Role of nitric oxide in fasting gastric fundus tone and in 5-HT$_1$ receptor-mediated relaxation of gastric fundus

BERNARD COULIE, JAN TACK, DANIEL SIFRIM, ANTONIUS ANDRIOLI, AND JOZEF JANSSENS

Center for Gastroenterological Research, University Hospital Gasthuisberg, Catholic University of Leuven, B-3000 Louvain, Belgium

Coulie, Bernard, J an Tack, Daniel Sifrim, Antonius Andrioli, and J ozef J ansSENS. Role of nitric oxide in fasting gastric fundus tone and in 5-HT$_1$ receptor-mediated relaxation of gastric fundus. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G373–G377, 1999.—Fasting gastric fundus tone is maintained by continuous cholinergic input. 5-Hydroxytryptamine-1 (5-HT$_1$) receptor activation decreases gastric fundus tone in humans. Whether this fundus-relaxing effect is mediated via inhibition of cholinergic input or via activation of a nitrergic pathway is unknown. The aim of the present study was to determine the effect of nitroglycerin inhibition on feline gastric fundus tone and on 5-HT$_1$ receptor-mediated relaxation of the fundus. Administration of N-nitro-L-arginine methyl ester (L-NAME) alone caused a significant decrease of the mean baseline volume (P < 0.005), which was restored completely by addition of L-arginine. Sumatriptan caused a dose-dependent relaxation of the gastric fundus (P < 0.0005). This relaxation was inhibited by L-NAME (P < 0.02) and was restored by prior administration of L-arginine. Buspirone did not cause any change in mean baseline volume, whereas the sumatriptan-induced relaxation was not affected by prior administration of NAN-190. Our data indicate that fasting fundus tone relies not only on continuous cholinergic input but also on continuous nitroglycerin input, implying that fasting fundus tone is maintained by the balance of a cholinergic and nitroglycerin drive. Furthermore, fundus relaxation via 5-HT$_1$ receptor activation is mediated through activation of a nitrergic pathway.

sumatriptan; feline gastric fundus; nonadrenergic, noncholinergic; serotonin

GASTRIC FUNDUS TONE in the fasted state is maintained by a vagally mediated cholinergic input (5). Gastric accommodation occurs during and after a meal, and this relaxation is mediated by a vagovagal reflex pathway, involving activation of nonadrenergic noncholinergic (NANC) neurons in the gastric wall (4, 20, 24). It is not known whether activation of NANC input to the stomach is solely involved in triggering postprandial fundus relaxation or whether it also contributes to resting gastric tone by counterbalancing excitatory cholinergic input.

5-Hydroxytryptamine (5-HT) is one of the neurotransmitters shown to be involved in vagally mediated gastric relaxation in both guinea pig and mouse (10). More recently, it was demonstrated that 5-HT-induced relaxations of the guinea pig stomach are mediated via the release of nitric oxide (NO) through activation of a 5-HT$_1$-like receptor (31).

5-HT$_1$ receptor activation using the 5-HT$_1$ receptor agonists sumatriptan and buspirone (13, 36) results in an immediate and profound relaxation of the gastric fundus in humans. The neural pathway involved in the inhibitory action of 5-HT$_1$ receptor activation on fundus tone is unknown. The actions of sumatriptan on the fundus must be mediated via an intrinsic pathway because sumatriptan poorly penetrates the blood-brain barrier (19). Moreover, sumatriptan relaxes the isolated guinea pig stomach, which argues against a central action (30). The acute relaxatory effect of sumatriptan on the gastric fundus suggests activation of an inhibitory pathway. However, it is possible that other mechanisms or pathways may be involved because sumatriptan may act via activation of presynaptic 5-HT$_1$ receptor on cholinergic motoneurons causing inhibition of ACh release at the neuromuscular junction.

The present study was undertaken to test the hypothesis that resting gastric fundus tone relies not only on excitatory cholinergic but also on inhibitory nitroglycerin input and that 5-HT$_1$ receptor activation by sumatriptan induces a relaxation of the gastric fundus via activation of a nitroglycerin pathway. Therefore, we developed an in vivo animal model that allowed us to study neural control and pharmacological modulation of gastric fundus tone using an electronic barostat in cats.

MATERIALS AND METHODS

Study subjects. Experiments were performed on seven cats of either sex, with a weight between 3 and 5 kg. The animals were fasted for at least 12 h before the start of the experiment, and light anesthesia was induced with ketamine chloride (Parke-Davis, Warner-Lambert, Zaventem, Belgium) 10–15 mg/kg intramuscularly for induction and 10 mg/kg intramuscularly every 30–45 min for maintenance. The ketamine anesthesia allowed the cats to tolerate intubation by a double-lumen polyvinyl tube with an intragastric plastic bag attached, while spontaneous breathing was preserved. Throughout the experiments a heating pad was used to maintain body temperature of the animal at 37°C.

Gastric barostat. A computer-driven programmable volume-displacement barostat (Synectics Visceral Stimulator, Stockholm, Sweden) was used in these studies to distend the gastric fundus. The barostat device can deliver pressure steps at different rates, while simultaneously monitoring intra-balloon pressure and volume at a sampling rate of 8 s$^{-1}$. Pressure is monitored within the inflation device. Both a pressure recording port and an air inflation port are independently connected by a double-lumen polyvinyl tube (Salem sump tube, 3.3 mm diam, Sherwood Medical, Petit Rechain, Belgium) to an intragastric plastic bag (60 ml capacity; 6.5 cm
maximal diam). The bag was tested before and after each experiment to ensure that there was no leak. In the present study the barostat produced gastric distensions at fixed pressures (isobaric). To produce fixed-pressure distensions, the barostat maintains a constant pressure level by an electronic feedback regulation of the air volume within the intragastric bag. The desired pressure level is set by means of a pressure selector dial, and the intragastric volume is recorded.

Study design. After an overnight fast of at least 12 h the polyvinyl tube with the adherent bag finely folded was introduced through the mouth. The position of the bag in the gastric fundus was secured by pulling back the inflated balloon once it was introduced into the stomach until a resistance was noted, indicating that the balloon was just distal to the lower esophageal sphincter. The position of tube and adherent bag was secured with a screw within a bite block. The polyvinyl tube was then connected to the barostat device. To unfold the intragastric bag it was inflated with a fixed pressure of 15 mmHg of air for 2 min.

The minimal distension pressure was defined as the pressure that resulted in a corresponding volume of >10 ml. During the experiment intragastric pressure was set at minimal distension pressure plus 2 mmHg. A stable baseline was recorded for at least 10 min before any drugs were administered.

During the experiment the following pharmacological agents were applied: sumatriptan (100, 200, 400, and 800 μg/kg and 1.5 mg/kg; Imitrex; Glaxo-Welcome, Brussels, Belgium), the NO synthase (NOS) inhibitor N-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg iv) and subsequent administration of sumatriptan (800 μg/kg sc). L-NAME provokes decrease of intraballoon volume, reflecting contraction of fundus, and inhibits sumatriptan-induced relaxation. Dashed lines, automatic baseline reconstruction as performed by computed algorithm.

RESULTS

Effect of inhibition of nitrergic pathway on resting gastric fundus tone. The effect of inhibition of the nitrergic pathway was tested in six cats. Administration of the NOS inhibitor L-NAME provoked an increase of the fundus tone expressed as a decrease of mean basal intraballoon volume (14.6 ± 3.4 vs. 24.9 ± 3.5 ml, P < 0.005; Fig. 1). The reversibility of the effect of L-NAME on resting fundus tone was tested by administration of the NO-precursor L-arginine in three cats. L-Arginine completely reversed the L-NAME-induced decrease of baseline volume (25.1 ± 3.2 vs. 16.1 ± 2.7 ml; P = 0.005).

Effect of sumatriptan on resting gastric fundus tone. Subcutaneous administration of sumatriptan in doses of 100 and 200 μg/kg did not provoke any change in baseline intragastric volume (n = 2, Fig. 2). A dose of 400 μg/kg resulted in a mean baseline volume increase of 11.8 ± 8.3 ml (n = 2, Fig. 2). Administration of 800 μg/kg sumatriptan resulted in all animals studied (n = 7) in an immediate increase of intragastric volume from a mean baseline volume of 14.6 ± 2 to 40.9 ± 4 ml (P < 0.0005), reflecting a profound relaxation of the gastric fundus (Figs. 1 and 2). Maximal volume was reached after 2.5 ± 0.3 min. Sumatriptan, 1.5 mg/kg, resulted in all animals studied in an immediate increase of intragastric volume from a mean baseline volume of 17.3 ± 1.6 to 42.1 ± 4.8 ml (P < 0.001, Fig. 2). Maximal volume was reached after 3.5 ± 0.6 min. Because 800 μg/kg sumatriptan elicited maximal relaxatory response, this dose was used in subsequent experiments.
Administration of the 5-HT₁A receptor agonist buspirone in a dose of 1 mg/kg (n = 6) did not cause any change in baseline volume (13.5 ± 1.5 vs. 15.9 ± 3 ml, not significant). Prior administration of the 5-HT₁A receptor antagonist NAN-190 in a dose of 100 µg/kg (n = 3) did not have any significant effect on the sumatriptan-induced relaxation (Fig. 3).

Effect of L-NAME on sumatriptan-induced gastric fundus relaxation. The NOS inhibitor L-NAME was administered 10 min before administration of sumatriptan in six cats in which the effect of 800 µg/kg sumatriptan had already been tested on a separate day. After administration of L-NAME the sumatriptan-induced increase of intraballoon volume was significantly inhibited (ΔV 11.1 ± 5.7 vs. 26.3 ± 2.9 ml, P < 0.02; Fig. 1).

To exclude nonspecific effects of L-NAME administration, the reversibility of the inhibition of the relaxatory response to sumatriptan was tested by prior administration of the NO-precursor L-arginine in three cats. L-Arginine was given 10 min after administration of L-NAME and subsequently sumatriptan was administered. Administration of sumatriptan resulted in an increase of intraballoon volume, which was reduced by addition of L-NAME (Fig. 3). Administration of L-arginine in the presence of L-NAME restored the size of the sumatriptan-induced increase of intraballoon volume to the control value (Fig. 3).

**DISCUSSION**

Our data show that inhibition of nitrergic input by L-NAME results in an increase in basal fundus tone reflecting contraction of the fundus. This suggests that resting gastric fundus tone is not only dependent on excitatory cholinergic input but that it also depends on a continuous inhibitory nitrergic drive.

Our observations also confirm earlier findings in humans that activation of 5-HT₁ receptors by sumatriptan profoundly alters gastric fundus tone. Sumatriptan causes a significant increase in mean basal intragastric volume, reflecting a relaxation of the fundus. This relaxation is reversibly blocked by inhibition of NOS, suggesting activation of a nitrergic pathway.

Gastric fundus tone is the result of tonic contraction of muscle fibers of the proximal stomach (3, 32). During fasting the proximal stomach is in a continuous state of tonic contraction that is maintained by vagally mediated cholinergic input (5, 35).

It is unknown whether the cholinergic drive is counterbalanced by an inhibitory drive. We observed that administration of L-NAME results in an increase of the resting fundus tone, an effect that is reversed by L-arginine. These data demonstrate that resting fundus tone is also subject to a continuous nitrergic input. Therefore, fasting fundus tone in the cat seems to be maintained by the balance of a cholinergic and a nitrergic drive.

Variations in gastric tone are instrumental in achieving the reservoir function of the stomach by regulating both gastric accommodation and gastric emptying (4, 16). The fundus functions primarily to receive and store food by receptive relaxation and accommodation (1). The neural pathway initiating the postprandial receptive relaxation is not entirely understood. Gastric relaxation induced by nutrient perfusion into the intestine is mediated through a vagovagally driven NANC mechanism (4). Receptive relaxation of the isolated guinea pig stomach is mediated through activation of a nitrergic pathway (20). Vagal stimulation results in a marked relaxation of the fundus, a phenomenon that is mediated by the release of a NANC neurotransmitter (2, 24, 25, 33). Efferent vagal preganglionic fibers synapse both on cholinergic excitatory and NANC inhibitory intrinsic neurons within the myenteric plexus of the fundus wall. The precise nature of the specific neurotransmitter released by the NANC neurons responsible for gastric relaxation is still debated. Both in vivo and in vitro studies suggest that the two main candidates are NO and vasoactive intestinal peptide (VIP) (6, 7, 17, 18, 20–22, 27–29, 38). Depending on the species studied, both inhibitory neurotransmitters can act concur-
NITRERIC CONTROL OF GASTRIC FUNDUS TONE

recently in mediating NANC relaxation of the fundus (6, 28, 38) or NO can be the only mediator of the NANC relaxation (20, 29, 31).

Data on the effect of 5-HT and the 5-HT receptor subtypes involved in the control of gastric fundus tone are very scarce. Bülbbring and Gershon (10) demonstrated that 5-HT acts as a neurotransmitter of intrinsic neurons in the vagally mediated gastric relaxation in mouse and guinea pig. More recent data demonstrated that 5-HT-induced relaxations of the guinea pig stomach are mediated via NO or a NO-related substance (31). These relaxations are not mediated via interaction with 5-HT₂, 5-HT₃, or 5-HT₄ receptors but probably via receptors that most resemble the 5-HT₁ receptor subtype (26, 31). Ondansetron does not affect gastric fundus tone in humans, confirming that 5-HT₃ receptors are less likely to be involved in the control of fundus tone (40, 41).

Sumatriptan displays weak affinity for the 5-HT₁ₐ receptor (23), which is within the myenteric plexus mainly located presynaptically on cholinergic nerve endings (37). Activation of this receptor mediates inhibition of fast synaptic cholinergic transmission by 5-HT and thus could account for the inhibitory effect of sumatriptan by decreasing cholinergic excitatory input to the gastric fundus. In isolated guinea pig stomach, sumatriptan causes a relaxation that is atropine sensitive and that is blocked by the 5-HT₁ᵐ receptor antagonist NAN-190, suggesting that at least in the guinea pig the mode of action of sumatriptan on gastric tone is via interaction with a 5-HT₁ᵐ receptor on cholinergic motor neurons, blocking ACh release (30). In our experiments, however, the selective 5-HT₁ᵐ receptor agonist buspirone did not mimic the sumatriptan-induced fundus relaxation in cats. Moreover, prior administration of NAN-190 did not affect the fundus relaxatory effect of sumatriptan. This suggests that the mechanism underlying the effect of sumatriptan on the gastric fundus and the 5-HT receptor subtype involved are species specific.

The observed inhibitory effect of NOS blockade on gastric relaxation by sumatriptan is not complete. This suggests that the fundus relaxation may only be partially mediated through activation of a nitrergic pathway and that other nonnitrergic mechanisms are involved. Neurally mediated fundus relaxation can also be produced by adrenergic stimulation or by other nonnitrergic inhibitory mechanisms.

Activation of adrenergic input to the gastric fundus cannot be entirely excluded. However, the relaxatory effect of 5-HT on the canine fundus and in other parts of the gastrointestinal tract is not affected by adrenergic receptor blockade or the sympathetic ganglion blocker guanethidine, making it rather unlikely that norepinephrine is involved as an inhibitory neurotransmitter (9, 11, 31).

In addition to NO, VIP has also been shown to act as a NANC neurotransmitter in the gastric fundus (18, 21, 22). Because VIP and NOS are colocalized in a subpopulation of gastric myenteric neurons (15, 34), stimulation of these neurons by vagal input is likely to result in the corelease of both transmitters. It is conceivable that when sumatriptan activates nitrergic-VIPergic intrinsic neurons, release of both transmitters occurs, resulting in inhibition of fundus tone. Hence, fundus relaxation will still occur when only one of the two components of the inhibitory pathway is blocked, although the kinetics of it may be affected.

In conclusion, our data demonstrate that resting tone of the gastric fundus is the result of a precisely regulated balance between excitatory (cholinergic) and inhibitory (partially nitrergic) neural inputs to the fundus.

From our observations both in humans and in cats, it is clear that activation of the 5-HT₁ receptor by sumatriptan results in an inhibition of fasting gastric fundus tone. This effect is partially mediated through activation of a NANC mechanism, involving NO as the inhibitory neurotransmitter. In cats the specific 5-HT₁ receptor subtype involved does not seem to be a 5-HT₁ₐ receptor. Further studies are warranted to characterize the receptor subtype using selective ligands for in vivo use, which are currently not available. Although it was initially thought that 5-HT₁ receptors were not critically involved in the regulation of gut motility (12), recent observations provide evidence for a role of all the 5-HT receptor subtypes present in the gut, including the 5-HT₁ₐ receptor, in the control of gastric motility (14, 39). Our observations certainly demonstrate that stimulation of 5-HT₁ receptors has a major impact on gastric fundus tone in vivo.

Portions of this study were presented at the American Gastroenterological Association Meeting, 1997, and was printed in abstract form (13).

Address for reprint requests: J. Tack, Dept. of Internal Medicine, Div. of Gastroenterology, Univ. Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium.

Received 3 April 1998; accepted in final form 26 October 1998

REFERENCES