Inducible Nitric Oxide Synthase (iNOS) and Obstruction Research Literatures

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Abstract: Nitric oxide synthases (NOSs, EC 1.14.13.39) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule that helps to modulate the vascular tone, insulin secretion, airway tone and peristalsis, etc, and NO is involved in angiogenesis and neural development. It may function as a retrograde neurotransmitter. NO is mediated in mammals by the calcium-calmodulin controlled isoenzymes eNOS (endothelial NOS) and nNOS (neuronal NOS). The inducible isoform, iNOS, is involved in immune response, binds calmodulin at physiologically relevant concentrations, and produces NO as an immune defense mechanism, as NO is a free radical with an unpaired electron. It is the proximate cause of septic shock and may function in autoimmune disease. Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. It can occur suddenly, or be a long-term problem. If the blockage comes on suddenly, kidney damage is less likely if the problem is detected and treated promptly, and the damage to the kidneys goes away normally. Long-term damage to the kidneys may occur if the blockage has been present for a long time. If the problem is caused by a blockage in the bladder, the bladder may have long-term damage, which may lead to problems emptying the bladder or leakage of urine. This article introduces recent research reports as references in the inducible nitric oxide Synthase (iNOS) and obstruction related studies.

Key words: inducible nitric oxide synthase (iNOS); Obstruction; life; cell

1. Introduction

Nitric oxide synthases (NOSs, EC 1.14.13.39) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule that helps to modulate the vascular tone, insulin secretion, airway tone and peristalsis, etc, and NO is involved in angiogenesis and neural development. It may function as a retrograde neurotransmitter. NO is mediated in mammals by the calcium-calmodulin controlled isoenzymes eNOS (endothelial NOS) and nNOS (neuronal NOS). The inducible isoform, iNOS, is involved in immune response, binds calmodulin at physiologically relevant concentrations, and produces NO as an immune defense mechanism, as NO is a free radical with an unpaired electron. It is the proximate cause of septic shock and may function in autoimmune disease.

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BACKGROUND: The current study evaluated whether biliary tract obstruction stimulates inducible nitric oxide synthase (iNOS) protein expression in the liver and analyzed the implication of lymphomononuclear cells and interleukin-4 (IL-4).

METHODS: Male Wistar rats were used. Bile flow interruption was achieved by a complete division of the extrapancreatic common bile duct. iNOS expression was determined by both the Western blot technique and immunohistochemistry. RESULTS: iNOS protein was markedly expressed in the liver 7 days after bile duct obstruction. Treatment with
thymostimulin (TP-1), a partially purified thymic extract, reduced the intensity of the expression of iNOS protein in the liver after bile duct ligation. Recent data have suggested that IL-4 attenuates iNOS protein expression. We then analyzed the involvement of this anti-inflammatory cytokine on the modulation of iNOS expression in the liver. The liver from rats that underwent bile duct ligation (BDL) showed a lower content of IL-4 than that of sham-operated (SO) rats. TP-1 treatment increased the content of IL-4 in the liver. Liver slices incubated in vitro with Escherichia coli lipopolysaccharide (LPS, 10 microg/mL) stimulated the expression of iNOS protein. The level of LPS-induced iNOS expression was reduced by lymphomononuclear cells obtained from sham-operated animals. However, lymphomononuclear cells isolated from BDL rats potentiated the induction of iNOS expression by LPS-stimulated liver. However, lymphomononuclear cells from TP-1-treated BDL rats failed to modify LPS-stimulated iNOS expression. The different effect of lymphomononuclear cells on the modulation of iNOS expression in the liver was associated with their ability to generate IL-4. CONCLUSIONS: The liver of jaundiced rats markedly expressed iNOS protein, which was associated to modifications in the content of IL-4 in the liver. Furthermore, lymphomononuclear cells modulate iNOS protein expression in the liver by a mechanism in which IL-4 is involved.


OBJECTIVES: To investigate the antiinflammatory activity of Serenoa repens (SeR), LY, and on proinflammatory phenotype in rat peritoneal macrophages (Ms) stimulated with Salmonella enteritidis lipopolysaccharide (LPS) and in the prostate of rats with partial bladder outlet obstruction. SeR, combined with other compounds, such as LY and Se is used to relieve symptoms associated with benign prostatic hyperplasia (BPH). Inflammation plays a pivotal role in the pathogenesis of BPH and represents a target for anti-BPH drugs. METHODS: After stimulation with 1 mug/mL of LPS, peritoneal rat MPhis were coincubated with LY (2 mug/mL), Se (0.03 mug/mL), and SeR (10 mug/mL), alone or in association (LY-Se-SeR) and with RPMI. Inducible cyclooxygenase (COX-2), 5-lipoxygenase (5-LOX), inducible nitric oxide synthase (iNOS), and inhibitor kappaBalpha (IkappaB-alpha) protein were evaluated by Western blot. Nuclear factor-kappa B (NF-kappaB) binding activity was measured by electrophoretic mobility shift assay. Tumor necrosis factor-alpha (TNF-alpha) gene expression was investigated by real-time polymerase chain reaction. We also evaluated malondialdehyde (MDA) and nitrite levels. RESULTS: LPS stimulation produced a proinflammatory phenotype in rat peritoneal Ms. LY, Se, and SeR inhibited the inflammatory cascade, but the Ly-Se-SeR association caused a greater inhibitory effect on the expression of COX-2, 5-LOX, and iNOS. The Ly-Se-SeR association showed a higher efficacy in reducing the loss of IkappaB-alpha, the increased NF-kappaB binding activity, the enhanced mRNA levels of TNF-alpha, the elevated MDA, and nitrite content. The Ly-Se-SeR association in vivo caused a greater inhibitory effect on prostate inflammation induced in rats by partial bladder outlet obstruction. CONCLUSIONS: The Ly-Se-SeR association might be useful in the treatment of BPH.


Nitric oxide (NO) is postulated to play a role in endotoxin-induced ileus. We investigated the effect of selective blockade of inducible NO synthase (iNOS) and guanylyl cyclase on endotoxin-induced ileus in mice. Thirty minutes before injection of lipopolysaccharides (LPS), mice were pretreated with L-NAME (N omega-nitro-L-arginine methyl ester, non-selective NOS inhibitor), 1400W (N-(3-(aminomethyl)benzyl)acetamide, selective iNOS inhibitor), ODQ (1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one, guanylyl cyclase inhibitor), dimethyl sulfoxide (DMSO, vehicle), or dexamethasone. After 18 h, general well being deteriorated and the mice developed hypothermia and a significant delay in gastric emptying and intestinal transit as measured by Evans blue. 1400W completely reversed the endotoxin-induced delay in gastric emptying, while L-NAME did not have these beneficial effects. On the contrary, even in control mice, L-NAME delayed gastric emptying. Dexamethasone, DMSO, and ODQ mimicked the effect of 1400W on endotoxin-induced delay in gastric emptying. The endotoxin-induced delay in transit was significantly improved only by 1400W. None of the drugs reversed the hypothermia. In LPS mice treated with L-NAME, the behavior scale increased even further, while it decreased after treatment with 1400W. In conclusion, selective inhibition of iNOS reverses the endotoxin-induced delay in gastric emptying and transit and improves general well being. The pathway used by NO, derived from iNOS, may
Nitric oxide (NO) can play an important role in the regulation of vascular tone and neurotransmission, as well as in non-specific immunoreactions and inflammation in a variety of tissues. Increased quantities of nitric oxide in expired air can be measured during inflammatory processes. However, the exact role and precise sources of NO under physiological and pathophysiological conditions within the airways remain to be defined. Three isoforms of NO-synthases can be distinguished: two constitutive (neuronal and endothelial) Ca(2+)-dependent eNOS and one inducible Ca(2+)-independent iNOS (NOS II). Constitutive NOS (NOS I and III) release a basal amount of NO under physiological conditions. The inducible form once expressed can catalyse the generation of large quantities of NO. Many kinds of cells, such as macrophages, neutrophils, endothelium and smooth muscle cells, are capable of expressing NOS II. Since all isoforms of NO-synthase seem to be present in nasal tissues and the expression of iNOS under inflammatory conditions seems to be responsible for excessive production of NO, the distribution of NOS-isoforms (especially NOS II) in normal and inflammatory nasal tissue, as well as the exact requirements for expression of iNOS remain to be proven. Non-inflamed fresh human nasal mucosa from the middle turbinate was compared immunohistologically with nasal mucosa having the typical findings of chronic polypoid rhinosinusitis (i.e., polypoid middle turbines and polyps of the middle nasal duct). In order to gain more information about the mechanisms of acute inflammation, non-inflamed vital turbinates were incubated in vitro with the proinflammatory substances bacterial lipopolysaccharides (LPS) and tumor necrosis-factor (TNF) for 30, 60, 90, 120, 180 and 240 min. Subsequent to exposure to NADPH-diaphorase and immunostaining with specific antibodies to each NOS-isoform, clearly increased or initiated expressions of inducible NOS (iNOS) in blood vessels, glands, macrophages and epithelium of chronically inflamed and LPS-incubated nasal tissue became apparent in comparison to the non-inflamed controls. In contrast, NOS III/NOS I seemed to be not affected. The onset of immunohistochemically recognizable NOS II expression was observed after 90 min incubation with LPS/TNF-alpha. Polypoid tissue showed a strong increase in submucosal thickness and a high infiltration of iNOS-positive leukocytes (granulocytes and macrophages) compared to the LPS-incubated non-inflamed specimens. These findings implicate NOS II generated nitric oxide as a key agent for causing nasal swelling, secretion and obstruction during nasal infections and allergic episodes.


Asthma results from allergen-driven intrapulmonary Th2 response, and is characterized by intermittent airway obstruction, airway hyperreactivity (AHR), and airway inflammation. Accumulating evidence indicates that inflammatory diseases of the respiratory tract are commonly associated with elevated production of nitric oxide (NO). It has been shown that exhaled NO may be derived from constitutive NO synthase (NOS) such as endothelial
Asthma results from an intrapulmonary allergen-driven Th2 response and is characterized by intermittent airway obstruction, airway hyperreactivity, and airway inflammation. An inverse association between allergic asthma and microbial infections has been observed. Microbial infections could prevent allergic responses by inducing the secretion of the type 1 cytokines, IL-12 and IFN-gamma. In this study, we examined whether administration of bacterial LPS, a prototypic bacterial product that activates innate immune cells via the Toll-like receptor 4 (TLR4) could suppress early and late allergic responses in a murine model of asthma. We report that LPS administration suppresses the IgE-mediated and mast cell-dependent passive cutaneous anaphylaxis, pulmonary inflammation, airway eosinophilia, mucus production, and airway hyperactivity. The suppression of asthma-like responses was not due to Th1 shift as it persisted in IL-12(-/-) or IFN-gamma(-/-) mice. However, the suppressive effect of LPS was not observed in TLR4- or NO synthase 2-deficient mice. Our findings demonstrate, for the first time, that LPS suppresses Th2 responses in vivo via the TLR4-dependent pathway that triggers NO synthase 2 activity.


BACKGROUND & AIMS: Endotoxin elicits an inflammatory response within the intestinal muscularis and causes intestinal muscle dysfunction. Our aims were to investigate intestinal muscle recovery after a single or repeated lipopolysaccharide (LPS) injections. We also investigated the ability of LPS to induce cross-tolerance to postoperative ileus. METHODS: Motility was measured in vivo and in vitro by transit and organ-bath techniques. Nuclear factor kappa-B, nuclear factor interleukin 6, and signal transducer and activator of transcription were quantified by using electrophoretic mobility shift assay, and tumor necrosis factor alpha, interleukin 6, inducible nitric oxide synthase, and cyclooxygenase 2 were measured with reverse-transcription polymerase chain reaction. Myeloperoxidase histochemistry for neutrophils was performed in jejunal muscularis whole mounts. RESULTS: Endotoxin-induced suppression of in vitro muscle contractility temporally recovered over 7 days with a similar profile whether after a single dose or during the continuous daily injection of LPS. Functional adaptation to continuous LPS was reflected in a significant blunting of transcription factor activation and cytokine messenger RNA up-regulation compared with the naive LPS-stimulated muscularis. Preconditioning of the...
muscularis showed significant cross-tolerance to the functional, molecular, and leukocytic sequelae of intestinal manipulation. CONCLUSIONS: The muscularis externa recovered and developed tolerance to endotoxin over 7 days, which conferred cross-tolerance to intestinal manipulation. Thus, preconditioning induces protective mechanisms to a subsequent insult within the muscularis externa.


AIM: To investigate the effect of biliary drainage on inducible nitric oxide synthase (iNOS), CD14 and TGR5 expression in rats with obstructive jaundice (OJ). METHODS: Male adult Sprague-Dawley rats were randomly assigned to four groups: OJ, sham operation (SH), internal biliary drainage (ID) and external biliary drainage (ED). Rat models were successfully established by two operations and succumbed for extraction of Kupffer cells (KCs) and liver tissue collection on the 8th and 15th day. KCs were isolated by in situ hepatic perfusion and digested with collagen IV, density gradient centrifuged by percoll reagent and purified by cell culture attachment. The isolated KCs were cultured with the endotoxin lipopolysaccharide (LPS) with and without the addition of ursodeoxycholic acid (UDCA). The expression of iNOS, CD14 and bile acid receptor-TGR5 protein in rat liver tissues was determined by immunohistochemistry. The expression of iNOS and CD14 messenger RNA (mRNA) on the isolated KCs was detected by reverse transcription polymerase chain reaction (PCR) and the TGR5 mRNA level in KCs was measured by real-time quantitative PCR.

RESULTS: The iNOS protein was markedly expressed in the liver of OJ rats, but rare expressed in SH rats. After relief of OJ, the iNOS expression was decidedly suppressed in the ID group (ID vs OJ, P < 0.01), but obviously increased in rats of ED (ED vs OJ, P = 0.004). When interfered only with LPS, the expression of iNOS mRNA by KCs was increased in the OJ group compared with the SH group (P = 0.004). After relief of biliary obstruction, the iNOS mRNA expression showed slight changes in the ED group (ED vs OJ, P = 0.71), but dropped in the ID group (ID vs OJ, P = 0.001). Compared with the simple intervention with LPS, the expressions of iNOS mRNA were significantly inhibited in all four groups after interfered with both LPS and UDCA (P < 0.01, respectively). After bile duct ligation, the CD14 protein expression in rat liver was significantly strengthened (OJ vs SH, P < 0.01), but the CD14 mRNA level by KCs was not up-regulated (OJ vs SH, P = 0.822). After relieving the OJ, the expression of CD14 protein was reduced in the ID group (ID vs OJ, P < 0.01), but not reduced in ED group (ED vs OJ, P = 0.591). And then the CD14 mRNA expression was aggravated by ED (ED vs OJ, P < 0.01), but was not significantly different between the ID group and the SH and OJ groups (ID vs SH, P = 0.944; ID vs OJ, P = 0.513, respectively). The expression of TGR5 protein and mRNA increased significantly in OJ rats (OJ vs SH, P = 0.001, respectively). After relief of OJ, ID could reduce the expression of TGR5 protein and mRNA to the levels of SH group (ID vs SH, P = 0.22 and P = 0.354, respectively), but ED could not (ED vs SH, P = 0.001, respectively). CONCLUSION: ID could be attributed to the regulatory function of activation of KCs and release of inflammatory mediators.


The pulmonary hypertensive response to pulmonary vascular obstruction caused by intravenously injected microparticles is amplified by pretreatment with N(omega)nitro-L-arginine methyl ester (L-NAME). The L-NAME prevents the synthesis of the potent vasodilator nitric oxide (NO) by inhibiting both the constitutive [endothelial NO synthase (eNOS or NOS-3)] and inducible [inducible NO synthase (iNOS or NOS-2)] forms of NO synthase. In the present study we used the selective iNOS inhibitor aminoguanidine (AG) to evaluate the role of iNOS in modulating the pulmonary hypertension (PH) triggered by microparticle injections. Experiment 1 was conducted to confirm the ability of AG to inhibit NO synthesis by iNOS in broiler peripheral blood mononuclear cells exposed to bacterial lipopolysaccharide (LPS, endotoxin). Mononuclear leukocytes treated with LPS produced 10-fold more NO than untreated (control) cells. The LPS-stimulated production of NO was partially inhibited by L-NAME and was fully inhibited by AG, thereby confirming that AG inhibits LPS-mediated iNOS activation in broilers. In Experiment 2 we evaluated the responses of male progeny from a base population (MP Base) and from a derivative line selected for one generation from the survivors of an LD50 microparticle injection (MP Select). The pulmonary arterial pressure (PAP) was lower in MP Select than in MP Base broilers. Both lines exhibited similar percentage increases in PAP after microparticles were injected, and AG modestly amplified the PH triggered by microparticles in both lines. In Experiment 3 we evaluated the responses of
male progeny from a second base population (PAC Base) and from a derivative line selected for 3 generations using the unilateral pulmonary artery clamp technique (PAC Select). The PAP was lower in PAC Select than in PAC Base broilers, and both lines exhibited similar percentage increases in PAP in response to the microparticles. The PH triggered by microparticles was not amplified by AG but was doubled by L-NAME. These experiments demonstrate that during the 30 min following pulmonary vascular entrapment of microparticles, iNOS modulated the PH elicited in broilers derived from the MP pedigree line, but not in broilers from the PAC pedigree line. Different NOS-mediated responses among broiler populations may affect pulmonary hemodynamic characteristics of broiler lines selected using i.v. microparticle injections.

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References