ROLE OF ENDOGENOUS NITRIC OXIDE IN THE CONTROL OF EXOCRINE AND ENDOCRINE PANCREATIC SECRETION

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L-Arginine (L-Arg), that is a substrate for nitric oxide (NO) synthase, stimulates the release of pancreatic islet hormones but the mechanism of this stimulation is unknown. The aim of this study was to determine the role of NO in the control of endocrine and exocrine pancreatic secretion in response to sham feeding (SF), ordinary meat feeding (F), duodenal perfusion with nutrients and i.v. infusion of gastrin releasing peptide (GRP) or urecholine in conscious dogs with chronic pancreatic fistulas. SF, F, duodenal nutrient and GRP and urecholine resulted in the stimulation of pancreatic secretion reaching, respectively, 50%, 50%, 40%, 85% and 20% of maximal response to caerulein (200 pmol/kg−h i.v.). Infusion of L-Arg (50 mg/kg+5 mg/kg−h i.v.) almost doubled the basal pancreatic protein secretion and significantly increased the secretory response to SF, F, and duodenal nutrient. After i.v. administration of L-NNA (2.5 mg/kg+0.5 mg/kg−h), an inhibitor of NO synthase, the pancreatic secretory responses to SF, F, duodenal nutrient, GRP and urecholine were significantly inhibited by about 74%, 70%, 70%, 80% and 30%, respectively. When L-Arg was combined with L-NNA, the reduction in pancreatic secretion induced by L-NNA was significantly attenuated. SF resulted in a marked rise in plasma insulin and glucagon and this response was completely abolished by L-NNA infusion. Insulin and glucagon levels were 2—3 folds increased by F and L-NNA infusion inhibited these responses while the addition of L-Arg partly reversed this inhibition. Duodenal nutrient produced several fold increase in plasma insulin and glucagon levels that were significantly reduced by L-NNA and this reduction was partially reversed by L-Arg. GRP also caused moderate rise in plasma insulin and glucagon levels which were significantly reduced by L-NNA and this was partially restored by L-Arg. We conclude that SF, F, duodenal nutrient, GRP or urecholine stimulate both the exocrine and endocrine pancreatic secretion and that these effects are mediated, at least in part, through the NO pathway.

Key words: Nitric oxide, pancreas, insulin, glucagon, gastrin

INTRODUCTION

Exocrine pancreatic secretion at low rate occurs under basal conditions in the absence of food but is greatly enhanced in response to a meal. The postprandial pancreatic secretion is controlled by interaction of neurohormonal factors on secretory cells. Until recently, this secretion was
attributed to the gut hormones such as CCK and secretin and to vagovagal reflexes activating cholinergic postganglionic neurons in the pancreas (1). Now it is clear that pancreatic secretory mechanisms also involve numerous neurons secreting various neurotransmitters and pancreatic islets hormones acting locally through paracrine pathway (1).

Recent studies have suggested that nitric oxide (NO) may be an important neurotransmitter in the pancreas. It has been shown that some neurons in the pancreatic ganglia receive nitroxidergic input and the others provide nitroxidergic innervation for acinar cells and islets (2—5). NO is the product of the oxidation of one of the guanidine nitrogen of L-arginine to L-citrulline by NO synthase (NOS). At least two different isoforms of nitric oxide synthase (constitutive and inducible) have been characterized. These isoforms are differentiated by gene expression and cofactor requirements. The activity of constitutive isoform of NOS depends on Ca$^{2+}$ and calmodulin, while the expression of inducible NOS results from the action of cytokines, and is independent of Ca$^{2+}$ and calmodulin. Constitutive isoform produces small amounts of NO which may function as a signaling molecule while inducible NOS generates large amounts of NO that may be cytotoxic to the target pancreatic secretory cells (6, 7).

The primary receptor for NO in effector cells appears to be soluble guanylate cyclase that stimulates the synthesis of cyclic GMP (cGMP). Increased levels of cGMP may activate cGMP-dependent protein kinase or act directly on ion channels (6). A better understanding of the physiological action of NO derives from the studies on the effects of L-arginine analogs such as, N$^G$-monomethyl-L-arginine (L-NMMA) or N$^G$-nitro-L-arginine (L-NNA) that suppress the NO synthase and prevent NO release (8, 9). We have previously shown that L-NNA inhibits exocrine pancreatic secretory response to various stimulants (10) and this finding was recently confirmed by others (11). This inhibition could be reversed in part by the application of L-arginine, the substrate for NOS (10, 11).

This study was designed to determine the possible role of NO in the regulation of vagal-cholinergic, postprandial and hormone-induced exocrine and endocrine pancreatic secretion in conscious dogs.

MATERIAL AND METHODS

Pancreatic secretory studies were carried out on ten mongrel dogs, weighing 18—22 kg and prepared surgically with gastric fistulas (GF), pancreatic fistulas (PF) and esophageal fistulas as previously described (12—14). The studies reported here started about 6 mo after surgery and were carried out for about one year. Before each experiment, the food but not water was withheld for at least 18 h before each test. Throughout the tests, except feeding and duodenal instillation of liquid mixed meal (Renutryl, Roussel Uclaf Nutrition Laboratoires, Sopharga, France) the GF was left
open to allow the draining of gastric juice to the exterior to prevent gastric acid from entering the duodenum and releasing endogenous hormones affecting pancreatic secretion.

Secretion from PF was collected continuously in 15 min aliquots. The volume was recorded to the nearest 0.1 ml and pancreatic protein concentrations and outputs were measured in each collected sample of the pancreatic juice and expressed in 15 or 30 min outputs. Several tests were performed on each animal and the experiments on the same animal were carried out not more often than once per week.

In tests with stimulated secretion (SF, ordinary feeding, duodenal nutrient or i.v. infusion of GRP or urecholine), the pancreatic secretion was first collected under basal conditions and then after the secretory stimulation. Intravenous saline infusion (40 ml/h) was maintained throughout the study period. In control experiments, the animals received the secretory stimulant alone. L-NNA (2.5 mg/kg bolus i.v. injection followed by infusion of 0.5 mg/kg -h), L-arginine (50 mg/kg bolus i.v. injection followed by infusion of 5 mg/kg -h) or L-NNA plus L-arginine in doses as above was added to i.v. infusion before and then during 15 min of SF and for 90 min after SF. During the SF, each dog was offered 500 g of cooked homogenized ground beef for 15 min, the ingested food fell from the esophageal fistula back into the feeding pan and was repeatedly reconsumed. Gastric and pancreatic juice was collected before, during and after SF.

In tests with ordinary feeding, the GF was closed and each dog was offered 500 g of cooked homogenized ground beef that was usually completely consumed. When the secretory response to a meal reached a well-sustained plateau, L-NNA 2.5 mg/kg + 0.5 mg/kg -h, L-arginine 50 mg/kg + 5 mg/kg -h or their combination was added to i.v. infusion in a constant dose for 60 min period. In experiments with duodenal nutrient, the GF was closed and Renutryl solution was instilled intraduodenally at rate of 160 ml/h through the hollow obturator of the cannula of PF. When the secretory rate plateaued, L-NNA 2.5 mg/kg + 0.5 mg/kg -h, L-arginine 50 mg/kg + 5 mg/kg -h or their combination was added to i.v. infusion in a constant dose for the remaining period of experiment. For the comparison, the maximal pancreatic protein secretion was elucidated in each dog by i.v. infusion of caerulein at a dose (200 pmol/kg -h) that was shown in our laboratory to induce such a secretion. In addition, in experiments with feeding the effects of different doses of L-NNA were tested.

In all tests, blood samples were taken from the peripheral vein for the determination of plasma insulin and glucagon, using radioimmunoassay as described previously (14). Peripheral blood pressure was measured in tests with L-NNA using modified sphygmomanometer applied on the front leg.

Results are expressed as means ± SEM. The significance of the difference between means was evaluated using a two-way analysis for repeated measures (two-way ANOVA) followed by Duncan's test with a level of confidence at P<0.05.

RESULTS

Effects of L-NNA, L-arginine or their combination on basal and stimulated exocrine pancreatic secretion.

Basal pancreatic protein secretion showed periodic fluctuations on the average of about 54±7 mg/15 min. L-NNA infused i.v. (2.5 mg/kg + 0.5 mg/kg -h i.v.) did not affect basal pancreatic protein secretion. The addition of L-arginine (50 mg/kg + 5 mg/kg -h i.v.) to i.v. infusion resulted in a significant rise in pancreatic protein output. When L-NNA added at a dose of 2.5 mg/kg + 0.5 mg/kg -h i.v. was combined with infusion of L-arginine
(50 mg/kg + 5 mg/kg–h) a significant reduction in protein secretion (as compared to that obtained with L-arginine alone) in protein secretion was observed (Table 1).

Table 1. Effects of L-NNA, L-arginine (L-Arg) or their combination on basal pancreatic protein secretion. Mean ± SEM of 8 tests on 8 dogs. Asterisk indicates significant change as compared to control value obtained with saline (vehicle) i.v. infusion. Cross indicates significant increase above the value obtained with L-NNA.

<table>
<thead>
<tr>
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<th>PROTEIN mg/15 min</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>L-NNA</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>L-Arg</td>
<td>110 ± 18*</td>
</tr>
<tr>
<td>L-NNA + L-Arg</td>
<td>72 ± 8*</td>
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</table>

SF carried out for 15 min caused an immediate rise in the protein output reaching peak of about 50% of caerulein maximum (Fig. 1). L-NNA infused i.v. in a dose (2.5 mg/kg + 0.5 mg/kg–h i.v.) for 15 min before, during and after the

Fig. 1. The increments (above basal) in pancreatic protein outputs (mean of two consecutive 15 min peak outputs) in response to sham-feeding without and with pretreatment with L-NNA, L-NNA + L-arginine (L-Arg) or L-arginine alone. For comparison, maximal pancreatic response to i.v. infusion of caerulein (200 pmol/kg–h) is presented. Means ± SEM of 6 tests on 6 dogs. Asterisk indicates significant decrease below the control value obtained with sham-feeding alone.

SF, resulted in a marked decrease in the protein response to SF. The peak protein secretion was reduced by L-NNA by about 74%. L-arginine (50 mg/kg + 5 mg/kg–h) added to i.v. infusion before the SF resulted in a significant increase in basal pancreatic protein output but failed to influence
the peak protein response to SF. When L-arginine (50 mg/kg + 5 mg/kg – h) was infused in combination with L-NNA, the increase in protein output during and after SF tended to reach higher value than that observed in tests with infusion of L-NNA alone but it was significantly lower than that in control tests with SF alone.

![Bar chart showing protein increment (mg/5 min) for different treatments: CONTROL, L-NNA, L-Arg, L-NNA + L-arginine (L-Arg), L-arginine (L-Arg)].

*Fig. 2. The increments (over basal) of pancreatic protein secretion (mean of two highest consecutive 15 min outputs) in response to meal feeding in tests without (control) and with i.v. administration of L-NNA, L-NNA + L-arginine (L-Arg) or L-arginine. Means ± SEM of 6 tests on 6 dogs. Asterisk indicates significant decrease below the control value obtained with meat feeding alone. Cross indicates significant increase above the value obtained with meat feeding + L-NNA.*

After meat feeding, the pancreatic protein secretion increased markedly reaching about 50% of caerulein maximum (*Fig. 2*). Intravenous infusion of L-NNA caused a prompt and marked reduction (by about 70%) in the postprandial protein output. When combined with L-arginine, L-NNA resulted in a significantly smaller decrease in this output falling only by about 20% below the control value. The effects of different doses of L-NNA on the postprandial pancreatic protein secretion is shown on *Fig. 3* and they demonstrate a dose dependent reduction by L-NNA in protein secretion induced be feeding. The threshold reduction in protein secretion was observed at a dose of 0.3 mg/kg L-NNA, about 40% reduction was achieved at a dose of 0.6 mg/kg and maximal (about 80%) reduction was reached at a dose of 5.0 mg/kg of L-NNA. Infusion of L-arginine alone did not influence significantly the protein response to feeding (*Fig. 3*).

Duodenal infusion of nutrient (Renutryl) resulted in an increase in pancreatic protein secretion that was somewhat smaller than that obtained with ordinary feeding (*Fig. 4*). Pretreatment with L-arginine did not affect significantly the secretory response to duodenal nutrient. After the
administration of L-NNA, the pancreatic secretory response was inhibited by 70% but when L-arginine was combined with L-NNA, the reduction in pancreatic secretion was significantly attenuated and protein output was similar to that achieved with duodenal nutrient alone.

Pancreatic response to GRP (0.5 µg/kg−h i.v.) was characterized by a well-sustained and a marked increase in protein secretion reaching about 85% of caerulein maximum (Fig. 5). Infusion of L-NNA during the GRP-stimulated protein secretion caused about 80% reduction in pancreatic
response to GRP. The addition of L-arginine to i.v. infusion of L-NNA resulted in a significantly higher pancreatic protein secretion than that observed after infusion of L-NNA alone but smaller than that achieved with GRP alone. L-arginine alone did not affect significantly pancreatic response to GRP.

Urecholine (50 μg/kg−h) caused a small but significant increase in pancreatic protein secretion reaching about 20% of caerulein maximum. Infusion of L-NNA decreased significantly the urecholine-stimulated secretion and this decrease was partially reversed by addition of L-arginine to L-NNA. L-arginine (50 mg/kg + 5 mg/kg−h) alone did not change significantly the pancreatic secretory response to urecholine (Fig. 6).
Effects of L-NNA, L-arginine or their combination on plasma concentrations of pancreatic hormones

Basal plasma insulin and glucagon concentrations were 3.4 ± 10.8 μU/ml and 123.0 ± 11.3 pg/ml, respectively. Infusion of L-arginine in a dose 50 mg/kg 5 mg/kg−h i.v. raised significantly plasma hormone levels over that observed in vehicle (saline) treated animals, whereas L-NNA infused at a dose 2.5 mg/kg + 0.5 mg/kg−h i.v. caused a significant decrease of insulin and glucagon levels. When L-arginine was added to L-NNA, plasma levels of both insulin and glucagon tended to increase but this increment was not statistically significant (Table 3 and 4).

SF resulted in a marked and significant elevation in plasma levels of both insulin and glucagon. L-NNA infusion almost completely abolished the increments of plasma insulin and glucagon in response to SF. Addition of L-arginine failed to influence the reduction in plasma insulin caused by L-NNA and L-arginine given alone did not affect significantly the increments in plasma insulin and glucagon levels recorded after SF (Table 3 and 4).

Ordinary feeding resulted in a marked rise of both glucagon and insulin levels, which were significantly reduced by L-NNA (Table 3 and 4). This reduction was partially reversed by the addition of L-arginine to L-NNA infusion. After infusion of L-arginine alone, the peaks glucagon and insulin in response to meat feeding were significantly higher than those observed after feeding alone. Effects of increasing doses of L-NNA on postprandial insulin and glucagon levels are shown on Fig. 7. With increasing doses of L-NNA, there was a gradual increase in the magnitude of the reduction in the postprandial rise in the plasma insulin and glucagon levels.

Fig. 7. The percent reduction in postprandial plasma insulin and glucagon level after i.v. infusion of various doses of L-NNA. Means ± SEM of 6 tests on 6 dogs.
Duodenal nutrient infusion produced a remarkable, about 2—3 folds increments of both insulin and glucagon levels (Table 3 and 4). Infusion of L-NNA significantly decreased, while infusion of L-arginine enhanced the increments in plasma insulin levels in tests without or with administration of L-NNA. Infusion of L-NNA had no significant effect on plasma levels of glucagon induced by duodenal nutrient in tests without or with L-arginine but L-arginine alone resulted in a small but significant rise in plasma levels of glucagon and in a marked rise in plasma insulin as compared to these obtained with duodenal nutrient alone.

For comparison, glucose infused i.v. in a 10% solution at a rate of 80 ml/h caused an immediate and marked rise in plasma insulin level but resulted in a decrease in plasma glucagon levels throughout the glucose infusion (Figs 8 and 9). Infusion of L-NNA decreased significantly plasma insulin level

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**Fig. 8.** Plasma insulin concentrations under basal conditions and following i.v. infusion of a constant dose of 10% glucose in tests without (control) and with i.v. administration of L-NNA, L-NNA + L-arginine (L-Arg) or L-arginine alone. Asterisk indicates significant decrease below the control value obtained with glucose alone. Cross indicates significant increase above the value obtained with L-NNA.

**Fig. 9.** Plasma glucagon level in tests as on Fig. 8.
and this response was partly reversed by the addition of L-arginine to infusion of L-NNA. L-arginine infusion tended to increase plasma insulin level above control values but this did not reach statistical significance. L-NNA alone did not affect significantly plasma glucagon levels in test with 10% glucose infusion without or with administration of L-arginine. Pancreatic protein secretion showed a small increase above basal rate in these tests with 10% glucose infusion and these results have not been included. After duodenal nutrient and intravenous glucose infusion, a significant rise in blood glucose level was observed. Infusion of L-NNA resulted in a small but significant fall in glucose concentration that was partially reversed when L-arginine was added to i.v. infusion (Table 2).

Table 2. Effect of L-NNA, L-arginine or their combination on plasma levels of glucose (mg%) in tests with 10% i.v. glucose infusion or duodenal nutrient (Renutryl) instillation. Means ± SEM of 8 tests on 8 dogs. Asterisk indicates significant changes compared to the control value with i.v. glucose or duodenal nutrient alone. Cross indicates significant increase above the value obtained with L-NNA.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>L-NNA</th>
<th>L-NNA + Arg</th>
<th>L-Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v. Glucose</td>
<td>121.8 ± 10.6</td>
<td>102.4 ± 9.5 *</td>
<td>116.3 ± 11.4 *</td>
<td>122.5 ± 10.1</td>
</tr>
<tr>
<td>Duodenal nutrient</td>
<td>163.3 ± 10.5</td>
<td>144.3 ± 11.0</td>
<td>155.9 ± 10.8 *</td>
<td>172.9 ± 16.9</td>
</tr>
</tbody>
</table>

Table 3. Effects of L-NNA, L-arginine or their combination on the insulin plasma levels (μU/ml). Asterisk indicates significant decrease below the control value. Cross indicates significant increase above the value obtained with L-NNA and double cross indicates significant increase above the control value.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Control</th>
<th>L-NNA</th>
<th>L-NNA + L-Arg</th>
<th>L-Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>3.7 ± 0.3</td>
<td>1.5 ± 0.2 *</td>
<td>2.5 ± 0.3</td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td>Sham-feeding</td>
<td>14.1 ± 1.7</td>
<td>4.1 ± 0.8 *</td>
<td>1.2 ± 0.3 *</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>Feeding</td>
<td>14.8 ± 1.7</td>
<td>6.1 ± 0.7 *</td>
<td>7.9 ± 8 +</td>
<td>15.7 ± 1.7 + +</td>
</tr>
<tr>
<td>Duodenal nutrient</td>
<td>30.7 ± 4.2</td>
<td>11.4 ± 1.6 *</td>
<td>23.7 ± 3.1 +</td>
<td>35.3 ± 4.3 + +</td>
</tr>
<tr>
<td>GRP</td>
<td>11.9 ± 1.2</td>
<td>4.4 ± 0.5 *</td>
<td>6.4 ± 0.7 +</td>
<td>15.3 ± 1.5 + +</td>
</tr>
<tr>
<td>Urecholine</td>
<td>24.4 ± 2.8</td>
<td>4.0 ± 0.6 *</td>
<td>22.3 ± 2.9</td>
<td>25.7 ± 2.8</td>
</tr>
</tbody>
</table>

GRP given in a dose 0.5 μg/kg – h i.v. resulted in a significant rise in plasma levels of both pancreatic hormones similar to that observed after feeding (Table 3 and 4). This rise was reduced by infusion of L-NNA given during the GRP stimulation and this reduction was more pronounced regarding insulin than glucagon level. The addition of L-arginine to L-NNA infusion resulted in significantly higher pancreatic hormone release than that observed after infusion of L-NNA alone. L-arginine alone without L-NNA caused
a significant and strong increase in plasma levels of both insulin and glucagon as compared to GRP alone. During urecholine infusion there was a marked rise in plasma insulin and moderate increase in plasma glucagon levels were observed (Table 3 and 4). L-NNA strongly decreased insulin but not glucagon plasma level. This reduction was almost completely reversed by addition of L-arginine. L-arginine alone added to urecholine infusion did not affect significantly the increase in plasma glucagon and insulin levels.

Table 4. Effects of L-NNA, L-arginine of their combination on the glucagon plasma levels (pM/L). Asterisk indicates significant decrease below the control value. Cross indicates significant increase above the value obtained with L-NNA and double cross indicates significant increase above the control value.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Control</th>
<th>L-NNA</th>
<th>L-NNA + L-Arg</th>
<th>L-Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>121.6 ± 13.1</td>
<td>65.0 ± 9.2*</td>
<td>120.3 ± 12.5</td>
<td>142.5 ± 14.9</td>
</tr>
<tr>
<td>Sham-feeding</td>
<td>181.6 ± 18.5</td>
<td>126.7 ± 14.1*</td>
<td>122.8 ± 13.8*</td>
<td>186.6 ± 18.7</td>
</tr>
<tr>
<td>Feeding</td>
<td>316.5 ± 33.7</td>
<td>145.5 ± 15.6*</td>
<td>291.5 ± 30.7*</td>
<td>386.6 ± 39.1**</td>
</tr>
<tr>
<td>Duodenal nutrient</td>
<td>303.1 ± 31.2</td>
<td>312.3 ± 34.3</td>
<td>316.7 ± 38.9</td>
<td>342.8 ± 31.7**</td>
</tr>
<tr>
<td>GRP</td>
<td>296.4 ± 28.8</td>
<td>230.1 ± 23.5*</td>
<td>247.3 ± 26.8*</td>
<td>426.4 ± 44.8**</td>
</tr>
<tr>
<td>Urecholine</td>
<td>256.6 ± 26.8</td>
<td>254.6 ± 28.1</td>
<td>254.4 ± 22.7</td>
<td>266.4 ± 25.6</td>
</tr>
</tbody>
</table>

Resting mean arterial pressure in our dogs averaged 105 ± 4 mm Hg and this was not significantly altered when secretory tests with endogenous or exogenous secretagogues or i.v. infusion glucose were used. Following the administration of L-NNA, the mean pressure showed a small but significant increase reaching about 125 ± 7 mm Hg. When L-arginine was added to infusion of L-NNA, the rise in the pressure was significantly smaller than that recorded with L-NNA alone but higher than that in control resting tests. In tests with infusion of L-arginine alone, the mean arterial pressure was not different from that recorded under control resting conditions.

DISCUSSION

This study demonstrates for the first time that the inhibition of the NO synthase by L-NNA in conscious animals strongly suppresses the pancreatic exocrine and endocrine responses to a variety of secretory stimulants suggesting that the mechanisms controlling the functions of pancreatic acini and pancreatic islets are highly integrated and that endogenous NO is involved in the regulation of both these functions. In the present study we have demonstrated that L-NNA not only inhibits pancreatic secretion induced by natural stimulants such as sham-feeding or ordinary meat feeding as it was
reported previously (10) but also suppresses the secretion induced by intraduodenally administrated nutrient, i.v. infusion of GRP or glucose. L-arginine added to L-NNA partially reversed the inhibition obtained with L-NNA. Our results remain in good agreement with recent study on isolated porcine pancreas (11) which demonstrated that L-NNA or L-NAME reduced the secretory response to electrical stimulation of vagal nerves or VIP infusion.

Pancreatic acinar cells synthesize and secrete a variety of digestive enzymes as well as a NaCl-rich pancreatic juice. The secretory mechanisms of the exocrine pancreas is thought to be activated by gut hormones, especially by CCK and secretin and by various neurotransmitters such as ACh, GRP and VIP released by intrapancreatic neurons (1). In addition, several regulatory peptides appear to modulate secretion and this includes insulin, glucagon, pancreatic polypeptide, somatostatin, all produced within the pancreas (1).

Actions of the secretagogues such as CCK, Ach, GRP involves binding to their receptors on acinar cells, activation of phospholipase C, generation of diacylglycerol and inositol triphosphate, mobilization of intracellular stored calcium, activation of protein kinase C and stimulation of enzyme secretion (15). Recent studies indicate that 1,4,5-IP$_3$ is responsible of releasing a portion of intracellular Ca$^{2+}$ in response to the stimulation of acinar cell by ACh or CCK. The activation of Ca$^{2+}$ influx is required for regulating free cytosolic Ca$^{2+}$ concentration, for refilling the internal calcium stores and for the secretory response of pancreatic acinar cells (16). The mechanism that underlies Ca$^{2+}$ influx is unknown (15) but it has been lately shown that cGMP can mediate receptor-activated Ca$^{2+}$ influx in pancreatic acinar cells (17).

Blocking the NO production by inhibitors of NOS abolished carbachol induced rise in cGMP and CaI$^{2+}$ influx in pancreatic acinar cells in a dose dependent manner. Carbachol increased both the nitrite levels and the production of NO by converting $^3$H-arginine to $^3$H-citruline (18). In study by Xu et al. (19) it has been shown that the depletion of intracellular Ca$^{2+}$ stores activated NOS to generate cyclic GMP and to regulate calcium influx in rat pancreatic cells. This study demonstrates also that the carbachol activated Ca$^2+$ entry and the increase in cellular cGMP was blocked by inhibitors of NOS or guanylate cyclase, and this inhibition was reversed by sodium nitroprusside and Bt2cGMP.

In the intact pancreas, in vivo, both endocrine and exocrine functions of the pancreas are highly interdependent and integrated in the process of assimilation of nutrients from the gut. Insulin secretion from pancreatic B cells is regulated by interactions between a variety of nutrients, hormones and neurotransmitters released by nerve terminals around the islets (20).

L-arginine mediates, in part, protein-induced insulin secretion and augments glucose-induced release of this hormone though the latter effect was
not found in our experiments (see Fig. 8). The mechanism of the stimulatory action of L-arginine on insulin release observed also in our study under basal conditions and after feeding, duodenal nutrient or i.v. GRP infusion, is not clearly understood but recently it has been postulated that insulinotropic effect may be explained by the induction of NOS (21). NOS has been shown in the pancreas not only in ganglia or nerve terminals innervating acinar tissue and islets but also in A and B cells (3,21,22). Islets cells were found to contain both the constitutive and inducible isoforms of NOS (22). Using NADPH-diaphorase as a probe for the presence of NOS, it was found that about 70% of islet cells stained for NOS activity (22). It has been demonstrated that exogenous cGMP triggers insulin release and that glucose or L-arginine increases cGMP in isolated pancreatic islets (23). Insulin release induced by glucose and L-arginine was decreased by the NOS inhibitors (21,24). However, it should be pointed out that in several in vitro studies NOS inhibitors stimulated rather than reduced insulin release (22,25-28) but in most of these studies interleukin-1 (IL-1) was used to activate insulin secretion. IL-1 appears to be linked to the release of NO (26,29). It was assumed that IL-1 induced massive NO secretion from intra-islet macrophages due to activity of inducible NOS and this NO then caused the islet cell destruction (29,30). It is possible that low, physiological levels of NO released by constitutive isoform of NOS acts as signaling molecule in endocrine B cells during L-arginine or glucose stimulated insulin secretion. These physiological responses are believed to be mediated by the direct activation of guanylate cyclase by NO with the formation of cGMP (21,23). In contrast, NO produced in much larger quantities by inducible isoform of NOS appears to function as an effector molecule that mediates cytostatic and cytotoxic effects and this seems to be independent of cGMP in islet cells (22,26).

So far, there is no report on the role of NO in the release of glucagon and insulin \textit{in vivo}. In our study on conscious dogs we observed that sham-feeding, ordinary feeding and duodenal nutrient significantly increased plasma insulin and glucagon levels. Infusion of L-NNA alone completely blocked the increments in plasma insulin and glucagon induced by sham-feeding and feeding and significantly attenuated the increments in plasma insulin but not glucagon increase after instillation of duodenal nutrient. These inhibitory effects of L-NNA probably resulted from the interference of the cholinergic innervation of the B-cells because direct cholinergic stimulation (by urecholine) of these cells was also blocked by L-NNA. These effects of L-NNA were partially reversed by the addition of L-arginine to L-NNA, while L-arginine by itself enhanced the plasma levels of pancreatic hormones. Intravenous glucose infusion markedly increased plasma insulin level and this response was also significantly reduced by L-NNA alone but less when L-arginine was added to L-NNA infusion. After intraduodenal nutrient and intravenous glucose

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infusion we observed a rise in plasma glucose level that probably directly activated the B-cells of pancreatic islets to release insulin.

Interestingly, the inhibitory effect of L-NNA on both endocrine and exocrine secretion was most pronounced after the vagal-cholinergic stimulation of the pancreas by sham-feeding. Because the secretory effects of this stimulation are mediated mainly via vagal nerves, these results may indicate that the mechanism involving NO in pancreatic secretions is predominantly related to vagal-cholinergic pathway. Since the pancreatic secretory responses to ordinary feeding or duodenal perfusion with mixed liquid meal may be also mediated by duodeno-pancreatic reflexes involving vagovagal reflexes, the potent inhibitory action of L-NNA on these responses could also be attributed to the reduction in vagally-mediated release of NO in the pancreas. Our data demonstrate that blockade of NO synthase by L-NNA decreased exocrine and endocrine pancreatic secretion in conscious dogs. This action appears to be associated with the inhibition of NO biosynthesis and it may depend on the vagal innervation but the possibility exists that NO may also have a direct influence on pancreatic secretory cells but this notion is unlikely because in our previous in vitro studies, the isolated pancreatic acini failed to respond either to exogenous NO released by NO donors or to the inhibitory action of L-NNA added to the incubation medium (10). On the other hand, the indiscriminate decrease of pancreatic exocrine secretion by L-NNA might be attributed to the common mechanism of action of this NO synthase blocker such as through the decrease of the pancreatic blood flow. Our previous ex vivo studies on anesthetized dogs (10) showed that NO exerts a tonic vasorelaxing effect on the pancreatic vasculature under resting conditions and following secretory stimulation, however, recent studies on isolated porcine pancreas it has been shown that pancreatic secretory response to vagal stimulation seems to be independent of its vascular effect (11). In the present study this NO synthase inhibitor elevated arterial blood pressure, and this effect was partially reversed by concurrent administration of L-arginine. It remains to be established whether the redistribution of the blood flow within the pancreas, that probably accompanies the rise in systemic arterial blood pressure, is responsible, at least in part, for the observed inhibition of exocrine and endocrine pancreatic secretion caused by the analogs of L-arginine suppressing the NO biosynthesis.

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