PHARMACOLOGIC STUDIES OF COCOA AND FLOUR DUST EXTRACTS IN ISOLATED GUINEA PIG TRACHEA

E. Schachter, E. Zuskin, M.G. Buck, S. Maayani, S.K. Goswami, N. Rienzi, Vincent Castranova, Michael Whitmer, Paul Siegel, Nawreen Satar and Arnab Das
Mount Sinai Medical Center
New York, NY
and the National Institute Occupational Safety and Health Morgantown, WV

Abstract

We studied the pharmacologic properties of water soluble extracts of confectionery dusts (Cocoa and Flour) using isolated guinea pig trachea. Dose response relationships between confectionery dusts and contractions of guinea pig tracheal smooth muscle were established. Cocoa and rye flour dusts were obtained from a plant manufacturing confectionery products. The two extracts were made by standard antigen preparation methods. The effects of pre-treatment with mediator blocking agents (pyrilamine, atropine) arachidonic acid metabolite inhibitors (indomethacin, NDGA, acivicin), the phospholipase A2 blocking agent pBPB and an intracellular calcium antagonist (TMB8) were examined and compared to results with similar experiments using cotton bract and wool extracts. The effect of pre-treatment with capsaicin a neuropeptide releasing agent was also assessed. Cocoa dust extract induced contraction was inhibited by atropine as well as acivicin, pyrilamine, indomethacin, pBPB, NDGA and TMB*. Similar findings were seen for rye flour extract. Pretreatment with capsaicin reduced the effects of both extracts. These studies suggest that water soluble extracts from the confectionery 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). Work in the confectionery industry involves the use of different food products. In particular, confectioners are often exposed to the dust of flour and cocoa as well as other food products. Houba et al (3) reported that asthma and other respiratory symptoms in bakery workers caused by exposure to wheat proteins are important occupational health problems. Hartman et al (4) studied 314 workers in an industrial bakery and found that 74% of the bakers complained of respiratory allergy to flour. Bonahada et al (5) showed that despite exposure to relatively low concentrations of respirable flour dust, bakers are at risk for developing both respiratory symptoms and airway hyper responsiveness. Matsmura et al reported atopic mechanisms in bakers contribute to wheat flower sensitization. in a recent study of 93 confectionery workers studied in Zagreb Croatia, Zuskin et al (6) found high prevalences of chronic respiratory symptoms in exposed workers and a prevalence of 5% for occupational asthma. Significant across shift lung function changes were documented in these workers as were high prevalences of positive skin tests: cocoa (60%), flour (26%) and serum IgE levels: cocoa (17.5%) and flour (18.7%). Bronchoprovocation testing demonstrated significant decreases in lung function following aerosol challenge with cocoa and flour extracts in workers with symptoms and large across shift changes in lung function. Pretreatment with cromolyn sodium protected workers against these lung function changes. The purpose of the current report is to further characterize acute respiratory effects of confectionery dust in an in vitro system using guinea pig tracheal rings.

Methods

The contractile response to cocoa and flour dust extracts was studied in isolated tracheas harvested from young 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 37ºC 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.

Cocoa and rye flour dust extracts were prepared from dust collected in a confectionery plant that manufactured confectionery products (cakes, cookies, chocolate milk powder etc.). This plant had been previously surveyed for respiratory findings in Zagreb, Croatia.

Cocoa and rye flour dust extracts were 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 both confectionery dust extracts (CDE). CDE 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 CDE-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

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:235-236 (1998)
National Cotton Council, Memphis TN

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 CDE 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 CDE in a dose dependant fashion, the second tissue was contracted with CDE following contraction with capsaicin (5 uM), a third tissue was contracted with CDE after 2 consecutive challenges with capsaicin, and in the fourth tissue capsaicin was added after paper dust.

The amount of endotoxin in the dust extracts was determined by the limulus lysate assay (7).

Results

A total of 36 guinea pigs underwent dose response studies with progressively increasing doses of CDE (10,30,100,300, 1000ul). The response characteristics of the dose response curve included an Emax of 119.8$\pm$ 1% for cocoa and 102.2 $\pm$ 2.2% for rye flour extract expressed as a percent (of baseline maximal carbachol response). An Emax of 42 $\pm$ 9% for CBE was documented in our previous studies (8). Comparison of Emax seen with individual blocking agents against their matched controls are detailed in Table 1. Significant attenuation of the cocoa response was seen for blocking agents with the exception of acivicin. Rye flour extract was attenuated by the same agents. Pretreatment of the tissue with capsaicin significantly reduced the response of tracheal contraction to cocoa and rye flour extracts. 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. These findings suggest that confectionery dusts exert some of their bronchoconstrictor effect through the release neuropeptides sensitive to capsaicin.

The results of the endotoxin analysis are detailed in Table 2. These indicate low values for cocoa and moderately low values for rye flour.

Discussion

These pharmacologic studies of confectionery dust extract on guinea pig tracheal smooth muscle imply a complex effect of these airway irritants. 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 finding that capsaicin pretreatment reduces the CDE effect suggests that neuromediators may be involved in this process. In comparison to similar studies with CBE and WDE (see Table 3) it would appear that rye and cocoa dust extracts induce constriction by their own unique pattern of mediator release.

Conclusions

1. Confectionery dust extracts cause dose dependant constriction of guinea pig trachea.

2. Both cocoa dust extract and rye flower dust extract are inhibited by similar mediator blocking agents.

3. Pretreatment with capsaicin reduces the effects of confectionery dust extracts.

4. 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.

References

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

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

Houba R, Van-Run P, Heederick D, Doekes G(1996). Wheat antigen exposure assessment for epidemiologic studies in bakeries using personal dust sampling and inhibition ELISA. Clin Exp Allerg 42:192-5.

Hartman AL, Wutrich B, Deflorin-Stolz R, Helfenstein U, Hewitt B, Guerin B (1985): Atopic-screening:Prick-multitest, gesamt-IgE oder Rast. Schweiz Med Wochen 115:466-75.

Bohadana SJ, Massin N, Kolopp MN, Tosmain JPJ(1994):Respiratory symptoms and airway responsiveness in apparently healthy workers exposed to fl;our dust. Europ Respir J 7:1070-7.

Zuskin E, Bozica K, Schachter EN, Godnic-Cvar J, Mustajbegovic J, Budak A: Respiratiory function and immunological status in cocoa and flour processing workers. Amer J Industr med (in press).

Olenchock SA (1990): Endotoxins. In Morey PR, Freeley JC< Otten JA (eds). Biological contaminants in Indoor

Environments. American Society for testing and Materials, Philadelphia. pp 190-200.

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. Env Res 1995; 69:90-95.

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	Cap	BPB
Cocoa dust	9*	65	52	67**	72 *	34**	56	64	74
Flour dust	11**	71	59	83**	64*	54	61	51	36

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= 10-5M; Cap=Captopril 10-5M.

Table 2. Endotoxin Results for Cocoa and Rye Flour Dust Extracts

Sample	EU/Mg Dust*
Rye Flour	181.95
Cocoa	46.21

* Typical endotoxin values from naturally occuring organic dusts:

Cotton Dust	1,000-2,000 EU/mg
Chopped Hay	4,000 EU/mg
Silage	55 EU/mg
Wood Compost	110 EU/mg
Nylon Fibers	11 EU/mg

Table 3. Comparisons of Pharmacologic Agents of the Dose-Response Characteristics of Two Textile Extracts

	CBE	WOOL	COCOA	FLOUR
Pyrilamine	+	+/-	+	+
Atropine	+	-	+	+
Indomethacin	X	-	+	+
BW 755 C	X	X		
LY 171883	+	X		
Acividin			-	-
NDGA			+	+
Verapamil			+	
TMB8		+	+	+
Capsaicin			+	+
Captopril			+	+

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