PHARMACOLOGIC STUDIES OF PAPER DUST EXTRACTS IN ISOLATED GUINEA PIG TRACHEA

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Abstract

We studied the pharmacologic properties of water soluble extracts of paper dust (PDE) using isolated guinea pig trachea. Dose response relationships between PDE's and contractions of guinea pig tracheal smooth muscle were established. Paper dust was obtained from separate sites in a paper re-cycling mill, early in the process (PD1) and late in the process (PD2). The effects of pre-treatment with mediator blockers (pyrilamine, atropine) arachidonic acid metabolite inhibitors (indomethacin, NDGA, LY 171883) 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. PD1 induced contraction was partially inhibited by all the mediator inhibiting agents as well as the receptor blocking agents with the exception of LY 171883. Similar findings were seen for PD-2 except that pyrilamine and indomethacin failed to block the bronchoconstrictor effect. Pretreatment with capsaicin enhanced the effects of PD-1 and 2. These studies suggest that water soluble extracts from the paper re-cycling 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). Thoren et al (3-5) and Deprez (6) reported that employment in paper mills was found to be associated with an increased risk of bronchial asthma and chronic obstructive pulmonary disease. Ericsson et al (7) reported a dose-dependent increase of upper respiratory symptoms in paper workers. Thoren et al (8) described significantly increased symptom prevalences in paper workers from both upper and lower airways as well as asthma but with no lung function impairment. Sigsgaard et al (9) described respiratory and mucosal symptoms (particularly chest tightness, itching of the nose, throat and eyes) among garbage handling and recycling workers suggesting an effect of this work environment on the respiratory system. In a study by Sigsgaard et al (10) recycling workers in a paper production mill had a fall of FEV1 over the work shift which was significantly associated with exposure to organic dust. Heederik et al (11) reported lower lung function tests with positive immediate intradermal reactions in workers exposed to soft-paper dust. Jarvholm et al (12) studied workers exposed to heavy concentrations of paper dust and found increased lung elastic recoil pressure, increased residual volume and a significant increase in symptoms of the lower respiratory tract. The purpose of the current report is to further characterize the respiratory effects of paper dusts in an in vitro system using guinea pig tracheal rings.

Methods

The contractile response to paper 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.

Paper dust extracts were prepared from dust collected at two sites in a paper recycling mill previously studied for respiratory findings in a small community in Croatia. The paper recycling industry manufactures different types of paper. The paper production process creates the potential for exposure to organic dust, particularly paper dust and talc, as well as to other respiratory irritants (chlorine gas, sulfur dioxide, chlorine dioxide, ammonia, caustic soda). Recycling begins with the classification of waste paper which is sorted by hand. Paper is then treated mechanically in hot water under alkaline conditions. After a number of screening and washing operations, the fiber is ready for reuse. Most of the material requires special chemical cleaning processes. Various chemicals (e.g. sodium hydroxide, alkaline soda, neutral-sulfite, sodium sulfite, sodium sulfide, hydrogen sulfide, chlorine gas, sulfur dioxide) and air are added in a specially designed flotation cell. by using chlorine dioxide, pulps can be bleached in several stages to a high brightness. Harmful exposures to the workers also include offensive mercaptan odors, talc dust, caustic lime dust, etc. After these treatments the paper is sprayed on the paper machines.

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The paper sheets are conveyed to a huge heated cylinder and scraped off. This procedure as well as hand sorting are particularly dusty. The paper is then rolled onto rolls which are divided and doubled in special re-rolling machines and cut. In between the mechanical process and fully cooked chemical pulps varying amounts of different chemicals are added. The two paper dusts collected for this study came from 2 different sections of the paper factory. Paper dust 1 (PD1) was collected early in the process before washing and bleaching of the recycled paper occurred. Paper dust 2 (PD2) was collected after the bleaching process. Paper dust extract was prepared in a weight to volume ratio of 1:10 by the standard method of Sheldon to the preparation of antigens. Measurements of the endotoxin content of the dust was performed Limulus lysote test.

Dose dependent contraction of tracheal smooth muscle was consistently shown for both paper dust extracts (PDE). PDE 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 PDE-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. A PDE 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 PDE in a dose dependant fashion, the second tissue was contracted with PDE following contraction with capsaicin (5 μM), a third tissue was contracted with PDE 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 PDE (10,30,100,300, 1000ul). The response characteristics of the dose response curve included an Emax of 97.1 $\pm$ 5.3% for PD1 and 112.8 $\pm$ 5.6% for PD2 (of baseline maximal carbachol response compared to 42.3 $\pm$ 9.3% for CBE in our previous studies (11). Comparison of Emax seen with individual blocking agents against their matched controls are detailed in Table 1. Significant attenuation of PD1 was seen for all blocking agents with the exception of LY 171883. PD2 was attenuated by all agents except pyrilamine and indomethacin. Pretreatment of the tissue with capsaicin enhanced the response of tracheal contraction to PDE. 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 PD1 or 2 did not result in an attenuated capsaicin response indicating that PDE's effect did not result in a significant release of endogenous neuropeptide mediators. The endotoxin levels for PD1 and PD2 are displayed in Table 3 (13).

Discussion

These pharmacologic studies of PDE on guinea pig tracheal smooth muscle suggest a complex effect of this airway irritant. These initial investigations suggest that many mediators (e.g. histamine, prostaglandin and leukotrienes) 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 a PDE 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 PDE induced constriction in its own unique pattern of mediator release.

Conclusions

1. Paper dust extracts from different processing areas in the paper recycling industry cause dose dependant constriction of guinea pig trachea.

2. Bronchoconstriction associated with paper dust from the early phase of the processing (PD1) is inhibited by the anticholinergic agent atropine, the antihistamine pyrilamine, the arachidonic acid mediator cascade inhibitors, indomethacin and NDGA as well as the calcium channel blocker TMB8.

3. Bronchoconstriction associated with paper dust extract from the later phase of the recycling process is attenuated by atropine, TMB-8, NDGA, LY 171883, but not by pyrilamine or indomethacin.

4. Treatment of guinea pig trachea by paper dust extracts does not deplete neuromediators released by capsaicin.

5. Pretreatment with capsaicin enhances the effects of PD1 and PD2.

6. These studies indicate that extracts of organic industrial products cause a non-specific release of airway mediators. The origin of these mediators is, as yet, not well defined.

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References

1. Brooks SM, Weiss MA, Bernstein IL (1985). Reactive airways dysfunction syndrome (RADS). Persistent asthma syndrome after high level irritant exposures. Chest 88:376-384.

2. Rylander R. (1994). Organic dusts- from knowledge to prevention. Scand J Work Environ Health 20:116-22.

3. Thoren K, Jarvholm B, Morgan U. (1989). Mortality from asthma and chronic obstructive pulmonary disease among workers in a soft paper mill; case referent study. Br J Industr Med 46:192-195.

4. Thoren K, Jarvholm B, Sallsten G, Gunnar G. (1994). Respiratory symptoms and asthma among workers exposed to paper dust: a cohort study. Am J Ind Med 26:489-496.

5. Thoren K, Sallsten G, Jarvholm B. (1991). Mortality from asthma, chronic obstructive pulmonary disease, respiratory system cancer, and stomach cancer among paper mill workers: a case referent study. Am J Industr Med 19:729-737.

6. Deprez RD, Oliver C, Halteman W. (1986). Variations in respiratory disease morbidity among pulp and paper mill town residents. J Occup Med 28:486-491.

7. Ericsson J, Jarvholm B, Norin F. (1988). Respiratory symptoms and lung function following exposure in workers exposed to soft paper tissue dust. Int Arch Occup Environ Health 60:341-345.

8. Thoren K, Sallsten G, Bake B, Drake U, Jarvholm B, Sahle W (1989a). Lung function and respiratory symptoms among workers in a soft paper mill. Int Arch Occup Environ Health 61:467-471.

9. Sigsgaard T, Abel A, Donbaek L, Malmros P. (1994). Lung function changes among recycling workers exposed to organic dust. Am J Industr Med 25:69-72.

10. Sigsgaard T, Malmros P, Nersting L, Petersen C. (1994a). Respiratory disorders and atopy in Danish refuse workers. Am J Respir Crit Care Med 149:1407-12.

11. Heederik D, Burdorf L, Boleij J, Willems H, Bilsen J. (1987). Pulmonary function and intradermal tests in workers exposed to soft-paper dust. Am J Industr Med II:637-645.

12. Jarvholm B, Thoren K, Brolin I, Ericsson J, Morgan U, Tylen U, Bake B (1988). Lung function in workers exposed to soft paper dust. Am J Ind Med 14:457-464.

13. Schachter EN, Zuskin E, Mustajbegovic J, Buck MG, Maayani S, Marom Z, Goswami SK, Rienzi N. Pharmacologic studies of wool dust extract in isolated guinea pig trachea. Proc Cotton Dust Research Conference (99-) pp.

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

Emax	A	P	I	L	N	TMB8	C	C+C
Paper dust 1	21.2**	68.5**	49.3**	77.2	62.5*	72.9**	146.8**	141.8**
Paper dust 2	2.9**	89.0	82.8	62.3**	77.4**	53.1**	93.6	140.4**

Statistical comparison are with untreated, paired controls using the paired t-test

*p<0.05; **p<0.01

A=atropine 10-6M; P=pyrilamine 10-6M; I=indomethacin 10-6M; L=LY171883 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	PD1	PD2
Pyrilamine	+	+/-	+	-
Atropine	+	-	+	+
Indomethacin	X	-	+	-
BW 755 C	X	X		
LY 171883	+	X	-	+
NDGA			+	+
Verapmil		+		
TMB8		+	+	+

- = no effect

+ = attenuation

X = attenuation at low concentrations of extract, enhancement at high concentrations

Table 3. Endotoxin Results for Paper Dust Extract

Sample	Description	Mg Dust Used	EU/Ml	EU/Mg Dust*
Paper Dust I	initial	21	85.98	40.94
Paper Dust II	end	33	543.75	164.77

*Calculated a (EU/ml) (10 fold dilution factor)/mg dust used