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Pharmacologic Characterization of Wool Dust Extract in Isolated Guinea Pig Trachea[1]

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Wool mill workers develop respiratory symptoms and lung function abnormalities associated with their work in the textile industry. As in other workplaces, which process organic materials, the dust generated in the manufacture of wool has been implicated as a cause of these respiratory problems. Pharmacologic studies of wool dust extract were performed *in vitro* on guinea pig tracheal (GPT) segments. A wool dust extract (WDE) was prepared from material collected from a mill previously surveyed. When the standardized WDE solution was added to an organ bath in increments of 10, 30, 100, 300, and 1000 μl it caused a consistent, dose-dependent constriction of GPT. Pretreatment of guinea pig tracheas, prior to WDE challenge, with atropine (10^{-6} M), pyrilamine (10^{-6} M), indomethacin (10^{-6} M), verapamil (10^{-6} M), TMB8 (10^{-6} M), BW755C (10^{-6} M), and LY171883 (10^{-6} M) was studied in order to evaluate receptor-dependent and -independent characteristics of WDE-induced constriction. WDE-induced bronchoconstriction was partially inhibited by the antihistamine pyrilamine. Atropine and leukotriene inhibitors (LY171883 and BW755C) were not found to have a significant protective effect on WDE-induced constriction. Both TMB8 and verapamil (intra- and extracellular calcium blocking agents) suppressed the effect of wool dust extract in the range tested. These findings suggest that in this model, WDE-induced airway constriction is only partly attributable to common mediators of bronchoconstriction (e.g., histamine). The airway effects of WDE may be modulated by calcium channel blocking agents.

INTRODUCTION

Obstructive airway disease in textile workers has been recognized since the eighteenth century when Ramazzini (1713) described diseases in workers who processed hemp and flax. The major syndrome associated with the respiratory system in these workers is byssinosis. Epidemiological studies have identified a high frequency of respiratory abnormalities among wool mill and carpet factory employees (Moll, 1933; Alardice *et al.*, 1983; Zuskin *et al.*, 1976; Love *et al.*, 1987a,b, 1988). Sigsgaard *et al.* (1992) found that the changes in lung function were greatest among atopic wool and cotton workers in comparison to other textile workers. The mechanism inducing airway disease in these workers is unknown. Clinical similarities between the respiratory effects of wool and cotton dust have been recently established by Ozesmi *et al.* (1987) who documented a 22% prevalence of symptoms similar to byssinosis among 303 carpet weavers exposed to wool dust. As with cotton bract extract (Cooper *et al.*, 1986), exposure of laboratory animals to wool dust has been shown to result in inflammation of the airways (Donaldson *et al.*, 1988). *In vitro* studies with hemp and cotton dust extracts (Zuskin *et al.*, 1992a,b) have demonstrated that these materials induce a dose-dependent constriction of airways.

The purpose of the current study is to characterize the airway effects of wool dust in an *in vitro* system using guinea pig tracheal rings and to compare these studies with results previously obtained with cotton bract extract in the same model (Schachter *et al.*, 1988).

METHODS

Wood Dust Extract Preparation

Wool dust was collected from carding machines in a wool mill in Zabok, Croatia where textile workers

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had been studied as part of an epidemiologic survey of respiratory health. The WDE was prepared by incubating wool dust (obtained from the workplace) in sterile distilled water (Abbott Laboratories, North Chicago, IL) in a weight to volume ratio of 1:10 (1 g of wool dust in 10 ml of sterile water) for 24 hr at +4°C. The solution was filtered through sterile gauze and centrifuged at 16,000 rpm for 60 min and the supernatant solution was decanted. The solution of extract thus prepared served as our stock and was stored at +4°C for subsequent use.

Protein and Endotoxin Determination

The protein content of the wool dust extract was determined by Lowry's method (Lowry *et al.*, 1951). The endotoxin content of the wool dust extract was assayed by the limulus test (Sigma, E-TOXATE concentrate Limulus Amebocyte Lysate kit No. 210-A) for detection and semiquantitation of endotoxin.

Guinea Pig Trachea Preparation

We used the tracheas of young albino Hartley male guinea pigs (300–390 g) purchased from Charles River Labs (Wilmington, MA). The animals were sacrificed by CO_2 asphyxiation for 5 min and the tracheas were removed within 3 min of sacrifice. The animal tissues were manually trimmed to remove connective and other tissues. Four segments ("rings" each 4–6 mm wide) were cut from a single trachea, and each was suspended between two L-shaped stainless steel hooks mounted in a 20-ml organ chamber containing Krebs–Hanseliet buffer of the following composition (μM): NaCl, 110.0 KCl, 4.80; $CaCl_2$, 2.35 $MgSO_4$, 1.20; $KHPO_4$, 1.20; $NaHCO_3$, 25.0; dextrose, 110.0; and Na_2 EDTA, 0.03, in glass-distilled water. Organ chambers were maintained at 36.5 ± 0.5°C and were continuously aerated with 95% O_2 and 5% CO_2 to maintain pH = 7.5 ± 0.1. The tissue segments were initially set to 2 g of tension and were allowed to stabilize for approximately 1.5 hr before the experiment began. During that period the tissue was washed at 15-min intervals. After the relaxation period, the tension in each tissue segment was readjusted to 2 g for all subsequent assays. Isometric contractions were recorded using a Grass FTO3C force displacement transducer attached to a Grass polygraph recorder. Before the contraction–response assay with WDE was performed, a challenge with 10^{-5} M carbachol was run. A dose–response curve with wool extract was obtained by adding increasing volumes of extract or Krebs (used as a control) into the tissue bath in progressive aliquots of 10, 30, 100, 300, and 1000 μl. The potency of the extract was determined by comparing its biological activity with the maximal contraction induced by carbachol (10^{-5} M) on the same tissue. The data were expressed as a percentage of the initial maximal carbachol contraction. In each experiment the responsiveness to maximal carbachol stimulation (10^{-5} M) was initially established. This was followed by washing, reestablishment of the baseline, and subsequently a dose–response challenge.

Steady-State Characterization of the WDE Dose–Response Curve

After equilibration, each tissue segment was maximally contracted with carbachol (10 μM). This response was measured in grams of tension and designated as the maximal carbachol response for that tissue (100%). All subsequent contractions of the segment were normalized to this maximal carbachol response and expressed as a percentage of maximal carbachol-induced contraction. Isometric contractions induced by WDE were measured to the sequential cumulative dose increments administered in ½-log unit dose steps. Concentration–response curves were constituted using the RS-1 software on an IBM-XT. Data points were fit by iteration to the logistic function

$$E = E_{max}/(1 + (EC_{50}/[A]^n),$$

where E is the observed muscle tension (grams above baseline), $[A]$ is the concentration of the agonist, EC_{50} is $[A]$ eliciting one-half of the maximal response, and n is the slope of the curve.

Statistical Methods

Mean values were compared between controls and drug-treated tissue using matched tracheal rings, by the paired t test–Statview (Brain Power Inc., Calabasas, CA).

Drug Treatment Protocol

In a typical drug experiment the tissue was washed and baseline reestablished after an initial contraction with 10^{-5} M carbachol demonstrated tissue viability and established maximal contractile tension. A specific blocking agent (or a control solution) was then added to the organ bath and incubated with the tissue for 20 min. A wool dust extract dose–response challenge was then performed. After the dose response the tissue was again washed and 10^{-5} M carbachol was added to verify the viability of the tissue. In the drug experiments, different drugs were added to the organ bath such as 10^{-6} M atropine ($N = 8$), 10^{-6} M pyrilamine ($N = 8$), 10^{-6} M indo-

methacin ($N = 7$), 10^{-6} M verapamil ($N = 8$), 10^{-6} M TMB8 [3,4,5-trimethoxybenzoic acid–8-(diethylamino) actyl ester] ($N = 8$), 10^{-6} M BW755C ($N = 8$), and 10^{-5} M LY171883 ($N = 8$).

RESULTS

Dose–response curves to wool dust extract were measured in 30 guinea pig tracheal rings (each obtained from a separate animal) and expressed as a percentage of maximal carbachol contraction. The mean response is shown in Fig. 1. The interrupted line connecting the mean values was extrapolated from the data using the equation described under Methods. WDE produces a dose–response curve similar to that seen with cotton bract extract (Zuskin *et al.*, 1992a) and hemp dust extract (Zuskin *et al.*, 1992b). The response parameters of the wood dose–response curve included an E_{max} of 57.9 ± 2.3% (of baseline maximal carbachol response) compared to 42.3 ± 9.3% for cotton bract extract in our previous studies (Schachter *et al.*, 1988). The EC_{50} for wool was 48 ± 6.5 μl and the slope of the curve was 0.915 ± 0.01.

The dose–response curves with pretreatment by each drug and their matched controls are presented for pyrilamine (10^{-6} M) in Fig. 2, for atropine (10^{-6} M) in Fig. 3, for indomethacin (10^{-6} M) in Fig. 4, for LY171883 (10^{-5} M) in Fig. 5, for BW755C (10^{-6} M) in Fig. 6, for verapamil (10^{-6} M) in Fig. 7, and for TMB8 (10^{-6} M) in Fig. 8. As can be appreciated, arachidonic acid metabolite inhibitors LY171883 and BW755C and the prostaglandin synthesis inhibitor indomethacin as well as atropine had no significant effect on the constriction induced by WDE. Pyrilamine partially blocked the effect of WDE at the highest WDE doses. Verapamil and TMB8 completely blocked the response to WDE in the range of WDE concentrations tested. The E_{max} seen with individual blocking agents, compared to their matched controls, are detailed in Table 1.

The pharmacologic pattern of response to specific antagonists appears to distinguish the effect of wool dust extract and cotton bract extract on airway smooth muscle. Table 2 presents a comparison of pharmacologic agents used in this model with WDE and cotton bract extract (CBE). As can be appreciated CBE-induced bronchoconstriction was significantly inhibited by a number of receptor and mediator blocking agents while WDE in general was not.

The protein content of this wool preparation was found to be 9.0 mg/ml. Measurement of endotoxin content revealed that endotoxin was essentially absent from this preparation (<0.015 EU/ml.

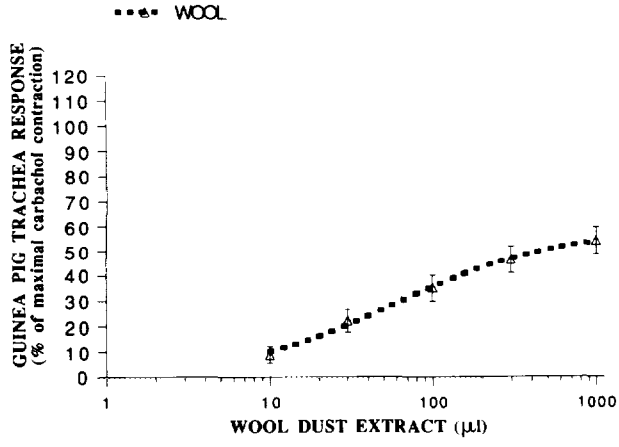

FIG. 1. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle as percentage of 10^{-5} M carbachol contraction.

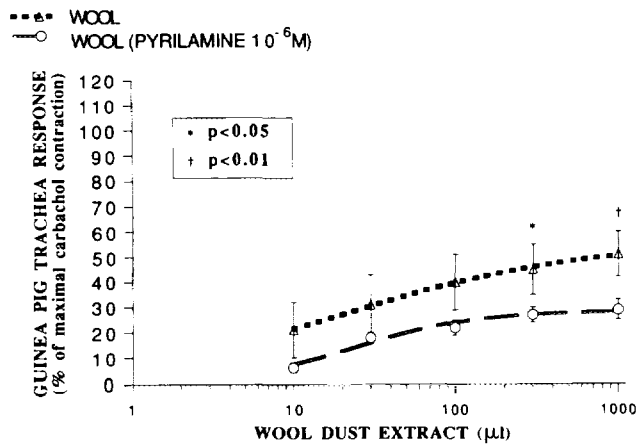

FIG. 2. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-6} M pyrilamine.

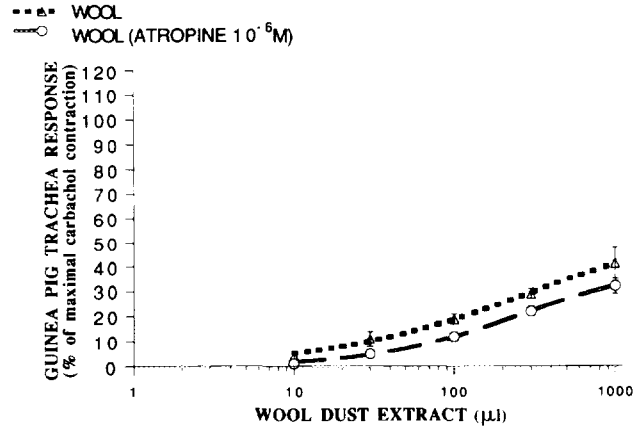

FIG. 3. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-6} M atropine.

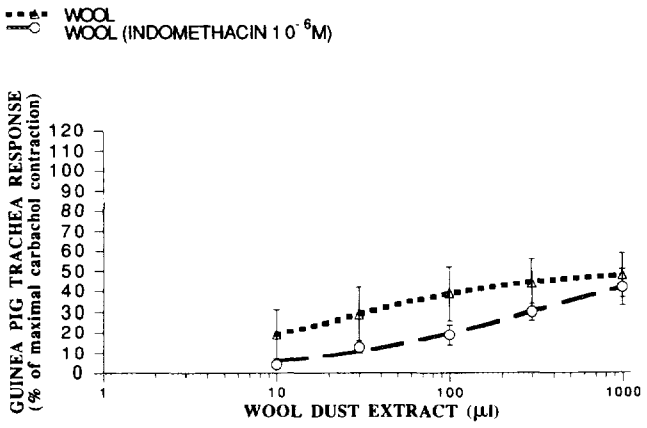

FIG. 4. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-6} M indomethacin.

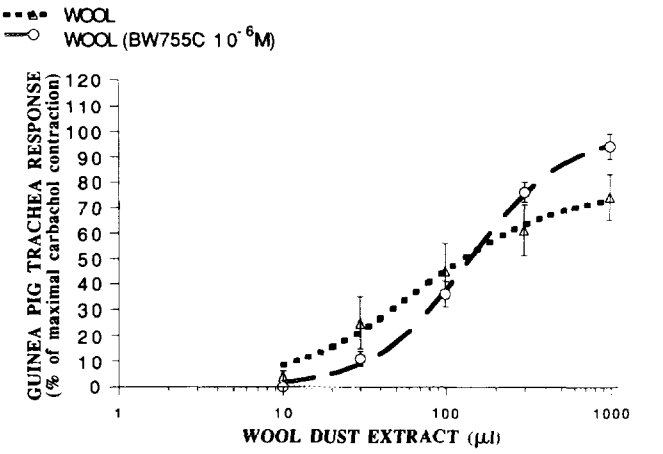

FIG. 6. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-6} M BW755C.

DISCUSSION

Pharmacologic studies of WDE-induced constriction of guinea pig tracheal smooth muscle suggest a possible complex mechanism for this airway irritant. These initial investigations indicate that at least one mediator (i.e., histamine) may be involved in the *in vitro* induced constriction. Such a mechanism is plausible since extracts from other textile dusts (e.g., sisal and cotton) have been shown to release histamine from pig and human lung tissue challenged *in vitro*. Studies with cotton bract extract (Schachter *et al.*, 1981) demonstrate that mediator-modifying drugs can inhibit induced airway constriction *in vivo*. Induced mediator release by these organic extracts is thus a possible mechanism for the constrictor effect seen both *in vivo* and *in vitro* with WDE. Histamine, in particular, has been postulated as a possible candidate for some of the acute workplace-associated ventilatory effects noted for sisal and cotton textile workers as well as for the effects seen in *in vitro* models (Nicholls, 1962; Nicholls *et al.*, 1973; Hitchcock *et al.*, 1973).

Wool dust has been associated with occupational lung disease. Epidemiologic studies (Zuskin *et al.*, 1976; Allardice *et al.*, 1983; Ozesmi *et al.*, 1987) have established the risk of lung disease in the wool industry. Clinical studies by Zuskin *et al.* (1976) reported that inhalation of wool dust extract causes a significant decrease in lung function in healthy subjects, suggesting that these dusts are active *in vivo* and may be associated with occupational disease. Donaldson *et al.* (1988) found that injection of wool dust into rat lung caused considerable pulmonary inflammation. These findings may be related to the constrictor effects we have demonstrated *in vitro*. Extrapolations of the *in vitro* results with organic

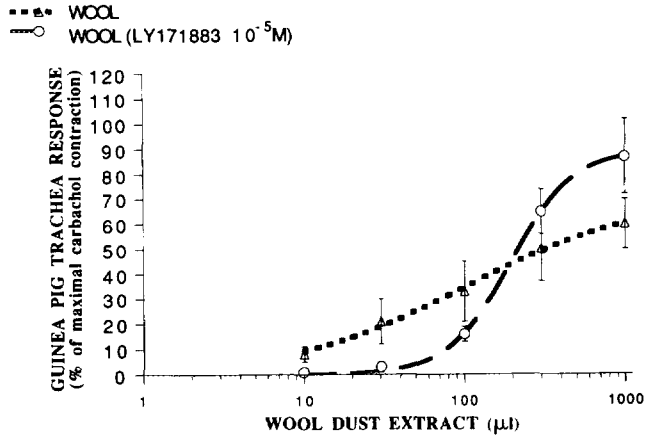

FIG. 5. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-5} M LY171883.

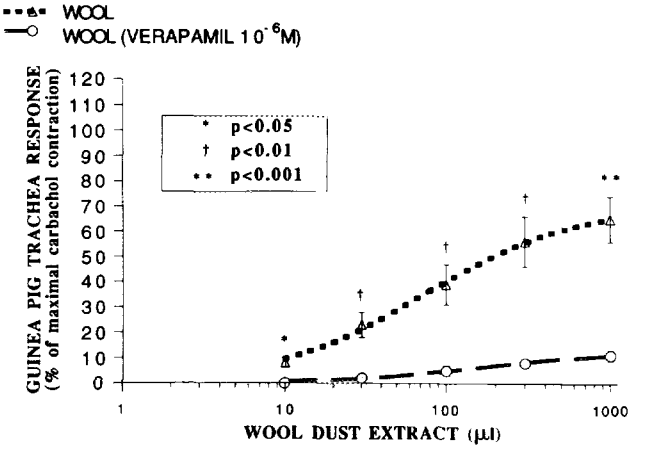

FIG. 7. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-6} M verapamil.

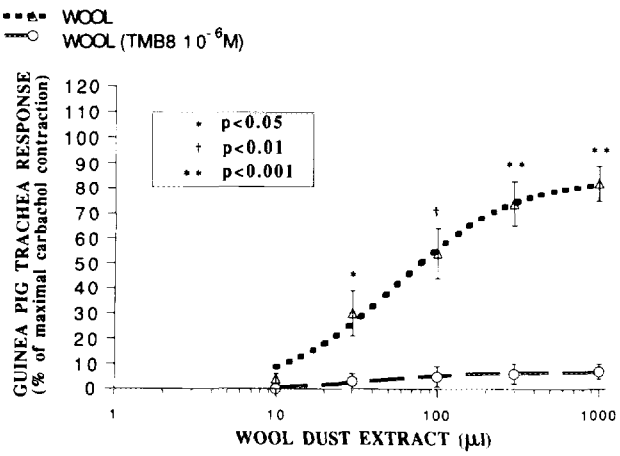

FIG. 8. Contractile activity of wool dust extract on isolated guinea pig tracheal smooth muscle following pretreatment with Krebs and 10^{-6} M TMB8.

dusts in general and WDE in particular to the *in vivo* situation must, however, be carefully qualified particularly in view of potential differences in exposure concentrations used in our *in vitro* model (and those actually occurring in the workplace), as well as chemical and physiological differences seen between natural dusts (e.g., cotton, wool, and hemp).

Endotoxin, which has been implicated as an active agent in many occupationally processed organic materials, was essentially absent in our extract preparation. Thus, endotoxin does not appear to be involved in the *in vitro* response studied with WDE.

The effect of WDE in isolated guinea pig trachea in our studies suggests that nonimmunologic (non-IgE) reactions may be involved in this model since the guinea pigs were not presensitized to WDE. This finding is similar to those seen with cotton bract extract (Schachter *et al.*, 1988), cotton dust extract (Zuskin *et al.*, 1992b), and hemp dust extract (Zuskin *et al.* 1992a). In particular, in the clinical study of Witek *et al.* (1988) and Schachter *et al.*

TABLE 1

Comparison of E_{max} for Drug-Treated Guinea Pig Tracheas with Matched Controls

Drug	Concentration (µl)	E_{max} Treated	E_{max} Control
Pyrilamine	10	28.9 ± 4.2	51.0 ± 9.7
Atropine	1	36.1 ± 5.7	37.7 ± 8.7
Indomethacin	1	41.7 ± 9.5	53.4 ± 11.6
LY171883	10	86.8 ± 17.1	60.3 ± 22.3
BW755C	10	74.3 ± 9.3	94.0 ± 15.2
Verapamil	10	10.8 ± 2.1	64.9 ± 10.3
TMB8	1	7.1 ± 4.3	82.0 ± 8.1

Note. Data are presented as means ± SE.

TABLE 2

Comparison of Pharmacological Agents of the Dose-Response Characteristics of Two Textile Extracts

Drug	Cotton bract extract	Wool dust extract
Pyrilamine	+	±
Atropine	+	–
Indomethacin	X	–
LY171883	+	–
BW755C	X	–

Note. –, no effect; +, attenuation; X, attenuation at low concentrations of extract, enhancement at high concentrations.

(1981) the tested subjects were naive volunteers not previously exposed to textile dusts. This precluded prior sensitization of the tested individuals. Reinforcing this observation is a study by Brown (1992) in which the author notes that skin prick tests for wool are nearly always negative in affected workers.

By analyzing the patterns of response to different drugs used in our experiments with wool dust extract we conclude that blockade of specific receptors other than the histamine receptor had little effect on WDE-induced constriction. In particular, atropine did not have a significant effect on E_{max}. For agents affecting the arachidonic acid pathway (indomethacin, LY171883, and BW755C) no significant differences were noted. This is in contrast to our studies with the cotton bract extract (Table 2) for which mediator-modifying agents were potent inhibitors.

Both verapamil and TMB8 (calcium channel blocking agents) significantly suppressed the wool dust extract effect. Both agents are calcium channel blockers capable of limiting the accumulation of intracellular calcium. An increase in intracellular Ca^{2+} occurs in many smooth muscle preparations induced to constrict by receptor and nonreceptor stimulation (Aleixandre *et al.*, 1993; Gustavsson and Nilsson, 1993). Calcium mobilization for the contractile mechanism may originate from intra- or extracellular stores. While this is probably a secondary event in the sequence leading to smooth muscle constriction, the role for intra- and extracellular blocking agents in the prevention of dust-related airway obstruction remains to be explored both in this model and in the clinical setting.

Our experimental data with wool dust extract as well as our previous studies with other textile dusts suggest that the clinical effects of wool dust are related to a non-IgE mechanism. Unlike other textile dust-induced bronchoconstriction *in vitro*, common mediators (e.g., prostaglandins and leukotrienes) may not play a central role in our model. Moreover, endotoxin does not appear to be involved in this *in*

vitro response. Histamine may nonetheless play a role in WDE-induced constriction *in vitro*. Such findings may have clinical and therapeutic implications for the acute and chronic respiratory symptoms and the lung function changes reported in epidemiological and clinical studies of wool textile workers.

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