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# Nerve-induced release of nitric oxide in the rabbit gastrointestinal tract as measured by *in vivo* microdialysis

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**1** Nitric oxide (NO) has been suggested as a gastrointestinal neurotransmitter, mediating the gastric receptive relaxation and the relaxation in the peristaltic reflex. The aim of the present study was to measure nerve-induced NO formation *in vivo* in the gastrointestinal tract.

**2** Formation of the nitric oxide oxidation products nitrite and nitrate during vagal nerve stimulation were measured in the anaesthetized rabbit. Microdialysis probes were inserted into the wall of the stomach and proximal colon, and nitrite and nitrate in dialysate measured by capillary electrophoresis.

**3** During bilateral vagal nerve stimulation there was an increase in nitrite and nitrate formation at the level of the stomach and in nitrite formation at the level of the colon. This increase was inhibited by intravenous administration of the NO synthase inhibitor $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME 30 mg $kg^{-1}$). Furthermore, L-NAME significantly increased nerve-induced gastric and colonic contractions, as well as spontaneous colonic contractions.

**4** In summary, we present a new methodological procedure for quantification of small changes in nitric oxide formation *in vivo*. This study provides evidence that nitric oxide is released in the stomach and colonic wall during vagal nerve activity, at concentrations able to cause inhibition of smooth muscle contractions *in vivo*.

**Keywords:** Nitric oxide; gastrointestinal tract; vagal nerve; capillary electrophoresis; microdialysis; nitrite; nitrate

## Introduction

A large number of studies have shown that nitric oxide (NO) functions both as a signalling molecule in endothelial cells and nerve cells, and as a mediator of host-defence reactions in activated immune cells (Moncada *et al*., 1991; Ånggård, 1994). Considerable evidence suggests that NO acts as a mediator of non-adrenergic non-cholinergic (NANC) autonomic neurotransmission (Sanders & Ward, 1992). Release of NO in the stomach wall is probably responsible for adaptive gastric relaxation (Desai *et al*., 1991), and nerve-induced relaxation mediated by NO has been demonstrated in the canine ileocolonic junction (Boeckxstaens *et al*., 1990), in the guinea-pig caecum (Shuttleworth *et al*., 1991), ileum (Gustafsson *et al*., 1990) and colon (Iversen *et al*., 1994b). In addition, immunohistochemical studies have shown that nitric oxide synthase (NOS) positive neurones constitute a large proportion of myenteric nerves (Bredt *et al*., 1991; Costa *et al*., 1992).

An impaired or absent NO formation has been suggested as the underlying pathophysiological mechanism in achalasia (Mearin *et al*., 1993), Hirschprung's disease (Vanderwinden *et al*., 1993), and infantile pyloric stenosis (Vanderwinden *et al*., 1992). Excessive NO synthesis in colonic mucosa in ulcerative colitis might contribute to impaired motility associated with toxic dilatation (Boughton-Smith *et al*., 1993).

Increased frequency and amplitude of jejunal spontaneous contractions, by inhibiting nitric oxide synthase (NOS) with $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) has previously been demonstrated in an *in vivo* study (Calignano *et al*., 1992). But nerve-induced gastrointestinal NO formation has never been quantified *in vivo*.

The aim of the present study was to quantify NO release *in vitro* in the stomach wall and colonic wall during vagal nerve stimulation, and to study its effects on gastrointestinal smooth muscle activity *in vivo*. Since NO has a short biological half-life the oxidation products of NO, nitrite ($NO_2^-$) and nitrate ($NO_3^-$), have been used to quantify NO formation during nerve stimulation *in vitro* (Iversen *et al*., 1994a, b; Wiklund *et al*., 1993). The present study demonstrates a new methodological procedure which makes it possible to measure very small changes in the formation of $NO_2^-$ and $NO_3^-$ *in vivo*.

## Methods

New Zealand White rabbits (2.4–2.6 kg) of either sex were anaesthetized with sodium pentobarbitone 50–60 mg $kg^{-1}$, i.v. Supplementary i.v. infusion of anaesthetic (sodium pentobarbitone, 30 mg $kg^{-1}$ 60 $min^{-1}$) as well as fluid substitution (6 ml $kg^{-1}$ 60 $min^{-1}$) were provided throughout the experiments. The animals were tracheotomized and ventilated by means of a Palmer small animal ventilator (BK Universal Sollentuna, Sweden) set to give 36 strokes per min and a stroke volume giving a minute volume of 250–300 ml $kg^{-1}$. Endtidal $PCO_2$ was continuously monitored by a Datex respiration monitor (Instrumentarium OY, Helsinki, Finland), and kept at 4.5–5.0 kPa. A positive end-expiratory pressure was set at 5 $cmH_2O$. Arterial blood gases were checked before and after administration of the NO synthase inhibitor L-NAME (30 mg $kg^{-1}$, 20 min i.v. injection). Systemic arterial blood pressure and heart rate were recorded via a catheter introduced into a carotid artery and by means of a Statham P 23AC transducer and a Grass model 7D polygraph (Grass Instruments, Quincy MA, U.S.A.). The abdomen was opened by a midline incision. Microdialysis probes were inserted into the stomach and colonic wall, respectively. The vagi were dissected lateral to the trachea and the central parts of the nerves were crushed. The vagi were then placed on platinum electrodes. Vagal stimulation with monophasic square wave pulses (0.5 ms, 15 V, 10 Hz for 1–10 min at 10–30 min intervals) were applied to both nerves by a Grass S44 stimulator. The

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stimulation parameters were chosen in order to obtain a significant NO release, and still get reproducible motor responses and a moderate heart rate reduction. It has previously been shown that intestinal NO release is frequency-dependent (Wiklund *et al*., 1993). When higher frequencies were applied more pronounced effects on heart rate were observed. The rectal temperature was maintained at 38°C by means of a heating pad connected to a temperature control unit. Experiments were started approximately 60 min after surgery was completed.

### *Mechanical recordings*

Contractions of circular gastric muscle fibres were recorded isometrically. The proximal part of the antrum was connected, via a suture located at the greater gastric curvature, to a Grass FT03C force transducer and stretched towards the lesser curvature by an adjustable hook at a load of 50–100 mN. Contractions in circular muscle fibres in the proximal colon were recorded isometrically. A suture was connected to a Grass FT03C force transducer and the colon was stretched in the opposite direction by another suture connected to an adjustable hook at a load of 20–30 mN.

### *Microdialysis procedure*

Microdialysis was performed as previously described in detail (Tossman & Ungerstedt, 1986). A rigid probe (CMA/10, CMA Medical AB, Stockholm, Sweden) with a $3 \times 0.7$ mm dialysis membrane (length and outer diameter) and a molecular weight cut-off at 20 kilodaltons was used.

In order to minimise the trauma when inserting the microdialysis probes, the serosa was first tangentially punctured by a needle. The probes were then gently inserted tangentially into the wall of the proximal antrum and proximal colonic wall, approximately at the site of mechanical recordings. The tip of the probes was positioned between the serosa and the mucosa. The stomach wall and colonic wall are quite thin and it is technically not possible to insert the probe so that the whole membrane is located in a certain histological layer. The probes were sutured at the surface of the stomach and the colon. Nerve-induced contractions did not cause rupture of the microdialysis membrane.

Saline was used as perfusion medium, and was delivered to the probe by a CMA/100 microinjection pump (CMA Medical AB, Stockholm, Sweden) at a flow rate of 1.0 $\mu$l min$^{-1}$. Dead space volume of the probe system was taken into account during sample collection which started after a 30 min equilibration period. In order to study variations in $NO_2^-$ and $NO_3^-$ concentrations consecutive samples were obtained at basal conditions. In the stomach wall ($n=7$) $NO_2^-$ and $NO_3^-$ concentrations were $9.5 \pm 2.1$ $\mu$M and $60 \pm 11$ $\mu$M at basal, and one hour later $9.4 \pm 2.2$ $\mu$M and $63 \pm 11$ $\mu$M, respectively. In the colonic wall ($n=5$) $NO_2^-$ and $NO_3^-$ concentrations were $8.2 \pm 2.7$ $\mu$M and $43 \pm 7.3$ $\mu$M at basal, and one hour later $9.3 \pm 2.5$ $\mu$M and $42 \pm 8.6$ $\mu$M, respectively. Dialysate was collected during the 10 min immediately before and during each stimulation period. The *in vitro* recovery for $NO_2^-$ and $NO_3^-$, by use of the microdialysis probe described above at a flow rate of 1.0 $\mu$l min$^{-1}$, was 33% and 31%, respectively. It has previously been shown that *in vivo* recovery is different from *in vitro* recovery (Jacobson *et al*., 1985; Lönnroth *et al*., 1987; Benveniste *et al*., 1989; Amberg & Lindefors, 1989; Ståhle *et al*., 1991). *In vitro* recovery is in most cases higher than *in vivo* recovery and, hence, results in an underestimation of *in vivo* concentration if the values are corrected for *in vitro* recovery. However, for the presently used probe dimensions and flow rate, *in vivo* recovery for compounds such as $NO_2^-$ and $NO_3^-$ will be in the range 10–40% (Ståhle, personal communication). Thus, the present data may be corrected for 31% and 33%, respectively, although the values obtained should be considered as being approximations to a limited degree.

### *Nitrite and nitrate determination*

Quantification of $NO_2^-$ and $NO_3^-$, in arterial plasma and micro dialysate was performed by capillary electrophoresis as described previously (Leone *et al*., 1994a). The samples were diluted 1:10 with ultra pure water (>18.2 MΩ MilliQ$^+$ water) and analysed on an Hewlett-Packard 3D capillary electrophoresis system (Hewlett Packard Ltd., Stockport, Cheshire, U.K.) with 104 cm fused silica capillaries of 75 $\mu$m i.d. (extended light path, 225 $\mu$m). The electrophoresis electrolyte consisted of 25 mM sodium sulphate containing 5% NICE-Pak OFM Anion-BT (Waters proprietary osmotic flow modifier). Samples were injected by electromigration for 20 s at $-6$ kV and analysed at an applied negative potential of 30 kV. Data were acquired at 214 nm onto an HP 3D CE Chem Station data system. The detection limit for $NO_2^-$ and $NO_3^-$ was 60 nM

### *Drugs*

N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) was purchased from Sigma Co. (St Louis, MO, U.S.A.). Sodium pentobarbitone was from Apoteksbolaget, Stockholm, Sweden.

### *Statistics*

Experimental data are expressed as mean values ± s.e.mean. Statistical significance was tested according to Student's *t* test for paired or unpaired observations.

## Results

### *Nitric oxide oxidation products in tissue dialysate*

Basal interstitial fluid tissue concentrations of $NO_2^-$ and $NO_3^-$ (corrected for 33% and 31% recovery, respectively) in the stomach wall were $10 \pm 2.7$ $\mu$M for $NO_2^-$, and for $NO_3^-$ $68 \pm 9.4$ $\mu$M ($n=10$). In the colonic wall it was $9.7 \pm 3.6$ $\mu$M for $NO_2^-$, and $58 \pm 14$ $\mu$M for $NO_3^-$ ($n=6$). Arterial plasma concentrations of $NO_2^-$ and $NO_3^-$ in anaesthetized rabbits were $3.9 \pm 0.9$ $\mu$M and $120 \pm 22$ $\mu$M, respectively. Basal interstitial tissue concentrations of $NO_2^-$ and $NO_3^-$ were unchanged 20–30 min after L-NAME infusion was initialized.

There was an increase in the concentration of NO oxidation products in the stomach wall during bilateral vagal nerve stimulation (Figure 1a). Stomach wall dialysate concentrations of $NO_2^-$ increased by $31 \pm 8\%$ ($P<0.01$) and of $NO_3^-$ by $23 \pm 9\%$ ($P<0.05$, $n=10$). In the presence of intravenously administered L-NAME there was no nerve-induced increased in $NO_2^-$ or $NO_3^-$ concentration (Figure 1a).

Enhancement of nitric oxide oxidation product concentration during vagal nerve stimulation was seen also in the wall of the proximal colon (Figure 1b). Tissue dialysate concentration of $NO_2^-$ increased by $82 \pm 23\%$ ($P<0.05$, $n=6$). This increase was prevented by L-NAME ($P<0.05$, $n=5$). There was no significant nerve-induced increase in $NO_3^-$ concentration in the colonic wall (Figure 1b).

### *Contractile activity in the stomach and colon*

Bilateral vagal nerve stimulation elicited highly reproducible contractions of the stomach and the proximal colon. Concomitantly there was a decrease in heart rate and arterial blood pressure (Figure 2). The force of stimulation-induced contractions was $90 \pm 18$ mN in the stomach, and $36 \pm 4$ mN in the colon. A 20 min infusion of L-NAME 30 mg kg$^{-1}$, i.v., enhanced stimulation-induced contractions in the stomach by $32 \pm 13\%$ ($P<0.05$, $n=4$), and in the colon by $67 \pm 15\%$ ($P<0.01$, $n=5$; Figure 2). L-NAME induced an increase in mean arterial blood pressure from its resting value ($103 \pm 5$ mmHg) by $19 \pm 4$ mmHg ($P<0.01$).

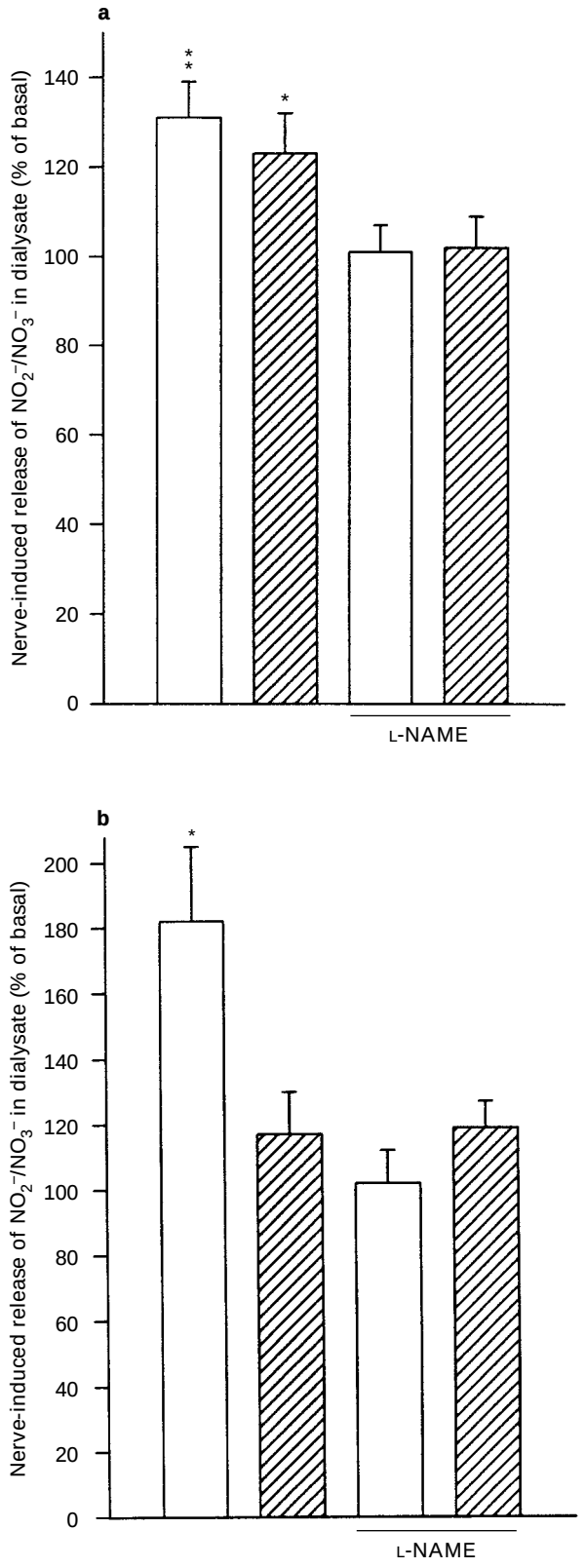


**Figure 1** Diagram showing effects on interstitial $NO_2^-$ (open columns) and $NO_3^-$ (hatched columns) concentrations of bilateral vagal nerve stimulation (0.5 ms, 15 V, 10 Hz for 10 min at 30 min intervals) in anaesthetized rabbits. Nerve-induced release of $NO_2^-$ and $NO_3^-$ in tissue microdialysate from (a) the stomach wall and (b) proximal colonic wall expressed as % of basal concentration before nerve stimulation. Dialysate was collected for 10 min before and during each stimulation period. Stimulations were also made after a 20 min i.v. infusion of L-NAME (30 mg $kg^{-1}$). Mean ± s.e.mean are shown. $^*P<0.05$, $^{**}P<0.01$ compared to basal concentration.

Spontaneous contractile activity was present in the colon (Figure 2) but only very weak spontaneous contractions were observed in the stomach. The frequency of spontaneous colonic contractions exceeding 10 mN was $6 \pm 2$ per 10 min. In the presence of L-NAME (30 mg $kg^{-1}$) the frequency of these contractions increased to $11 \pm 2.6$ per 10 min ($P<0.05$, $n=5$). Furthermore L-NAME increased the maximum force of spontaneous contractions by $128 \pm 37\%$ ($P<0.05$, $n=5$). No effect of L-NAME on basal tone was found either in the stomach or colon.

## Discussion

In this study we demonstrated an increase in NO oxidation products in the rabbit stomach and colonic wall as a consequence of vagal nerve stimulation. $NO_2^-$ and $NO_3^-$ formation as an indicator of NO release during vagal nerve stimulation was detected by means of an *in vivo* microdialysis technique. This technique constitutes a useful method for the *in vivo* sampling of substances in the extracellular fluid (Ungerstedt, 1991) and it has previously been used in the gastrointestinal tract (Meirien *et al*., 1986). NO is a reactive and unstable molecule and its biological half-life is estimated to a few seconds in biological systems *in vitro* (Palmer *et al*., 1987; Bult *et al*., 1990; Iversen *et al*., 1994a,b). Thus, the two main NO oxidation products $NO_2^-$ and $NO_3^-$ are commonly used as markers of NO generation. Consequently, previous *in vitro* studies have shown that nerve-induced NO formation can be measured as $NO_2^-$ and $NO_3^-$ formation (Wiklund *et al*., 1993; Iversen *et al*., 1994b; Leone *et al*., 1994b). *In vivo* detection of NO release as measured by determination of its oxidation products, combined with the microdialysis technique, has previously been performed in the rat brain (Luo *et al*., 1993; Ohta *et al*., 1994).

In the present study $NO_2^-$ concentration increased in dialysate collected from the gastric and colonic wall during vagal nerve stimulation. In the presence of the NOS inhibitor (L-NAME) there was no increase in $NO_2^-$ concentration during vagal nerve stimulation. Thus, the nerve-induced increase in $NO_2^-$ is probably due to NOS activity. Dialysate concentration of $NO_3^-$ was also increased in the stomach wall. However, no significant increase in $NO_3^-$ was seen in dialysate from the proximal colonic wall. Since basal plasma and interstitial fluid concentrations of $NO_3^-$ as compared to $NO_2^-$ are about 30 and 6 times higher, respectively, a small change in $NO_3^-$ formation is likely to be masked by natural variation in tissue $NO_3^-$ concentration. This could explain the lack of significant nerve-induced increase of $NO_3^-$ formation in the colon. Thus, $NO_2^-$ formation as measured by microdialysis is more useful as a marker for minute changes in endogenous NO production.

NO is rapidly oxidized to nitrite and nitrate in blood where $NO_2^-$ is further oxidized to $NO_3^-$ by oxyhaemoproteins (Leaf *et al*., 1990). Nitrite and nitrate are subsequently excreted in the urine giving a plasma half-life of approximately 8 h (Wennmalm, 1995). In aqueous solutions lacking haemoproteins the principal spontaneous oxidation product of NO is $NO_2^-$ (Ignarro *et al*., 1993). With the present experimental procedure where dialysate without haemoproteins is collected, $NO_2^-$ was approximately 2.5 times higher in the extracellular fluid as compared to plasma. This is probably due to a larger proportion of locally released NO being oxidized to $NO_2^-$ rather than $NO_3^-$, and then 'protected' by the microdialysis membrane from oxidation by oxyhaemoproteins. This contributes to the usefulness of $NO_2^-$ as a marker for NO formation in our experiments. In fact, the actual extracellular concentration of $NO_2^-$ may be even higher than the estimated value since it was calculated from the *in vitro* recovery. This probably results in an underestimation as mentioned in the methods section.

Bilateral cervical vagal stimulation causes increased gastrointestinal blood flow in cat (Martinsson, 1965). Such an increase in blood flow could affect the extracellular con-

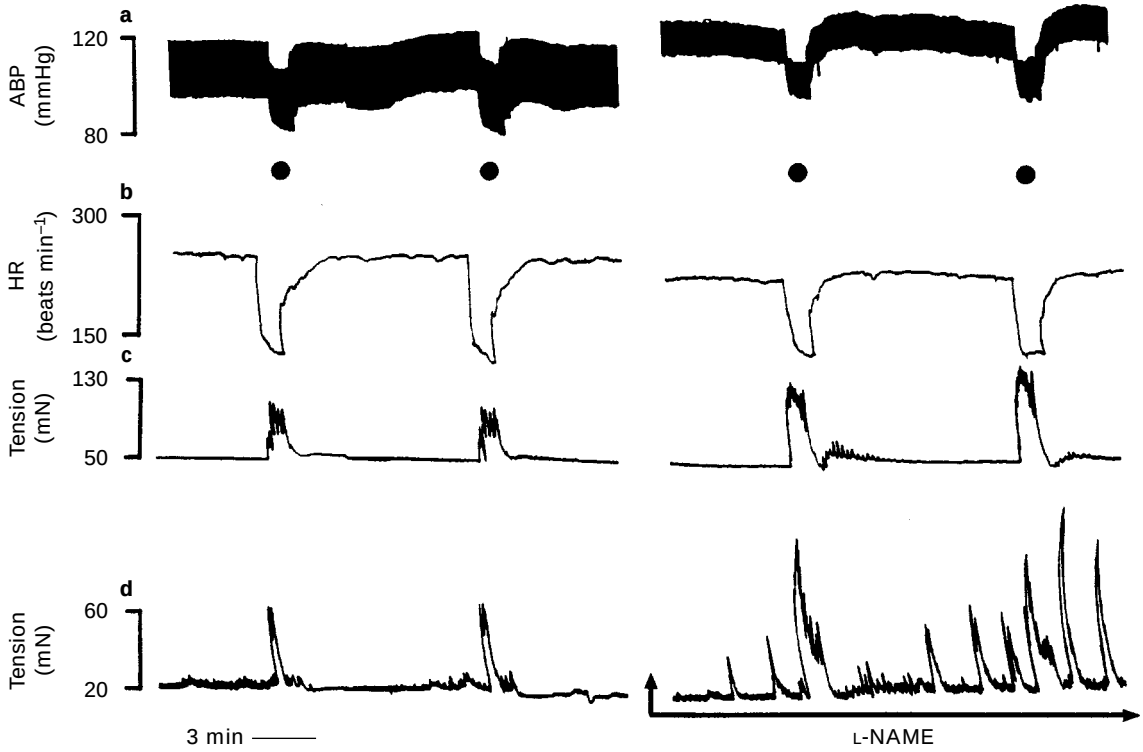


**Figure 2** Records of (a) arterial blood pressure (ABP), (b) heart rate (HR), and contractile activity in the (c) stomach and (d) proximal colon of an anaesthetized rabbit. Bilateral cervical vagal nerve stimulation (indicated as ●) elicited gastric and colonic contractions which were enhanced by the NOS inhibitor L-NAME. Administration of L-NAME (30 mg $kg^{-1}$, i.v.) also increased spontaneous contractile activity in the colon, as well as arterial blood pressure.

centrations of $NO_2^-$ and $NO_3^-$. However, since the basal extracellular level of $NO_2^-$ was higher than the plasma level an increase in blood flow cannot contribute to the observed increase in $NO_2^-$ concentration in dialysate during vagal nerve stimulation.

Vagally induced gastric and colonic contractions were enhanced by L-NAME administration, suggesting that vagal nerve activity released endogenous NO in amounts sufficient for smooth muscle inhibitory activity. Amplitude and frequency of spontaneous contractions in the proximal colon were also significantly increased by L-NAME administration. This is in agreement with data from a previous study showing increased frequency and amplitude of jejunal spontaneous contractions after inhibition of NOS with L-NAME (Calignano *et al*, 1992). Nitric oxide has previously been suggested to mediate the relaxing component in the colonic peristaltic reflex (Hata *et al*., 1990). By inhibiting endogenous NO formation L-NAME might thus interrupt normal peristaltic activity and intramural reflexes. Furthermore, decreased NO synthesis by myenteric nerves has been suggested as an underlying pathophysiological mechanism in achalasia (Mearin *et al*., 1993), Hirschprung's disease (Vanderwinden *et al*., 1993), and infantile pyloric stenosis (Vanderwinden *et al*., 1992). To evaluate the role for NO in gastrointestinal pathophysiology the present method for *in vivo* microdialysis quantification of intramural NO formation could be used.

In conclusion, we present a new methodological procedure for quantification of minute changes in NO formation *in vivo*. This study provides evidence that vagal regulation of the stomach and colon involves inhibition of smooth muscle activity by endogenous NO.

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## References

AMBERG, G. & LINDEFORS, N. (1989). Intracerebral microdialysis: II. Mathematical studies of diffusion kinetics. *J. Pharmacol. Methods*, **22,** 157–183.

ÄNGGÅRD, E. (1994). Nitric oxide: mediator, murderer, and medicine. *Lancet*, **343,** 1199–1206.

BENVENISTE, H., HANSEN, A.J. & OTTOSEN, N.S. (1989). Determination of brain interstitial concentrations by microdialysis. *J. Neurochem*., **52,** 1741–1750.

BOECKXSTAENS, G.E., PELCKMANS, P.A., BULT, H., DE MAN, J.G., HERMAN, A.G. & VAN MAERCKE, Y.M. (1990). Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. *Eur. J. Pharmacol*., **190,** 239–246.

BOUGHTON-SMITH, N.K., EVANS, S.M., HAWKEY, C.J., COLE, A.T., BALSITIS, M., WHITTLE, B.J.R. & MONCADA, S. (1993). Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Lancet*, **342,** 338–340.

BREDT, D.S., HWANG, P.H. & SNYDER, S.H. (1991). Localization of nitric oxide synthase indicating a role for nitric oxide. *Nature*, **351,** 714–718.

BULT, H., BOECKXSTAENS, G.E., PELCKMANS, P.A., JORDAENS, F.H., VAN MAERCKE, Y.M. & HERMAN, A.G. (1990). Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature*, **345,** 346–347.

CALIGNANO, A., WHITTLE, B.J.R., DI ROSA, M. & MONCADA, S. (1992). Involvement of endogenous nitric oxide in the regulation of rat intestinal motility *in vivo*. *Eur. J. Pharmacol*., **229,** 273–276.

COSTA, M., FURNESS, J.B., POMPOLO, S., BROOKS, S.J.H., BORNSTEIN, J.C., BREDT, D.S. & SNYDER, S.H. (1992). Projections and chemical coding of neurons with immunoreactivity for nitric oxide synthase in the guinea-pig small intestine. *Neurosci. Lett*., **148,** 121–125.

DESAI, K.M., SESSA, W.C. & VANE, J.R. (1991). Involvement of nitric oxide in the reflex relaxation of the stomach to accomodate food or fluid. *Nature*, **351,** 477 – 479.

GUSTAFSSON, L.E., WIKLUND, C.U., WIKLUND, N.P., PERSSON, M. & MONCADA, S. (1990). Modulation of neuroeffector transmission by nitric oxide in guinea pig ileum. *Biochem. Biophys. Res. Commun.*, **173,** 106 – 110.

HATA, F., ISHII, T., KANADA, A., YAMANO, N., KATAOKA, T., TAKEUCHI, T. & YAGASAKI, O. (1990). Essential role for nitric oxide in descending inhibition in the rat proximal colon. *Biochem. Biophys. Res. Commun.*, **172,** 1400 – 1406.

IGNARRO, L.J., FUKUTO, J.M., GRISCAVAGE, J.M., ROGERS, N.E. & BYRNS, R.E. (1993). Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: Comparison with enzymatically formed nitric oxide from L-arginine. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 8103 – 8107.

IVERSEN, H.H., GUSTAFSSON, L.E., LEONE, A.M. & WIKLUND, N.P. (1994a). Smooth muscle relaxing effects of NO, nitrosothiols and a nerve-induced relaxing factor released in guinea-pig colon. *Br. J. Pharmacol.*, **113,** 1088 – 1092.

IVERSEN, H.H., WIKLUND, N.P. & GUSTAFSSON, L.E. (1994b). Nitric oxide-like activity in guinea pig colon as determined by effector responses, bioassay and chemiluminescence analysis. *Acta Physiol. Scand.*, **152,** 315 – 322.

JACOBSON, I., SANDBERG, M. & HAMBERGER, A. (1985). Mass transfer in brain dialysis devices - a new method for estimation of extracellular amino acids concentration. *J. Neurosci. Methods*, **15,** 263 – 268.

LEAF, F.D., WISHNOK, J.S. & TANNENBAUM, S.R. (1990). Nitric oxide: the dark side. In *Nitric Oxide from L-Arginine: a Bioregulatory System*. ed. Moncada, S. & Higgs, E.A. pp. 291 – 299. Amsterdam: Elsevier.

LEONE, A.M., FRANCIS, P.L., RHODES, P. & MONCADA, S. (1994a). A rapid and simple method for the measurement of nitrite and nitrate in plasma by high performance capillary electrophoresis. *Biochem. Biophys. Res. Commun.*, **200,** 951 – 957.

LEONE, A.M., WIKLUND, N.P., HÖKFELT, T., BRUNDIN, L. & MONCADA, S. (1994b). Release of nitric oxide by nerve stimulation in the urogenital tract. *NeuroReport*, **5,** 733 – 736.

LUO, D., KNEZEVICH, S. & VINCENT, S.R. (1993). N-methyl-D-aspartate-induced nitric oxide release: An *in vivo* microdialysis study. *Neuroscience*, **57,** 897 – 900.

LÖNNROTH, P., JANSSON, P.-A. & SMITH, U. (1987). A microdialysis method allowing characterization of intercellular water space in humans. *Am. J. Physiol.*, **253,** E228 – E231.

MARTINSSON, J. (1965). The effect of graded vagal stimulation on gastric motility, secretion and blood flow in the cat. *Acta Physiol. Scand.*, **65,** 300 – 309.

MEARIN, F., MOURELLE, M., GUARNER, F., SALAS, A., RIVEROS-MORENO, V., MONCADA, S. & MALAGELADA, J.-R. (1993). Patients with achalasia lack nitric oxide synthase in the gastro-oesophageal junction. *Eur. J. Clin. Invest.*, **23,** 724 – 728.

MEIRIEN, O., PAIRET, M., SUTRA, J.F. & RUCKEBUSCH, M. (1986). Local release of monoamines in the gastrointestinal tract: An in vivo study in rabbits. *Life Sci.*, **38,** 827 – 834.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43,** 109 – 142.

OHTA, K., ARAKI, N., SHIBATA, M., HAMADA, J., KOMATSUMOTO, S., SHIMAZU, K. & FUKUUCHI, Y. (1994). A novel in vivo assay system for consecutive measurement of brain nitric oxide production combined with the microdialysis technique. *Neurosci. Lett.*, **176,** 165 – 168.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of the endothelium-derived relaxing factor. *Nature*, **327,** 524 – 526.

SANDERS, K.M. & WARD, S.M. (1992). Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. *Am. J. Physiol.*, **262,** G379 – G392.

SHUTTLEWORTH, C.W.R., MURPHY, R. & FURNESS, J.B. (1991). Evidence that nitric oxide participates in non-adrenergic inhibitory transmission to intestinal muscle in guinea-pig. *Neurosci. Lett.*, **130,** 77 – 80.

STÅHLE, L., SEGERSVÄRD, S. & UNGERSTEDT, U. (1991). A comparison between three methods for estimation of extracellular concentrations of exogenous and endogenous compounds by microdialysis. *J. Pharmacol. Methods*, **25,** 41 – 52.

TOSSMAN, U. & UNGERSTEDT, U. (1986). Microdialysis in the study of extracellular levels of amino acids in the rat brain. *Acta Physiol. Scand.*, **128,** 9 – 14.

UNGERSTEDT, U. (1991). Microdialysis-principles and applications for studies in animals and man. *J. Intern. Med.*, **230,** 365 – 373.

VANDERWINDEN, J.-M., DE LAET, M.-H., SCHIFFMANN, S.N., MAILLEUX, P., LOWENSTEIN, C.J., SNYDER, S.H. & VANDERHAEGHEN, J.-J. (1993). Nitric oxide synthase distribution in the enteric nervous system of Hirschprung's disease. *Gastroenterology*, **105,** 969 – 973.

VANDERWINDEN, J.-M., MAILLEUX, P., SCHIFFMANN, S.N., VANDERHAEGHEN, J.-J. & DE LACT, M.H. (1992). Nitric oxide synthase activity in infantile hypertrophic pyloric stenosis. *N. Engl. J. Med.*, **327,** 511 – 515.

WENNMALM, Å. (1995). Metabolism and excretion of nitric oxide in man: basal studies and applications. *Agents & Actions* (*Suppl.*), **45,** 129 – 138.

WIKLUND, N.P., LEONE, A.M., GUSTAFSSON, L.E. & MONCADA, S. (1993). Release of nitric oxide evoked by nerve stimulation in guinea-pig intestine. *Neurosci.*, **53,** 607 – 611.

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