Papel del óxido nítrico en la función exocrina del páncreas

Role of nitric oxide on pancreatic exocrine function

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RESUMEN

El óxido nítrico (NO) es un gas sintetizado enzimáticamente, con una vida media corta. El NO tiene importantes funciones en los sistemas cardiovascular (actuando como vasodilatador) y nervioso (como neurotransmisor). Sin embargo, el NO no puede ser considerado un mensajero intercelular típico, ya que tiene una gran capacidad de difusión y no se une a receptores celulares. En este trabajo, revisaremos las acciones del NO en el páncreas exocrino. Los estudios mas clásicos demostraban que el NO estimula el flujo sanguíneo pancreático y por tanto, la secreción exocrina del páncreas; además, podría tener algún efecto estimulante sobre las células acinares y ductulares. Más recientemente se ha descrito que las células acinares pancreáticas pueden producir NO tras estimulación con secretagogos. El NO tiene un efecto modulador sobre la estimulación de la secreción enzimática con secretagogos e influye sobre la fosforilación en tirosina de algunas proteínas del citoesqueleto acinar. Finalmente el NO parece estar implicado en el daño tisular derivado de la pancreatitis.

PALABRAS CLAVE: Oxido nítrico, páncreas exocrino

SUMMARY

Nitric oxide (NO) is a free gas enzymatically synthetized and with a short half life. NO has important functions in the cardiovascular (vasodilator) and nervous (neurotransmitter) systems. However, NO is not a typical intercellular messenger since it is highly diffusible and does not bind to cellular receptors. In this paper, we will review the actions of NO on the exocrine pancreas. The most classical studies demonstrated that NO stimulates pancreatic blood flow and thereby exocrine pancreatic secretion, but, additionally it might have some stimulatory effects on pancreatic acinar and ductal cells. It has been recently reported that acinar pancreatic cells can produce NO after secretagogue stimulation. NO has a modulatory effect on secretagogue stimulation of pancreatic enzyme secretion and it influences tyrosine phosphorylation of some acinar cytoskeletal proteins. Finally, NO seems to be involved in tissue damage derived from pancreatitis.

KEY WORDS: Nitric oxide, exocrine pancreas

INTRODUCTION

Nitric oxide (NO) is a free gas enzymatically synthetized, subjected to strong regulation and with a short half life. The first reference about the production of NO_3^- in mammals was reported by Mitchell et al (1) in 1916. This observation was ignored for a long time until the study of Green et al (2, 3), describing the endogenous production of NO_3^- after infection and during carcinogenesis in humans, and after endotoxin treatment in rodents; finally, Stuerg and Marletta (4) reported the release of NO_3^- from activated macrophages.

Hibbs et al, in 1987, (5) reported that L-arginine was the substrate for NO_2^- and NO_3^- synthesis in mouse activated macrophages. In this same year, Palmer et al (6) identified the endothelium derived

relaxing factor (EDRF) with nitric oxide; later on, Palmer et al (7) also reported that L-arginine was the substrate for the NO synthesis, NO being the biological intermediary between L-arginine and NO_2^- and NO_3^-. Identical conclusions were reached in mouse activated macrophages (8).

The enzymes which are responsible for NO synthesis from L-argininine are called NO synthases (NOS) and were described in 1989 (9), purified in 1990 (10) and cloned in 1991 (11). There are three NOS, coded by three different genes. Two of them are constitutives (nNOS, originally identified in neuronal cells and eNOS, first described in vascular endothelial cells). The third type of enzyme depends on cytokine induction; it is the inducible NOS (iNOS). The synthesis of NO from L-arginine needs NADPH and produces citrulline, according to the following chemical reaction:

L-arginine + NADPH + O_2 ----> L-citrulline + $NADP^+$ + H_2O

NOS requires nicotinamide adenine dinucleotide phosphate (NADPH) and other cofactors. The oxidation of NADPH to $NADP^+$ can be detected by a histochemical technique, the NADPH-diaphorase reaction. Therefore, in mammals, this histochemical method has been used as a marker for NOS-containing neurons.

NO synthases are highly regulated enzymes. They are phosphorylated in serine residues by a protein kinase C, or by protein kinase II, dependent on Ca^{2+}/calmodulin (12). Stimulation of phosphoinositol metabolism in cells containing cNOS has two opossite effects on its activity. On the one hand, the increase in cytosolic calcium enhances the activity of the enzyme, whereas on the other hand, the increase in diacylglicerol stimulates protein kinase C, which inhibits the synthesis of NO. Moreover, it has been described that several cytokines (IL-4, TNF-â and IL-10) inhibit the iNOS, whose activity is calcium-independent. Therefore, hormones and secretagogues can activate or inhibit the activity of NOS, through the intervention of cytosolic free calcium or other intracellular messengers.

NO has important functions in the cardiovascular system, being an important vasodilator and avoiding platelet aggregation. Furthemore it has a noteworthy role in the central and peripheral nervous system, acting as a neurotransmitter. However, NO can not be considered as a classical intercellular messenger since it is highly diffusible and does not bind to cellular receptors. The target enzyme, which mediates many of its effects is guanylyl cyclase (13). NO synthases studies have been addressed using substances which are inhibitors to these enzymes; the most widely used are arginine derivatives, such as N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA) or its methyl ester (L-NAME).

In this review we will focus on the actions of NO on the exocrine pancreas. Most of the knowledge on the physiological role of NO on exocrine pancreatic secretion deal with the effects of NO acting as a vasodilator. Moreover, some work has been done about the effects of NO on neurons of the intrapancreatic ganglia. Finally, scarce results have been reported concerning the actions of NO as intracellular messenger on the acinar cells. In the next pages we will summarize the most remarkable results on this topic.

SOURCES OF NO IN THE PANCREAS

It has been reported the existence of NOS in neurons and nerve fibres of rat pancreas (14). Potencial targets of these intrapancreatic axons, which present NADPH diaphorase activity, are acini, ducts, islets and blood vessels (15).

Furthermore, it is likely that vascular endothelium could also be an important source of pancreatic NO (16), in a similar way to that described in many other tissues.

Finally, it has been recently reported that acinar pancreatic cells can produce NO after secretagogue stimulation (17).

ACTIONS OF NITRIC OXIDE IN THE EXOCRINE PANCREAS

Role of NO in the pancreatic blood flow and secretion

The correlation between local blood flow and exocrine pancreatic secretion has been recognized for a long time (18). So, the administration of vasopressin (19), C4 leucotriene (20) or peptide YY (21) produces a decrease in pancreatic blood flow and thereby, an important inhibition of exocrine pancreatic secretion.

The endogenous production of NO in the canine pancreas produces a tonic vasodilatory influence on basal and stimulated pancreatic circulation (16); so, the inhibition of NO synthesis by L-NNA resulted in a marked reduction in the postprandial and hormonally-stimulated pancreatic secretion in dogs (16). Accordingly, endogenous NO may affect exocrine pancreatic secretion probably through changes in the vascular bed. However, more recently, in cats (22) it has been shown that the effects of NO may be, at least in part, independent on changes in pancreatic blood flow. In particular, it has been demonstrated that L-NMMA, though reducing the increase in blood flow associated with cholecystokinin stimulation, had no effect on that associated with secretin; however, in both cases it inhibited the stimulated exocrine pancreatic secretion. Therefore, it seems that NO has a selective role in mediating changes in pancreatic secretion and this points to the existence of some stimulatory effects of NO which are not mediated by an increase of the pancreatic blood flow. There are also differences depending on the species: Holst et al (23) reported that, in the pig, the formation of NO is essential for the stimulatory actions of the vagus nerve on fluid secretion, but it did not mediate the vagus nerve-induced vasodilation. Therefore, in summary, nitric oxide has an stimulatory effect on pancreatic blood flow, which increases exocrine pancreatic secretion and, moreover, it may have stimulatory effects on pancreatic acinar and ductal cells. In the next section, we will focus on the mechanisms of action of nitric oxide, considering its role as intracellular messenger.

Role of nitric oxide as intracellular messenger

At the intracellular level, NO may act on several targets, but one of the most important is the enzyme guanylyl cyclase (13). Previous work had revealed that cyclic GMP (cGMP) induces relaxation of smooth muscle, such as vascular, airway and intestinal smooth muscle (24). Therefore, the term nitrovasodilators may be applied to those agents which generate NO, and, thereby, activate guanylyl cyclase and increase the production of cGMP.

The increase of cGMP is an early event in hormone or neurotransmitter-induced cascade of reactions in pancreatic acinar cells (25); this increase reflects the activation of the cytosolic soluble enzyme guanylyl cyclase. Moreover, it has been reported that synthesis of cGMP occurred in pancreatic acinar cells stimulated by carbachol (17) and that not only the cGMP increase, but also the arginine to citrulline conversion is blocked by NO synthase inhibitors (17). This suggested that NO might play some role in the stimulus-secretion coupling mechanisms of cholinergic agonists on pancreatic acinar cells.

Concerning the role of Ca^{2+} in this process, Gukovskaya and Pandol (17) also reported that the blockade of NO production inhibited the carbachol-induced increase in $^{45}Ca^{2+}$ uptake in both guinea pig and rat pancreatic acinar cells. Moreover, the concentration-response curves for inhibition by L-NMMA of $^{45}Ca^{2+}$ uptake and cGMP formation were superimposable. According to these authors, these findings indicate the involvement of NO in the regulation of cGMP production and Ca^{2+} transport in pancreatic acinar cells. Therefore, the production of NO and the formation of cGMP seem to be necessary for the carbachol-activated Ca^{2+} influx. Moreover, Xu et al (26), using permeabilized acini, reported that when the internal calcium stores were maintained loaded, increasing calcium concentration in medium up to 2.5 μM only modestly increased NOS activity, but, on the other hand, when the calcium stores were depleted, this markedly increased NOS activity, independent of the calcium concentration of the medium. Therefore, according to these authors, NOS senses both cytosolic free calcium concentration and internal Ca^{2+} stores load. So, these authors concluded that the activation of calcium entry involves an agonist-mediated Ca^{2+} release from internal stores which activates a cellular pool of NOS to generate cGMP, which then modulates the Ca^{2+} entry pathway in the plasma membrane. Cyclic GMP has, therefore, a

dual role, depending on the cGMP concentration: low concentrations activate whereas high concentrations inhibit Ca^{2+} entry and this could provide the cells with a negative feed-back mechanism, which inhibits the calcium entry when internal calcium concentration is high. Finally, Gukovskaya and Pandol (27) reported the existence of regulatory actions of intracelular calcium on cGMP. In pancreatic acinar cells of the guinea pig, Ca^{2+} regulates cGMP production in opposite directions by activating NOS and inhibiting guanylyl cyclase.

Other secretagogues, different from carbachol, such as cholecystokinin (CCK), have also been reported to increase the production of NO and the levels of cGMP in rat pancreatic tissue (28), which points to the existence of the CCK-NO-cGMP pathway; on the other hand, other pancreatic secretagogues which uses intracellular Ca^{2+}-independent pathways, such as vasoactive intestinal peptide (VIP), have no relationship with NOS activity and thereby with cGMP (29).

In a very recent paper, Yoshida et al (30) reported that the NO/cGMP system does not appear to be significantly involved in the mediation of Ca^{2+} signalling and amylase secretion stimulated by carbachol and CCK. Though these results could be understandable concerning CCK, they seem rather confusing for carbachol, since these authors have used L-NAME to inhibit the NOS activity and it is known that L-NAME is also an antagonist of muscarinic receptors (31). In fact, we have recently shown (Fig 1) that the dose-response curve of amylase secretion to carbachol, in rat pancreatic acini, was shifted to the right when L-NAME was added at a 0.32 mM concentration.

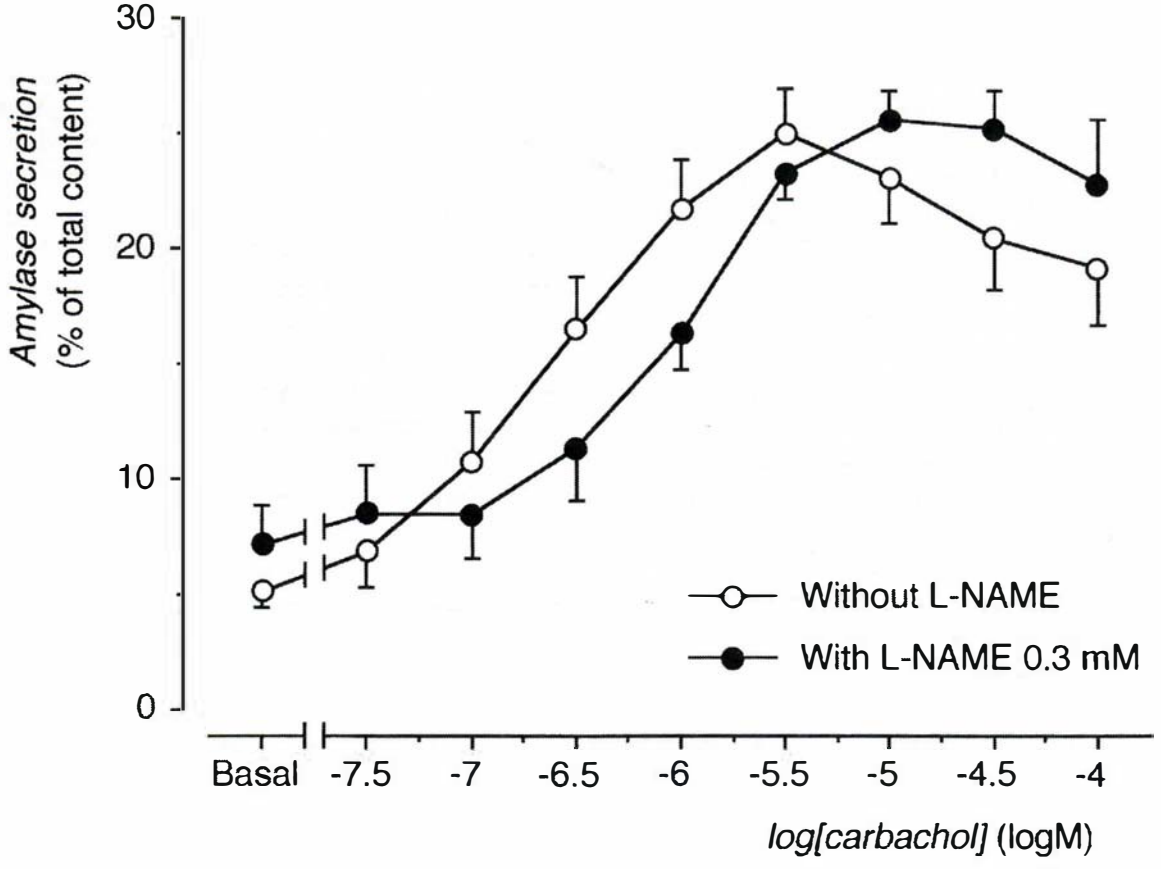

Figure 1: Dose-response curves of amylase secretion to carbachol or carbachol + 3.2×10^{-4}M L-NAME. Results are expressed as means ± SEM (n=7) of the percentage of amylase secretion compared to the total amylase content (100%).

Moreover, the EC_{50} of carbachol is significantly lower in the presence of L-NAME (Fig 2).

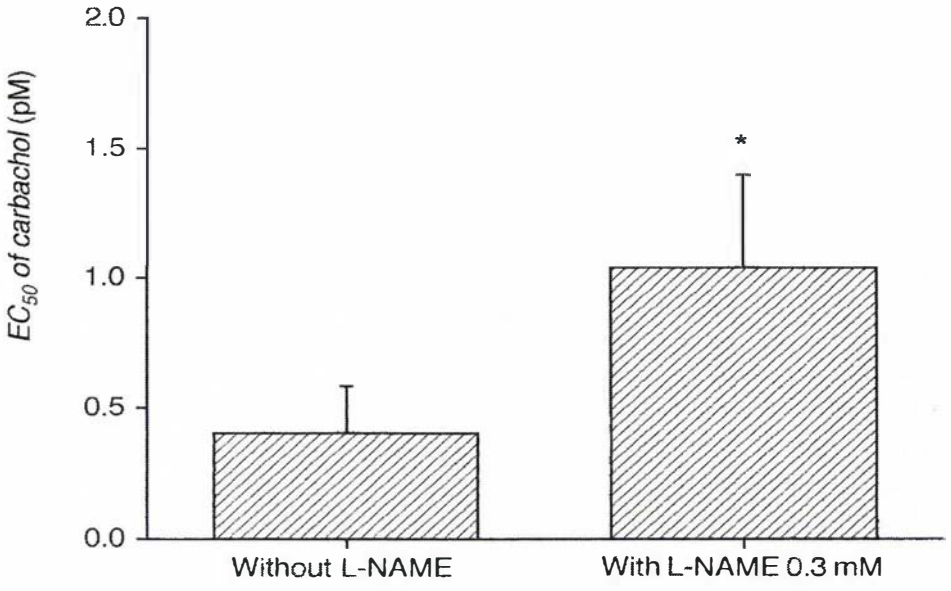

Figure 2: EC_{50} of carbachol (pM) on amylase secretion with or without $3.2x10^{-4}$M L-NAME. Results are expressed as means ± SEM (n=7).
*: statistically significant differences ($p<0.05$) regarding the absence or presence of L-NAME.

Regarding CCK, when pancreatic segments were perfused with this peptide the secretion of trypsinogen was significantly inhibited by L-NAME (32). The maximal secretion of trypsinogen was elicited by CCK 0.3 nM and averaged 1.45 ± 0.23 and 0.90 ± 0.11 trypsin units/mg pancreatic tissue in pancreatic segments perfused with CCK alone or CCK + L-NAME 0.1 mM, respectively (Fig 3).

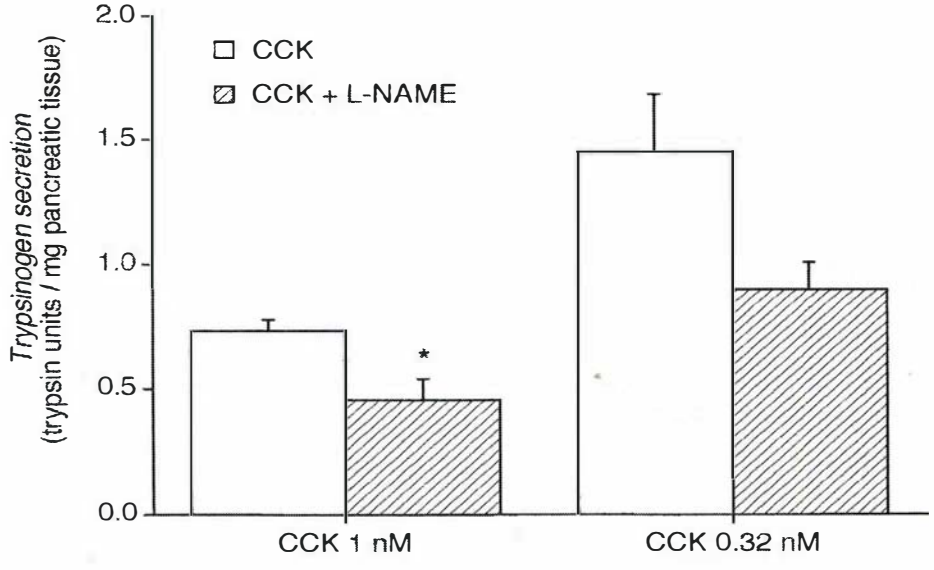

Figure 3: Trypsinogen secretion (trypsin units/mg pancreatic tissue) from pancreatic segments after CCK stimulation, in the presence or absence of 10^{-4}M L-NAME. Results are expressed as means ± SEM (n=7).
*: statistically significant differences ($p<0.05$) when comparing the effect of CCK alone or CCK + L-NAME.

Therefore, it can be concluded that synthesis of NO modulates trypsinogen secretion after CCK stimulation.

Finally, a direct action of NO on amylase secretion in pancreatic acinar cells might be discarded, since the incubation of rat acini with different concentrations of sodium nitroprusside or hydroxylamine, two typical NO donors, dit not produce amylase secretion (Fig 4).

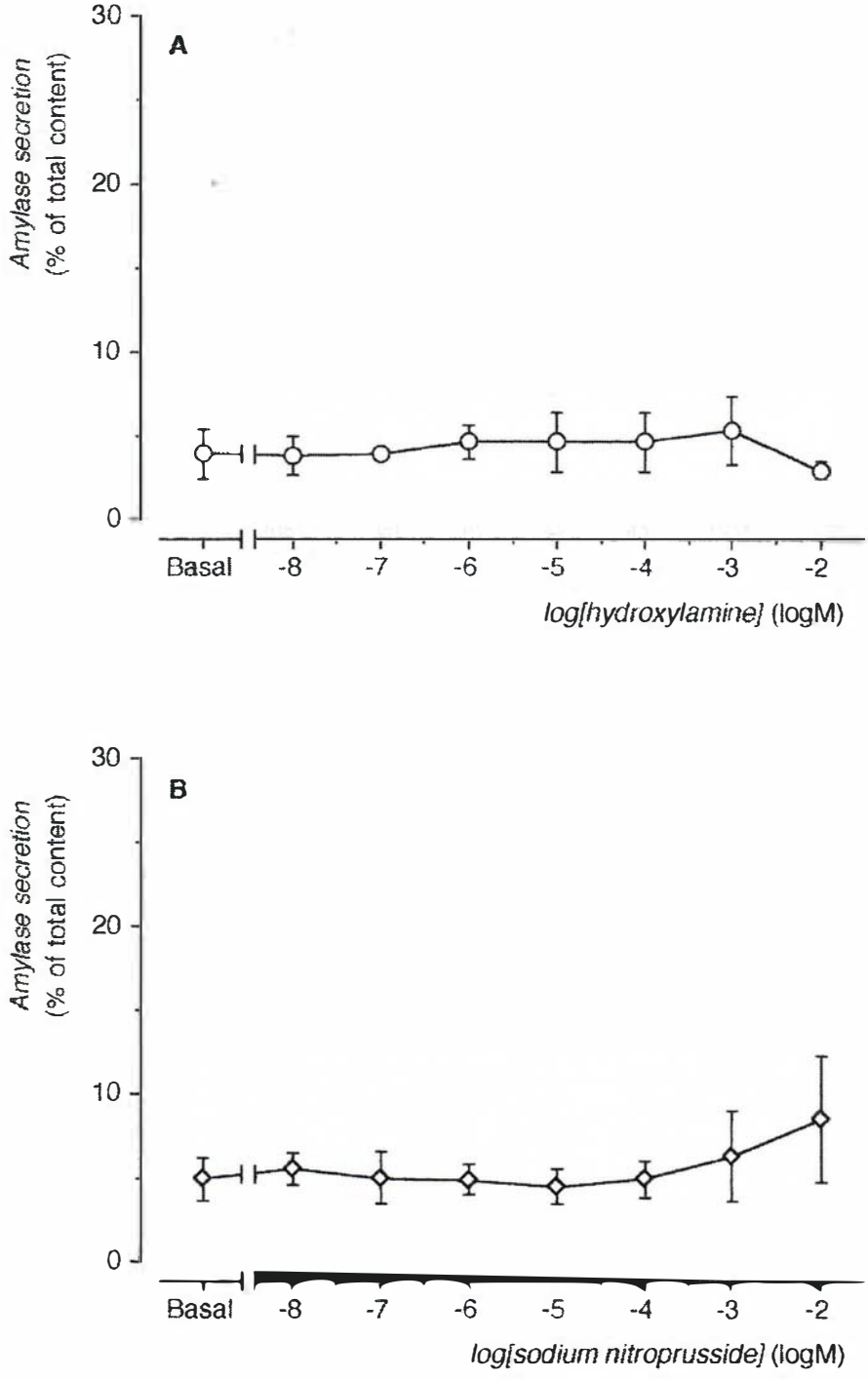

Figure 4:

A. Amylase secretion from pancreatic acini after incubation with increasing concentrations of hydroxylamine. Results are expressed as means ± SEM (n=3) of the percentage of amylase secretion compared to the total amylase acinar content (100%).

B. Amylase secretion from pancreatic acini after incubation with increasing concentrations of sodium nitroprusside. Results are expressed as means ± SEM (n=3) of percentage of amylase secretion compared to the total amylase acinar content (100%).

However, the intracellular role of NO can not only be circumscribed to its modulatory effect on enzyme secretion and we have recently demonstrated that NO may play some role in the tyrosine phosphorilation of two proteins, p125 focal adhesion kinase and paxillin, which are involved in the cytoskeletal organization (33). The intracellular pathways which can be activated in this process are not yet known, but it seems a promising research field for a near future.

In summary, the role of NO as an intracellular messenger in pancreatic acinar cells is not yet clear, and though a direct action on enzyme secretion does not seem plausible, an indirect, modulatory effect seems very probable. Additionally, NO may have other important funtions which are not yet known.

Role of NO in the pancreatic pathophysiology

Most of the studies in this topic has been focused on the role that NO might play in the characteristic inflammatory reactions which can be observed during pancreatitis. In the cerulein model of experimental pancreatitis (34), it has been shown a rise in pancreatic blood flow, mediated by NO, which might have a protective role in the development of the disease (35). This is in agreement with that reported by Tanaka et al (36) in the rat pancreas and with the results of Benz et al (37), which demonstrated that NO generated after pancreatic ischemia-reperfusion may have a protective role. On the other hand, Said (38) reported that excess of NO provokes tissue injury in inflamatory processes, and, furthermore, Hotter et al (39) have demonstrated that the inhibition of NOS reverses the increase in edema and eicosanoid production in rats with pancreas transplantation, which points to the existence of a close relationship between NO and eicosanoid production in the ischemia-reperfusion injury.

The role of NO production in tissue damage is, therefore, not yet clear, but a possible explanation was recently suggested by Kikuchi et al (40). According to these authors, NO derived from cNOS in physiological conditions may have a protective role in edematous pancreatitis but, when pancreatitis is accompanied by bacterial infection, the situation may be aggravated by excessive production of NO, derived from inflammatory activated cells, which express iNOS.

Severe pancreatitis can lead to systemic complications and multiple organ failure. In agreement with that reported by Kikuchi et al (40), Lomis et al (41) had pointed to a potential utility of NOS inhibitors for the treatment of severe pancreatitis, which suggests that excessive NO production can damage the organ. In fact, we have recently demonstrated that, during taurocholate-induced necrotizing pancreatitis in the rat, the decrease of arterial pressure can be reversed after the infusion of L-NAME, (42), which demonstrates the involvement of NO in the hypotension, characteristic of this disease. However, this novel research field needs more work to shed some light on these complex mechanisms.

In summary NO has an important role in exocrine pancretic physiology. Some of this role is that related to the classical cardiovascular functions first described for this agent; secondly the neurotransmitter role seems evident and, finally, important intracellular actions are now starting to be described, this being a promising research field for a near future.

REFERENCES

MITCHELL, H.H., SHONLE, H.A., GRINDLEY, H.S.: *J Biol Chem* (1916), **24**: 461.
GREEN,L.C., RUIZ DE LUZURIAGA, K., WAGNER, D.A., RAND, W., ISTFAN, N., YOUNG, V.R., TANNEBAUM, S.R.: *Proc Natl Acad Sci USA* (1981), **78**: 7764-7768.
GREEN, L.C., TANNEBAUM, S.R., GOLDMAN, P.: *Science* (1981), **212**: 56-58.
STUEHR, D.J., MARLETTA, M.A.: *Proc Natl Acad Sci* (1985), **82**: 7738-7742.
HIBBS, J.B.,TAINTOR, R.R., VAVRIN, Z.: *Science* (1987), **235:** 473-476.
PALMER, R.M.J., FERRIGE, A.G., MONCADA, S.: *Nature* (1987), **327**: 524-526.
PALMER, R.M.J., ASHTON, D.S., MONCADA, S.: *Nature* (1988), **333**: 664-666.
MARLETTA, M.A., YOON, P.S., IYENGAR, R., LIAF, C.D., WISHNOK, J.S.: *Biochem J* (1988), **27**: 8706-8711.
KNOWLES, R.G., PALACIOS, M., PALMER, R.M.J., MONCADA, S.: *Proc Natl Acad Sci USA* (1989), **86**: 5159-5162.
BREDT, D.S., SNYDER, S.H.: *Proc Natl Acad Sci USA* (1990), **87**: 682-685.
BREDT, D.S., HWANG, P.M., GLATT, C.E., LOWESTEIN, C., REED, R.R., SNYDER, S.H.: *Nature* (1991), **351**: 714-718.

BREDT, D.S., FERRIS, C.D., SNYDER, S.H.: *J Biol Chem* (1991), **267**: 10976-10981.
MURAD, F. *Recent Prog Horm Res* (1994), **49**: 239-248.
SHIMOSEGAWA, T., ABE, T., SATOH, A., ASAKURA, T., MASARU, D., TAKAYOSHI, T.: *Neurosci Lett* (1992), **148**: 67-70.
KIRCHGESSNER, A.L., LIU, M.T., GERSHON, M.D. : *J Comp Neurol* (1994), **342**: 115-130.
KONTUREK, S.J., BILSKI, J., KONTUREK, P.K., CIESZKOWSDI, M., PAWLIK, W.: *Gastroenterol* (1993), **104**: 896-902.
GUKOVSKAYA, A., PANDOL, S. : *Am J Physiol* (1994), **266**: G350-G356.
TANKEL, H.I., HOLLANDER, F.: *Gastroenterol* (1957), **32**: 633-641.
BEIJER, H.J.M., BROUWER, F.A.S., CHARBON, G.A. : *Scand J Gastroenterol* (1979), **14**: 295-300.
KONTUREK, S.J., PAWLIK, W., CZARNOBILSKI, K., GUSTAW, P., JAWOREK, J., BECK, G., JENDRALLA, H.: *Am J Physiol* (1988), **254**: G849-G855.
INOUE, Y.,HOSOTANI, R., TATEMOTO, K., YAJIMA, H., TOBE, I.: *Dig Dis Sci* (1988), **33**: 828-832.
PATEL, A. G., TOYAMA, M. T., NGUYEN, T. N., COHEN, G. A., IGNARRO, L.J. REBER H. A.: *Gastroenterol* (1995), **108**: 1215-1220.
HOLST, J.J., RASMUSSEN, T.N., SCHMIDT, P.: *Am J Physiol* (1994), **266**: G206-G213.
KATSUKI, S., ARNOLD, W., MITTAL, C.K., MURAD, F.: *J Cyclic Nucl Res* (1977), **3**: 23-35.
HOOTMAN, S.R., WILLIAMS, J.A. In: *Physiology of the Gastrointestinal Tract* (1987) pp,1129-1146, Raven Press, New York.
Xu, X, STAR, R.A., TORTORICI, G., MUALLEM, S.: *J Biol Chem* (1994), **269**:12645-12653.
GUKOVSKAYA, A.S., PANDOL, S.J.: *Am J Physiol* (1995), **268**: G900-G907.
SEO, D-W., NAM, S.W., NAM, T.K., LEE, Y.J., KO. Y.K., LEE, H.W.: *Arch Pharm Res* (1995), **18**: 434-439.
NAM, T.K., HAN, J.W., NAM, S.W., SEO, D-W., LEE, Y.J., KO, Y.K., LEE, H.W.: *Arch Pharm Res* (1996), **19**: 201-206.
YOSHIDA, H., TSUNODA, Y., OWYANG, CH.: *Eur J Physiol* (1997), **434**: 25-37.
BUXTON , I.A.L., CHEEK, D.J., ECKMAN, D., WESTFALL, D.W., SANDERS, K.M., KEEF, K.D.: *Circ Res* (1992), **72**: 387-395.
HERNÁNDEZ-GUIJO, J.M., ACOSTA, J.J., GARCÍA-BENITO, M., LÓPEZ, M.A., SAN ROMÁN, J.I., CALVO, J.J.: *Digestion* (1996), **57**: 236.
CALVO, J.J., GARCIA-BENITO, M., ACOSTA, J.J., SAN ROMAN, J.I., LOPEZ, M.A., GARCIA, L.J.: *Digestion* (1997), **58** S2: 8.
LAMPER, M., KERN, H.F.: *Virchows Arch A* (1977), **373**: 97-117.
SATOH,A., SHIMOSEGAWA, T., ABE,T.: *Pancreas* (1994), **9**: 574-579.
TANAKA, S., KAMIIKE, W., KOSAKA, H., ITO, T., KUMURA, E., SHIGA, T., MATSUDA, H.: *Am J Physiol* (1996),**34**: G405-G409.
BENZ, S., WEBER, H., PFEFFER, F., WIESNER, R., BREITENBUSCH, P., HOPT, U.T.: *Digestion* (1997), **58** S2: 8.
SAID, S.I.: *Biomed Res* (1994), **15** S2: 79-84.
HOTTER, G., CLOSA, D., PI, F., PRATS, N., FERNÁNDEZ-CRUZ, L., ORIOL, B., GELPI, E., ROSELLO-CATAFAU, J.: *Transplantation* (1995), **59**: 417-421.
KIKUCHI Y., SHIMOSEGAWA, T., SATOH, A., ABE, R., ABE, T., KOIZUMKI, M., TOYOTA, T.: *Pancreas* (1996), **12**: 68-75.
LOMIS, T., SIFFRING, C.W., CHALASANI, S., ZIEGLER, D.W., LENTZ, K.E., STAUFFER, K.E., MCMILLAN, A., AGARWAL, N., LOWENSTEIN, C. RHOADS, J.E.: *Am Surg* (1995), **61**: 7-10.
SAN ROMÁN, J.I., RODRÍGUEZ-NODAL, F., BRAGADO, J., LÓPEZ-NOVOA, J.M., LÓPEZ, M.A., CALVO, J.J.: *Digestion* (1995), **56**: 314-315.