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Lead Acetate Induces Epithelium-Dependent Contraction of Airway Smooth Muscle

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Abstract: The effect of lead acetate on tracheal smooth muscle (TSM) of dog pups was investigated in this study. In addition we studied the role of epithelium and involvement of nitric oxide (NO) in counteracting the effects of lead acetate on TSM as well as the modifying effects of lead acetate on contractile responses of TSM to acetylcholine (ACh) . Tracheal rings were excised and placed in *in vitro* organ baths. In vitro administration of lead acetate in increasing concentrations (10^{-7}–10^{-3} M) induced concentration-dependent contraction of TSM. Prior treatment of tissues with a single dose of lead acetate increased the contractile responses of TSM to acetylcholine (ACh) (10^{-10}–10^{-6} M). In additional tracheal rings the epithelium was denuded, then preparations were treated with lead acetate. Denudation of the epithelium resulted in an increase in lead acetate-induced contraction of TSM. Another set of tracheal rings were preincubated in bathing medium containing bradykinin (BK, 0.4 mM), to activate NOS. Presence of BK reduced contractile responses of TSM to lead acetate. Our data suggest that subacute exposure to lead, induces epithelium-dependent contraction of airway smooth muscle (ASM) probably via modulation of nitric oxide (NO) release.

Keywords: *airway smooth muscle, contraction, epithelium, lead acetate, nitric oxide, tracheal smooth muscle.*

Introduction

Lead is a toxic metal known to elicit pathophysiological effects in different organ systems. Earlier studies revealed that lead directly induces contraction of vascular smooth muscle (Watts et al., 1995; Tomera & Harakal, 1986; Valencia-Hernandez *et al*., 2001) and gastrointestinal system smooth muscle (Janin et al., 1985). Lead potentiates sensitivity of the anococcygeus muscle to contractile stimuli in rats (Shah *et al*. 1987) as well as aortic smooth muscle (Le-Feng et al., 2005; Heydari et al., 2006) of adult rats in response to different stimuli. Recent studies have shown that lead has inhibitory effects on relaxation of smooth muscle to vaso and bronchodilators (Le-Feng *et al*., 2007; Gupta & Fahim, 2007). There are some proposed and studied possible mechanisms of the action of lead. Chronic exposure to lead inhibits non-adrenergic non-cholinergic (NANC) relaxation in the rat gastric fundus probably via modulation of NO release from NANC nerves and/or by interacting with intracellular signaling mechanisms (Santos et al., 2006). Another publication speculates that lead-induced cytotoxicity in PC12 cells may be mediated by overproduction of NO (Sharifi *et al*., 2005). Lead also exhibits direct effects on smooth muscle of blood vessels, mediated by inhibition of the activity of the Na-K-ATPase and increases in intracellular Ca^{2+} levels as well as interacting with protein kinase C (Hwang et al., 2001; Kramer *et al*., 1986; Watts *et al*., 1995).

The airway epithelium has been implicated as a possible modulator of airway smooth muscle tension. It acts as a protective barrier for the underlying ASM to guard against noxious substances and it can release relaxant factors such as NO and prostaglandin E_2 (PGE_2), to counteract excessive contractions of ASM (Folkerts, 1995). NO is an important endogenous bronchodilator and is generated from the semi-essential aminoacid L-arginine by the enzyme NO synthase (NOS), of which three different isoforms have been identified in the airways. Constitutive NOS (cNOS) isoforms are mainly

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expressed in iNANC neurons (nNOS), endothelium (eNOS), and epithelium (nNOS and eNOS) of airways, whereas inducibile isoform of NOS (iNOS) induced by proinflamatory cytokines during airway inflammation, is mainly expressed in macrophages and epithelial cells (Ricciardolo, 2003).

Among heavy metals, lead should be stressed as pollutant in Kosovo, in Mitrovica Region, respectively. There is a study which has shown that blood lead level was found to be higher than permissible limits (Musliu *et a*l., 2008). It is known that majority of lead enters the body through inhalation (Rabinowitz *et al*., 1997) and children are the most affected because of their higher sensitivity to lead (Victery et al., 1988; Chamberlain et al. 1978). Little attention has been paid to the toxic effects that lead may have on airways. A recent study has shown a constrictive effect of lead in adult rats TSM (Gupta & Fahim, 2007). However, the effect of lead on ASM and the mechanisms of its action during postnatal maturation are not well understood. Therefore we have undertaken an *in vitro* study to investigate the effects of lead on TSM in a dog pup model and its role on the sensitivity of TSM to contractile stimuli. In addition we sought to show the role of epithelium and epithelium-derived relaxant molecules such as NO in modulating lead effects in the airway.

Materials and Method

Tissue preparation

Experiments were performed on the trachea obtained from 20 mongrel dog pups at age one month of either sex. This study was performed in accordance with the regulations and approval of Institutional Animal Care and Use Committee of the University of Prishtina. On the day of the experiment, animals were anesthesized by inhalation of diethyl-ether and euthanized with exusanguination. Trachea was removed and placed in ice-cold (4 ˚C) Krebs-Hensleit (KH) solution containing (in mM): 118.2 NaCl; 24.8 $NaHCO_3$; 4.6 KCl; 2.5 $CaCl_2$; 1.2 $MgSO_4$; 1.2 KH_2PO_4 and 10 % dextrose, pH=7.4. The trachea was cleaned of surrounding connective tissues and several tracheal rings were dissected from the middle part of trachea as described previously (Jakupaj et al., 1997). Only one tracheal ring was placed in an organ bath, filled with 20 mL KH solution, at 37 ˚C and continuously bubbled with mixture of 95% O_2 + 5% CO_2. Rings were mounted on a pair of stainless-steel hooks; one of which was fixed to an L-shaped rod inside the chamber and the other to the force displacement transducer (FORT 10 Transducer, World Precession Instruments, Inc). The output signals were recorded on four-channel recorder (Watanabe 6000, Germany). Tissues were allowed to equilibrate under a final force of 0.5 g for a period of 45 min, with bathing medium renewed every 15 min.

Experimental protocol

A cumulative dose-response curve was obtained by adding increasing concentrations of lead acetate ($2.6x10^{-7}$ - $2.6x10^{-3}$ M) at 5 min intervals, in an organ bath with tracheal ring (n=10). The selection of the concentrations of lead acetate we have chosen was based on preliminary studies where we found that the minimal dose to induce detectable response of tissue was $2.6x10^{-7}$ M, and the dose at which tissue response reached the plateau was $2.6x10^{-3}$ M. To test the effect of lead acetate on sensitivity of TSM to other bronchoconstrictive agents, tracheal rings were treated with cumulative doses of ACh ($5.5x10^{-10}$ – $5.5x10^{-6}$ M, n=9), after an incubation in a single dose of lead acetate ($2.6 x10^{-7}$) for 5 min.

In order to define the role of epithelium of TSM response to lead acetate, epithelium-denuded tracheal rings and those with intact epithelium were used (n=10 in each group). Both, epithelium-denuded and intact tracheal rings were obtained from the same animal. Epithelium-denuded tracheal rings were prepared by removing epithelium with gentle rubbing of the luminal surface of the trachea with cotton tipped applicators. After an equilibration period in KH solution a full dose response curve with lead acetate was obtained. To study the role of NO, additional tracheal rings (n = 10) were used. The preparations were treated with cumulative doses of lead acetate, then washed and stabilized for 30 min. After stabilization, tissues were incubated in the NOS stimulator bradykinin at 0.4 mM (the lowest dose which has shown effect on tissues' responses) for 15 min and then lead acetate dose response curve was repeated.

Statistics

Data are expressed as arithmetic mean ± SEM. For statistical analysis two-way ANOVA with repeated measurements was used. In all cases, $p < 0.05$ was considered statistically significant.

Results and Discussion

As shown in Figure 1, *in vitro* administration of lead acetate at increasing concentrations ($2.6x10^{-7}$–$2.6x10^{-3}$ M) induced concentration-dependent contraction of TSM of dog pups (Figure- 1). Lead has been shown to induce contraction of TSM of adult rats (Gupta & Fahim, 2007). Incubation of rat aorta segments in lead reduced relaxant responses to ACh (Le-Feng *et al.*, 2007). Inhibition of relaxation was also shown in lead-incubated rat' TSM response to isoproterenol (Gupta & Fahim, 2007).

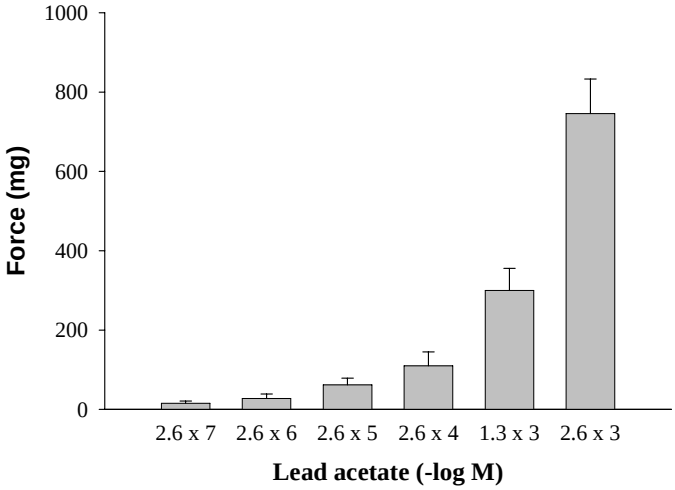

Figure 1. Lead acetate induces contraction of TSM in a dose-dependent fashion.

Figure- 2 illustrates the effect of lead acetate on TSM response to Ach. Incubation of tissues in a single dose of lead acetate ($2.6 x10^{-7}$ M) and then treatment with increasing concentrations of ACh ($5.5 x 10^{-10} – 5.5 x 10^{-6}$ M) resulted in significantly increased ($p<0.001$) contarctile responses of TSM to ACh when compared to control responses where ACh was administered alone (Figure- 2).

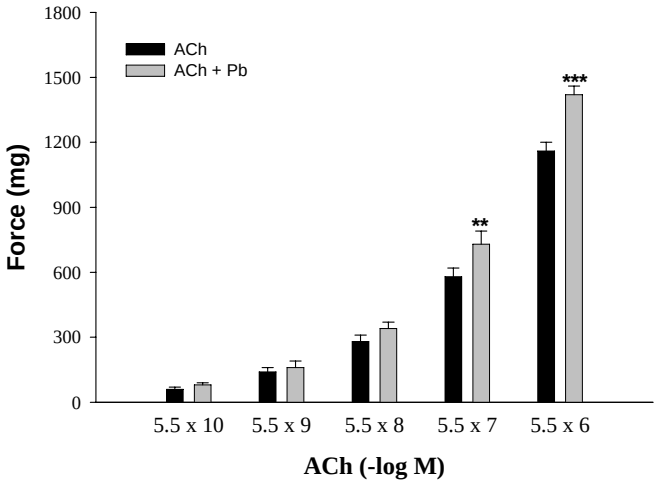

Figure 2. The effect of lead acetate on TSM responses to ACh. The presence of lead acetate ($2.6x10^{-7}$ M), increased TSM sensitivity to ACh (** $p<0.01$; *** $p<0.001$).

Shah et al., (1987) have shown that chronic exposure of adult rats to lead induced hypersensitivity of anococcygeus muscle in response to different agonists, such as noradrenaline, dopamine, carbachol and 5-hydroxytryptamine (5-HT). This increase in sensitivity is related to mechanisms of Ca^{2+} action (Shah et al. 1987). Enhanced contraction was also shown in rat aortic segments in response to vasoconstrictors (5-HT and norepinephrine) after incubation in lead at different duration (Le-Feng et al., 2005). This increased sensitivity to 5-HT might be because of an increase in the number of $5\text{-}HT_{2A}$ type receptors (Le-Feng *et al*., 2005).

It is known that lead binds sulfhydrile groups (SH) of proteins that are a constitutive component of muscarinic receptors, increasing the sensitivity to agonists of these receptors (Ryden & Walsh, 1987). Also it has been shown that lead interacts with Ca^{2+} and protein kinase C (Watts et al., 1995; Markovac et al., 1998) initiating intracellular mechanisms of contraction. In contrast to our findings, Gupta & Fahim (2007) have shown that incubation of rat tracheal preparations in lead reduced the constrictive responses to ACh at lower concentrations but not at higher concentrations (Gupta & Fahim, 2007). This difference between our findings and Gupta's might be attributed to the differences that exist between species. Therefore, more experiments are needed to better quantify and understand the mechanism of the effects of lead exposure at different ages among different species.

To explain the role of airway epithelium in responses of TSM to lead acetate exposure in our experiments we employed intact tracheal rings and those with epithelium-denuded. Denudation of epithelium significantly increased (p<0.001) contraction of TSM at higher concentration of lead acetate (1.3×10^{-3} and 2.6×10^{-3}) compared to responses of tracheal rings with intact epithelium (Figure 3). There are some contradictory data for the role of epithelium in modifying of the lead-induced contraction of ASM. Incubation of endothelium-denuded rat aortic segments in lead, attenuated the contractile responses to 5-HT (Le-Feng *et al*., 2005). Yet, epithelium-denudation of rat tracheal rings did not affect the contractile responses to lead (Gupta & Fahim, 2007). These differences between our results and others might be because of changes that the epithelium undergoes during maturation, in response to lead, as well as differences among the species. The mechanism by which the epithelial cells modulate airway responsiveness depends upon their ability to release relaxant factors which then counteract the bronchoconstriction induced by a variety of spasmogens (Nijkamp *et al*., 1993).

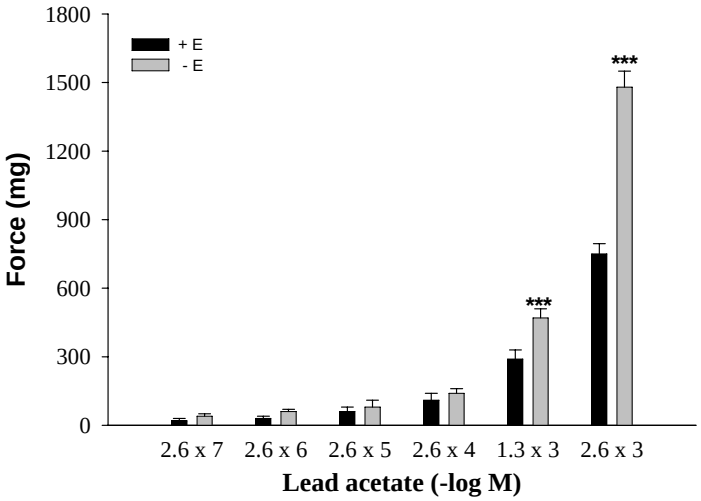

Figure 3. TSM responses to lead acetate obtained from tracheal rings with intact epithelium (+E) and epithelium-denuded (-E). Denudation of epithelium greatly increased TSM response to lead acetate (***p<0.001).

In order to study the role of NO in attenuating contractile responses of TSM to lead acetate, tissues were incubated in BK and then lead acetate was administered in cumulative fashion. Preincubation of tissues in BK significantly reduced (p<0.001) contractile responses of TSM (Figure-

4). Hypertension induced by chronic exposure to lead was associated with reduced NO and increased levels of reactive oxygen species (ROS) (Gonick *et al*., 1997; Vaziri *et al*., 1997). Chronic exposure of animals to lead also resulted in inhibition of NANC release of NO by the gastric fundus (Santos et al., 2006). Lead decreases the expression of soluble guanylate cyclase, an enzyme needed for cGMP production leading to NO-induced vasodilatation (Marques *et al*., 2001).

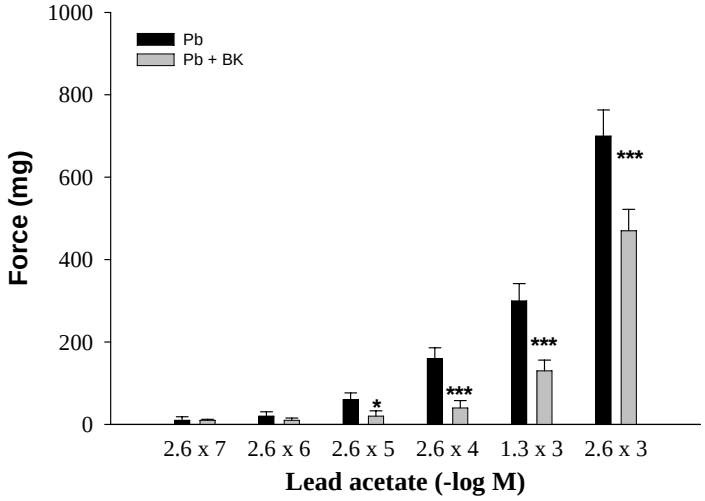

Figure 4. The effect of BK on attenuation of lead acetate-induced contraction of TSM. Incubation of tissues in BK significantly reduced contractile responses (* $p<0.05$; *** $p<0.001$).

Conclusions

In summary our results demonstrate that lead induces contraction directly in ASM and increases response to common bronchoconstrictors. For the first time our results have shown that airway epithelium plays an important role in attenuation of the constrictive effects of lead in airway smooth muscle via NO release as relaxant molecule. The role of Ca^{2+}, calcium influx through various channels, change in NOS expression in airways of animals exposed to lead and after lead-induced changes in ASM *in vitro* are beyond the scope of this work but bear further investigation.

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