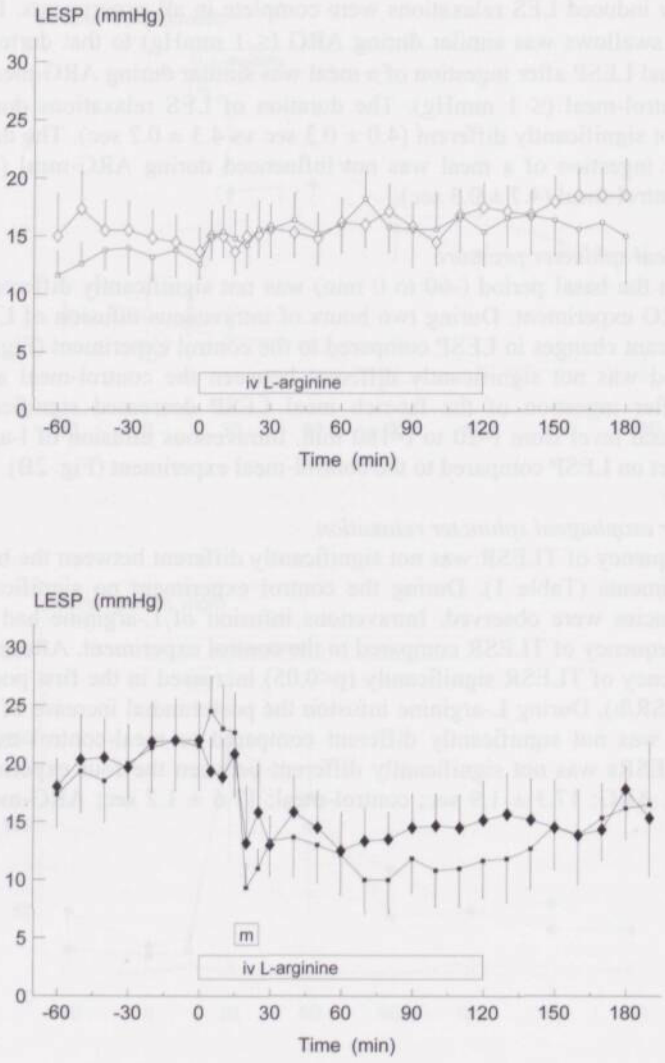


Table 1. *Frequency of TLESR (number/hour; mean±SEM) one hour before (basal) and during three hours of intravenous infusion of saline (control) or arginine (ARG) in six healthy subjects under fasting and postprandial conditions. Asterisks denote a significant ($p<0.05$) increase compared to basal.*

TLESR	control	ARG	control-meal	ARG-meal
Basal	3.0±0.8	1.5±0.6	2.7±0.7	2.8±1.1
Infusion				
I	1.5±0.4	2.7±0.6	4.5±0.8*	5.3±1.3*
II	2.7±0.5	2.7±0.6	3.5±0.7	3.0±1.2
III	2.7±0.3	2.8±0.5	2.8±1.0	3.2±1.0

Mechanisms of gastroesophageal reflux

As expected, the number of reflux episodes and the percentage of time pH<4 was low in the healthy volunteers even after meal ingestion. In all experiments the predominant mechanism of reflux were TLESRs (80%). (Table 2).

Table 2. *Mechanisms of gastroesophageal reflux during three hours of intravenous infusion of saline (control) or arginine (ARG) in six healthy subjects under fasting and postprandial conditions.*

Mechanisms of GER	control	ARG	control-meal	ARG-meal
% time pH <4	0.28%	0.58%	0.55%	0.57%
TLESR	11 (69%)	19(83%)	21(73%)	15(100%)
Swallow induced LESR	2(13%)	0(0%)	3(10%)	0(0%)
LES pressure drift	1(6%)	4(17%)	2(7%)	0
Absent LES pressure	0	0	1(3%)	0
Abdominal strain	1(6%)	0(0%)	1(3%)	0(0%)
Other	1(6%)	0(0%)	1(3%)	0
Total	16(100%)	23(100%)	29(100%)	15(100%)

DISCUSSION

Ingestion of a fat-rich meal significantly reduced LESP and significantly increased the postprandial frequency of TLESR. Intravenous infusion of L-arginine did not significantly influence LES pressure or TLESRs neither under fasting nor under postprandial conditions. Swallow induced relaxations of the LES and TLESRs were not affected by L-arginine.

The amount of L-arginine that can be administered intravenously without vascular effects is limited. The total dose of L-arginine we administered may affect blood pressure or heart rate when administered in a 30 min period [17]. We have administered the total dosage in 120 min to avoid systemic effects that might influence LES. L-arginine administration resulted in increases in the plasma levels of L-citrulline pointing to increased NO production.

It is currently not possible to constitute in vivo direct proof of stimulation of NO synthesis by L-arginine infusion in man, because of the short half-life of NO caused by oxidation to NO2- and NO_3^- [18].

The effect of L-arginine may very much depend on the study conditions of L-arginine may, under certain circumstances, increase agonist-stimulated NO synthesis. In animal experiments LES relaxation due to electrical nerve stimulation is antagonized by inhibitors of NO biosynthesis. The effect of NO synthase inhibitors is prevented by L-arginine. These studies provided evidence that NANC relaxations are mediated by NO. Similar experiments with human smooth muscle specimens taken from the esophagogastric junction showed consistent results that inhibitory NANC responses to electrical stimulation of nerves in human LES are mediated by a product of L-arginine/nitric oxide pathway.

Willis *et al* [19] have studied the effect of a NO donor (Molsidomin) on LES. The NO donor decreased basal LESP and slightly reduced amplitude and peristaltic velocity of dry swallows. However LES responses to wet swallows were not affected. These results are consistent with our findings that infusion of the precursor of NO, L-arginine did influence esophageal motility after wet swallows. Luiking *et al* [20] have studied the effect of long-term oral L-arginine supplementation on LES motility. A daily dose of 30 g for 8 days suppressed the late postprandial LESP increase. The frequency of TLESR was not affected by L-arginine but the mean duration of TLESR was prolonged. Plasma levels of L-arginine after oral intake of L-arginine were much lower compared to intravenous infusion. In the present study, even during acute increases of plasma L-arginine we did not find significant changes in TLESR characteristics. TLESRs are spontaneous LES relaxations not related to swallowing. The exact mechanism of stimulation of TLESR is not known. Fat containing meals provoke TLESR. This effect may be mediated via CCK while ingestion of fat increases plasma CCK levels. The putative role of CCK on TLESR is under investigation. CCK-33 significantly reduced LES pressure but the postprandial frequency of TLESR was not influenced by intravenous CCK-33 [21]. CCK-8 increased the frequency of TLESR during gastric distension with an intragastric barostat bag [22] whereas a CCK-A receptor antagonist inhibited the frequency of TLESR [22] Boulant *et al* reported that triggering of TLESR by gastric distension is inhibited not only by CCK-A receptor antagonist but also by a NO synthase inhibitor under stimulatory conditions [13]. It was than suggested that CCK may be involved in the occurrence of TLESR through peripheral CCK-A receptors and an L-arginine nitric oxide pathway. This study shows that, after stimulation of endogenous CCK, addition of the precursor of NO, L-arginine, did not increase the frequency of TLESR.

In conclusion, we have studied the effect of intravenous infusion of L-arginine on LES characteristics. Neither under fasting nor under postprandial conditions did L-arginine significantly influence LESP, TLESRs or esophageal motility.

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