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Influence of the L-arginine-nitric oxide pathway on vasoactive intestinal polypeptide release and motility in the rat stomach in vitro

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Abstract

Endogenous nitric oxide (NO) plays an important role as non-adrenergic, non-cholinergic inhibitory transmitter in the gastrointestinal tract, especially in sphincter regions. The aim of this study was to investigate the influence of NO on pyloric motility and on the release of vasoactive intestinal polypeptide (VIP) in the isolated perfused rat stomach in vitro. Therefore, pyloric motility was continously recorded by a special sleeve manometry catheter placed in the pyloric region and the concentration of VIP was determined in the venous effluent of the portal vein. Arterial perfusion with the nitrate agonist sodiumnitroprusside led to a dose-dependent reduction of the pyloric motility index (basal 166 ± 48 mm Hg/min; sodiumnitroprusside 10^{-6} M 30 ± 20 mmHg/min; sodiumnitroprusside 10^{-4} M 0; $n = 8$, $P < 0.001$) while VIP release was not influenced significantly. Inhibition of endogenous NO production by the NO-synthase inhibitor N^G-nitro-L-Arg (L-NNA) significantly increased pyloric motility (basal motility index 175 ± 28 mmHg/min; L-NNA 10^{-4} M 348 ± 48 mmHg/min; $n = 8$, $P < 0.05$). This effect was completely blocked by addition of L-Arg 10^{-3} M (125 ± 45 mm Hg/min; $n = 8$, $P < 0.01$). L-NNA and L-Arg both did not influence VIP release. Stimulation of the vagal nerve (VS; 20 V, 20 Hz, 1 ms) led to a significant decrease of the pyloric motility index (basal 181 ± 15 mmHg/min; VS 143 ± 21 mmHg/min; $n = 7$, $P < 0.05$), which was consistent even after addition of L-NNA 10^{-4} M (basal 338 ± 58 mmHg/min; VS 228 ± 30 mmHg/min; $n = 7$, $P < 0.05$). Vagal stimulation increased VIP release significantly (basal 14.9 ± 1.4 pmol/l; VS 20.1 ± 2.6 pmol/l; $n = 7$, $P < 0.05$) while L-NNA had no influence on vagally induced VIP release. From these data, we conclude that the pylorus of the rat is under a tonic inhibition by endogenously released NO. Under the conditions studied, NO seems not to mediate the inhibitory effect of vagal stimulation exclusively and there seems to be no interaction between NO and VIP in the rat pylorus.

Keywords: Nitric oxide (NO); VIP (vasoactive intestinal polypeptide); NANC (non-adrenergic non-cholinergic); Pylorus, rat; Motility

1. Introduction

Throughout the gastrointestinal tract, there is a non-adrenergic non-cholinergic (NANC) inhibitory and excitatory innervation (Daniel, 1985). In sphincteric regions, this NANC inhibitory innervation seems to be of major physiological importance for the relaxation of sphincter regions (Anuras et al., 1974).

There is recent evidence that nitric oxide (NO), a metabolite of L-Arg, is a putative inhibitory NANC mediator in the whole gastrointestinal tract (Stark and Szurszewski, 1992; Meulemans and Schuurkes, 1993). Enzymatic blockade of NO production using Arg methyl esters reduced or blocked the inhibitory NANC response in the lower esophageal (Tottrup et al., 1991; Anand and Paterson, 1994), ileocecal (Boeckxstaens et al., 1990) and internal anal sphincter (Rattan and Chakder, 1991) in vitro and in the canine pylorus in vivo and in vitro (Allescher et al., 1992). Furthermore, recent electrophysiological experiments confirmed NO to be the final inhibitory transmitter in the canine pyloric sphincter (Bayguinov and Sanders, 1993).

Vasoactive intestinal polypeptide (VIP) has also been proposed as a possible NANC inhibitor in various sphincter regions (Goyal and Rattan, 1980; Biancani et al., 1985) and a potent inhibitory effect of VIP was also shown in the canine pylorus in vivo (Allescher et al., 1989). In contrast, VIP showed only an inconsistent effect on the pyloric

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muscle in vitro (Allescher et al., 1989, 1992). Vagal stimulation caused a potent inhibitory effect in the pylorus which was almost completely reversed by the NO-synthase inhibitor N^G-nitro-L-Arg methyl ester (L-NAME) whereas the VIP effect was not affected (Allescher et al., 1992). However, after cessation of vagal stimulation, there appeared to be an additional inhibitory component, which was not affected by L-NAME and a possible role of VIP was speculated.

There is only little information on the interaction of NO with VIP in the pyloric region. Inhibition of NO production by Arg methyl esters did not affect VIP-induced relaxation in the canine pylorus in vivo, indicating that any action of VIP is unlikely to require obligatory NO synthesis (Allescher et al., 1992). On the other hand, Grider and Makhlouf reported that NO amplifies the relaxant effect of VIP and possibly enhances VIP release in isolated gastric muscle cells and in smooth muscle from the rat colon (Grider et al., 1992; Grider, 1993).

Therefore, the aim of the present study was to investigate the possible role of NO as NANC mediator in the rat pylorus in vitro and to examine its possible interactions with VIP release.

2. Materials and methods

2.1. Experimental set-up and data analysis

Experiments were carried out in the isolated arterially perfused rat stomach. Male Wistar rats weighing 300 g–340 g were anesthetized with pentobarbital sodium (40 mg/kg i.p.). The abdomen was opened and preparation of the stomach was carried out like described elsewhere (Schusdziarra et al., 1983). In brief, the stomach was isolated and perfused via the gastric artery. The vagal nerve was carefully dissected and preserved along the oesophagus. For sampling of the venous gastric effluent, the portal vein was cannulated distally and ligated proximal of the gastric vein. Then, the stomach was removed with its intact vascular structures and placed in oxygenated Krebs solution by 37°C (NaCl 115.5 mM, KCl 4.16 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.16 mM, $NaHCO_3$ 21.9 mM, glucose 11.1 mM). It was constantly perfused via the gastric artery with a modified Krebs solution (NaCl 115.5 mM, KCl 4.16 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.16 mM, $NaHCO_3$ 21.9 mM, glucose 11.1 mM, human albumin 0.2%, dextran T 70 4%) by a pulsatory roller pump (Desaga, Germany, perfusion rate 2 ml/min), which was maintained at 37°C in a thermostatic water bath. The venous effluent in the isolated portal vein was collected in 1-min portions and immediately stored on ice for later determination of concentrations of VIP. After an incision in the gastric fundus, a special sleeve manometry catheter with a diameter of 1 mm was placed in the pyloric region. The right catheter position was controlled visually by translumination. Pressure changes were continuously recorded with spectramed pressure transducer and were amplified and recorded on a Beckman R 611 chart recorder. For vagal stimulation, the vagal nerve was prepared at the distal esophagus and carefully attached to platinium hook electrodes. Stimulation impulses were applied via a Grass S 9 stimulator (Grass, Quincy, MA, USA) with standard parameters of 20-V pulse strength, 20-Hz pulse frequency and 1-ms pulse width.

The amplitudes of the pyloric contractions were measured as changes in pressure from the mean baseline pyloric pressure to the peak of the contractions and the pyloric motility index (mean amplitude × frequency of contractions (mmHg/min)) was calculated for the whole period. Pressure changes of < 2 mmHg were not taken into account.

2.2. Experimental design

After an equilibration period of 20 min without sampling of the venous efflux, the experiments involving sodiumnitroprusside began with a control period of 10 min with perfusion with Krebs solution like in the equilibration period. The next 10 min the stomach was perfused with sodiumnitroprusside 10^{-6} M dissolved in oxygenated Krebs solution. After a control period of 10 min with normal Krebs perfusion, there was a further 10-min period with sodiumnitroprusside 10^{-4} M, followed by a last 10-min control period.

The experiments involving N^G-nitro-L-Arg (L-NNA) began with a 15-min basal period after 20 min of equilibration. The next 15 min, the stomach was perfused with L-NNA 10^{-4} M dissolved in oxygenated Krebs solution. In the last 15 min, it was perfused with oxygenated Krebs solution containing L-NNA 10^{-4} M and L-Arg 10^{-3} M.

For vagal stimulation, the equilibration period was followed by a 10-min basal period and then a vagal stimulation period of 10 min duration. Then, the perfusion medium was changed and perfusion was carried out with Krebs solution containing L-NNA 10^{-4} M. With this perfusion medium, there was again a control period of 10 min and a second vagal stimulation period of 10 min.

2.3. Radioimmunoassays

VIP immunoreactivity was determined by radioimmunoassay with a porcine VIP antibody obtained from S.R. Bloom (London, UK). Antibody and VIP standard or unknown samples were incubated at 4°C for 72 h in 0.06 M phosphate-EDTA buffer (pH 7.2) containing 0.6% bovine serum albumin. 1 fmol [^{125}I]VIP (Amersham, UK) was added and bound and unbound tracer were seperated by addition of dextran-coated charcoal (6 mg/tube; BDH, Norit GSX) suspended in assay buffer containing 0.25% gelatin. The detection limit of the assay was 0.5 fmol/tube or 5 fmol/ml. The antibody showed no interaction with N-terminal fragments of VIP and no interaction with se-

cretin, glucagon, peptide histidine isoleucine, growth hormone-releasing factor, gastric inhibitory peptide or pituitary adenylate cyclase activating peptide. Differences in the amount of the venous efflux could be excluded as reasons for varying peptide concentrations.

2.4. Drugs

The drugs used in this study were L-Arg, L-NNA and sodiumnitroprusside. The drugs were dissolved in saline on the day of the experiment and further diluted in Krebs solution. All drugs, except L-NNA (Bachem, Heidelberg, Germany) and human albumin (Behring, Germany), were obtained from Sigma (St. Louis, MO, USA).

2.5. Statistics

Data are given as means ± S.E.M.; n indicates the number of independent experiments in different animal preparations. A paired Student's t-test was used to compare the mean values and a probability (P) of $< 5\%$ was considered to be significant.

3. Results

3.1. Effect of sodiumnitroprusside

Under basal conditions, pyloric contractions with a mean amplitude of 34.5 ± 5.9 mmHg and a frequency of

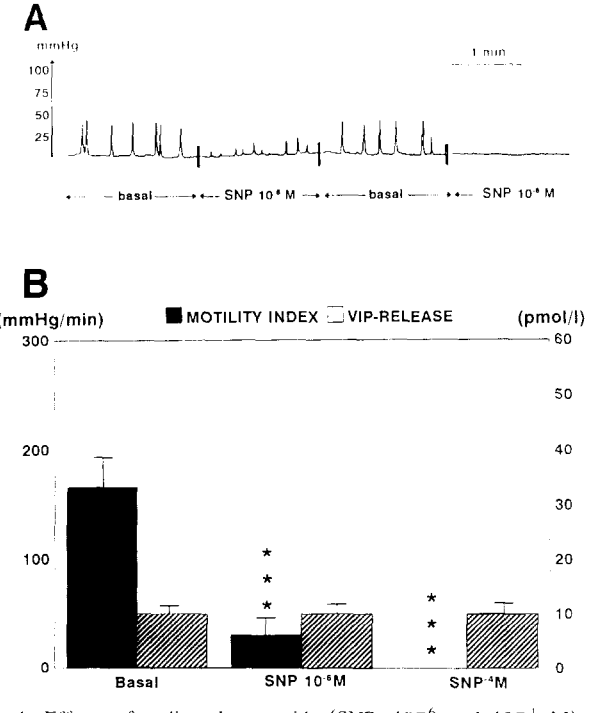

Fig. 1. Effects of sodiumnitroprusside (SNP; 10^{-6} and 10^{-4} M) on pyloric motility and VIP release. (A) Representative tracing of pyloric motility. (B) values are means ± S.E.M. (* * * $P < 0.001$; $n = 8$; Student's t-test).

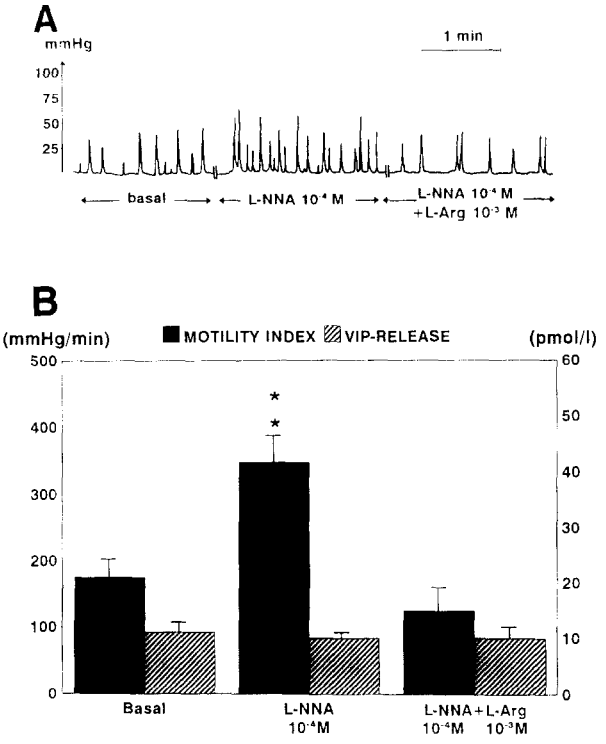

Fig. 2. Effect of blockade of endogenous NO synthesis with L-NNA 10^{-4} M and its reversal with L-Arg 10^{-3} M on pyloric motility and VIP release. (A) Representative tracing of pyloric motility, (B) values are means ± S.E.M. (* * $P < 0.01$; $n = 8$; Student's t-test).

8/min (motility index 166 ± 48 mmHg/min) were recorded. Arterial perfusion with the nitrate agonist sodiumnitroprusside led to a significant dose-dependent reduction of the mean amplitude (sodiumnitroprusside 10^{-6} M 16 ± 8 mmHg, SONP 10^{-4} M 0 mmHg), contraction frequency (sodiumnitroprusside 10^{-6} M 2/min, SONP 10^{-4} M 0/min) and motility index (sodiumnitroprusside 10^{-6} M 30 ± 20 mmHg/min, SONP 10^{-4} M 0 mmHg/min; $P < 0.001$, $n = 8$) (Fig. 1A,B). Simultaneously, VIP release was not influenced significantly (basal 9.6 ± 1.9 pmol/l, sodiumnitroprusside 10^{-6} M 10.6 ± 1.9 pmol/l; sodiumnitroprusside 10^{-4} M 10.4 ± 2.0 pmol/l) (Fig. 1B).

3.2. Effect of L-NNA

Inhibition of endogenous NO production by the NO-sythase inhibitor L-NNA in a concentration of 10^{-4} M significantly increased the pyloric motility index (basal 176 ± 28 mmHg/min, L-NNA 348 ± 48 mmHg/min; $P < 0.05$, $n = 8$) by an increase of contraction frequency (basal 6 ± 2/min, L-NNA 9 ± 2/min; $P < 0.05$, $n = 8$) and mean contraction amplitude (basal 30 ± 5 mmHg, L-NNA 39 ± 3 mmHg; $P < 0.05$, $n = 8$). This effect was completely blocked by additional perfusion with L-Arg 10^{-3} M (Fig. 2A,B). L-NNA and L-Arg both did not influence VIP release (basal 11.3 ± 1.7 pmol/l; L-NNA 10.3 ± 1.5 pmol/l; L-Arg 10.3 ± 1.8 pmol/l; N.S.) (Fig. 2B).

Table 1
Influence of pulse frequency of VS (20 V, 1 ms) on gastric release of VIP

Stimulation frequency	Basal	2 Hz	5 Hz	10 Hz	20 Hz
VIP release [pmol/l]	7.3 ± 0.8	7.7 ± 0.6	8.7 ± 1.1	23.0 ± 2.4 [a]	25.5 ± 3.5 [a]

Values are means ± S.E.M.
[a] $P < 0.05$, $n = 10$, Student's t-test.

3.3. Effect of vagal stimulation

During the basic characterization of the model electrical vagal stimulation without motility recording was performed at various stimulation frequencies (2, 5, 10 and 20 Hz) and the release of VIP was determined. It turned out that a stimulation frequency of 20 Hz led to the most effective release of VIP in this model (Table 1). Additional experiments with a stimulation frequency of 10 Hz showed no different responses of pyloric motility from the results with 20 Hz. Therefore, this frequency was used for further experiments.

Stimulation of the vagal nerve (VS) lead to a significant decrease of the pyloric motility index (basal 181 ± 15 mmHg/min; VS 143 ± 21 mmHg/min; $n = 7$, $P < 0.05$). The subsequent perfusion with L-NNA 10^{-4} M led again to an increase of the motility index when compared to the basal period (338 ± 58 mmHg/min), but the inhibitory effect of VS was still present after application of L-NNA (228 ± 30 mmHg/min; $P < 0.05$, $n = 7$) (Fig. 3A,B). This effect was due to a decreased contraction frequency (basal 10 ± 2/min, VS 6 ± 1/min, L-NNA 11 ± 1/min, L-NNA + VS 7 ± 1/min; $P < 0.05$) while mean contraction amplitudes did not change significantly (basal 18 ± 4 mmHg, VS 24 ± 7 mmHg, L-NNA 34 ± 9 mmHg, L-NNA + VS 30 ± 7 mmHg; N.S.). VS increased VIP release significantly (basal 14.9 ± 1.4 pmol/l; VS 20.1 ± 2.6 pmol/l; $n = 7$, $P < 0.05$) while L-NNA had no influence on vagally induced VIP release (Fig. 3B).

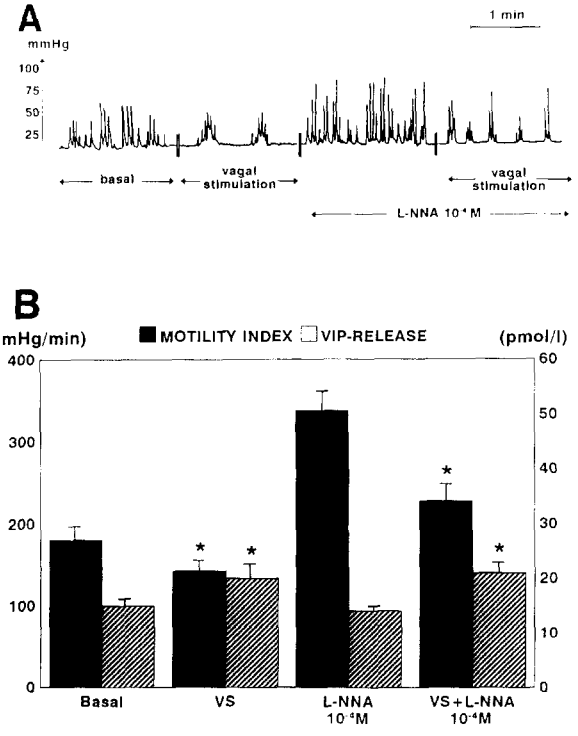

Fig. 3. Effects of VS (20 V, 20 Hz, 1 ms) on pyloric motility and VIP release. (A) Representative tracing of pyloric motility, (B) values are means ± S.E.M. (* $P < 0.05$; $n = 7$; Student's t-test).

4. Discussion

Previous studies have shown that the pylorus is characterized by a potent non-adrenergic non-cholinergic innervation, which can be demonstrated in vitro and in vivo (Mir et al., 1979; Anuras et al., 1974; Allescher et al., 1989, 1992). The nature of the neurotransmitter which mediates this NANC inhibition has been unknown until recently. VIP, which causes a direct and tetrodotoxin-insensitive inhibition in vivo showed only a very inconsistent effect in vitro, especially when the pylorus was precontracted with an agonist, whereas the inhibitory effect of electrical field stimulation was unaltered under these conditions (Allescher et al., 1989). Furthermore, VIP did not mimic the actions of the final NANC mediator electrophysiologically (Daniel et al., 1983; Biancani et al., 1985) and biochemically (Torphy et al., 1986). These results indicate that VIP can not be the only final mediator causing this inhibitory response.

NO as a product of the enzymatic conversion of L-Arg has been shown to be an important inhibitory transmitter in most regions of the gastrointestinal tract (Palmer et al., 1987; Boeckxstaens et al., 1990; Tottrup et al., 1991; De Man et al., 1991; Allescher et al., 1992; Stark and Szurszewski, 1992). From in vitro and in vivo studies in dogs, it was suggested that there is an inhibitory tone exerted by continous release of NO in the pyloric region. It was demonstrated furthermore that both intrinsic and extrinsic neural pathways seem to use NO as mediator (Allescher et al., 1992).

The question arises whether there is an interaction of NO with other mediators, especially VIP in this region or within the neural pathways. Grider et al. (1992) reported that NO enhances VIP-induced relaxation in isolated gastric muscle cells of the guinea-pig. In vivo, intra-arterial injection of VIP caused a potent direct inhibition of pyloric motor activity which could not be blocked by L-NAME, a potent inhibitor of endogenous NO synthesis. There was even a tendency to slightly increased inhibitory responses of VIP after L-NAME. This indicates that the direct inhibitory effect of VIP occurs independently of NO synthesis and release and, therefore, an independent mechanism must be present. In vitro, VIP had no or inconsistent

effects on the canine pylorus (Allescher et al., 1989). These differences between in vivo and in vitro results are still unexplained. Until now, neither the roles of VIP and NO nor their interactions concerning the NANC inhibition are sufficiently resolved.

In this study, the effect of NO on pyloric motility and VIP release in the isolated arterially perfused rat stomach was investigated. The imitation of high endogenous NO levels by administration of the NO donor sodiumnitroprusside significantly reduced and blockade of endogenous NO release by L-NNA significantly increased basal pyloric motility, suggesting that there is a tonic inhibitory effect of endogenous NO on basal pyloric motor activity. Basal VIP output was not influenced by L-NNA or by addition of L-NNA + L-Arg. Additionally, there was no effect of exogenous infusion of sodiumnitroprusside on VIP release. This indicates that there is no tonic effect of endogenous NO production on VIP release and no effect of exogenous NO on VIP release in the stomach. This could be in contrast to other regions of the gastrointestinal tract, where a possible interaction of NO and VIP was postulated (Allescher and Daniel, in press). VS led to a significant decrease of the pyloric motility index. This effect was even present after blockade of endogenous NO synthesis by L-NNA, indicating that NO is not mandatory for this inhibition. On the other hand, VS led to a NO-independent increase of gastric VIP output. These data support the view that VIP and NO are released independently by VS. This opinion is supported by preliminary results from the isolated porcine antrum, where no interaction between VIP and NO could be found (Schmidt et al., 1993). Another study reported that VIP and NO are released by independent mechanisms in response to VS and that NO might account for the immediate inhibition whereas VIP could account for the prolonged inhibition of the rat stomach in vitro (Takahashi and Owyang, 1995). However, on the basis of the existing data, an interaction between VIP and NO release cannot be ruled out.

Putting all this information together, one could speculate that endogenous NO and VIP are important NANC inhibitory neurotransmitter, which act independently in the rat pylorus. It seems that the pylorus of the rat is controlled by a tonic inhibition of endogenously released NO. In contrast to the canine pylorus, NO does not mediate the inhibitory effect of VS at the frequency parameters used. In our preliminary experiments, there was a strong correlation of VIP release and stimulation frequency. These data are corresponding with the results of Takahashi and Owyang (1995), who reported a frequency-dependent VIP release in the portal vein after VS with a maximum at 10 Hz. On the other hand, the maximum of NO release was found at 2.5 Hz (Takahashi and Owyang, 1995). In accordance to the latter results, Boeckxstaens et al. (1992) reported that, in longitudinal muscle strips of the rat, gastric fundus NANC mediated transient relaxation at low stimulation frequencies were mediated mainly by NO whereas, at higher frequency, NO, together with a peptide (probably VIP), were responsible for long-lasting NANC relaxations. From this, one could suggest different frequency-dependent regulation pathways using different transmitters after VS in the rat pylorus with emphasis of NO at lower and VIP at higher stimulation frequencies. The different roles of VIP and NO for the immediate and prolonged inhibition of the stomach and especially its functional consequences remain to be further characterized.

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