CHARACTERIZATION OF ORGANIC DUSTS FROM THE TOBACCO INDUSTRY

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Abstract

We have previously used guinea pig tracheal rings to analyze the effect of cotton and other organic dusts on airway smooth muscle (ASM) contraction. In a recent epidemiologic study we documented that workers exposed to dust in the tobacco industry developed a high frequency of respiratory symptoms and ventilatory changes similar to those of textile workers. Water soluble extract of dust obtained from samples of tobacco leaves and associated dust were prepared as a 1:10 w/v solution by the method of Sheldon. Dose related contractions of nonsensitized GPT were demonstrated using the extracts. Pharmacologic studies were performed with indomethacin, pyrilamine, atropine, NDGA, acivicin, BPB, captopril and capsaicin. We also analyzed the contractile properties of different tobacco extract fractionated by molecular weight and lipid content. We suggest that tobacco extracts cause ASM contraction by non-immunological mechanisms involving mediators.

Introduction

Tobacco processing workers are exposed to aerosols of organic compounds. Previous experience with workers in this industry indicate that they are at risk for developing respiratory problems. Respiratory disorders among tobacco workers have been reported by many authors. As early as 1948, McCormick et al (1) reported that because of the character of the manufacturing operations in the tobacco industry, certain potential health hazards exist for tobacco workers. Several investigators (2-5) found significant decreases in FVC and FEV1 as well as constriction of smaller airways. The duration of exposure to dust in the tobacco industry did not appear to be a factor.

York et al (6) demonstrated that cigarette tobacco extract affected the O2 consumption of pulmonary macrophages. Tobacco extract produced a biphasic effect on macrophage respiration: a stimulatiuon at low concentrations and an inhibition at high concentrations. Bernal-Madrazo et al (7) studied leukocyte migration inhibition factor in healthy individuals using an antigen of tobacco dust and found that this immunogen is capable of sensitizing a high proportion of the healthy population both smokers and non-smokers. Gleich and Welsh (8) showed that extracts of green tobacco leaf and cured tobacco leaf contain antigens that stimulate antibody formation in laboratory animals. The antigens in these extracts were acid proteins. Experimental studies with tobacco extract demonstrated that application of the extract on rat lip mucosa caused irritational hyperplasia which was related to the level of irritation. it was greatest in those rats subjected to the longest period of application of the tobacco extract with the greatest concentration of tar (9)

The purpose of the current report is to further characterize acute respiratory effects of tobacco dust in an in vitro system using guinea pig tracheal rings.

Methods

The contractile response to tobacco 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.

Tobacco dust extracts were prepared from dust and leaves collected on machines in a tobacco processing indusrtry located in Zagreb, Croatia. This plant had been previously surveyed for respiratory findings in workers.
Tobacco dust extract was prepared in a weight to volume ratio of 1:10 by the standard method of Sheldon to the preparation of antigens.

Dose dependent contraction of tracheal smooth muscle was consistently shown for tobacco dust extracts (TDE). TDE 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

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was then added to the organ bath and incubated with the tissue for 20 minutes. An TDE 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 TDE in a dose dependant fashion, the second tissue was contracted with TDE following contraction with capsaicin (5 μM), a third tissue was contracted with TDE after 2 consecutive challenges with capsaicin, and in the fourth tissue capsaicin was added after TDE.

Additionally the TDE was separated into tw fractions, one greater than 10 Kd and one less. These extracts were further sepsarated in two fractions one in which the lipid content was removed, and the other in which the lipid content was preserved. The separation was performed using butanol-di-isopropyl ether 40:60 v/v by the method of Cham and Knowles (10). Protein was determined by the method of Lowry (1951).

Endotoxin was measured using the limulus lysate method.

Results

A total of 21 guinea pigs underwent dose response studies with progressively increasing doses of TDE (10,30,100,300, 1000ul). The response characteristics of the dose response curve included an Emax of101 $\pm$ 3% for TDE and an EC50 of 84 $\pm$ 10%. An Emax of 42 $\pm$ 9% for CBE was documented 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 TDE response was seen for blocking agents with the exception of pyrilamine. Pretreatment of the tissue with capsaicin did significantly reduce the response of tracheal contraction to TDE. 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.

Dose response studies with the high molecular weight TDE (>10Kd) resulted in significant attenuation of the TDE response. The low molecular weight extract retained its activity (see Table 2). Removal of the lipid content of the erxtract had no effect on TDE's contractile respinse but the lipid fraction was inert (see Table 2).

Analysis of the TDE fior endotoxin showed that the extract had large quantities of bacterial contamination (Table 3).

Discussion

These pharmacologic studies of TDE 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 presence of a capsaicin effect on TDE indicates that neuromediators are possibly involved. In comparison to similar studies with CBE and WDE (see Table 2) it would appear that TDE induces constriction by its own unique pattern of mediator release.

Conclusions

1. Tobacco dust extract causes dose dependant constriction of guinea pig trachea.
2. TDE is inhibited by a large number of mediator blocking agents.
3. Treatment of guinea pig trachea by capsaicin reduces the contractile potency of TDE suggesting the involvement of neuromediators.
4. The active agant of TDE appears to reside in the non-lipid low molecular weight fraction of this agent.
5. Tobacco dust extracts are heavily contaminated by bacterial endotoxin.

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Table 1. Summary data of Emax values (expressed as a percentage of the control induced Emax) obtained for Tobacco Dust Extract under different pretreatment conditions.

Emax	A	P	I	Ac	N	TMB8	BPB	C
TDE	0**	74	53*	57*	59*	77*	64*	55*

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 BPB=Bromophenacyl Bromide 10-5M

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

	CBE	WOOL	TDE
Pyrilamine	+	+/-	-
Atropine	0	-	0
Indomethacin	X	-	0
BW 755 C	X	X	
LY 171883	0	X	
Acividin			0
NDGA			0
Verapmil	0		
TMB8		0	0

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

Table 3. Endotoxin content of the TDE samples (2 separate measurements).

LAB #	Mg Dust	EU/ml	EU/mg
1	213	111,328	5227
2	217	107,422	4950