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Influence of N^G-nitro-L-arginine methyl ester on vagally induced gastric relaxation in the anaesthetized rat

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1 The influence of the nitric oxide (NO) biosynthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on the gastric relaxation induced by peripheral vagal stimulation was investigated in the anaesthetized rat.

2 Peripheral vagal stimulation (10 Hz, 10 V, 1 ms for 20 s) induced a reproducible biphasic response: a short-lasting increase followed by a more pronounced decrease in intragastric pressure. This response also occurred in reserpinized animals (5 mg kg^{-1}, i.p., 24 h before the experiment) while atropine (1 mg kg^{-1}, i.v.) abolished the initial increase in intragastric pressure.

3 L-NAME (1–30 mg kg^{-1}, i.v.) induced an increase in arterial blood pressure. L-NAME (1 mg kg^{-1}, i.v.) had no influence on the vagally induced gastric response while L-NAME (10 and 30 mg kg^{-1} i.v.) significantly changed it: the initial increase in intragastric pressure was enhanced while the decrease in intragastric pressure was reduced or abolished. N^G-nitro-L-arginine (L-NNA, 10 mg kg^{-1}, i.v.) had the same effect.

4 An i.v. infusion of phenylephrine (10 μg kg^{-1} min^{-1}) inducing a pressor response similar to that produced by L-NAME (30 mg kg^{-1}, i.v.) did not influence the vagal gastric response. Infusion of L-arginine (300 mg kg^{-1} bolus, then 100 mg kg^{-1} h^{-1}) starting 30 min beforehand, reduced the pressor effect and prevented the influence of L-NAME (10 mg kg^{-1}, i.v.) on the vagal gastric response. After injection of both atropine (1 mg kg^{-1}, i.v.) and L-NAME (30 mg kg^{-1}, i.v.), the vagally induced decrease in intragastric pressure was similar to that obtained under control conditions.

5 These results are consistent with NO being released and inducing gastric relaxation during peripheral vagal stimulation. In addition to NO, another inhibitory non-adrenergic non-cholinergic neurotransmitter is released.

Keywords: Rat stomach; gastric relaxation; non-adrenergic non-cholinergic; vagal stimulation; N^G-nitro-L-arginine methyl ester (L-NAME); nitric oxide

Introduction

Nitric oxide (NO) has been recognized as an endothelium-derived relaxing factor (Palmer *et al.*, 1987; Ignarro, 1990). Inhibition of NO biosynthesis by L-arginine analogues such as N^G-mono-methyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) upon intravenous administration of these compounds induces pressor responses in rats (Rees *et al.*, 1990; Gardiner *et al.*, 1990), guinea-pigs (Aisaka *et al.*, 1989) and rabbits (Rees *et al.*, 1989). These results suggest that the formation of NO has an important role in the regulation of blood pressure. Recent *in vitro* data suggest that NO is also a neurotransmitter of peripheral inhibitory non-adrenergic non-cholinergic (NANC) neurones. Indeed, in the anococcygeus muscle of the rat (Gillespie *et al.*, 1989) and the mouse (Gibson *et al.*, 1990), the ileocolonic junction of the dog (Boeckxstaens *et al.*, 1990), and the guinea-pig trachea (Li & Rand, 1991), the NO synthesis inhibitors L-NMMA, L-NAME and/or N^G-nitro-L-arginine (L-NNA) inhibited the relaxation, induced by electrical stimulation of the NANC neurones. Furthermore, in a superfusion bioassay, the release of a vasorelaxant factor with NO characteristics was shown upon stimulation of the NANC nerves in the canine ileocolonic junction (Bult *et al.*, 1990) and the presence of NO synthase has been shown in myenteric plexus neurones of the rat intestine (Bredt *et al.*, 1990).

In the rat gastric fundus, vasoactive intestinal polypeptide (VIP) has been proposed as inhibitory NANC neurotransmitter (De Beurme & Lefebvre, 1987; Kamata *et al.*, 1988) but the non-blockade of the initial relaxation, induced by electrical stimulation of the NANC neurones, by VIP-antiserum suggested the involvement of a non-VIP component (De Beurme & Lefebvre, 1988). There is now evidence that NO might be the co-transmitter with VIP in this preparation, as L-NMMA reduced NANC relaxations elicited by short periods of field stimulation (Li & Rand, 1990; Boeckxstaens *et al.*, 1991). The inhibitory NANC neurones of the gastric fundus represent the final step of the vagal inhibitory pathway involved in gastric relaxation (Abrahamsson, 1986). In studies *in vivo*, stimulation of the peripheral cut end of the vagus, especially after atropine treatment, induces NANC gastric relaxation in the guinea-pig (Ohta *et al.*, 1985), the cat (Martinson, 1964) and the dog (Jahnberg, 1977). In the present study, we investigated the influence of L-NAME on gastric relaxation induced by efferent vagal stimulation in the anaesthetized rat. Our results provide *in vivo* evidence for the involvement of NO in the vagal inhibitory NANC pathway to the stomach. A preliminary account of the results has been given to the British Pharmacological Society (Lefebvre *et al.*, 1991).

Methods

Preparation of animals

Male Wistar rats (230–460 g) were fasted for 24 h with water available *ad libitum*. The animals were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.); anaesthesia was maintained by bolus administration (3 mg kg^{-1}, i.v.) when required. A tracheotomy was performed and a tracheal tube inserted, through which the animals breathed room air spontaneously. Catheters containing heparinized (50 units ml^{-1}) saline were inserted into the right carotid artery and the right external jugular vein for blood pressure measurement and intravenous administration of drugs respectively. The arterial catheter was connected to a PDCR 75 S/N 1684 or a Statham P23AA pressure transducer and mean arterial blood pressure was derived from the direct measurement. Intragastric pressure was measured by use of a rubber balloon inserted into the stomach via the mouth. The balloon was connected to a

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Statham P23BB or a PDCR 75/1 S/N 430 pressure transducer and filled with water at 37°C (1.1–1.6 ml). This volume was determined before the use of each new balloon as the quantity of water, that just did not induce pressure in the balloon, outside the body. After each experiment, the exact localization of the balloon was verified. Blood pressure and intragastric pressure were registered on a Beckman Dynograph recorder, type R.

The cervical vagal nerves were carefully isolated and bilaterally sectioned. The peripheral cut end of the left cervical vagus was placed on a bipolar electrode and covered with liquid paraffin. The electrode was connected to an electric stimulator (Braun Type I, HSE). In a few animals, the peripheral cut end of the right cervical vagus was stimulated, as no response was obtained upon stimulation of the left vagus. Except for the preliminary experiments, stimulation was with square wave pulses of 1 ms, 10 V and 10 Hz for 20 s.

Experimental protocols

After section of the vagal nerves, a stabilization period of 20 min was allowed. In a first series of experiments ($n = 24$), vagal stimluation was performed 3 times at an interval of about 1 h. Before the second vagal stimulation, an i.v. bolus injection of saline or L-NAME (1, 10 or 30 mg kg^{-1}) was given. The interval between the saline injection and the second vagal stimulation was 10 min; when L-NAME was injected, the second vagal stimulation was performed at the time of the maximal blood pressure increase induced by L-NAME. The gastric response to vagal stimulation was also studied before and after injection of L-NNA (10 mg kg^{-1}, i.v.; $n = 6$).

The influence of vagal stimulation on intragastric pressure was also studied before and 10 min after i.v. bolus injection of pentolinium (2 mg kg^{-1}; Howe *et al.*, 1986; $n = 8$) and before and during i.v. infusion of phenylephrine (10 μg kg^{-1} min^{-1}; $n = 8$); vagal stimulation was performed when the phenylephrine-induced pressor response was maximal. The influence of L-NAME (30 mg kg^{-1}) was also studied in atropinized ($n = 8$) and reserpinized ($n = 5$) rats. Atropine (1 mg kg^{-1}, i.v.) was administered 10 min before the second vagal stimulation. Fifty min after the second vagal stimulation, the atropine injection was repeated, followed after 10 min by the i.v. injection of L-NAME (30 mg kg^{-1}). When the blood pressure increase induced by L-NAME was maximal, a third vagal stimulation was performed. Reserpine (5 mg kg^{-1}) was administered i.p. 24 h before the experiment.

In the final series of experiments, the influence of pretreatment with L-arginine on the effect of L-NAME was investigated. Vagal stimulation was performed 3 times with an interval of at least 1 h between each stimulation. Fifty min after the first vagal stimulation, an i.v. bolus injection of L-arginine (300 mg kg^{-1}) was given, followed after 10 min by L-NAME (30 mg kg^{-1}) as an i.v. bolus ($n = 6$). In another group of rats, L-arginine was infused (300 mg kg^{-1} bolus, 100 mg kg^{-1} h^{-1} infusion) for 30 min before i.v. bolus administration of 10 ($n = 6$) or 30 mg kg^{-1} ($n = 3$) L-NAME. In a similar way, the influence of D-arginine (300 mg kg^{-1} bolus, 100 mg kg^{-1} h^{-1} infusion for 30 min) was tested versus L-NAME (10 mg kg^{-1}; $n = 8$).

Drugs

The following drugs were used: atropine sulphate (Boehringer Ingelheim, Germany), D-arginine hydrochloride (Sigma, St. Louis, Mo, U.S.A.), L-arginine hydrochloride (Sigma), N^G-nitro-L-arginine (Sigma), N^G-nitro-L-arginine methyl ester hydrochloride (Sigma), pentolinium tartrate (Janssen Chimica, Geel, Belgium), phenylephrine (Winthrop, Brussels, Belgium), reserpine (Aldrich Chemie, Brussels, Belgium).

Drugs were dissolved or diluted with sterile saline. For phenylephrine, commercially available ampoules were used. A stock solution of reserpine was prepared from powder (5 mg ml^{-1} dissolved in 10% ascorbic acid). The substances were injected in volumes of 0.1 ml/100 g, flushed in with 0.2 ml saline. Infusions were given at a rate of 0.1 ml min^{-1}.

Statistical analysis

Results are given as mean ± s.e.mean. Responses to vagal stimulation after a given treatment were compared to those before by means of the signed-ranks test. $P < 0.05$ was taken as statistically significant.

Results

During preliminary experiments ($n = 5$), the vagal nerve was stimulated at 5 min intervals for 20 s at a frequency of 2 or 10 Hz; the voltage was stepwise increased from 10 to 50 V. Stimulation at 2 Hz tended to increase intragastric pressure (corresponding to gastric contraction), while stimulation at 10 Hz yielded a decrease in intragastric pressure (corresponding to gastric relaxation) or a biphasic response, i.e. a small increase followed by a more pronounced decrease in intragastric pressure. From these results, vagal stimulation at 10 Hz, 10 V, 1 ms for 20 s was chosen for further experiments.

Influence of L-NAME and L-NNA on the vagally induced gastric response

Vagal nerve stimulation generally induced a decrease in mean arterial blood pressure (Figure 1). In the first series of experiments ($n = 24$), the response of intragastric pressure to vagal stimulation was biphasic, i.e. increase followed by decrease, in 23 rats (Figure 1) while in one rat only a decrease occurred. Except for 2 rats, the decrease in intragastric pressure was always clearly more pronounced than the increase. In 14 experiments out of 24, stopping vagal stimulation was followed by a rebound contraction (Figure 1). Even when a rebound contraction occurred, intragastric pressure quickly decreased to a lower level than before vagal stimulation. Within the 1 h before the following vagal stimulation, intragastric pressure slowly returned to its original level. Bolus injection of saline did not influence mean blood pressure nor intragastric pressure; the gastric response to vagal stimulation performed 10 and 70 min after the saline injection was the same as that to the first vagal stimulation (Figure 2).

Bolus administration of L-NAME (1, 10 and 30 mg kg^{-1}) significantly increased mean blood pressure from 105 ± 6 to 121 ± 5 mmHg ($n = 6$), from 110 ± 8 to 126 ± 12 mmHg ($n = 6$) and from 123 ± 10 to 148 ± 9 mmHg ($n = 6$) respectively. Intragastric pressure was not influenced by the administration of L-NAME or tended to decrease (−0.1 ± 0.3, −0.4 ± 0.1 and −0.3 ± 0.1 cmH_2O for 1, 10 and 30 mg kg^{-1} L-NAME respectively). L-NAME (1 mg kg^{-1}) had no influence on the gastric response to vagal stimulation but the higher doses markedly changed this response (Figures 1 and 2). The vagally induced increase in intragastric pressure was increased in 5 rats out of 6 in each group, while the vagally induced decrease in intragastric pressure was abolished or greatly reduced in all rats. Although the relaxation during vagal stimulation was reduced or abolished, intragastric pressure quickly decreased after stopping the stimulation similar to that observed before administration of L-NAME. The effect of L-NAME on the vagally induced gastric response persisted for more than 1 h as can be seen from the response to the third vagal stimulation in Figure 2.

L-NNA (10 mg kg^{-1} i.v.) increased blood pressure from 110 ± 9 to 140 ± 9 mmHg ($n = 6$); intragastric pressure slightly decreased from 7.1 ± 0.8 to 6.8 ± 0.8 cmH_2O. L-NNA (10 mg kg^{-1}, i.v.) had the same influence on the vagally induced gastric response as L-NAME (10 and 30 mg kg^{-1}, i.v.): the vagally induced increase in intragastric pressure was

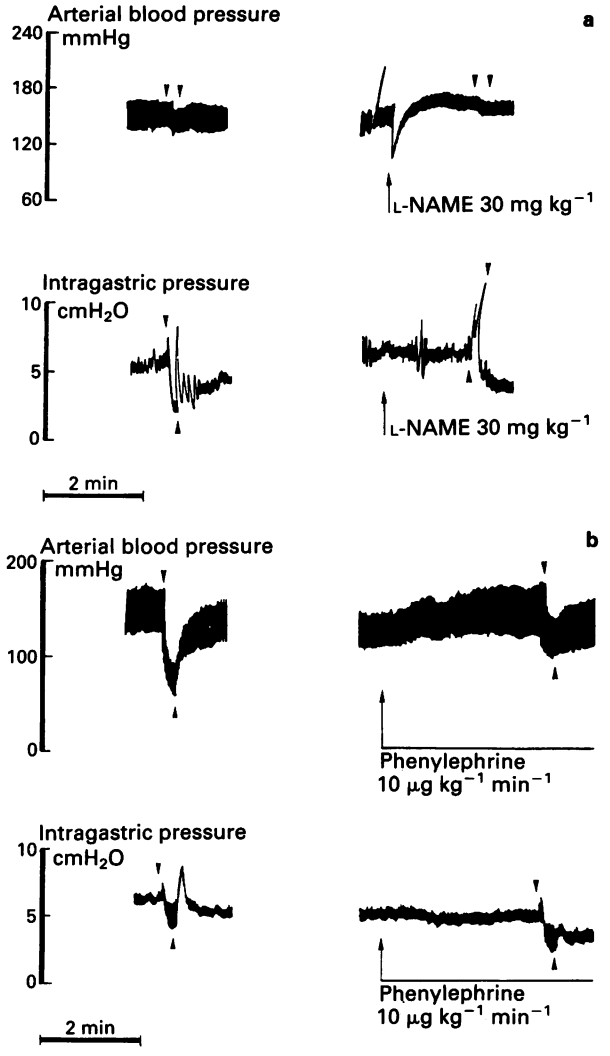

Figure 1 Original recordings showing the influence of an i.v. bolus injection of 30 mg kg^{-1} N^G-nitro-L-arginine methyl ester (L-NAME) (a) and an i.v. infusion of 10 μg kg^{-1} min^{-1} phenylephrine (b) on the gastric response induced by vagal stimulation (10 Hz, 10 V, 1 ms, 20 s) in 2 different rats. The arrows indicate the beginning and the end of the stimulation.

enhanced from 3.5 ± 0.7 to 9.0 ± 0.4 cmH_2O ($n = 6$, $P < 0.05$) while the decrease (−5.0 ± 0.8 cmH_2O) was abolished ($P < 0.05$).

In 6 experiments out of 8, a single i.v. administration of pentolinium (2 mg kg^{-1}) abolished or greatly reduced the gastric response to vagal stimulation. The initial increase in intragastric pressure was reduced from 0.4 ± 0.3 to 0.04 ± 0.04 cmH_2O while the vagally induced decrease in intragastric pressure was reduced from 2.7 ± 0.4 cmH_2O before administration of pentolinium to 0.3 ± 0.1 cmH_2O in its presence ($n = 6$, $P < 0.05$). The injection of pentolinium reduced blood pressure by 55 ± 5 mmHg from 113 ± 8 to 58 ± 5 mmHg ($n = 6$); it did not manifestly change basal intragastric pressure (−0.3 ± 0.1 cmH_2O, $n = 6$). In the 2 other experiments, the dose of pentolinium needed to be increased to 4 and 7 mg kg^{-1} respectively before the vagally induced responses were clearly reduced. These doses of pentolinium reduced blood pressure by 60 and 52 mmHg.

Infusion of phenylephrine (10 μg kg^{-1} min^{-1}) increased blood pressure by 25 ± 3 mmHg from 127 ± 6 to 152 ± 5 mmHg ($n = 8$). This pressor response did not influence the gastric response to vagal stimulation (Figure 1). The vagally induced increase and decrease in intragastric pressure was 0.4 ± 0.1 and 1.8 ± 0.3 cmH_2O before and 0.5 ± 0.1 and 1.6 ± 0.1 cmH_2O during the phenylephrine infusion ($n = 8$).

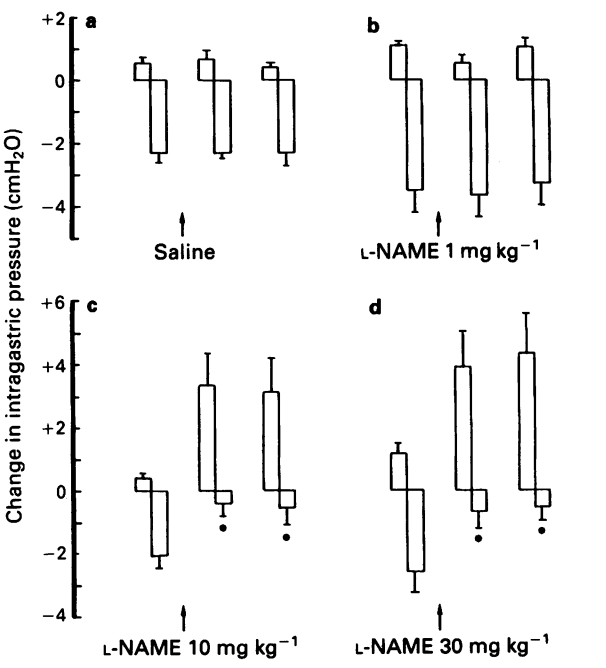

Figure 2 Influence of vagal stimulation (10 Hz, 10 V, 1 ms, 20 s) on intragastric pressure in 4 groups of rats (a, b, c, d). Vagal stimulation was performed 3 times; before the second stimulation, saline (a) or N^G-nitro-L-arginine methyl ester (L-NAME) 1 (b), 10 (c) or 30 (d) mg kg^{-1} was administered i.v. The columns show mean values with the s.e.mean (vertical bars) of $n = 6$, except for the third vagal stimulation in group (b) where $n = 4$. * $P < 0.05$, significantly different versus the response during the first vagal stimulation. The enhancement of the vagally induced increase in intragastric pressure after i.v. administration of 10 and 30 mg kg^{-1} L-NAME did not reach statistical significance at the two tail level because it only occurred in 5 of the 6 rats.

Influence of L-NAME in atropinized and reserpinized rats

Before administration of atropine, vagal stimulation induced the usual biphasic response although the mean decrease in intragastric pressure in this series was less pronounced than in the other series (Figure 3). The i.v. injection of atropine did not manifestly change blood pressure or intragastric pressure. In 5 rats out of 6, intragastric pressure decreased somewhat giving a mean reduction of intragastric pressure from 6.3 ± 0.7 to 5.6 ± 0.5 cmH_2O ($n = 6$). After administration of atropine, vagal stimulation no longer induced an increase in intragastric pressure while the vagally induced decrease in intragastric pressure was not significantly influenced (Figure 3). In all rats of this group, a rebound contraction occurred after the first vagal stimulation but this rebound contraction was absent after the injection of atropine.

The i.v. injection of L-NAME (30 mg kg^{-1}), after atropine had been administered again increased blood pressure by 26 ± 6 mmHg from 101 ± 11 till 127 ± 15 mmHg ($n = 6$). The decrease in intragastric pressure, induced by vagal stimulation, was not significantly reduced by L-NAME (Figure 3). Also in reserpinized animals, vagal stimulation induced an initial short-lasting gastric contraction, followed by a more pronounced gastric relaxation. The amplitude of the gastric relaxation was similar to that induced by the first vagal stimulation in the group where atropine was administered (Figure 3). In the reserpinized rats, L-NAME (30 mg kg^{-1}, i.v.) increased the blood pressure from 91 ± 8 to 134 ± 4 mmHg ($n = 5$) and changed the gastric response to vagal stimulation in a similar way to non-treated rats i.e. the vagally induced increase in intragastric pressure was greatly enhanced while the decrease in intragastric pressure was completely abolished (Figure 3). Ten min before a third vagal stimulation, atropine

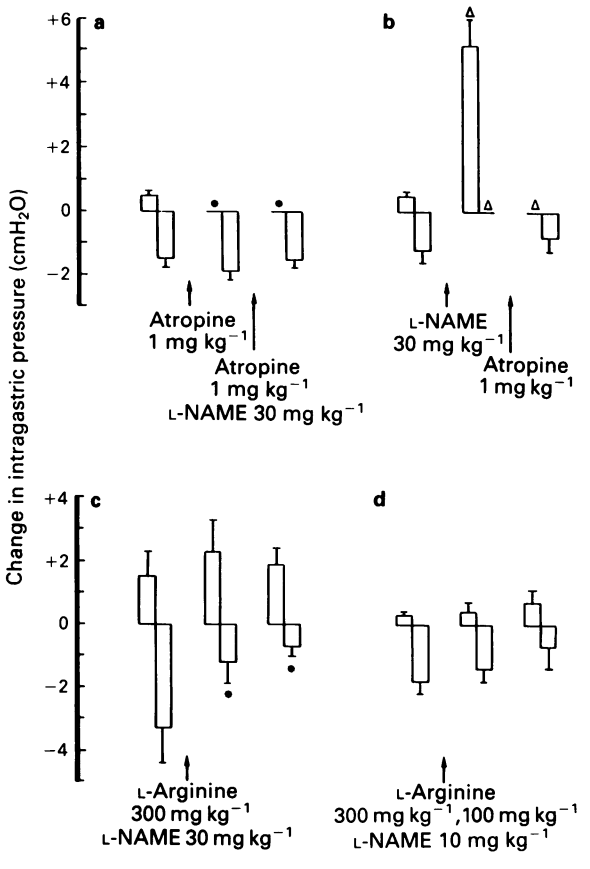

Figure 3 Influence of vagal stimulation (10 Hz, 10 V, 1 ms, 20 s) on intragastric pressure in 4 groups of rats (a, b, c, d). Gr (b) was reserpinized (5 mg kg^{-1}, i.p., 24 h before the experiment). Substances administered in between the vagal stimulation are indicated. The columns show mean values with s.e.mean (vertical bars) of $n = 6$ except for the reserpinized group where $n = 5$. $^{*}P < 0.05$; $^{\Delta}P < 0.05$ (one tail), significantly different versus the response during the first vagal stimulation.

(1 mg kg^{-1}) was injected i.v. Vagal stimulation no longer induced an increase in intragastric pressure but in 3 rats a small decrease and in 2 rats a more pronounced decrease in intragastric pressure occurred (mean decrease = 0.8 ± 0.4 cmH_2O, $n = 5$, Figure 3).

Influence of L-arginine and D-arginine on the effect of L-NAME

Preliminary experiments showed that an i.v. bolus injection of arginine (100 mg kg^{-1}) did not prevent the pressor effect of L-NAME (30 mg kg^{-1}) nor its influence on the vagally induced change in intragastric pressure. Also when the dose of L-arginine was increased to 300 mg kg^{-1}, the effect of L-NAME (30 mg kg^{-1}) was not reversed since blood pressure increased from 119 ± 7 to 145 ± 9 mmHg ($n = 6$). The vagally induced gastric response after administration of L-arginine and L-NAME is shown in Figure 3. The increase in intragastric pressure was enhanced although to a smaller extent than in other groups. The vagally induced decrease in intragastric pressure was reduced and remained reduced after 1 h.

In another group of 9 rats, L-arginine was given as an i.v. bolus of 300 mg kg^{-1} followed by an infusion of 100 mg kg^{-1} h^{-1} for 30 min. In 3 of these rats, L-NAME (30 mg kg^{-1}) was then injected. Only in one of these rats, was the influence of L-NAME on the vagally induced gastric response inhibited. In contrast, the effect of L-NAME (10 mg kg^{-1}) was prevented (Figure 3). During the infusion of L-arginine, blood pressure was not manifestly influenced (110 ± 6 mmHg before the infusion, 106 ± 10 mmHg just before the injection of 10 mg kg^{-1} L-NAME, $n = 6$), while intragastric pressure tended to decrease (from 5.0 ± 0.7 to 4.2 ± 0.5 cmH_2O, $n = 6$). L-NAME (10 mg kg^{-1}, i.v.) moderately increased blood pressure (from 106 ± 10 to 114 ± 11 mmHg, $n = 6$) and did not influence intragastric pressure (maintained at 4.2 ± 0.5 cmH_2O). The injection of L-NAME (10 mg kg^{-1}) did not influence the vagally induced gastric response in 5 rats out of 6, while the vagally induced decrease in intragastric pressure was abolished in one rat. However, during another vagal stimulation 60 min later, the inhibitory effect of L-NAME (10 mg kg^{-1}) was no longer prevented by L-arginine as the vagally induced decrease in intragastric pressure was abolished in 4 and greatly reduced in one of the 6 rats studied.

During the infusion of D-arginine, the blood pressure increased from 111 ± 10 to 117 ± 10 mmHg while the intragastric pressure decreased from 8.4 ± 1.0 to 7.5 ± 1.1 cmH_2O ($n = 8$). The i.v. injection of L-NAME (10 mg kg^{-1}) increased blood pressure by 30 ± 5 mmHg; intragastric pressure showed a moderate increase (0.3 ± 0.3 cmH_2O). After administration of D-arginine and L-NAME, the vagally induced increase in intragastric pressure was consistently enhanced (from 1.9 ± 0.5 to 2.9 ± 0.5 cmH_2O, $n = 8$, $P < 0.01$). The vagally induced relaxation was decreased in 6 experiments out of 8 but slightly increased in the 2 others, yielding a mean response of 2.6 ± 0.8 cmH_2O (versus 4.1 ± 0.5 cmH_2O before D-arginine and L-NAME, $n = 8$, $P < 0.05$, one tail).

Discussion

Recent *in vitro* data have provided evidence that NO and VIP are co-transmitters of the inhibitory NANC neurones of the rat gastric fundus (Li & Rand, 1990; Boeckxstaens *et al.*, 1991). In different mammals such as the guinea-pig (Ohta *et al.*, 1985), the cat (Martinson, 1964) and the dog (Jahnberg, 1977), it has been established that the vagal nerve carries 2 types of preganglionic fibres i.e. those that synapse with postganglionic cholinergic neurones and induce an increase in gastric tone upon stimulation and those that synapse with postganglionic NANC neurones and induce a reduction in gastric tone upon stimulation. The presence of both pathways has also been shown in the rat (Delbro, 1989). The aim of the present study was therefore to investigate *in vivo* the influence of the NO biosynthesis inhibitor L-NMMA on the vagally induced NANC relaxation of the rat stomach.

The inhibitory vagal fibres have a higher excitation threshold than the excitatory ones (Martinson & Muren, 1963; Jansson & Martinson, 1965). Our preliminary experiments revealed that peripheral vagal stimulation at 10 Hz, 10 V and 1 ms induced a clear reduction of intragastric pressure even in the absence of atropine. The train duration of stimulation was limited to 20 s as *in vitro* studies in the rat gastric fundus showed that the short-lasting relaxation induced by this type of stimulation of the inhibitory NANC neurones was greatly reduced by the NO synthesis inhibitors (Boeckxstaens *et al.*, 1991). The major part of our experiments was done in the absence of atropine to avoid the pronounced decrease in gastric tone, that has been observed in other species and that can interfere with the registration of inhibitory responses (Martinson, 1964). However, the experiments with atropine later showed that it only moderately decreased intragastric pressure, suggesting that the intrinsic cholinergic neurones contribute only moderately to the maintenance of rat gastric tone in our experimental conditions. Atropine blocked the initial increase in intragastric pressure, induced by vagal stimulation, illustrating the cholinergic nature of this component of the response, but did not influence the vagally induced decrease in intragastric pressure; it also blocked the rebound contraction. The relaxation also occurred in reserpinized animals (Lefebvre, 1986), confirming the NANC nature of the vagally induced gastric relaxation. The ganglion-blocking agent, pentolinium (Taylor, 1990) greatly reduced or abolished the vagally induced decrease in intragastric pressure, confirming the presence of nicotinic synapses between

the preganglionic vagal fibres and the inhibitory NANC neurones (Roman & Gonella, 1987). The gastric response to vagal stimulation was perfectly reproducible after i.v. administration of saline.

The i.v. injection of L-NAME increased the mean arterial blood pressure as expected, although there was no clear dose-dependency as reported by Rees *et al.* (1990) for L-NAME in anaesthetized rats. The two higher doses of L-NAME (10 and 30 mg kg^{-1}) reduced or abolished the vagally induced decrease in intragastric pressure, suggesting that NO release is essential for this response. This was confirmed by the study of another inhibitor of NO-synthesis, L-NNA. When NO release is blocked, the cholinergic contractile response becomes predominant during vagal stimulation. The fact that cholinergic neurones are activated during the whole course of stimulation at our parameters but are overruled by the simultaneously released relaxant agent is also illustrated by the cholinergic rebound contraction that occurred in many animals after stimulation. The increase in mean arterial blood pressure is not the mechanism by which L-NAME influences the vagally induced gastric response, as the same pressor response as for the highest dose of L-NAME evoked by phenylephrine infusion had no such effect. Although L-NAME (1 mg kg^{-1}) also increased blood pressure, it had no influence on the vagal gastric response. Similarly, it was reported that a lower dose of L-NMMA increased arterial blood pressure but did not reduce gastric mucosal blood flow while higher doses did (Pique *et al.*, 1989). The finding that pretreatment with an L-arginine infusion reversed the action of L-NAME (10 mg kg^{-1}), both on blood pressure and on the vagally induced gastric relaxation, provides further evidence that NO is involved in the latter response. Neither bolus injections nor infusion of L-arginine, however, prevented the pressor effect and the reduction of the vagal gastric relaxation by 30 mg kg^{-1} L-NAME. It might be difficult to reverse the effect of this high dose of L-NAME, which was shown to be the most potent in increasing blood pressure amongst the NO synthesis inhibitors available (Rees *et al.*, 1990). In our hands, a 300 mg kg^{-1} bolus plus a 100 mg kg^{-1} h^{-1} infusion of L-arginine prevented the effect of 10 mg kg^{-1} L-NAME but 1 h after stopping the L-arginine infusion, the inhibitory effect of 10 mg kg^{-1} L-NAME was again observed. We have no explanation as to why D-arginine prevented the influence of L-NAME on vagally induced relaxation in 2 experiments. Recently, it has been shown that the D-enantiomer of N^G-nitro-arginine can also interfere with the L-arginine/NO pathway, although this effect was not prevented by D-arginine (Wang *et al.*, 1991).

L-NAME did not reduce the vagally induced gastric relaxation after injection of atropine. This seems to indicate that another relaxant transmitter besides NO is released during vagal stimulation. This seems also corroborated by the observation that in the absence as well as in the presence of L-NAME, intragastric pressure stayed decreased after stopping vagal stimulation. Vagal stimulation at our parameters would thus yield release of acetylcholine, NO and the second relaxant transmitter, the overall response being relaxation. When NO release is prevented, the cholinergic contraction becomes predominant; when the effect of acetylcholine is antagonized, a relaxation due to the second relaxant transmitter occurs. This transmitter is certainly not noradrenaline as L-NAME also abolished the vagal gastric relaxation in reserpinized rats, but after injection of atropine a vagally induced gastric relaxation again occurred. A suitable candidate for the second NANC neurotransmitter is VIP, which was proposed as co-transmitter of NO in studies *in vitro* in the rat gastric fundus (Li & Rand, 1990; Boeckxstaens, 1991). In those studies in atropinized conditions, the relaxation of rat gastric fundus strips induced by transmural stimulation at low frequencies was completely abolished by NO synthesis inhibitors, while the response to higher frequency stimulation (5 Hz, Li & Rand, 1990; 16 Hz, Boeckxstaens, 1991) was only partially reduced and evidence for the involvement of VIP was provided.

Twenty four h after reserpinization, the arterial blood pressure of the rats was lower than in the non-reserpinized animals confirming previous reports (Kisin & Yuzhakov, 1976). The pressor effect of L-NAME (30 mg kg^{-1}) tended to be higher (43 mmHg) than in non-treated rats (25 mmHg). This contrasts with the report of Vargas *et al.* (1990) that the pressor effect of L-NMMA was attenuated in rats devoid of sympathetic tone by pithing or ganglionic blockade. We have no explanation for this difference although it should be kept in mind that the experiments of Vargas *et al.* (1990) were conducted acutely, while ours were performed 24 h after reserpinization.

The administration of L-NAME and L-NNA did not increase intragastric pressure; it even tended to decrease it. L-NMMA and L-NNA *in vitro* increase the resting tension of rat gastric fundus strips, suggesting a tonic release of NO; however, non-neurogenic sources of NO or a direct action of L-NMMA and L-NNA at the smooth muscle cells could not be excluded (Li & Rand, 1990; Boeckxstaens *et al.*, 1991).

In conclusion, the present results are consistent with NO being released and inducing gastric relaxation during vagal stimulation: the NO synthesis inhibitor L-NAME reduced the vagally induced gastric relaxation, and this effect of L-NAME was prevented by pre-administration of L-arginine. This finding provides *in vivo* evidence for NO being an inhibitory NANC neurotransmitter at this site in the gastrointestinal tract. The pathway might be involved in adaptive relaxation of the stomach; it was recently shown in the guinea-pig isolated stomach that adaptive relaxation, induced by stimulation of ganglionic nicotinic receptors, is NO-dependent (Desai *et al.*, 1991).

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