Experimental Study

Interaction between nitric oxide and prostanoids in the respiratory system

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Abstract

Background: **Prostaglandins and nitric oxide are important mediators of different physiological and pathophysiological processes. So far, is not characterized clearly their relationship in the conditions of airways hyperreactivity.**
Objectives: **We tried to detect the relationship of interaction NOS-COX in conditions of exogenous irritant-induced experimental bronchial hyperreactivity.**
Methods: **Male guinea pigs were used in the experiment. Animals received agent that inhibits COX activity – diclofenac in a dose of 10 mg/kg i.m. or direct NO donor – molsidomine in a dose of 2 mg/kg i.p. Agents were administered singly (10 days) or in combination (last 3 days). Then animals were exposed to the toluene vapours for two hours over each of three consecutive days to provoke hyperreactivity. Then we recorded the reactivity changes to cumulative doses of histamine or acetylcholine (10^{-8}–10^{-3} mol/l).**
Results: **The administration of NO donor (10 days) and consecutive COX inhibition (3 days) increased the reactivity of both observed preparations in comparison to agents administered single. COX inhibition during 10 days and consecutive treatment with NO donor (3 days) evoked different changes of tracheal smooth muscle and lung tissue smooth muscle response but had more beneficial effect on the airways reactivity on the whole.**
Conclusion: **It is possible to suppose some participation of both followed enzymatic systems and theirs interaction in our experimental conditions since airways reactivity was affected the by used agents *(Fig. 7, Ref. 32)*.**
Key words: airway hyperreactivity, nitric oxide, prostaglandins, interaction

Different systems of mediators are involved in the regulation of physiological functions of respiratory system as well as in pathophysiology of pulmonary diseases. So far, the relationship of two important systems, the system that participates in the production of prostanoids and system that produces nitric oxide (NO) has not been characterized clearly. Enzymes that participate in the biosynthesis of each of these significant mediators show certain common characteristics regardless of their dissimilarities and specificities. Nitric oxide is produced by NO synthases (NOS) from L-arginine, and prostaglandins (PGs) are produced from arachidonic acid by activity of cyclooxygenases (COX). Both NOS and COX enzymes occur in two forms. Constitutive isoforms (cNOS, COX-1) are situated in the quantity of cells and the products of their activities (NO and PGs) are important for the regulation of the physiological functions of different systems. In the majority of cases inducible isoforms (iNOS, COX-2) are responsible for the augmentation of pathophysiological processes and are activated, for example, by proinflammatory mediators (1, 2, 3).

Nitric oxide is produced by constitutive isoforms (nNOS – neuronal and eNOS – endothelial) and has different effects in the airways. It is a significant neurotransmitter of the inhibitory nonadrenergic noncholinergic nervous system; it causes bronchodilation and vasodilatation; it participates in the regulation of gas changes, blood flow, mucociliary transport, and surfactant production; and it represents important non-specific defence

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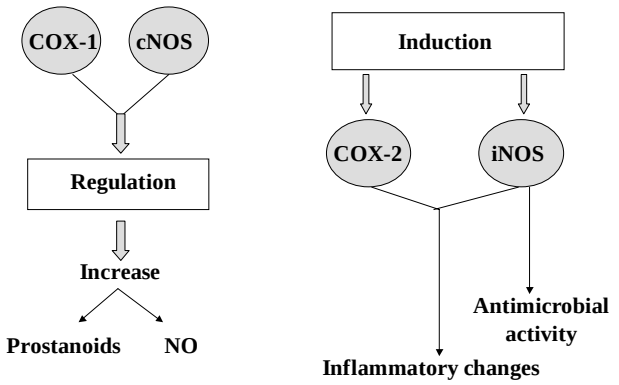

Fig. 1. Role of constitutive and inducible NOS and COX isoforms in organisms (Martin et al, 2001).

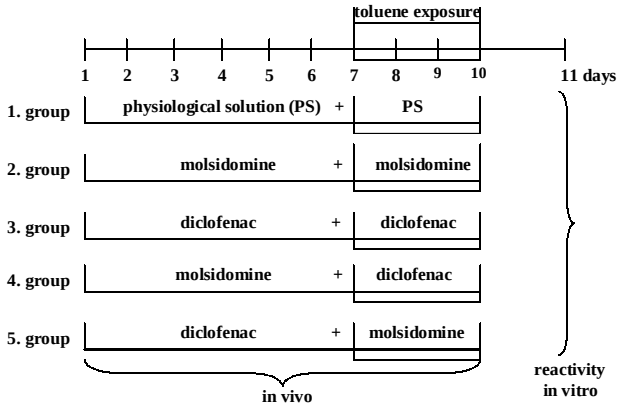

Fig. 2. Schedule of experiments.

mechanism in the airways. Different cells can be the source of NO – nervous, endothelial, epithelial, cells of vascular and bronchial smooth muscle as well as inflammatory cells. Increased amounts of NO produced by inducible NOS may contribute to development of various respiratory illnesses (asthma, chronic obstructive pulmonary disease, tuberculosis, tumours, virus infections etc (4, 5).

The lipid mediators, prostaglandins, are formed from membrane phospholipids by acting of two isoforms of cyclooxygenases – COX-1 and COX-2. Both isoforms are located in the endoplasmic reticulum of the cell but COX-2 is located also in the nuclear membrane of the cell, which determines the possible participation in the changes of gene expression. Prostaglandins produced by COX-1 have protective effects, they participate in the control of the bronchial and vascular smooth muscle tone, permeability of alveolar epithelium or surfactant homeostasis. The COX-2 induction evokes the production of PGs involved e.g. in the pathogenesis of respiratory inflammatory diseases (6, 7). The definitive localization of both COX isoforms was not exactly determined in the human respiratory system although these were identified in lung homogenates. The epithelium is regarded to be the most important source of cyclooxygenases and their products. PGE_2 is the dominant product of the epithelial layer that has significant inhibitory effect on the activation of inflammatory cells and contractility of airway smooth muscles.

Both PGs and NO are regarded to be paracrine modulators of the cells functions and they are frequently released simultaneously. They participate in the neurotransmission of intracellular signals via cyclic nucleotides – second messenger cAMP or cGMP. NOS as well as COX are hemoproteins with homodimer characteristics that show bifunctional catalytic activity. Both enzymes occur either constitutively (cNOS, COX-1) or they are induced (iNOS, COX-2) by different impulses. The constitutive isoforms are involved in the synthesis of two important products at least – NO a PGI_2 that affect synergically the regulation of the physiological functions of organism. Inducible isoforms are exprimed and cooperate, e.g. in inflammatory conditions (8, 9). There is interesting information on the PGs and NO dual effects (beneficial, deleterious) (Fig. 1). The results of studies regarding this problem are complicated and contradictory.

We tried to detect the link of NOS-COX in conditions of exogenous irritant-induced experimental bronchial hyperreactivity because it can be an important pathogenetic mechanism and may provided target for therapeutic measures. We used an indirect method based on the induction of PGs and NO level changes in the organism. We administered to animals an agent that inhibits its COX activity – diclofenac or a direct NO donor – molsidomine. Our motivation was also the fact that these groups of agents are used simultaneously in the clinical practice.

Material and methods

Animals and agents

Five groups of pathogen free male Trick guinea pigs (250–350 g) were used in our study. The animals were housed in individual cages in climate-controlled animal quarters and received water and food ad libitum. The pre-treatment with chosen agents lasts 10 days. Agents were administered single (10 days) or in combination (7±3 days). The treatment schedule is showed in Figure 2.

Group 1 (n=8) was a control group and inhaled toluene vapours for 3 consecutive days during 2 hour without pre-treatment agents. The control group received physiological solution.

Group 2 (n=8) received direct single NO donor – molsidomine (Corvaton inj. Hoechst Marion Roussel) in a dose of 2 mg/kg intraperitoneally (i.p.) during 10 days.

Group 3 (n=8) was treated with a COX inhibitor – single diclofenac (Veral inj. Slovakofarma) in a dose of 10 mg/kg intramuscularly (i.m.) during 10 days.

Group 4 (n=8) received for 10 days only molsidomine and during the last 3 days and 30 minutes before toluene exposure received molsidomine with diclofenac.

To *Group 5* (n=8) we administered during 10 days single diclofenac and during last 3 days 30 minutes before toluene exposure was administered a combination of diclofenac with molsidomine.

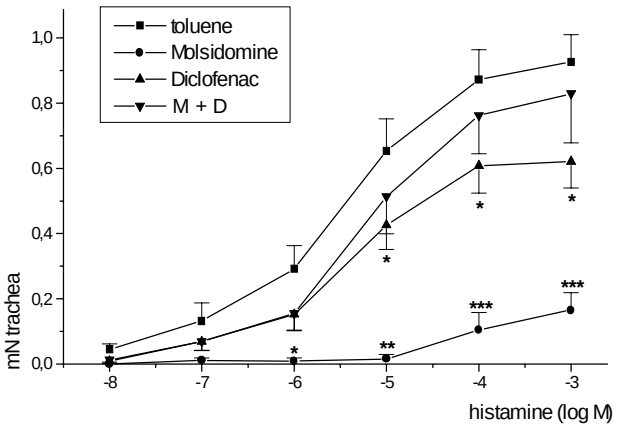

Fig. 3. Effect of the pretreatment with molsidomine – M (●), diclofenac – D (▲) or the combination of both agents – M + D (▼) on the tracheal smooth muscle reactivity to histamine after exposure to the toluene compared with toluene group (■). The curves represent the average values of the contraction amplitude with mean ±S.E.M. Axis x – the concentration of histamine in log M, axis y – the amplitude of contraction in mN. Significance p<0.05 – one asterisk, p<0.01 – two asterisks, p<0.001 – three asterisks.

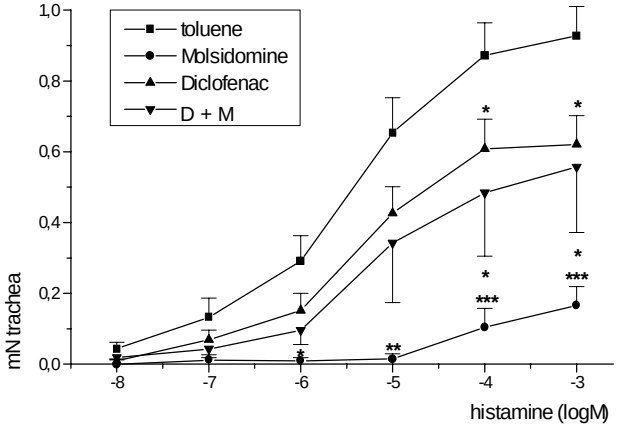

Fig. 5. Changes in tracheal smooth muscle reactivity to histamine after pretreatment with single molsidomine (●), single diclofenac (▲) and diclofenac + molsidomine – D + M (▼) compared with the toluene group (■). The curves represent average values of the contraction amplitude with mean±S.E.M. Axis x – the concentration of histamine in log M, axis y – the amplitude of contraction in mN. Significance p<0.05 – one asterisk, p<0.01 – two asterisks, p<0.001 – three asterisks.

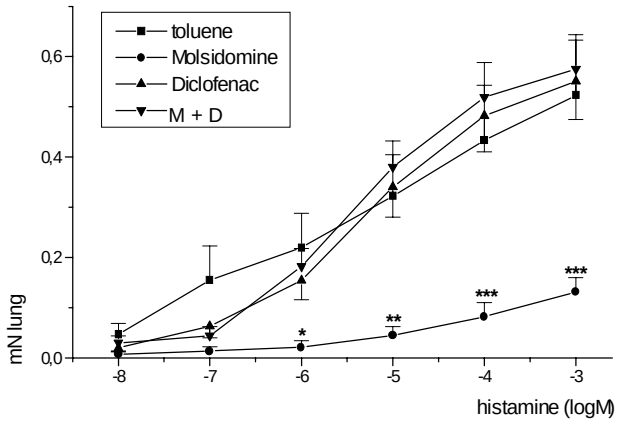

Fig. 4. Changes of lung tissue smooth muscle reactivity to histamine after pretreatment with single molsidomine (●), single diclofenac (▲) and molsidomine + diclofenac – M + D (▼) compared with the toluene group (■). The curves represent average values of the contraction amplitude with mean±S.E.M. Axis x – the concentration of histamine in log M, axis y – the amplitude of contraction in mN. Significance p<0.05 – one asterisk, p<0.01 – two asterisks, p<0.001 – three asterisks.

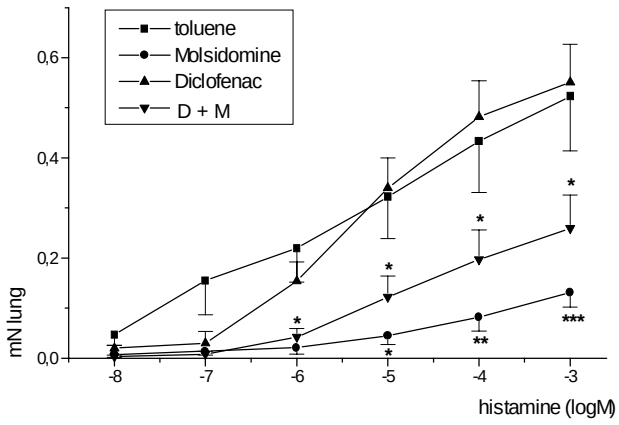

Fig. 6. Changes in lung tissue smooth muscle reactivity to histamine after pretreatment with single molsidomine (●), single diclofenac (▲) and diclofenac + molsidomine – D + M (▼) compared with the toluene group (■). The curves represent the average values of the contraction amplitude with mean±S.E.M. Axis x – the concentration of histamine in log M, axis y – the amplitude of contraction in mN. Significance p<0.05 – one asterisk, p<0.01 – two asterisks, p<0.001 – three asterisks.

Toluene exposure

Animals were exposed to the toluene vapours after pre-treatment with chosen agents or their combination. Toluene inhalation was carried out in a special exposure chamber made of Plexiglas. The chamber consisted of a compressor, flow-meter, vaporizer and exposure cage. The device was situated in the fume-cupboard at 22 °C. Toluene vapours were delivered to the cage with constant flow of 4 l/min. The average concentration of the toluene was 6 mg/l (1600 ppm). The duration of exposure was two hours in each of three consecutive days.

Airways responsiveness

Animals were killed 24 hours after the last toluene exposure. Strips from trachea and lung tissue were prepared and placed into organ bath with Krebs-Henseleit solution (110.0 mol/l NaCl, 4.8 mol/l KCl, 2.35 mol/l $CaCl_2$, 1.20 mol/l $MgSO_4$, 1.20 mol/l

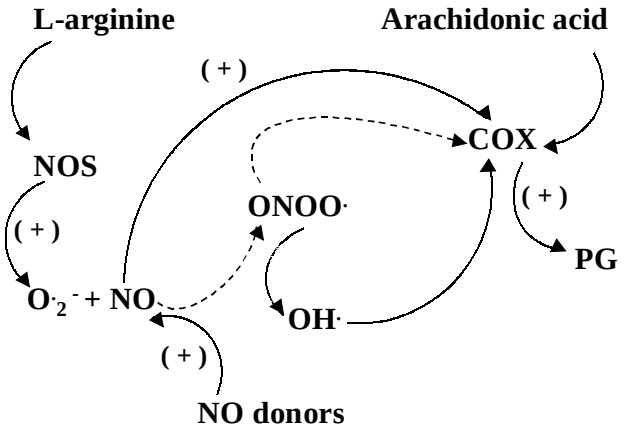

Fig. 7. Effects of nitric oxide and its metabolites on the production of prostaglandins (Martin et al, 2001).

$KHPO_4$, 25.0 mol/l $NaHCO_3$ and 4 g glucose in glass-distilled water). The solution was continuously aerated with mixture of 95 % O_2 and 5 % CO_2 at pH 7.5± 0.1 and temperature 36±0.5 °C. One of the strip endings was connected to a force transducer (TSR 10 G, Vývoj Martin, Slovakia) and an amplifier (M1101 SUPR, Mikrotechna Praha, Czech republic) and tension records were made on a Line Recorder TZ 4620 (Laboratorní přístroje Praha, Czech republic). The tissue strips were exposed initially to a tension of 4 g (30 minutes – loading phase). Thereafter, the tension was readjusted to a baseline of 2 g (30 minutes – adaptation phase). The Krebs-Henseleit solution was changed every 10 minutes. The strips were contracted by cumulative doses of histamine or acetylcholine (10^{-8}–10^{-3} mol/l). Statistical analysis was performed using ANOVA test. Differences were considered statistically significant when p-value was below 0.05. All results are expressed as mean±SEM.

The Ethical Committee of Jessenius Faculty of Medicine approved the study protocol.

Results

The effect of molsidomine (●), diclofenac (▲) or the combination of both agents (▼) on the reactivity of tracheal smooth muscle increased by toluene exposure is compared on the Figure 3. The toluene effect without pre-treatment with chosen drugs is showed by line (■). We observed most expressive changes after pre-treatment with single molsidomine (●) where the contraction amplitude decreased in all concentration of histamine. The pre-treatment with single diclofenac (▲) evoked also a statistically significant diminution of reactivity in the concentration of histamine 10^{-5}–10^{-3} mol/l. Simultaneous administration of NO donor (10 days) and COX inhibitor with donor (3 days during exposure) (▼) did not evoke significant difference in reactivity when comparing to animals inhaling irritant without agents pre-treatment.

The response of lung tissue smooth muscle to all used histamine concentration (Fig. 4) was again most expressive in animals receiving single molsidomine (●) for 10 days before toluene exposure in the comparison with control group (■). We did not observe statistically significant reactivity changes after pre-treatment with single diclofenac (▲) or molsidomine + diclofenac (▼).

We observed the decrease of tracheal smooth muscle reactivity to histamine after administration of the inverse combination (▼) – first COX inhibitor diclofenac (10 days) then molsidomine with diclofenac (3 days during toluene exposure) in comparison to control group (■). We recorded similar statistically significant decrease of reactivity after treatment with single diclofenac in concentration of 10^{-4}–10^{-3} mol/l histamine (▲). Single molsidomine induced the most expressive effect in concentration of 10^{-6}–10^{-3} mol/l histamine (●) (Fig. 5).

We also observed a more expressive effect in the lung tissue smooth muscle (Fig. 6) after administration of diclofenac (10 days) + molsidomine with diclofenac (3 days during toluene exposure) that provoked a decrease of reactivity to histamine in concentrations of 10^{-6}–10^{-3} mol/l in the comparison to single diclofenac (▲) or control group (■). Single molsidome (●) showed the most expressive effect.

Discussion

The interactions of enzymatic systems COX and NOS (Fig. 7) are interesting in different parts of the human organism (10, 11).

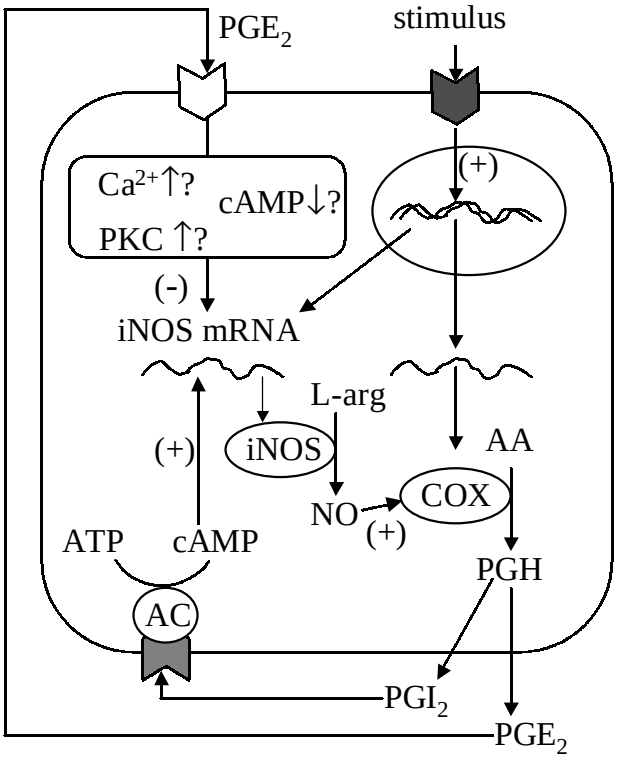

Fig. 8. Model for the interaction between COX and NOS pathways. AC – adenylate cyclase, PKC – protein kinase C, AA – arachidonic acid, L-arg – L-arginine (Tetsuka et al, 1994).

Although it is recognized that there is "cross-talk" between products of the NOS and COX pathways, until now the question of their reciprocal action in the respiratory tree in the physiological as well as pathological conditions has not been studied. There are not enough papers in the literature dealing with this problem (12, 13). It was the essence of our experiment for study of airways reactivity changes. We used agents involved to synthesis or modulation of the levels of NO and prostaglandins. We were interested in the modulating effect of an agent increasing NO levels in the organism (direct NO donor molsidomine) and agent blocking PGs synthesis (COX inhibitor diclofenac) in the airway hyperreactivity conditions. We presumed that hyperreactivity evoked by organic solvent via production of reactive metabolites (14) could be accentuated via inhibition of synthesis of protective prostaglandins. NO donor could compensate the reactivity changes.

The administration of single NO donor significantly decreased the tracheal and lung tissue smooth muscle reactivity to histamine in our experimental conditions. The treatment of animals with single diclofenac decreased the amplitude of contraction of the tracheal smooth muscle, too. However, lung tissue smooth muscle responded statistically with a non-significant increase in the amplitude of contraction to histamine concentrations of 10^{-4}–10^{-3} mol/l in animals receiving single diclofenac.

Our results show that administration of an NO donor (10 days) and consecutive COX inhibition (3 days) increased the reactivity of both observed preparations on the whole as compared to administered single agents. To the contrary, COX inhibition during 10 days and consecutive treatment with NO donor (last 3 days) evoked different changes in the tracheal smooth muscle and lung tissue smooth muscle response but had a more beneficial effect on the airway reactivity on the whole. This finding may be related to the effect of NO released from another source as epithelium and airway smooth muscle. It is necessary to speculate about participation of NO released from vascular endothelium in case of lung tissue (15).

It is known from the literature that there exists a relationship between nitric oxide and products of arachidonic acid metabolism – prostaglandins and so between enzymes that catalyse their production (16, 17). It results from some studies that the inhibition of NO synthases activity by prostaglandins can decrease NO synthesis and activity (18). The evidence for reciprocal interaction results from studies (19, 20) where COX inhibitors decreased in inflammatory conditions, not only PGE_2 levels but iNOS expression, too and prevents the induction of this enzyme (Fig. 8). Tanaka et al (21) discovered that COX-1 inhibition increases COX-2 expression. Prostaglandins produced by this enzyme may suppress detrimental manifestation of COX-1 inhibition e.g. overproduction of NO mediated iNOS, too.

COX products modulate iNOS activation probably by increasing cAMP level in the cells expriming iNOS (22). To the contrary, endogenous NO produced by iNOS activates COX and so increases prostaglandins production (10), but simultaneously inhibits lipooxygenase activity (23). It follows from this information that there is a possibility of reciprocal modulation of the reaction cascade catalysed by NOS and COX.

The change in the activity of one enzyme may change in a compensatory way the expression and/or activity of another enzyme. The decrease of PGs levels by the inhibition of COX-2 increases the NO synthesis. NO has a dual effect on the COX-2 activity in this process and this is dependent on the NO reactivity or on the presence of its reactive forms, mainly peroxynitrite. Low concentration NO and peroxynitrite stimulate PGs synthesis although high concentration of peroxynitrite inhibits COX-2 (24). PGE_2 show also such dual effects mediated by cAMP that may have stimulatory or inhibitory effect on iNOS activity and expression (Fig. 8). Lower cAMP levels stimulate iNOS expression but high cAMP levels inhibit the release of cytokines increasing iNOS expression (25, 26). The type of enzyme which is influenced by NO is also important. Clancy et al (27) reported that NO activates COX-1 but inhibits COX-2-derived prostaglandin production.

So far, there are no known mechanisms of the interaction of systems producing NO and PGs and the literature present possible alternatives to their reciprocal influence (28). The affect of NO on bivalent iron in the COX molecule is one possible mechanism (1, 29). The majority of NO mediated effects in any case result from its interaction with enzymes containing this element in the molecule. COX is one of these enzymes too, so COX may be the target for NO. The radical nature of this small molecule determining the production of its reactive metabolites (peroxynitrite) is regarded one of mechanisms of COX activation by nitric oxide. These reactive forms may subsequently nitrosylate free groups of cysteine. Since COX contains some of cysteine residue theirs nitrosylation has expressive effect on the COX activity. The modulation of transcription and posttranscription of COX genes by NO is another possible mechanism according to the type of cells and signal way modulated by NO (30). The stimulation of phospholipase A_2 activity quotes Hamilton a Warwer (1, 31) as one of possibilities of NO influence. The result of this affect is an increased release of arachidonic acid or an increase in its bioavailability. It was observed the differences in the NO effect on the PG production depending on the type of cell with different rates of PGs synthesis, on the manner of the activation of these cells and on the amount of COX and NOS proteins in the cell. The species differences are important, too. It is necessary to take all of these facts into consideration in the study of the NOS – COX interaction.

In conclusion, in our experiments we discovered the modulation of exogenous irritant-induced airway hyperreactivity by agents influencing the cascade of the reactions that participate in the production of nitric oxide and prostaglandins. It is possible to suppose some participation of both enzymatic systems (nitric oxide synthases and cyclooxygenases) and their interaction in our experimental conditions. Generally, NOS and COX enzymes may represent a regulatory step in the interaction between pro- and anti-inflammatory factors. This could present a new opportunity for therapeutic intervention.

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