Contrasting effects of circulating nitric oxide and nitrergic transmission on exocrine pancreatic secretion in rats

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Abstract

Background—Nitric oxide (NO) blockade by L-nitroarginine methyl ester (L-NAME) inhibits pancreatic secretion in vivo and aggravates caerulein induced pancreatitis. Nitric oxide synthase (NOS) is present in pancreatic islets, endothelium, and nerve fibres. L-NAME blocks all known NOS isoforms.

Aim—To investigate the source of NO blocked by L-NAME that inhibits amylase secretion.

Methods—Amylase output was measured in rats in response to caerulein (0.1–50 µg/kg) alone or with indazole. Baseline secretion and the response to supramaximal caerulein were also examined after administration of indazole, L-NAME, haemoglobin, or aminoguanidine under continuous blood pressure measurement. In separate experiments, pancreatic secretion was measured after blockade of afferent nerve fibres by either systemic or local capsaicin. The effect of neural NOS inhibition on caerulein induced pancreatitis was also investigated.

Results—L-NAME, haemoglobin, and supramaximal caerulein (10 µg/kg) increased blood pressure, whereas indazole and suboptimal caerulein (0.1 µg/kg) did not. Indazole and capsaicin decreased basal amylase output. L-NAME and haemoglobin reduced basal amylase output to a lesser extent and potentiated the inhibitory response to supramaximal caerulein. In contrast, full neural NOS inhibition by L-NAME partially reversed the expected caerulein induced suppression of amylase output. This effect was reproduced by indazole and capsaicin. Indazole did not alter responses to either optimal (0.25 µg/kg) or suboptimal (0.1 µg/kg) caerulein, nor, in contrast with L-NAME, aggravate the outcome of caerulein induced pancreatitis.

Conclusions—Reduction of circulating NO availability, probably of endothelial origin, is responsible for the decrease in amylase secretion observed in the early response to L-NAME. Nitrergic neurotransmission plays an important role in the control of pancreatic secretion and may induce opposite effects to endothelial NOS activity.

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Inhibition of endogenous nitric oxide (NO) synthesis by L-nitroarginine methyl ester (L-NAME) decreases basal and stimulated pancreatic secretion in vivo. This process has been shown in several species including the rat, pig, cat, and dog,^{1–4} but its mechanism of action is poorly understood.

In the normal pancreas, nitric oxide synthase (NOS) is present in the endothelium, nerve fibres, and islets of Langerhans.^{5–7} Immunostaining techniques do not show the presence of NOS in exocrine cells,⁷ whereas functional studies indicate that NOS activity is present.^{1–8} In fact, NOS may be involved in the control of agonist stimulated calcium influx.⁸ However, in dispersed pancreatic acini, blockade of NO production by L-NAME has not proven to be detrimental to amylase secretion,¹ an observation that is consistent with the lack of effect of intracellular modifications of cGMP concentration on amylase release.⁹

L-NAME (and most other structurally related compounds) exerts an inhibitory effect over all NOS isoforms, being effective on a wide variety of cell types. Thus L-NAME elicits a powerful and sustained vasopressor response, increasing mean arterial pressure (MAP)¹⁰ (as the result of endothelial NOS blockade), inducing anti-nociception by inhibiting neural NOS (nNOS) preventing NO mediated neurotransmission (nitrergic transmission),¹¹ and disrupting the regulation of islet hormone secretion^{6,12} by interfering with NOS activity in the islets of Langerhans.

We have previously shown that L-NAME inhibits in vivo pancreatic secretion stimulated by optimal and supramaximal doses of caerulein when both drugs are infused simultaneously.¹ However, L-N^G-nitroarginine (the compound that results from hydrolysis of L-NAME and often regarded as equivalent to L-NAME)¹³ shows progressive and irreversible inhibition of nNOS.^{14,15} We hypothesised that selective NOS inhibition may reveal the specific role of various NOS sites in pancreatic exocrine secretion. The aim of the present study was to establish the source of NO that, when blocked, is associated with decreased pancreatic secretion in vivo. A pharmacological strategy was employed: the pattern of amylase secretion was examined in the anaesthetised rat in response to selective compounds that interact with the normal physiology (trafficking) of NO.

Indazole inhibits nNOS without interfering with endothelial NOS activity in vivo.¹⁶ Thus MAP is not modified by systemic indazole. In

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Table 1 Summary of study groups, route of drug administration, dose and schedule of caerulein challenges

Experimental group	Dose, route of administration	Time before caerulein stimulation
Control (vehicle)	i.v./i.p.	—
L-NAME	30 mg/kg, i.v.	2 min
L-NAME 25	30 mg/kg, i.v.	25 min
Indazole	50 + 50 mg/kg, i.p.	50 and 2 min
Indazole 100	100 mg/kg, i.p.	2 min
Systemic capsaicin	150 mg/kg, s.c.	5 days
Local capsaicin	0.1%, perivagal + 1% intraduodenal	5 days
Caerulein alone	0.1–50 µg/kg, i.v.	—
Haemoglobin	500 + 500 mg/kg, i.v.	2 min
Aminoguanidine	40 mg/kg, i.v.	60 and 2 min

i.v., intravenous; i.p., intraperitoneal; s.c., subcutaneous.

our study, indazole was used to investigate the effects of selective inhibition of nNOS on pancreatic secretion.^{16–20} Capsaicin is a sensory neurotoxin with highly selective effects on a subset of primary afferent sensory neurons, including nociceptive neurons. Systemic administration or high concentration local application produces anti-nociceptive effects.^{21–23} NO has been shown to be formed and released in capsaicin sensitive afferent fibres.^{24–25} Thus to demonstrate the contribution of nitrenergic fibres to the control of pancreatic secretion without interference from inhibition of other NO sources, amylase output was measured in response to caerulein after either inhibition of neural transmission of NO by indazole or afferent sensorial nerve fibre ablation by capsaicin.

Since a selective inhibitor for endothelial NOS is not currently available, intravenous (i.v.) infusion of haemoglobin was used to decrease plasmatic free NO acutely. Reduced haemoglobin avidly binds free NO,^{26–27} and it has been used as an NO scavenger to investigate the effects of acute NO removal from blood.²⁸ It appears to have little or no effect on nitrenergic transmission²⁶ in vivo. The effects of inhibition of the inducible isoform of NOS (non-neural, non-endothelial) by aminoguanidine on pancreatic secretion were also examined.

In a previous study¹ we have shown that L-NAME, besides potentiating the inhibition of pancreatic secretion elicited by supramaximal doses of caerulein, also aggravates some of the parameters commonly used to assess pancreatic injury in caerulein induced pancreatitis. In addition, L-NAME has also been shown to increase tissue levels of trypsinogen activation peptide, an index of the amount of tissue necrosis.²⁹ To determine whether specific inhibition of nNOS may play a role in the development of acute pancreatitis in this model, the effects of indazole on three distinct features of acute pancreatic injury (amylasaemia, tissue oedema, and polymorphonuclear infiltration) were evaluated.

Materials and Methods

MATERIALS

Ketamine was from Parke-Davis SL (EL Prat de Llobregat, Spain). Caerulein, L-NAME, bovine haemoglobin, bovine serum albumin, aminoguanidine, capsaicin, and sodium dithionite were from Sigma Chemical Co

(Alcobendas, Spain). Indazole was purchased from Boehringer (Mannheim, Germany).

ANIMAL PREPARATION

Experiments were conducted on a total of 214 male Sprague-Dawley rats weighing between 220 and 320 g. After an overnight fast, rats were anaesthetised with ketamine (100 mg/kg body weight intraperitoneally (i.p.)), with subsequent intramuscular (i.m.) doses used as necessary to maintain anaesthesia. A jugular vein was cannulated (polyethylene catheter PE-10; Clay Adams, Parsippany, New Jersey, USA) for drug administration and continuous saline perfusion at 3 ml/h by means of a syringe pump. A heparinised (10 U/ml) catheter (PE-50) was placed into the carotid artery to monitor blood pressure and to facilitate blood sampling. A pressure transducer was connected to the carotid catheter for continuous recording of MAP in the anaesthetised resting state and in response to administration of drugs.

Animals were then laparotomised and the biliopancreatic duct was cannulated through its duodenal opening (PE-50). The abdominal wound was covered with a saline moisturised gauze, and body temperature was maintained with a heating lamp.

EXPERIMENTAL PROTOCOLS

Measurement of pancreatic secretion

Pancreatic secretion was measured as previously described.¹ Briefly, after completion of surgical procedures, biliopancreatic secretion was allowed to drain freely for 30 minutes before initiation of the experiments (stabilisation period). Then secretion was collected in tared vessels in separate 10 minute fractions for 60 minutes and volumes estimated by weight. Amylase output was calculated by determining amylase concentration in the collected fractions. The mean amylase output obtained from two consecutive 10 minute fractions after the stabilisation period was taken as the initial “basal” secretion. Separate experiments were performed to calculate the secretory response to vehicle, NOS inhibitors, haemoglobin, or caerulein at the indicated doses, administered as an i.v. bolus injection at the end of the 20 minutes of basal collection (time 0). Table 1 summarises the various groups entering the study.

Basal secretions tend to fluctuate spontaneously over time,³⁰ and bursts of secretion occur in a cyclic pattern every two hours or less.³¹ Accordingly, when unstimulated basal secretion was analysed, data on amylase output were standardised by expressing results as a percentage of the variation from initial basal output.

NOS blockade by L-NAME

The effects of L-NAME were investigated after i.v. administration of 30 mg/kg at time 0. Neural NOS inhibition by i.p. L-NAME is best manifested starting 15 minutes after its administration,¹¹ at a time when there are no further increments in arterial blood pressure. Thus, to investigate most effectively the effect of nNOS inhibition by L-NAME on pancreatic

secretion, amylase output was measured in response to supramaximal caerulein (10 µg/kg), infused either 2 or 25 minutes after L-NAME (30 mg/kg).

Specific inhibition of neural NOS

Indazole (100 mg/kg, i.p.) was dissolved in one part of ethanol and five parts of peanut oil (v/v) to a maximal volume of 1 ml. Pancreatic secretion was measured as described above. Indazole or vehicle was administered i.p. at the end of the initial two 10 minute basal periods (time 0).

Since the effects of L-NAME on pancreatic secretion were analysed both at 2 and 25 minutes from its administration, two protocols were conducted to examine the effects of indazole on caerulein stimulated pancreatic secretion. Indazole (or vehicle) was given in two equally divided doses (100 mg/kg total, i.p.) 50 and 5 minutes before juice collection. Pancreatic secretion was then measured as described above in independent experiments in response to 0.1, 0.25, 1, 10, or 50 µg/kg caerulein (bolus i.v.). In a second set of experiments, indazole (100 mg/kg) or vehicle was administered 2 minutes before 10 µg/kg caerulein.

Ablation of sensorial afferent nerve fibres by capsaicin

To investigate the effects of functional destruction of afferent nerve fibres on pancreatic secretory response to supramaximal caerulein, capsaicin was administered according to two different protocols (systemic or local administration) five days before measurement of pancreatic secretion in response to i.v. caerulein (10 µg/kg).

Systemic capsaicin administration

Capsaicin as a 1% (w/v) solution was dissolved in ethanol/Tween 80/saline (10:10:80, by vol.). Under light ether anaesthesia, rats were injected subcutaneously (s.c.) with capsaicin in three divided doses on three consecutive days for a total dose of 150 mg/kg in a regimen shown to induce functional ablation of primary afferent sensitive fibres.³² Rats were pretreated with terbutaline (0.1 mg/kg, i.m.) and aminophylline (10 mg/kg, i.m.) before capsaicin injections to prevent acute respiratory distress. On the day before the experiments, defunctionalisation of afferent neurons was ascertained by evaluating the reduction of wiping movements in response to intraocular instillation of a 0.1% solution of capsaicin. A reduction of more than 80% compared with control rats was considered an adequate sensitive defunctionalisation.³³

Local capsaicin administration

Rats were anaesthetised with ketamine. After a midline incision, both subdiaphragmatic vagal trunks were exposed and surrounded with a small piece of gauze soaked with 100 µl 0.1% capsaicin for 30 minutes. In addition, a gauze soaked with 200 µl 0.5% capsaicin was advanced to the duodenum from the stomach through an antral incision and applied to the duodenal mucosa for 30 minutes. When treat-

ment was completed, all of the gauzes were removed and the incisions sutured. Animals were allowed to recover from surgery and experiments conducted five days later.

Removal of circulating NO

Reduced haemoglobin was prepared as previously described³⁴ by adding to a 1 mmol/l solution of haemoglobin in distilled water, a 10-fold molar excess of the reducing agent, sodium dithionite. The sodium dithionite was then removed by dialysis against 100 vol of saline for two hours at 4°C. This solution was warmed to 37°C before infusion.

Reduced haemoglobin was infused through the venous catheter at time 0. A dose of 500 mg/kg in 1 ml saline was administered as a bolus injection, followed by an infusion of the same amount over two minutes. To prevent acute changes in intravascular volume, an equal amount of blood from the arterial catheter was withdrawn. MAP was continuously recorded and pancreatic secretions collected in 10 minute fractions.

In separate studies, the effect of haemoglobin on pancreatic response to supramaximal caerulein was investigated. Haemoglobin was administered as described above. Two minutes after the end of haemoglobin infusion, caerulein (10 µg/kg) was administered and pancreatic juice collected for measurement of amylase output.

To exclude possible effects of an increase in oncotic pressure, albumin was infused in amounts that matched the oncotic capacity of haemoglobin.³⁵ Albumin (485.5 mg/kg) in 1 ml saline was administered in the same way as haemoglobin, and MAP and amylase output measured.

The secretory pancreatic response was also examined after aminoguanidine treatment. Aminoguanidine (40 mg/kg body weight, i.v.) inhibits the inducible NOS isoform and was used as a control for inhibition of non-neural non-endothelial NOS.

Induction of acute pancreatitis

Acute pancreatitis was produced by administering a total of four i.p. injections of caerulein at the dose of 20 µg/kg body weight at one hour intervals in ketamine anaesthetised rats (n = 5 per group). Indazole (50 mg/kg) was solubilised by sonication and warming in 4% ethanol in 0.5% Na₂CO₃, pH 7.4, and administered i.p. 30 minutes before induction of acute pancreatitis. To maintain adequate levels of indazole throughout the experiment, doses of 10 mg/kg indazole were administered i.m. at one hour intervals. Control rats received either vehicle alone or vehicle plus caerulein. Blood was obtained by cardiac puncture for determination of amylase activity nine hours after the first caerulein injection. Amylase activity was determined by the alpha amylase EPS test (Boehringer) for BM/Hitachi system 717. Aliquots of volume 200 µl of 1:800 to 1:2000 dilutions of the sample were used in the assay.

To measure pancreatic oedema, excised pancreata were wet weighed, desiccated at 160°C for 24 hours and reweighed. Pancreatic

Figure 1 Effect of single doses of L-NAME (30 mg/kg, i.v.), haemoglobin (1 g/kg, i.v.), supramaximal caerulein (10 µg/kg, i.v.), haemoglobin plus supramaximal caerulein, albumin (970 mg/kg), indazole (100 mg/kg, i.p.), and suboptimal caerulein (0.1 µg/kg, i.v.) on mean arterial pressure (MAP) in anaesthetised rats ($n = 8$ for each experimental condition). Bars represent average percentage variation from control in peak MAP induced by drug administration. * $p < 0.05$ compared with L-NAME group, and † $p < 0.05$ compared with all other groups.

water content was calculated as a percentage of total wet weight.

Tissue myeloperoxidase (MPO) activity was used as a biochemical marker for polymorphonuclear infiltration. It was determined using a previously described method^{36,37} that measures MPO activity as the result of H_2O_2 dependent oxidation of 3,3',5,5'-tetramethylbenzidine and expressed as units per mg protein.

STATISTICAL ANALYSIS

Results are presented as means (SEM). For statistical evaluations of differences between two groups of animals, Student's t test for unpaired data was used. Differences between more than two groups of animals were

Figure 2 Time course of the effect of single bolus injections of L-NAME, haemoglobin, albumin, indazole, or vehicle on amylase output expressed as percentage of variation from basal. Each point is the mean (SE) from five separate experiments for each experimental condition. Doses were as in fig 1. * $p < 0.05$ compared with vehicle. † $p < 0.05$ compared with all groups.

evaluated by analysis of variance. Statistical comparisons of data in fig 2 were performed using repeated measures analysis of variance followed by Fisher's protected least significant differences test. Differences were regarded as significant when $p < 0.05$.

Results

EFFECT OF NOS INHIBITORS, REDUCED HAEMOGLOBIN, AND CAERULEIN ON MAP

Basal MAP was 113 (2) mm Hg. Infusion of reduced haemoglobin produced a sharp and marked increase (fig 1). This effect was reversible, with MAP returning to baseline values in 11 (0.5) minutes. Supramaximal caerulein in 200 µl saline also induced a sharp rise in MAP which matched that induced by haemoglobin, but with shorter duration (5.25 (0.4) minutes). In contrast, infusion of caerulein at submaximal doses of 0.1 µg/kg in the same amount of saline (200 µl) induced a small but significant and transient (5 (0.7) minutes) reduction in MAP. The effect of i.v. bolus infusion of L-NAME was characterised by a progressive increase in MAP during the first 7.6 minutes, followed by a slow decline to 1.6 times baseline values by the end of the observation period. Peak change in MAP was greater after L-NAME infusion than after haemoglobin or caerulein infusion alone. Interestingly, the peak rise in MAP was enhanced by the combined treatment of supramaximal caerulein and haemoglobin, which then became similar to the peak change in MAP observed after L-NAME infusion.

Treatment with albumin in amounts that matched the expected oncotic capacity of haemoglobin raised MAP to a much lesser extent and duration (6.25 (0.25) minutes) than the response obtained by reduced haemoglobin or L-NAME. Indazole given either i.m. or i.p. showed no significant effects on MAP.

EFFECT OF NOS INHIBITORS AND REDUCED HAEMOGLOBIN ON BASAL AMYLASE OUTPUT

As previously described,¹ an i.v. L-NAME bolus of 30 mg/kg decreased basal amylase output (fig 2). Removal of circulating NO by infusion of reduced haemoglobin caused a decrease in amylase output of similar magnitude. In contrast, i.v. infusion of iso-oncotic amounts of albumin did not inhibit basal secretion. In fact, albumin produced a slight, but significant, increment in amylase output.

Systemic capsaicin treatment virtually abolished the eye wiping response to a local irritant, indicating effective defunctionalisation of sensory fibres. Inhibition of nNOS by indazole (fig 2) or capsaicin treatment (both systemically or locally applied) resulted in a reduction in basal amylase output. Capsaicin treatment reduced unstimulated amylase output relative to that of control rats (47.4 (8) v 85.8 (11) U/10 min; $p < 0.05$), which was not statistically different from amylase output measured after indazole treatment (42.7 (7) U/10 min).

Aminoguanidine, a non-neural non-endothelial inhibitor of NOS, had no detectable effect on basal amylase output ($n = 5$).

Figure 3 Amylase output in response to independent bolus doses of caerulein alone or caerulein plus indazole (100 mg/kg). Each bar represents percentage increment from baseline amylase output 40 minutes after caerulein infusion (means (SE)). Six or more separate experiments were conducted for each experimental condition. * $p < 0.05$ compared with caerulein alone.

EFFECT OF nNOS INHIBITION ON CAERULEIN STIMULATED PANCREATIC SECRETION

Indazole did not modify amylase output in response to suboptimal and optimal doses of caerulein (fig 3). However, indazole treatment partially reversed the inhibitory effect of a supramaximal (10 µg/kg) dose of caerulein. Increasing the dose of caerulein to 50 µg/kg reduced the secretory response in indazole treated rats, suggesting that nNOS blockade induces a desensitisation of the inhibitory pathway activated by high doses of caerulein. No differences were observed in the amylase response to supramaximal caerulein, whether indazole was administered 2 or 50 minutes before caerulein (data not shown).

Figure 4 Amylase output in response to an intravenous bolus dose of caerulein (10 µg/kg). Caerulein was infused alone or after L-NAME (30 mg/kg), haemoglobin (1 g/kg), or indazole (100 mg/kg) administration or after afferent nerve ablation by local application of capsaicin. Drugs were given 2 minutes before caerulein except for L-NAME* (25 minutes) or capsaicin (five days before the experiments). Each bar represents percentage increment from baseline amylase output 40 minutes after caerulein infusion (means (SE)). Six or more separate experiments were conducted for each experimental condition. * $p < 0.05$ compared with caerulein alone.

Sensorial nerve ablation by capsaicin reproduced the effects of indazole treatment and rendered rats more sensitive to doses of caerulein that would ordinarily suppress amylase secretion (fig 4). This type of response was readily observed in all rats treated with capsaicin, whichever way capsaicin was administered, that is either via systemic or local application.

As expected, co-infusion of L-NAME and supramaximal caerulein induced a further reduction in amylase output as compared with caerulein alone. However, when nNOS was fully inhibited by L-NAME (administered 25 minutes before supramaximal caerulein) a paradoxical response occurred, since L-NAME counteracted the suppression of amylase output elicited by supramaximal caerulein alone (fig 4), in sharp contrast with the profound inhibition of amylase output observed when L-NAME was co-administered with caerulein.

EFFECT OF L-NAME AND REDUCED HAEMOGLOBIN ON PANCREATIC RESPONSE TO SUPRAMAXIMAL CAERULEIN

The decrease in circulating NO caused by reduced haemoglobin or L-NAME, when administered 2 minutes before supramaximal caerulein, potentiated the inhibition of amylase secretion produced by these doses of caerulein (fig 4). Thus removal of circulating NO is clearly detrimental to normal amylase secretion by the intact pancreas in vivo.

Inhibition of the inducible form of NOS by aminoguanidine did not modify the pancreatic response to supramaximal caerulein (260 (43) U/40 min for caerulein alone *v* 192 (27) for caerulein plus aminoguanidine; $p > 0.05$; $n = 6$).

EFFECT OF nNOS INHIBITION ON CAERULEIN INDUCED ACUTE PANCREATITIS

Repeated i.p. caerulein (20 µg/kg each) increased serum amylase (32.2 (3.6) U/ml) and pancreatic water content (81.3 (0.9)% of pancreatic weight) as compared with unstimulated rats (4.3 (0.4) U/ml and 71.2 (1.3)% respectively). Indazole treatment failed to either ameliorate or aggravate caerulein induced increments in these two parameters of early pancreatic injury (28.2 (2.6) U/ml and 82 (1.1)%). However, indazole reduced the increase in MPO activity induced by caerulein (1300 (148) *v* 793 (115) mU/mg protein; $p < 0.05$), indicating a putative permissive role for neural NO in polymorphonuclear infiltration to the pancreas.

Discussion

The issue investigated in our studies is which NOS isoform blocked by the non-specific inhibitor L-NAME is responsible for the observed inhibition of amylase output. To address this problem, we followed a pharmacological approach: pancreatic secretory pattern was measured in response to NOS antagonists.

To inhibit nNOS activity, indazole, a specific inhibitor, was employed. Indazole and certain related derivatives have been reported to selectively inhibit nNOS in vivo.^{16 17} They show no effects on endothelial NOS and accordingly do not raise arterial blood pressure.^{19 20} Certainly,

indazole did not modify MAP in our experiments. However, impairment of nitroergic neurotransmission by nNOS inhibition reduced baseline amylase output. In the experimental design of the present work, biliopancreatic secretion is diverted from the duodenal lumen for 50 minutes before addition of drugs (stabilisation period plus two consecutive 10 minute periods to calculate "basal" secretion). Therefore basal secretion was most likely the result of endogenous cholecystokinin stimulation at picomolar (physiological) concentrations.³⁸ Our results on basal pancreatic secretion after indazole treatment are in close agreement with those obtained by Li *et al.*³⁸ in response to biliopancreatic juice diversion after truncal vagotomy or perivagal application of capsaicin.

Although L-NAME inhibits all NOS isoenzymes, its effect on nNOS appears to be delayed with respect to endothelial NOS, since it is fully expressed after 15 minutes of systemic administration,¹¹ whereas MAP (resulting from endothelial NOS inhibition) starts to increase in two to three minutes. This may explain why indazole had a greater impact on baseline amylase secretion, reflecting a regulatory function of nitroergic transmission in the maintenance of the neural pathways that control the normal physiology of baseline pancreatic secretion.

Indazole treatment showed no measurable effect on the secretory response to suboptimal and optimal doses of caerulein, but partially reversed the suppression of secretory activity elicited by supramaximal doses of caerulein, just as L-NAME when fully inhibiting nNOS. The pattern of response to indazole was reproduced by capsaicin. Basal secretion was reduced and supramaximal inhibition partially reversed. Both capsaicin application and nNOS inactivation result in an anti-nociceptive effect and prevention of neuropeptide release.^{11 20 22-24} This suggests that impairment of neural NO transmission shifts the caerulein dose-response curve to the right, rendering anaesthetised rats less sensitive to cholecystokinin; they do not respond to low cholecystokinin concentrations (as induced by biliopancreatic juice diversion) but are capable of secretory responses to supramaximal doses of caerulein that would otherwise suppress amylase output.

We observed a dual effect of L-NAME on pancreatic secretion: when it is evaluated immediately after L-NAME infusion, inhibition of basal, optimal, and supramaximal caerulein stimulation occurs, as previously described.¹ In contrast, when the pancreas is stimulated allowing a short lag period after L-NAME administration (25–35 minutes), the pattern of response parallels that observed after indazole or capsaicin treatment, two independent pharmacological approaches that impair sensorial nerve function. Subsequently, no further increases in arterial pressure are recorded (there is actually a slow decline in MAP) but L-NAME accomplishes full irreversible inhibition of nNOS.¹¹ This suggests that nNOS inhibition may suppress or totally reverse the effects of endothelial NOS blockade on pancreatic secretion.

To obtain further support for this hypothesis we acutely removed NO from the intravascular bed by infusion of haemoglobin, an NO scavenger. Reduced haemoglobin avidly binds and inactivates NO,^{27 39} but does not interfere with intracellular NO pathways or prevent neural NO transmission *in vivo*.²⁶

An inhibitory effect of haemoglobin on NO mediated neurotransmission *in vitro* has been reported.⁴⁰ However, our current understanding of the pharmacokinetics of native or artificially modified haemoglobins and their relation to NO binding argues against the conclusions drawn by some investigators on the effects of i.v. haemoglobin on NO neurotransmission *in vivo*.⁴¹ Owing to its high affinity for NO, intravenously infused haemoglobin rapidly reacts with circulating free NO (the reaction is essentially instantaneous) to form nitrate and met-haemoglobin, which is the basis for a well known NO assay.⁴²

Removal of free NO from the circulation induces a transient increase in MAP and an increase in peripheral vascular resistance.^{43 44} To scavenge NO close to any synaptic cleft *in vivo*, where NO might be released, haemoglobin should reach the extravascular space while still keeping its NO binding capacity. However, free circulating haemoglobin is likely to be rapidly processed into modified metabolites that may lose their NO affinity. In this regard, some haemoglobin derivatives even show vasodilator activity after having been nitrosylated.⁴⁴ It is conceivable that some of the reported effects of haemoglobin on neurotransmission *in vivo* are related to effects caused by the activities of its catabolic products.^{45 46}

Under basal conditions, circulating NO mostly reflects endothelial NOS activity. NO is a major physiological regulator of basal systemic blood vessel tone. Continuous release of NO by vascular endothelial cells controls MAP in the resting state.⁴⁷ Variations in MAP in response to NO inhibitors reflect, to a large extent, variations in the concentration of circulating free NO of endothelial origin. Indeed, haemoglobin infusion induced a sharp rise in MAP and a parallel reduction in amylase output. In contrast, iso-oncotic amounts of albumin resulted in a smaller increment in MAP while pancreatic secretion actually increased. The reason for this divergent effect may reside in the different interactions between both haemoglobin and albumin and free NO. Haemoglobin scavenges and inactivates NO^{27 39 44} whereas albumin acts as a carrier for NO making it available to the microvasculature.⁴⁸ Amylase output in response to supramaximal caerulein stimulation was further reduced when haemoglobin was given i.v., just as L-NAME does when it is co-administered with caerulein, and in sharp contrast with the pattern of secretion induced by indazole or capsaicin.

Interestingly, supramaximal caerulein induced an increase in MAP and potentiated the peak increase in MAP elicited by haemoglobin. Suboptimal doses of caerulein (which would induce plasma caerulein concentrations that

match postprandial plasma cholecystokinin) did not increase MAP but, on the contrary, induced a transient reduction in MAP.

The effects of caerulein on the microvasculature have been known for a long time, but the mechanisms underlying this vasoreactivity are poorly understood. In dogs, i.v. bolus of caerulein at doses that effectively stimulate pancreatic secretion induce a fall in blood pressure.⁴⁹ Likewise, the transient increase in MAP after 10 µg/kg caerulein is in agreement with reports of an increase in MAP after these doses of caerulein.⁵⁰

It has been shown that NOS inhibition by L-NAME aggravates caerulein induced pancreatitis.^{1, 29} Since indazole counteracted the suppression of amylase secretion in response to doses of caerulein that induce the development of pancreatitis, we examined whether or not indazole treatment could prevent pancreatic injury.

Moreover, NO has been involved in the pathogenesis of oedema formation in animal models of neurogenic inflammation.²⁰ If neural NO contributes to oedema formation in caerulein induced pancreatitis, liquid accumulation in the pancreas would be expected to be prevented, at least in part, by indazole treatment.

In contrast with L-NAME effects when it is co-infused with caerulein,¹ impairment of nitrgic transmission by indazole did not increase hyperamylasaemia in caerulein induced pancreatitis. Also, indazole had no measurable effect on liquid accumulation in the pancreas, suggesting that nitrgic transmission is not significantly involved in oedema formation in this model of inflammation. The fact that indazole reduced MPO activity is once again in opposition to the previously described effects of L-NAME in this situation, since L-NAME has been shown to increase MPO activity.¹

NOS is widely distributed in the body and may well be present in several cell types in a given organ, as in the case of the pancreas. General blockade of NOS by universal inhibitors such as L-NAME may give rise to contrasting effects in organ function, depending on the predominant pathway or cell function that is disrupted and on alternative activation of compensatory mechanisms. We should reconsider conclusions drawn from in vivo experiments, especially those in which L-NAME was given in repeated doses or for long periods of time. The use of inhibitors that selectively target different forms of NOS (best if cell selective) should be recommended, since they would produce more meaningful results.

We conclude that nitrgic nerve fibres play an important role in the control of pancreatic secretion, independently of other pancreatic NO sources. Pancreatic effects of nNOS inhibition differ from the effects resulting from endothelial NOS blockade, which we believe is the NO related event that produces inhibition of basal and stimulated secretion in vivo and the aggravation of acute pancreatitis.

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