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INTERACTIONS OF A WATER EXTRACT OF COTTON BRACT WITH DOG ISOLATED AIRWAY SMOOTH MUSCLE

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The inhalation of cotton dust by cotton-mill workers causes byssinosis, a disease characterized by chest tightness and other symptoms indicative of bronchoconstriction. In the present study the dog isolated trachealis smooth muscle preparation was employed to examine the ability of a crude, water extract of cotton bracts (CBE) to (1) cause contractile responses mediated by receptors, (2) modify responses of the tissue to a number of excitatory and inhibitory agents, and (3) alter responsiveness of the preparations to electrical field stimulation mediated by intrinsic cholinergic, excitatory nerves and adrenergic, inhibitory nerves. CBE evoked contraction of the tissues by an action that did not involve muscarinic, histamine, or 5-hydroxytryptamine receptors. The maximum tissue responses to exogenous histamine, 5-hydroxytryptamine, and isoproterenol were enhanced significantly in the presence of CBE, while those to KCl and methacholine were unaffected. The sensitivities (EC50 values) of the tissues to these agents were not, however, altered by CBE. Contractions induced by cholinergic nerve stimulation were increased in the presence of CBE, via a mechanism that appears, at present, to result from a prejunctional facilitation of acetycholine release. Relaxations to adrenergic nerve stimulation were not affected by CBE. Results suggest that, in addition to its direct, contractile activity, CBE can alter indirectly the responsiveness of the tissue to a number of endogenous mediator substances. Intrinsic, cholinergic excitatory nerves may be a target for the acute action of CBE as well.

INTRODUCTION

Byssinosis, an occupationally related respiratory disease occurring in cotton-mill workers as a result of the inhalation of workplace dust, is characterized by symptoms—i.e., chest tightness, wheezing (Parkes, 1982)—that are indicative of increases in airway smooth-muscle tone (National Research Council, 1982). The etiologically important agent(s) involved in the disease may be found in the highest concentration in

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the cotton bract (Morey et al., 1976). The inhalation by humans of aqueous extracts of cotton bract (CBE) causes a decrement in pulmonary function that resembles the Monday morning respiratory distress experienced by mill workers (Buck and Bouhuys, 1981).

Research into the mechanisms of byssinosis has been centered around the identification of the causative agent(s); a large number of candidate agents have been proposed (Mundie et al., 1983). Several types of in vitro preparations of respiratory and nonrespiratory smooth muscles have been used to characterize the stimulant activities contained in plant and dust extracts. Bract extracts generally produce contraction of these muscles (Davenport and Paton, 1962; Nicholls, 1962; Nicholls and Skidmore, 1975; Cinkotai and Franklin, 1975; Battigelli et al., 1977). Only recently (Russell et al., 1982; Mundie et al., 1983) have attempts been made to understand the pharmacological nature of the contractile response to cotton extracts. This information cannot be obtained merely by using respiratory smooth muscles for their ability to contract in bioassay (screening) experiments (see, for example, Cinkotai and Franklin, 1975; Battigelli et al., 1977; Russell et al., 1983).

The level of airway smooth-muscle tone is influenced by the balance of the effects of intrinsic excitatory and inhibitory autonomic nerves (Suzuki et al., 1976; Russell, 1980; Leff et al., 1983). It is well known that the release of neurotransmitters from such nerves may be regulated physiologically, so as to enhance or reduce release, by several endogenous and exogenous substances (Vermeire and Vanhoutte, 1979; Grundström et al., 1981; Vanhoutte et al., 1981; Sheller et al., 1982; Hadhazy et al., 1982). A reasonable hypothesis, not previously considered, would be that cotton dusts and extracts, either acutely or chronically, evoke bronchoconstriction via an indirect effect on the activity or function of intrinsic nerves.

The twofold purpose of this study was to characterize the direct effects of a water extract of cotton bract on the dog isolated trachealis muscle preparation and to determine the possible influence of CBE on neurogenic excitatory and inhibitory responses of the tissue. No a priori assumptions were made regarding the nature of the active agent(s) in CBE. No purifications of CBE were performed, therefore, so as to leave open the possibility that there could exist a synergism between the actions of chemically unrelated natural products, as might occur in a workplace setting. A preliminary account of some of this work has been reported (Fedan et al., 1983).

METHODS

Preparation of CBE

A crude water extract of CBE was used in this study. Frost-killed bracts, typical of those incorporated into seed cotton, were collected

within a 5-mile radius of Idalou, Tex., during the first week of December, 1977. The bracts (220 g) were ground to powder in a Wiley mill by sequential passage through mesh sizes 20, 40, and 60. To each of two 100-g aliquots of powder was added 1 l of sterile, nonpyrogenic water, and the suspensions were extracted at 23°C with vigorous shaking for 2 h. The suspension was filtered under vacuum through Whatman number 1 filter paper. The residue was discarded and the filtrate was filtered again under vacuum through 0.45-μm cellulose acetate filters. The residue was discarded and the filtrate was lyophilized to dryness. The resulting powder (32.8 g) was dissolved (333 mg/ml final concentration) in sterile, nonpyrogenic 9% NaCl, and aliquots were stored frozen (−20°C) until use. With the exception of the grinding of the bracts with the mill, all steps were carried out under sterile conditions.

Preparation of Dog Isolated Trachealis Muscles

These experiments were in adherence with the "Guiding Principles in the Care and Use of Animals" (Office of Science and Health Reports, DPR/NIH, 1978) and other national and international codes. Male mongrel dogs (14–21 kg) were anesthesized with sodium pentobarbital (40 mg/kg, iv) and the trachea was removed, placed in modified Krebs–Henseleit solution (composition below), and cleaned. The trachealis muscle from individual ring segments, beginning with the sixth ring from the larynx and extending posteriorly, was divided, in a direction parallel to the fiber bundles, into two paired strips (1.5–2 mm in width by 5–6 mm in length). The strips were tied at one end to a holder, placed in organ chambers containing modified Krebs–Henseleit solution (37°C), and attached at the other end to force-displacement transducers for the measurement of isometric tension responses. The bath volumes were 3 ml for concentration-response determinations and 8 ml for frequency-response determinations (see below). The tissues were equilibrated under 2 g resting tension for 1 h prior to the beginning of concentration-response and frequency-response determinations.

Concentration-Response Studies

Concentration-response relationships for agonists (and CBE) that induce contraction were obtained following their cumulative addition to the organ bath containing a tissue under resting tension. Relaxation responses to isoproterenol were obtained following its cumulative addition to preparations in which active tension had first been induced with 30 m*M* KCl; this concentration of KCl approximates its EC50. Only one concentration-response relationship was obtained from each preparation. Test agents were evaluated in one muscle strip from a ring segment, while the other strip served as control. Antagonist drugs, when present, were added to the organ baths 30 min prior to the ad-

dition of agonists or CBE. When the effects of CBE on agonist concentration-response curves was evaluated, CBE was added to the baths 20 min prior to beginning the concentration-response determination.

Frequency-Response Studies

To obtain neurogenic tension responses to electric field stimulation (EFS), holders were used in which the tissues were placed longitudinally within a pair of platinum ring electrodes. EFS with stepwise increasing frequencies was produced using 10-s trains of rectangular-wave stimuli (50 V, 0.5 ms duration) at 7-min intervals. This interval was determined to be sufficient for individual EFS-induced responses to return to the preexisting level of tension. Two experimental protocols were employed. In the first, two frequency-response relationships were obtained for each preparation. For the test tissues the first served as control while the second, obtained 30 min after the first, was obtained in the presence of test agents. Test agents were added immediately after the end of the first determination and thus were present for 30 min before their effects on responses were evaluated. For control tissues, the second frequency-response curve was obtained in the absence of test agents. In the second protocol, the tissues were first contracted with methacholine or histamine before EFS-induced responses were evoked. Due to gradual deterioration of tension with time under these conditions, only one frequency-response relationship was obtained for each tissue. In these experiments, one muscle strip from a tracheal ring segment was used as control, while the paired strip from the same ring was used to evaluate the effects of test agents. For both protocols, CBE was added to the baths 20 min prior to evaluating its effects on frequency-response curves.

Test Agents of Antagonists

The drugs used in the present study were the α-adrenoceptor antagonist phentolamine (10^{-6} *M*), the β-adrenoceptor antagonist propranolol (10^{-6} *M*), the adrenergic neuron blocker guanethidine (10^{-5} *M*), the muscarinic receptor antagonist atropine (10^{-7} *M*), the histamine H_1-receptor antagonist pyrilamine (3×10^{-6} *M*), and the 5-hydroxytryptamine (5-HT) receptor antagonist methysergide (10^{-7} *M*). Phentolamine, propranolol, and guanethidine were always added together; the combination is referred to as PPG. All of the drugs used were from Sigma Chemical Co. (St. Louis, Mo.).

The modified Krebs–Henseleit solution contained (m*M*) NaCl (113), KCl (4.8), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $NaHCO_3$ (25), and glucose (5.7), and was gassed with 95% O_2–5% CO_2 to give pH 7.4 (37°C).

Data Analysis

Each tissue was used either for a concentration-response or a frequency-response determination. The tissues were weighed at the end of the experiment to normalize tension responses in terms of grams wet tissue weight (gww), or g tension/gww, as appropriate. The results are expressed as means ± SEM. Geometric mean EC50 values (the concentration of agonist that produces 50% of the maximum response) were determined using linear regression analysis of probit-transformed data. The data were evaluated for differences using Student's *t*-test for paired or nonpaired samples, as appropriate. The 0.05 level of probability was considered significant; *n* is the number of separate experiments.

RESULTS

Concentration-Response Studies

The concentration-response curve for CBE-induced contractile responses is compared in Fig. 1 to those for other agents that produce contraction of the tissue. Concentrations of CBE higher than those shown resulted in foam production (due to constant aeration of the bath with 95% O_2–5% CO_2) that caused the frothy solution to overflow from the baths. Consequently, the true maximum responses to CBE

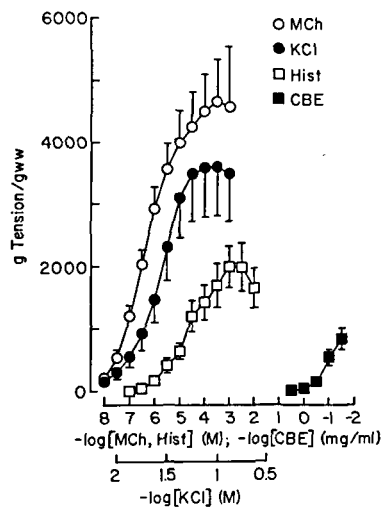

FIGURE 1. Comparison of concentration-response curves for methacholine (MCh; *n* = 8), KCl (*n* = 7), histamine (Hist; *n* = 9), and CBE (*n* = 9) in dog trachealis smooth muscle. The concentration of CBE is expressed in terms of mg/ml, since the molecular weight(s) of the active species is not known. In this and in the remaining figures the vertical bars denote the SEM unless it is enclosed within the symbol.

could not be achieved. Nevertheless, the inflection in the slope of the CBE curve, in the concentrations used, indicates that maximum response was nearly achieved (this was more evident in the raw data of the individual experiments than in the average results shown in the figure). These findings would indicate that the maximum response to CBE is substantially less than that produced by methacholine, KCl, and histamine. Moreover, the maximum response to CBE also would appear to be less than that to 5-HT (~1200 g tension/gww; see Fig. 4). In tissues contracted with methacholine or histamine, the subsequent addition of CBE led to an additional contractile response; relaxation to CBE was not observed ($n = 8$; data not shown).

Atropine (10^{-7} *M*), pyrilamine (3×10^{-6} *M*), and methysergide (10^{-7} *M*) produced 88.7-, 19.7-, and 19.5-fold shifts of the methacholine, histamine, and 5-HT concentration-response curves, respectively, to the right of control (results expressed as the effects of the antagonists on EC50 values; not shown). These antagonists, in the same concentrations, had no effect on responses of the tissues to CBE (Fig. 2).

Since the ability of CBE to evoke responses was not striking and since responses to CBE appeared not to be mediated by receptors for these agents, we reasoned that CBE might exert an influence on the smooth muscle to otherwise modify its reactivity. A series of experiments were performed to test this possibility. In these studies the concentration of CBE (2.1 mg/ml) used was just above the threshold value for producing contraction. Table 1 shows that responses to methacholine obtained in the presence of CBE were not different from those obtained in its absence. In contrast, the maximum responses of the tissues to histamine (Fig. 3) and 5-HT (Fig. 4) were elevated in the presence of CBE; the EC50 values were not affected (Table 1). The maximum

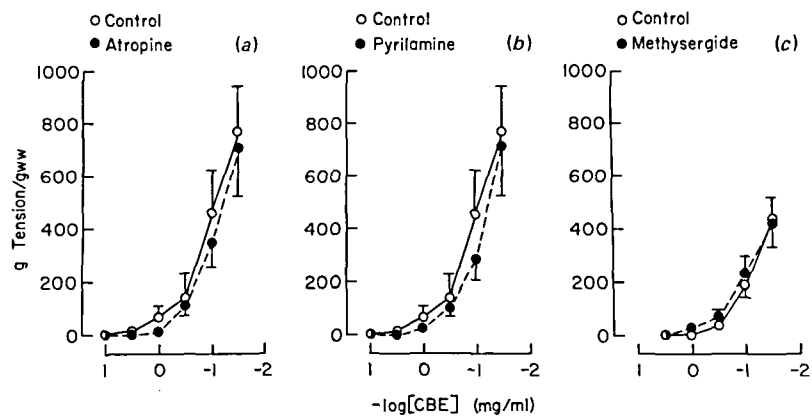

FIGURE 2. Lack of effect of (a) atropine (10^{-7} *M*; $n = 6$), (b) pyrilamine (3×10^{-6} *M*; $n = 6$), and (c) methysergide (10^{-7} *M*; $n = 6$) on the CBE concentration-response curve of dog trachealis smooth muscle.

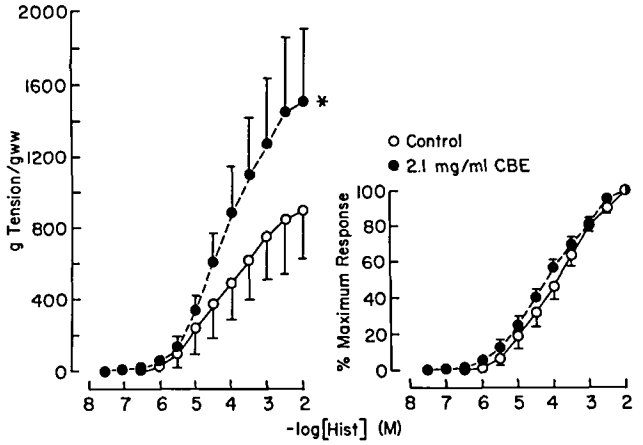

FIGURE 3. Comparison of histamine concentration-response curves of dog trachealis smooth muscle obtained in the absence and presence of CBE (2.1 mg/ml; $n = 7$). Asterisk indicates maximum response significantly larger than control.

response to KCl seemed elevated by CBE (Fig. 5), but this effect was not significant; CBE had no effect on the EC50 value for KCl (Table 1). CBE potentiated the maximum relaxation response to isoproterenol, but the sensitivity to this agent was not changed (Fig. 6; Table 1).

Frequency-Response Studies: Neurogenic Contraction

Responses of these preparations to EFS were blocked by tetrodotoxin (3×10^{-7} *M*) and were therefore neurogenic in nature and not the result of direct electrical stimulation of the muscle.

In resting muscles, EFS produced frequency-related contractile responses (Fig. 7). These responses were abolished in the presence of

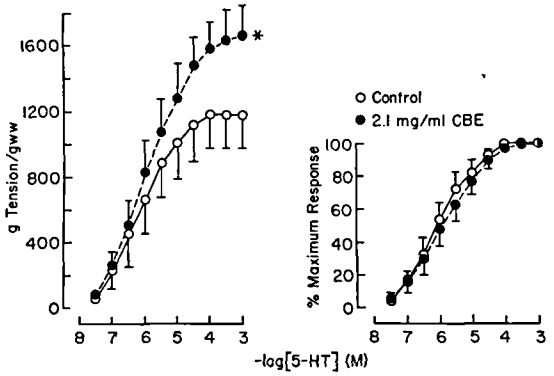

FIGURE 4. Comparison of 5-HT concentration-response curves of dog trachealis smooth muscle obtained in the absence and presence of CBE (2.1 mg/ml; $n = 6$). Asterisk indicates maximum response significantly larger than control.

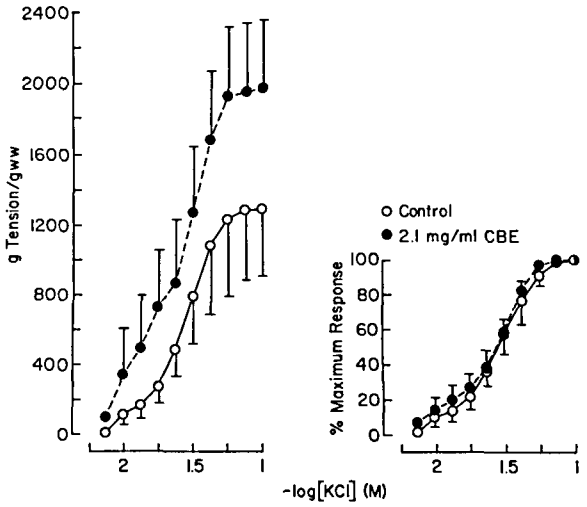

FIGURE 5. Comparison of KCl concentration-response curves of dog trachealis smooth muscle obtained in the absence and presence of CBE (2.1 mg/ml; $n = 6$). The maximum response obtained in the presence of CBE was not different from the control level.

atropine (10^{-7} *M*), except at the 30-Hz frequency of stimulation where small residual responses remained ($n = 4$; data not shown). EFS-induced contractions, therefore, are mediated by the release of acetylcholine from parasympathetic nerves.

The effects of CBE on neurogenic, contractile responses were assessed in the absence and presence of PPG. The presence of PPG eliminates the participation of adrenergic inhibitory nerves in the development of the tissue response[1] (see also Suzuki et al., 1976; Russell, 1980; Leff et al., 1983). In control tissues (in the absence or presence of PPG) there was routinely a small diminution in the level of responses obtained in the second frequency-response determination, which must be taken into account when interpreting the effect of CBE, which was evaluated during the second determination. Figure 7 illustrates that CBE, in the near-threshold concentration of 2.1 mg/ml, potentiated EFS-induced contractions, both in the absence (Fig. 7*a* and *b*) and presence (Fig. 7*c* and *d*) of PPG, resulting in an approximate twofold shift of the frequency-response curve to the left of control.

Methysergide (10^{-7} *M*) alone had no effect on EFS-induced responses (Fig. 8*a* and 2*b*), in agreement with the findings of Russell et

[1] In preliminary experiments ($n = 3$; data not shown), the effectiveness of PPG in eliminating adrenergic inhibitory neurogenic responses was assessed. In control tissues that were precontracted with histamine, frequency-dependent relaxation responses (see Fig. 9) were elicited. In the presence of PPG, EFS-induced relaxations were inhibited completely.

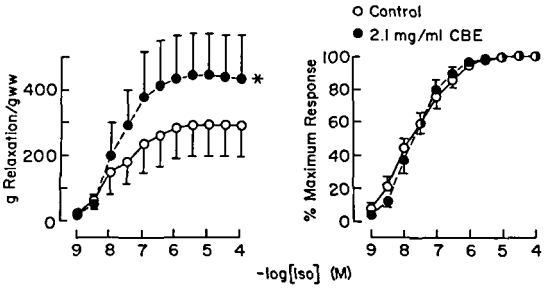

FIGURE 6. Comparison of isoproterenol concentration-response curves of dog trachealis smooth muscle in the absence and presence of CBE (2.1 mg/ml; $n = 6$). In these experiments with isoproterenol, both the control and test preparations were precontracted with 30 m*M* KCl in the absence of CBE. CBE was added when the responses had reached a plateau level. The concentration-response determination was begun 5 min later. This protocol eliminated an artifact that might have been introduced had CBE been added before KCl, in view of the nonsignificant trend of effect shown in Fig. 5. *Asterisk indicates maximum response significantly larger than control.*

al. (1982). The two- to threefold shift of the frequency-response curve to the left of control observed in the presence of CBE (2.1 mg/ml) was unaffected by methysergide (10^{-7} *M*; Fig. 8c and *d*).

Frequency-Response Studies: Neurogenic Relaxation

In these experiments, atropine (10^{-7} *M*) was present to prevent neurogenic contraction. Contractile tension was induced in the prep-

TABLE 1. Effect of CBE (2.1 mg/ml) on Responses of Dog Isolated Trachealis Muscle to Various Agents

Agent	−log EC50 (*M*)	Maximum response (g tension/gww)[a]
Methacholine (6)[c]		
Control	6.37 ± 0.25	1698 ± 192
+CBE	6.26 ± 0.22	1620 ± 216
Histamine (7)		
Control	3.91 ± 0.16	900 ± 271
+CBE	4.15 ± 0.11	1517 ± 405[b]
5-HT (6)		
Control	6.01 ± 0.25	1181 ± 198
+CBE	5.85 ± 0.24	1659 ± 200[b]
KCl (6)		
Control	1.57 ± 0.06	1301 ± 411
+CBE	1.62 ± 0.06	1993 ± 383
Isoproterenol (6)		
Control	7.62 ± 0.14	291 ± 92
+CBE	7.68 ± 0.19	433 ± 133[b]

[a] Results expressed as g relaxation/gww for isoproterenol.
[b] Significantly larger than control.
[c] Value of *n* in parentheses.

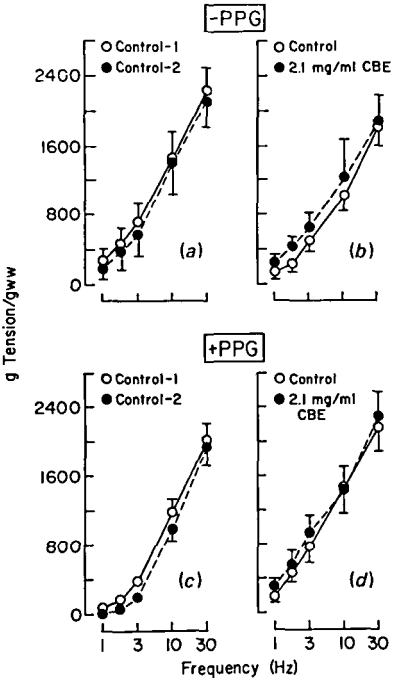

FIGURE 7. Effect of CBE (2.1 mg/ml) on frequency-response curves of dog trachealis smooth muscle. Upper panels (*a* and *b*): Experiments were conducted in the absence of PPG. Lower panels (*c* and *d*): PPG was present during the first ("Control-1" or Control) and second ("Control-2" or 2.1 mg/ml CBE) frequency-response determinations. These results show data for contractile responses of resting tissue. The rightward shifts in the second frequency response curves of the controls (compare Control-2 to Control-1 in *a* and *c*) are inherent in the leftward shifts caused by CBE (*b* and *d*). For each panel, $n = 6$.

arations with 10^{-5} *M* histamine. When the responses had reached a plateau level, 2.1 mg CBE/ml was added. Occasionally, a very small increment in tone was produced. Frequency-response determinations were initiated 20 min after the addition of CBE. The results are shown in Fig. 9, where it can be seen that CBE had no effect on relaxation responses induced by stimulation of adrenergic nerves. A lack of effect was evident when the results were expressed as the absolute magnitude of the relaxation responses (Fig. 9, left panel) or in terms of the fractional inhibition of histamine-induced tension (Fig. 9, right panel). We considered the possibility that the presence of histamine, to induce tone, may have masked a possible effect of CBE on EFS-induced relaxation responses. The experiments were, therefore, repeated using methacholine (10^{-6} *M*) to induce tone. Similar results were found, namely, that CBE had no effect on the neurogenic inhibitory responses ($n = 4$; data not shown).

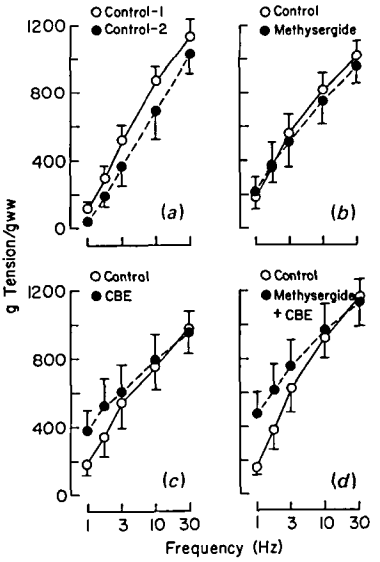

FIGURE 8. Lack of effect of methysergide (10^{-7} *M; a* and *b*) on frequency-response curves, and lack of effect of methysergide on CBE-induced potentiation of EFS-induced contractile responses (*a, c,* and *d*), in dog trachealis smooth muscle. PPG were absent in these experiments. For each panel, $n = 7$.

DISCUSSION

These results indicate that CBE obtained from frost-killed botanical material elicits contractile responses that are not antagonized by atropine, pyrilamine, or methysergide, and that are not, therefore, mediated by muscarinic, histamine H_1-, or 5-HT receptors. A number of

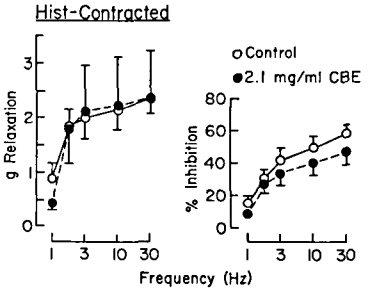

FIGURE 9. Lack of effect of CBE (2.1 mg/ml) on frequency-response curves of dog trachealis smooth muscle. Histamine (10^{-5} *M*) was used to contract the tissues. At the plateau of response, CBE was added (for the reason described in Fig. 8); field stimulation was begun 5 min later. In the absence of any antagonists, when tone is induced the response to EFS is a transient initial contraction (parasympathetic in origin) followed by relaxation (adrenergic in origin). Atropine (10^{-7} *M*) was present in these experiments to eliminate the contractile portion of the response; $n = 8$.

previous reports (Table 2) have indicated that responses of a variety of smooth muscle preparations to crude cotton extracts are blocked by one or more antagonists of these receptor classes. We know of no other earlier work in which contractile responses to cotton extracts are not, at least in part, ascribable to actions on these receptors. The other effects of CBE observed in the present study are due, apparently, to some other action(s) of the active agent(s) that are independent of these receptors. It is of interest that the magnitude of responses to CBE (Fig. 1) was nevertheless comparable to those obtained by Russell et al. (1983), and by Rohrbach et al. (1984), for extracts of senescent bracts, responses that were sensitive to methysergide but unaffected by atropine, and that were elicited by a more purified preparation of CBE [an acetone extraction was used by Rohrbach et al. (1983)] than employed in our studies.

It is evident from the present study that not only is CBE capable of eliciting contractile responses itself, but it also modifies the effects of other stimulant agents. Thus, CBE potentiated responses to histamine and 5-HT. It could be envisaged that CBE might potentiate the actions of these mediators after their release from mast cells, and enhance the bronchoconstrictive effects of these amines. Since cotton-dust extracts also evoke histamine release from lung tissue (Bouhuys and Lindell, 1961; Hitchcock et al., 1973), the effects of released histamine could be amplified. A compelling reason for considering this possibility is that responses to CBE per se were not blocked by pyrilamine or methysergide, and were not, therefore, mediated by released histamine or 5-HT.

A curious aspect of the ability of CBE to potentiate responses to excitatory agents is the relative specificity of the effect for certain agents. That is, responses to KCl, which are initiated by membrane depolarization, were not potentiated to a significant extent (Fig. 5), although a trend was evident in the data. The absence of an effect of CBE on methacholine-induced contractions is not, however, equivocal. It may be concluded, therefore, that the potentiating effect of CBE does not result from the induction of a generalized "hyperresponsive" state.

The physiological expression and meaning of the potentiation of isoproterenol-induced relaxation by CBE is difficult to assess. Were this interaction to occur in vivo, it might be expected that the effect of circulating epinephrine would be increased. The resultant effect on airway smooth muscle—i.e., bronchodilation—is not a characteristic of byssinosis.

The finding that CBE potentiated neurogenic contractile responses (Figs. 7 and 8) has a number of implications. Since responses to methacholine were not affected by CBE, this observation indicates that CBE acted prejunctionally to facilitate or enhance the release of acetylcholine. An alternative explanation, that the neurogenic contractile re-

TABLE 2. Effects of Antagonists on Contractile Responses of Smooth Muscles to Crude Extracts of Cotton, as Reported in the Literature

Botanical material	Muscle preparation	Receptor antagonism tested	Result	Reference
Dust	Intestine, trachea	5-HT	Antagonism[a]	Davenport and Paton (1962)
		Histamine	No effect	
		Muscarinic	No effect	
Dust	Intestine	5-HT	No effect	Nicholls (1962)
		Histamine	Antagonism	
		Muscarinic	No effect	
Bracts	Trachea	5-HT	Antagonized	Russell et al. (1982)
		Histamine	Weak antagonism	
		Muscarinic	Weak antagonism	
Dust, Bracts, plant parts	Stomach	5-HT	Antagonized[b]	Mundie et al. (1983)
		Histamine	Weak antagonism	
Bracts	Trachea	5-HT	Antagonized[c]	Rohrbach et al. (1983)
		Muscarinic	Antagonized[c]	
Bracts	Trachea	5-HT	No effect	Present study
		Histamine	No effect	
		Muscarinic	No effect	

[a] Effect was tissue-dependent.
[b] Extent dependent on plant part.
[c] Extent temporally dependent on age/senescence.

sponses were larger because of an inhibitory action of CBE on adrenergic, inhibitory nerves, can be eliminated for the reasons that (1) CBE potentiated neurogenic contractions in the presence, as well as in the absence, of PPG and (2) CBE had no effect on relaxation responses to adrenergic nerve stimulation (Fig. 9). This interaction between CBE and cholinergic nerves could increase the level of bronchoconstriction in response to a given level of parasympathetic input to the airways.

The broad conclusion of this study, that CBE can interact with a number of mechanisms that regulate or affect airway smooth muscle tone, provides no information about the manner whereby CBE exerts these effects. It has been reported recently (Mundie et al., 1983) that cotton extracts caused the release of $PGF_{2\alpha}$ from rat stomach smooth muscle. This prostanoid itself elicits contraction in airway smooth muscle. Conceivably, CBE may have promoted the formation of $PGF_{2\alpha}$ in dog trachealis muscle, and $PGF_{2\alpha}$ may have mediated the potentiation of responses to histamine and 5-HT. $PGF_{2\alpha}$ also has been reported to potentiate acetylcholine-induced contractile responses of rabbit isolated tracheal smooth muscle (Hadhazy et al., 1982). The induction of prostaglandin formation by cotton extracts is of interest for the additional reason that PGE_2, which inhibits airway smooth muscle contractility, exerts a prejunctional inhibitory effect on cholinergic neurotransmission in dog trachealis muscle (Walters et al., 1984). Perhaps CBE interferes with the balance of the neuromodulatory actions of excitatory and inhibitory prostaglandins.

The results of these early studies indicate that additional evaluations of the effects of CBE with important regulators of airway smooth-muscle tone—e.g., prostaglandins, leukotrienes, platelet activating factor, etc.—are warranted.

REFERENCES

Batigelli, M. C., Craven, P. L., Fischer, J. J., Morey, P. R., and Sasser, P. E. 1977. The role of histamine in byssinosis. *J. Environ. Sci. Health* A12:327–339.

Bouhuys, A., and Lindell, S. E. 1961. Release of histamine by cotton dust extracts from human lung tissue in vitro. *Experientia* 17:211–215.

Buck, M. G., and Bouhuys, A. 1981. A purified extract from botton bracts induces airway constriction in humans. *Chest* 79(Suppl.):435–495.

Cinkotai, F. F., and Franklin, D. W. 1975. An evaluation of effect of airborne dust from a cotton mill on the guinea-pig ileum with reference to byssinosis. *Br. J. In. Med.* 32:239–243.

Davenport, A., and Paton, W. D. M. 1962. The pharmacological activity of extracts of cotton dust. *Br. J. In. Med.* 19:19–32.

Fedan, J. S., Franczak, M. S., Cahill, J. F., Kosten, C. J., and Morey, P. 1983. Effects of cotton bract extract (CBE) on airway smooth muscle and nerves. In *Proc. Seventh Cotton Dust Research Con.*, eds. P. J. Wakelyn, and R. R. Jacobs, pp. 93–96. Memphis, Tenn.: National Cotton Council.

Grundström, N., Anderson, R. G. G., and Wikberg, J. E. S. 1981. Prejunctional alpha$_2$ adrenocep-

tors inhibit contraction of tracheal smooth muscle by inhibiting cholinergic neurotransmission. *Life Sci.* 28:2981–2986.

Hadhazy, P., Van B. Q., Malomvölgyi, B., and Magyar, K. 1982. Nanomolar concentrations of prostaglandin $F_{2\alpha}$ potentiate cholinergic contractions of rabbit isolated tracheal muscle. *Eur. J. Pharmacol.* 83:207–212.

Hitchcock, M., Piscitelli, D. M., and Bouhuys, A. 1973. Histamine release from human lung by a component of cotton bracts. *Arch. Environ. Health* 26:177–182.

Leff, A. R., Munoz, N. M., and Hendrix, S. G. 1983. Parasympathetic and adrenergic contractile responses in canine trachea and bronchus. *J. Appl. Physiol.* 55:113–120.

Morey, P. R., Sasser, P. E., Bethea, R. M., and Kopetzky, M. T. 1976. Botanical trash present in cotton before and after saw-type lint cleaning. *Am. Ind. Hyg. Assoc. J.* 37:407–411.

Mundie, T. G., Cordova-Salinas, M., Bray, V. J., and Ainsworth, S. K. 1983. Bioassays of smooth muscle contracting agents in cotton mill dust and bract extracts: Arachidonic acid metabolites as possible mediators of the acute byssinotic reaction. *Environ. Res.* 32:62–71.

National Research Council. 1982. *Byssinosis: Clinical and Research Issues,* pp. 9–16. Washington, D.C.: National Academy Press.

Nicholls, P. J. 1962. Some pharmacological actions of cotton dust and other vegetable dusts. *Br. J. Ind. Med.* 19:33–41.

Nicholls, P. J., and Skidmore, J. W. 1975. Comparative study of the smooth muscle contractor activity of airborne dusts and of dustiness in cotton, flax, and jute mills *Br. J. Ind. Med.* 32:289–296.

Parkes, W. R. 1982. Occupational asthma (including byssinosis). In *Occupational Lung Disorders,* pp. 415–453. London: Butterworth.

Rohrbach, M. S., Rolstad, R. A., Fischer, J. J., and Russell, J. A. 1984. Comparison of the changes in levels of bio-active components of cotton bracts accompanying senescence. In *Proc. Eighth Cotton Dust Research Conf.,* eds. P. J. Wakelyn, and J. J. Jacobs, pp. 129–131. Memphis, Tenn: National Cotton Council.

Russell, J. A. 1980. Noradrenergic inhibitory innervation of canine airways. *J. Appl. Physiol.* 48:16–22.

Russell, J. A., Gilberstedt, M. L., and Rohrbach, M. S. 1982. Constrictor effect of cotton bract extract on isolated canine airways. *Am. Rev. Respir. Dis.* 125:727–733.

Russell, J. A., Gilberstedt, M. L., and Rohrbach, M. S. 1983. The effects of aqueous bracts extracts from several varieties of cotton on the behavior of airway smooth muscle and platelets. In *Proc. Seventh Cotton Dust Research Conf.,* eds. P. J. Wakelyn and R. R. Jacobs, pp. 90–92. Memphis, Tenn.: National Cotton Council.

Sheller, J. R., Holtzman, M. J., Skoogh, B. -E., and Nadel, J. A. 1982. Interaction of serotonin with vagal- and ACh-induced bronchoconstriction in canine lungs. *J. Appl. Physiol.* 52:964–966.

Suzuki, H., Morita, K., and Kuriyama, H. 1976. Innervation and properties of the smooth muscle of the dog trachea. *Jpn. J. Physiol.* 26:303–320.

Vanhoutte, P. M., Verbeuren, T. J., and Webb, R. C. 1981. Local modulation of adrenergic neuroeffector interaction in the blood vessel wall. *Physiol. Rev.* 61:151–247.

Vermeire, P. A., and Vanhoutte, P. M. 1979. Inhibitory efffects of catecholamines in isolated canine bronchial smooth muscle. *J. Appl. Physiol.* 46:787–791.

Walters, E. G. O'Bryne, P. M., Fabbri, L. M., Graf, P. D., Holtzman, M. J., and Nadel, J. A. 1984. Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. *J. Appl. Physiol.* 57:129–134.

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