EXPERIMENTAL STUDY

Nitric oxide and airway reactivity

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Abstract

Background: **Nitric oxide is a neurotransmitter of the inhibitory nonadrenergic noncholinergic mediation in the respiratory system. Its participation in the regulation of airways functions is determined by its level in the organism.**

Main purpose: **We examined participation of nitric oxide in the changes of the airway reactivity evoked by toluene exposure as the source of the free radicals. The changes of nitric oxide level in the organism were evoked by administration of its indirect donor isosorbide dinitrate. Thiol groups were provided by administration of antioxidative mucolytic N-acetylcysteine.**

Methods: **Used drugs — isosorbide dinitrate (5 mg/kg b.w.) and N-acetylcysteine (300 mg/kg b.w.) were administered intraperitoneally or by inhalation 30 minutes before each exposure to the toluene vapours. The control group was not treated with drugs. After toluene exposure (2 hours in each of 3 consecutive days) tracheal and lung strips smooth muscle reactivity to histamine was observed under in "in vitro" conditions.**

Results: **The administration of isosorbide dinitrate decreased especially the lung strip smooth muscle reactivity to histamine. We revealed more expressive effect of the pretreatment with intraperitoneally administered isosorbide dinitrate in the comparison with inhalation. Simultaneous pretreatment with N-acetylcysteine intensified beneficial effect of isosorbide dinitrate probably by increasing of the intracellullary level of thiols.**

Conclusion: **In our experimental conditions possible participation of nitric oxide in changes of airways smooth muscle reactivity after exposure to the toluene follows from results, as well as the importance of thiol groups for the activity of its indirect donors. (Fig. 6, Tab. 3, Ref. 35.)**

Key words: toluene, hyperreactivity, nitric oxide, isosorbide dinitrate, N-acetylcysteine.

In 1980 Furchgott a Zawadzki demonstrated that endogenous vasodilators — e.g. acetylcholine did not affect smooth muscle in the vessels directly but through diffusible factor released from endothelium. In the search of its chemical identity it was found out that the actions of this factor and the actions of nitric oxide (NO) were substantially similar (Ignarro et al., 1987). There is much information explainig the present position of NO in a human organism as a result of intensive studies (Barnes, 1996; Folkerts et al., 2000).

At the present time it is known that NO synthesis is catalysed by a family of nitric oxide synthases (NOS) which are localised in the cells of the smooth muscle, epithelium, nerves, endothelium, vessels and inflammatory tissue (tab. 1). The activity of a constitutive isoforms (neuronal — nNOS and endothelial — eNOS) depends on the intracellular calcium level and produce picomolar amounts of NO. Nitric oxide in this concentration is involved in the governing of the physiological regulatory mechanisms — neuronal transmission, neuroendocrine activity, blood pressure, congestion, adhesion, aggregation of the trombocytes, a tone of the airways smooth muscle, mucocilliary transport, vessel permeability etc.

Inducible calcium-independent form of this enzyme (iNOS) activated e.g. by inflammatory or other defence reactions of the organism produces NO in the nanomolar amounts. In these higher concentrations NO has the antimicrobial, antitumorous and cytotoxic effects connected with a reactive free radicals production (Singh and Evans, 1997; Bergendi et al., 1999). Increased amounts of NO produced by inducible isoform NOS contribute to the development of pulmonary pathological processes (Barnes and Belvisi, 1993).

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Tab. 1. Characteristics of NO synthase isoforms.

Type of enzyme	Ia nNOS	Ib eNOS	Ic cNOS	II iNOS	III eNOS	IV iNOS
	neurones	endothelium	neutrophiles	macrophages endothelium vascular smooth muscle	endothelium platelets	macrophages
Localization	cytosolic	cytosolic	cytosolic	fibroblasts cytosolic	membrane	membrane
Dependence on Ca^{2+}	+	+	+	−	+	−

The changes in the airway smooth musle tone and in the response to the different mediators are manifestations of the abnormality production and the activity of NO in the airways. It was demonstrated that exogenous NO administration or administration of NO releasing substances, possibly influencing the activity its synthetising enzymes, might contribute to the modification of the processes regulated by NO (Jörres, 2000).

We demonstrated the increase of the airway smooth musle reactivity in animals after their exposure to toluene in our previous experiments (Strapková et al., 1995, 1996). This organic solvent elevates free radicals production and evokes membrane phospholipids peroxidation (Mattia et al., 1991) with pathological changes in the respiratory tract. We were interested in the influence of NO releasing substances during these processes. We used isosorbide dinitrate (ISDN) as an indirect NO donor which was administered in two ways before toluene exposure. Mucolytic agent N-acetylcysteine (NAC) was administered simultaneously as a source of sulfhydryl groups as it is known that the presence of these groups is important for NO activity and for nitrates effects. Another reason for NAC utilisation was its antioxidant effect and the ability to affect positively on increased airway reactivity in exposed animals (Strapková et al., 1999).

Materials and methods

Male guinea-pigs weighing 300—450 g were used in the experiments. Used agents were administered thirty minutes before each exposure to the toluene. Isosorbide dinitrate (Isoket inj. Schwarz Pharma) was administered in a dose of 5 mg/kg body weight (b.w.) intraperitoneally (i.p.) or by inhalation. N-acetylcysteine (Broncholysin inj. Léčiva) was administered by inhalation in a dose of 300 mg/kg b.w simultaneously with intraperitoneal administration of isosorbide dinitrate to other group of animals. We used inhalator of the firm Pari designed for child patients (Pari Boy) to inhale used agents. The control group inhaled toluene vapours without the pretreatment with chosen drugs. The animals were exposed to the toluene vapours for 2 hours on each of 3 consecutive days. In "in vitro" conditions the response of tracheal and lung strips of smooth muscles to increasing histamine concentrations was observed during twenty-four hours after toluene exposure. The method of Bánovčin et al. (1986) was used. Method specifications of the toluene exposure and observing reactivity changes is introduced in paper Strapková et al. (1995). Statistical analysis was performed using Student *t*-test.

Results

The administration of isosorbide dinitrate (ISDN) in a dose of 5 mg/kg b.w. intraperitoneally before the exposure to toluene doesn't result in significant changes of the tracheal smooth muscle contraction amplitude. There is the tendency of the amplitude decrease in the histamine concentrations 10^{-4} and 10^{-3} mol/l, however (Fig. 1). ISDN showed more expressive effect on lung tissue smooth muscle (Fig. 2). The significant decrease of the contraction amplitude ($p<0.05$) was recorded even in the histamine concentration 10^{-5} mol/l.

A very similar picture of the tracheal smooth muscle reactivity changes was observed after aerosol ISDN pretreatment. Again, there is the tendency of the reactivity decrease in the highest histamine concentrations (Fig. 3). Lung tissue smooth muscle reactivity didn't change significantly contrary to i.p. administration (Fig. 4).

The administration of isosorbide dinitrate in a dose of 5 mg/kg b.w. intraperitoneally together with N-acetylcysteine in a dose of 300 mg/kg b.w. by inhalation half hour before toluene exposure didn't result in significant changes in the tracheal smooth muscle reactivity (Fig. 5). On the other hand simultaneous pretreatment with these agents led to significant decrease of the lung tissue smooth muscle reactivity (Fig. 6) even in the histamine concentration 10^{-5} mol/l ($p<0.001$)

The EC_{50} values (effective concentration of histamine leading to the decrease of the contraction amplitude to the half value in comparison with contraction values evoked by highest used histamine concentration) are in the Table 2. These values reflect the airway smooth muscle sensitivity to the used mediator of the bronchospasm.

The ratio of lung tissue wet weight and dry weight (desiccated 24 hours in 60° C temperature) can be seen in the Table 3. The acquired coeficient enables to assess the presence of increased fluid amount in the lungs. The higher coeficient values are found in toluene exposed animals whereas the pretreatment with used agents diminishes its values (except for NAC). Although they didn't reach the values of animals inhaling air, is possible to consider this effect to be positive.

Discussion

Nitric oxide is one of the most atypical neurotransmitters. Its "receptor" is iron at the guanylate cyclase active place. The relaxation of the smooth muscle is mediated by the activation of this
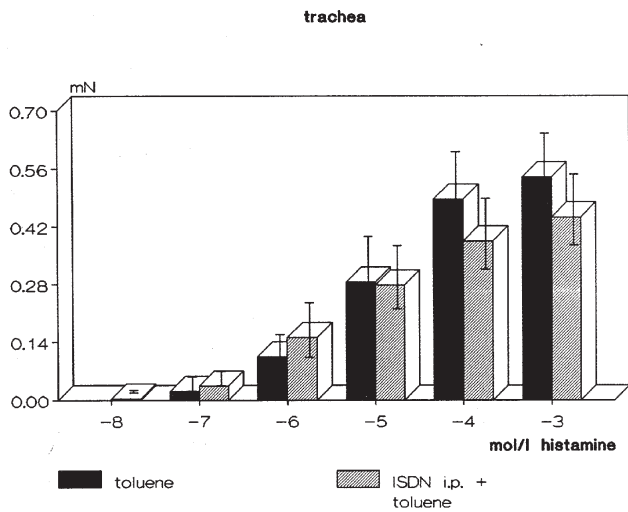
Fig. 1. The effect of the pretreatment with isosorbide dinitrate intraperitoneally (striped columns) on the tracheal strip smooth muscle reactivity to histamine after exposure to the toluene compared with toluene group (black columns). The columns represent average values of the contraction amplitude with mean average mistake ±S.E.M. Axis x — the concentration of the histamine in mol/l, axis y — the amplitude of the contraction in mN.

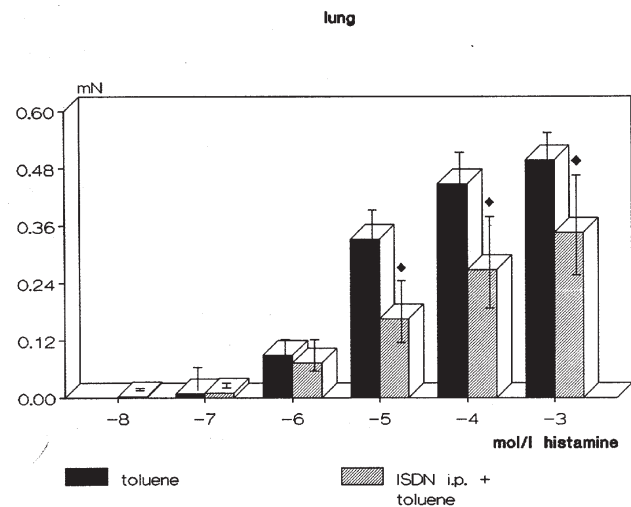
Fig. 2. The effect of the pretreatment with isosorbide dinitrate intraperitoneally (striped columns) on the lung strip smooth muscle reactivity to histamine after exposure to the toluene compared with toluene group (black columns). The columns represent average values of the contraction amplitude with mean average mistake ±S.E.M. Axis x — the concentration of the histamine in mol/l, axis y — the amplitude of the contraction in mN. Significance P<0.05 — marked with a dot.
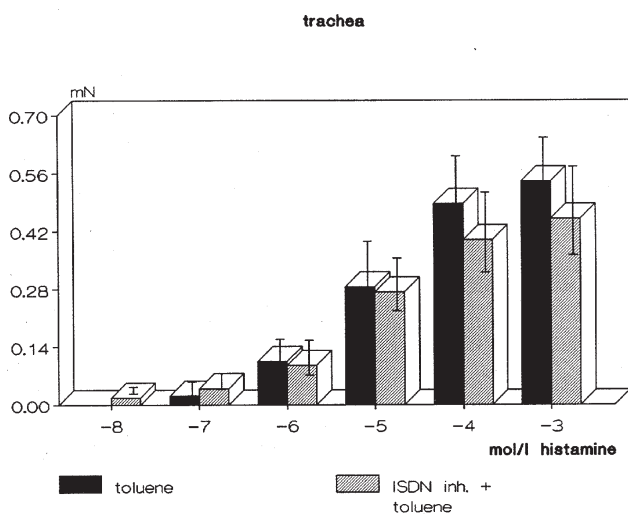

Fig. 3. The effect of the pretreatment with inhalation of the isosorbide dinitrate on the tracheal strip smooth muscle reactivity after the toluene exposure.
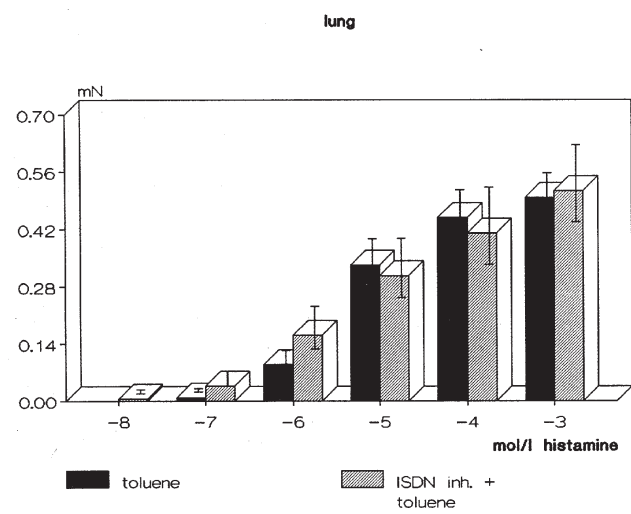

Fig. 4. The effect of the pretreatment with inhalation of the isosorbide dinitrate on the lung strip smooth muscle reactivity after the toluene exposure.

enzyme and by the following elevation of cGMP level. NO is considered a basic neurotransmitter of the inhibitory nonadrenergic noncholinergic neurotransmission (iNANC) in the airways. Nitric oxide acts as a vasodilator, contributes to the regulation of the pulmonary gases exchange, airway and pulmonary blood flow, mucocilliary transport in addition to bronchodilator activity and it is an important nonspecific defence mechanism in the airways (Barnes, 1996; Aizawa et al., 2000; Trifilieff, 2000).

Nitric oxide appears to act as a functional antagonist or "braking" mechanism of histamine and acetylcholine in the airway smooth muscle (Nijkamp and Folkerts, 1994; Schulling et al., 1998). This effect is manifested by bronchodilation in asthmatic patients after NO inhalation (Folkerts and Nijkamp, 1998; Taylor et al., 1998). Similarly a modification of the airway response is provoked by the administration of NO donors. Resulting effect depends on substances diversity which release NO, on variation
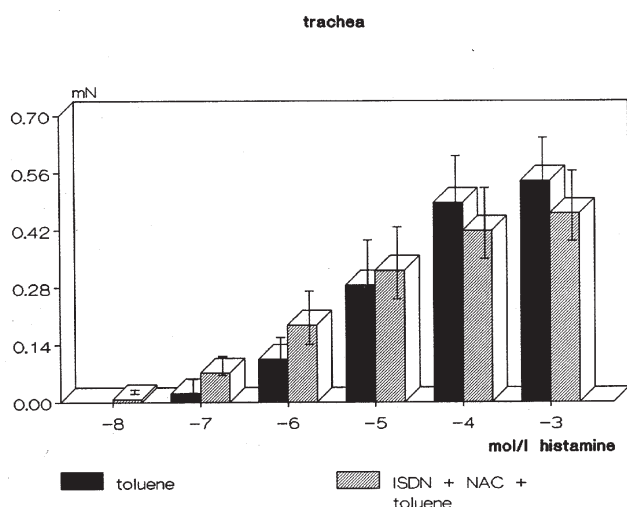
Fig. 5. The effect of the pretreatment with isosorbide dinitrate intraperitoneally and with inhalation of N-acetylcysteine on the tracheal strip smooth muscle reactivity after the toluene exposure.
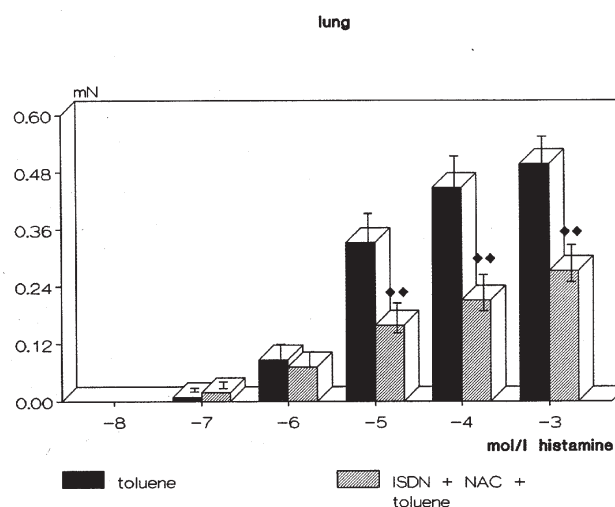

Fig. 6. The effect of the pretreatment with inhalation of the isosorbide dinitrate on the lung strip smooth muscle reactivity after the toluene exposure. Significance p<0.001 — two dots.

Tab. 2. EC50 values in the trachea and lung strip smooth muscle in animals inhaling air, toluene and toluene after pretreatment with isosorbide dinitrate and N-acetylcysteine.

Drug	EC_{50}	
	trachea	lung
air	1.14	2.29
toluene	1.43	0.98
ISDN i.p.+toluene	9.09	10.59
ISDN inh.+toluene	0.64	4.88
ISDN+NAC+toluene	1.17	1.34

Tab. 3. Ratio wet weight/dry weight of lung tissue in animals inhaling air, toluene and toluene after pretreatment with isosorbide dinitrate and N-acetylcysteine.

Drug	Wet weight
	Dry weight
air	3.81±0.18
toluene	5.56±0.46
ISDN i.p.+toluene	4.90±0.37
ISDN inh.+toluene	4.98±0.35
ISDN+NAC+toluene	5.33±0.28

of releasing speed and also on the possibility of releasing other free radicals (Marczin et al., 1997; Feelisch, 1998; Lamaz et al., 1998). The formation of a cytotoxic acting metabolits is a potential danger of NO action in an presence of oxygen or in the inflammatory cells activation. The most reactive metabolit is peroxynitrite that further decomposes to biologically destructive hydroxyl radical, which is toxic for basic substrates — nucleic acids, proteins, lipids and e.g. for surfactant, too (Butler et al., 1995).

We investigated the influence of the pretreatment with isosorbide dinitrate on the airways smooth muscles reactivity changes in animals exposed to the toluene vapours. It is known that exposure to this organic solvent elicits pathological changes in the mucous of the nose cavities, pharynx, larynx, causes irritation of upper airways, irritation resulting in the cough as well as the changes of the intensity and duration of voluntary cough (Sadloňová et al., 1986; Folkerts and Nijkamp, 1998). Under our conditions the exposure of animals to the toluene vapours in vivo caused an increase of the airways reactivity in vitro (Strapková et al., 1995). The administration of ISDN as NO indirect donor which is considered a potent lipids peroxidation inhibitor (Rubbo et al., 2000) resulted in the contraction amplitude fall in the tracheal and also in lung tissue smooth musle. The resulting effect depended on drug administration method. The pretreatment with isosorbide dinitrate administered intraperitoneally induced more expressive effect on the contraction amplitude than aerosol administration. It is possible that in the inhalation a local concentration of released NO is increased up to the levels exceeding protectively acting amounts. The activity of a exogenous and endogenous factors may contribute to this situation, too (Saleh et al., 1998). Under our conditions we can include in these factors such as a direct destructive action of the toluene on the delicate structure of the respiratory tract tissue, or a release of free radicals by activated inflammatory cells.

The effect of isosorbide dinitrate was on the whole more expressive on the lung tissue smooth muscle. This finding may also be determined by several factors. According to the literature NO modulates the response to histamine more in peripheral lungs than in the large airways (Dewachter et al., 1997). Neurons of iNANC neurotransmission are localized mainly in a deeper layers of the airways parenchyma and majority of NO is produced close to alveoli. It is probable that large airways are less sensitive to NO effects because tracheal smooth muscle manifests smaller increase of cGMP activity in comparison with lung tissue smooth muscle after NO substitution. According to the literature this fact is con-

nected more or less with different blood flow of tissue (Byrnes et al., 1997; Taylor et al., 1998).

Simultaneous administration of isosorbide dinitrate and N-acetylcysteine potentiated mainly a decrease of the lung tissue smooth muscle reactivity. A supposed reason of this finding can be found in the different activity of a natural antioxidant mechanisms on particular levels of the respiratory system, which can be changed after agent administration. Mucolytic N-acetylcysteine manifests antioxidant activity provoked by two mechanisms. First is the direct reduction of hydrogen peroxide, superoxid anion and hydroxyl radical to the less reactive forms. Second mechanism considered as prime is connected with a NAC deacetylation to cysteine, which is a precursor of the most important airways antioxidant system — glutathione redox system (Kelly et al., 1995). Thus NAC contributes to the inhibition of the cytotoxic peroxynitrite derivate production which supports lipid and sulfhydryl oxidation in the oxidants exposure conditions (Kanazawa et al., 1999). Besides that cysteine created by NAC biotransformation elevates glutathione level. This phenomenon was observed only in the patients with deficiency of this antioxidant substance after treatment with NAC, whereas more expressive effect was not recorded in individuals with its optimal level (Gosset et al., 1999). The formation of intermediate products which gradually release NO molecule and which are more stable, is another beneficial action of intracellular thiols increased levels (Feelisch, 1998).

There are different explanations concerning the position of NO in the regulation of vessel permeability changes as one of airways inflammation symptom. It is supposed that NO is involved in the process of the plasma exudation evoked mainly by activation of a neurokinin receptor (NK_1), less probably by activation of a histamine and leukotrienes receptors (Kageyama et al., 1997). The formation of a peroxynitrite radical causing impairment of the vessels and plasma leakage is another possible reason of increased permeability (Ohuchi, 1998).

We recorded edema development in all animal groups except for air inhaling group in our experimental conditions. Although coeficient values (expressing ratio wet and dry weight of the lung tissue determining a presence of the edema) slightly decreased in the animals pretreated with isosorbide dinitrate (intraperitoneally or by inhalation) they didn't reach the level reached in animals, which were not influenced by the oxidant or drugs. The ratio of wet and dry weight of the lung tissue in animals with simultaneous isosorbide dinitrate and N-acetylcysteine pretreatment was close to the value measured in the group exposed to the toluene vapour. Final effect may result from mucolytic action of NAC and from accumulation of the mucus in higher quantity in investigated tissue.

In our experiment the effect of isosorbide dinitrate manifested as a decrease mainly in lung tissue reactivity under the conditions of exogenous oxidant exposure — toluene. This fact is in compliance with some of literature information according to which NO modulates the response to the histamine more in peripheral regions than in the large airways. Simultaneous administration of N-acetylcysteine potentiated beneficial effect of isosorbide dinitrate probably by increasing of intracellular thiols level. The possibility of a nitric oxide participation in the airways changes reactivity after the exposure to toluene follows from achieved results

and importance of sulfhydryl groups for the activity of its indirect NO donors also in our experimental conditions.

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