PHARMACOLOGIC STUDIES OF LATEX DUST EXTRACTS IN ISOLATED GUINEA PIG TRACHEA

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Abstract

We studied the pharmacologic properties of water soluble extracts of latex dust (LDE) using isolated guinea pig trachea. Dose response relationships between LDE's and contractions of guinea pig tracheal smooth muscle were established. Latex dust was obtained from a plant manufacturing rubber gloves and other rubber products. Two extracts were made by standard antigen preparation methods. The first extract LD1 was prepared from native latex, and the second from a processed form of latex which was relatively free of soluble proteins (LD2). The effects of pre-treatment with mediator blocking agents (pyrilamine, atropine) arachidonic acid metabolite inhibitors (indomethacin, NDGA, acivicin) and an intracellular calcium antagonist (TMB8) were examined and compared to results with similar experiments using cotton bract extracts. The effect of pre-treatment with capsaicin a neuropeptide releasing agent was also assessed. LD1 induced contraction was inhibited by atropine as well as acivicin and NDGA. Similar findings were seen for LD2. Pretreatment with capsaicin did not enhance the effects of LD1 or 2. These studies suggest that water soluble extracts from the latex industry cause airway smooth muscle constriction via mediator release.

Introduction

Obstructive airway diseases in workers exposed to organic aerosols have been recognized since the eighteenth century when Ramazzini described diseases of workers who processed hemp and flax. Substances of plant origin such as wood products have been reported as a cause of airway disease in industrial workers (1,2). Occupational asthma due to latex has been reported in 2.5% of hospital employees by Vandenplast et al. (3). It has been demonstrated that water-soluble protein allergens from latex gloves may act as aeroallergens causing rhinitis and asthma (4-6). Jaeger et al. (7) reported that the powder of latex gloves are potent aeroallergens which can cause severe immediate-type allergic reactions of the upper and lower respiratory tract, particularly rhinitis and asthma. Marcos et al. (8) suggested that latex present in rubber gloves acts as inhalant allergen producing occupational asthma in exposed subjects, probably by means of an IgE-mediated mechanism. Chronic upper respiratory symptoms are common in latex workers. Kanny et al. (9) reported a case of rhinitis and asthma-type dyspnea in a female cook who used rubber gloves at work. Similarly, Marcos et al. (8) described asthma and rhinitis in a nurse due to the use of latex surgical gloves. Allmers et al. (10) reported that in health-care workers the first symptoms of an immediate-type sensitization to latex (urticaria and allergic symptoms of the lower respiratory tract) were noticed after an average of 5 years. Tarlo et al. (11) described acute spirometric changes during the work shift associated with a fall of 15% or greater in FEV1 accompanied by symptoms of occupational asthma in workers employed in a latex surgical glove manufacturing plant. Methacholine challenge in these workers indicated airway hyperresponsiveness. Pisati et al. (12) reported that nebulization of nonpowdered latex glove extract induced immediate bronchoconstriction. The authors suggested that airborne powder from latex gloves can be an occupational hazard. Latex, absorbed onto cornstarch powder in latex gloves and then aerosolized when the gloves are handled, is felt to be the causative agent of the respiratory events described. The purpose of the current report is to further characterize acute respiratory effects of latex dust in an in vitro system using guinea pig tracheal rings.

Methods

The contractile response to latex dust extracts was studied in isolated trachea from male Hartley-Albino guinea pigs. Guinea pigs were sacrificed by CO2 narcosis. Tracheas were trimmed of fat and connective tissue. Four 4 to 6 mm rings were cut and suspended between two L-shaped stainless steel hooks mounted in 20 ml organ baths containing Kreb's buffer. The buffer in each bath was maintained at 37°C and continuously aerated with 5% CO2 in oxygen. Tracheal rings were initially set at 2 grams tension and were allowed to relax for about 2 hours before experimentation. During this time, the tissue was washed with Kreb's buffer every 30 minutes. Isometric contractions were measured with Grass FT103C force displacement transducers attached to a Grass polygraph recorder. A total of 12 organ baths were connected by transducers to a 12 channel recorder.

Latex dust extracts were prepared from dust collected in a rubber processing plant that manufactured rubber gloves and other products (LD1). A second dust extract was prepared from the native latex which was processed so as to partially remove water soluble proteins (LD2). This plant had been previously surveyed for respiratory findings in a small community in Croatia.

Latex dust extract was prepared in a weight to volume ratio of 1:10 by the standard method of Sheldon to the preparation of antigens.

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Dose dependent contraction of tracheal smooth muscle was consistently shown for both latex dust extracts (LDE). LDE was added in amounts of 10,30,100,300,1000 ul to the organ bath. The tension developed by the smooth muscle was normalized for different tissues by relating the LDE-induced contraction of individual tracheal rings to the baseline maximal contraction of these rings by carbachol 10^{-5} molar. In each experiment the responsiveness to maximal carbachol stimulation with 10^{-5} molar was initially established. This was followed by washing, reestablishing the baseline, followed by a dose response reaction.

In a typical drug experiment the tissue was washed and baseline reestablished after an initial contraction with carbachol. A specific blocking agent or a control solution was then added to the organ bath and incubated with the tissue for 20 minutes. An LDE dose response was then performed. After the dose response the tissue was again washed and carbachol 10^{-5} was used to verify the viability of the tissue.

Additional experiments were performed to assess the role of endogenous neuropeptides in this contractile response. A set of replicate experiments using four rings from a single GP was done. The first ring was treated with LDE in a dose dependant fashion, the second tissue was contracted with LDE following contraction with capsaicin (5 μM), a third tissue was contracted with LDE after 2 consecutive challenges with capsaicin, and in the fourth tissue capsaicin was added after paper dust.

Results

A total of 36 guinea pigs underwent dose response studies with progressively increasing doses of LDE (10,30,100,300, 1000ul). The response characteristics of the dose response curve included an Emax of 94 $\pm$ 5% for LD1 and 79 $\pm$ 6% for LD2 expressed as a percent (of baseline maximal carbachol response). An Emax of 42 $\pm$ 9% for CBE was documented in our previous studies (13). Comparison of Emax seen with individual blocking agents against their matched controls are detailed in Table 1. Significant attenuation of LD1 response was seen for blocking agents with the exception of pyrilamine and idomethacin. LD2 was attenuated by the same agents. Pretreatment of the tissue with capsaicin did not significantly enhance the response of tracheal contraction to LDE. Capsaicin alone induced a transient contraction of guinea pig trachea which did not occur after a second challenge with capsaicin. This suggests that at the concentration used, capsaicin resulted in complete release of capsaicin sensitive mediators. Treatment with LD1 or 2 did not result in an attenuated capsaicin response indicating that LDE's effect did not result in a significant release of endogenous neuropeptide mediators.

Discussion

These pharmacologic studies of LDE on guinea pig tracheal smooth muscle imply a complex effect of this airway irritant. These initial investigations suggest that mediators (e.g. cholinergic and leukotriene) may be involved in this effect. The suppression of constriction by calcium blockers may simply reflect the reliance of this response on intracellular calcium mobilization. The absence of an LDE effect on capsaicin indicates that neuromediators are not primarily involved. In comparison to similar studies with CBE and WDE (see Table 2) it would appear that LDE induces constriction by its own unique pattern of mediator release.

Conclusions

1. Latex dust extracts with and without soluble protein cause dose dependant constriction of guinea pig trachea.
2. Both LD1 and 2 are inhibited by similar mediator blocking agents.
3. Treatment of guinea pig trachea by latex dust extracts does not deplete neuromediators released by capsaicin.
4. Pretreatment with capsaicin does not enhance the effects of LD1 and LD2.
5. These studies indicate that extracts of organic industrial products cause a non-specific release of airway mediators unrelated to pre-sensitization. The origin of these mediators is, as yet, not well defined.

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Table 1. Summary data of Emax values (expressed as a percentage of the control induced Emax) obtained for latex dust extract 1 and 2 under different pretreatment conditions.

Emax	A	P	I	Ac	N	TMB8	C	C+C
Latex dust 1	11**	63	58	57**	65**	84**	100	97
Latex dust 2	3**	70	95	32**	44*	55	147	109

Statistical comparison are with untreated, paired controls using the paired t-test. Each drug experiment has its own control.
*p<0.05; **p<0.01
A=atropine 10-6M; P=pyrilamine 10-6M; I=indomethacin 10-6M; Ac=Acividin 10-5M;N=NDGA 10-5M; TMB8 10-5M; C=capsaicin 5x10-6M; C+C=capsaicin[x2]5x10-6M

Table 2. Comparisons of Pharmacologic Agents of the Dose-Response Characteristics of Two Textile Extracts

	CBE	WOOL	LD1	LD2
Pyrilamine	+	+/-	-	-
Atropine	+	-	+	+
Indomethacin	X	-	-	-
BW 755 C	X	X		
LY 171883	+	X		
Acividin			+	+
NDGA			+	+
Verapmil	+			
TMB8		+	+	-

= no effect
+ = attenuation
X = attenuation at low concentrations of extract, enhancement at high concentrations