EFFECTS OF A WATER EXTRACT OF COTTON BRACT (CBE) IN ISOLATED GUINEA-PIG TRACHEALIS SMOOTH MUSCLE (GPT)

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Abstract

We characterized further the interactions of a crude, water extract of cotton bract with the smooth muscle of the GPT from two aspects: 1) Based on our previous finding that CBE potentiates relaxation responses induced by stimulation of intrinsic nonadrenergic, noncholinergic inhibitory nerves, which may use as a transmitter ATP or its breakdown product adenosine, we examined the effect of CBE on ATP- and adenosine-induced responses. The relaxation responses to these agents were potentiated. CBE may thus act at, or facilitate responses mediated by, purinergic receptors. 2) We compared contractile responses to crude CBE with those to dialyzed or ashed CBE. Most of the contractile response-inducing activity was dialyzable, and was retained in ashed samples. Inorganic constitutents, i.e., minerals, might contribute to bronchoconstrictor activity in cotton bract.

Introduction

We reported previously (Fedan et al., 1983) that among several direct acute effects of a water extract of cotton bract (CBE) in the isolated airway smooth muscles of dogs and guinea pigs was its ability to potentiate in the latter species relaxation responses elicited with electrical field stimulation of intrinsic inhibitory nerves. Through the use of appropriate pharmacological agents it was concluded that these nerves are of a type referred to as "nonadrenergic, noncholinergic" ("NANC"). These nerves may utilize ATP as a neurotransmitter (Burnstock, 1979, 1981). It is possible that the potentiation of NANC-induced inhibitory responses by CBE occurred by a prejunctional action, i.e., to facilitate the release of the NANC neurotransmitter, or by a postjunctional action whereby the action of the neurotransmitter is enhanced at the level of the smooth muscle. Considering the possibility that ATP or adenosine, its breakdown product, might mediate via purinergic receptors (Burnstock, 1981) the inhibitory response to NANC nerve stimulation, one purpose of the present studies was to evaluate the ability of CBE to modify the relaxation responses to added ATP and adenosine.

A second purpose of the present studies was to begin a characterization of the CBE we have been using. Using the reasoning that textile workers breath a cotton dust which is a mixture of a large number of botanical ingredients, our basic approach to byssinosis studies has been to characterize the effects of extracts which are left intentionally crude. We have thus made no assumptions about the identity of possible active agents. Therefore, samples of crude CBE were subjected to dialysis or ashed prior to testing to allow a comparison of the remaining activities with that contained in the crude water extract.

Materials and Methods

The bract samples and preparation of CBE were the same as described previously (Fedan et al., 1983). Bracts were collected during the first week of December, 1977 within a 5 mile radius of Idalou, TX. The bracts are representative of frost-killed material that was incorporated into seed cotton during the 1977 harvest process. Bracts (220 g) were ground into a powder with a Wiley mill by passing the material sequentially through meshes size 20, 40 and 60. Batches (100 g) of the ground material were added to 1 l of sterile, non-pyrogenic water and shaken vigorously for 2 hr at 23°C. The extract was filtered under vacuum through filter paper (Whatman #1) and the residue was discarded. The filtrate was filtered again through 0.45 micron cellulose acetate filters. The residue was discarded and the filtrate was lyophilized to dryness. The resulting powder, referred to hereafter as "CBE" was dissolved in sterile non-pyrogenic water to give a solution of 333 mg CBE/ml, aliquots of which were stored frozen until use. All procedures for the preparation of CBE were done under sterile conditions, with the exception of the grinding step.

Dialyzed samples of CBE were prepared by placing 10 ml of the above CBE solution into dialysis tubing (Spectropor Membrane Tubing, Spectrum Medical Industries, Inc., Los Angeles, CA; molecular weight cutoff 12,000-14,000) and dialyzing against 2 l of sterile, nonpyrogenic water for 24 hours at 4°C. The contents of the dialysis sack and the dialyzate were lyophilized to dryness. For a quantitative comparison of the activities in the dialysis sack and in the dialyzate, both samples were dissolved subsequently in 10 ml of sterile, nonpyrogenic water for use.

To prepare an ashed sample of CBE, approximately 2.65 g of CBE (from the 0.333 g/ml CBE stock solution) was lyophilized to dryness and ashed at low temperature (LTA-600 low temperature asher, LFE Corporation, Waltham, MA; 200 watts setting) for two days. The resulting ash, which was 63.4% of the original weight of CBE, was dissolved in the original volume of sterile, nonpyrogenic water for use to allow a direct comparison with the CBE stock. Interestingly, a substantial amount of heat, and an irritating vapor, were produced when the water was added to the ashed CBE.

Male guinea pigs (350-500 g; Camm Research Institute, Wayne NJ) were anesthetized and the trachea was removed, placed in modified Krebs-Henseleit physiological solution (O'Donnell et al., 1981), and cleaned. Rings consisting of two adjacent segments were prepared and opened by cutting through the cartilage wall opposite the trachealis muscle layer. Ligatures were tied to each end of the cut cartilage of the opened rings. The preparations were tied at one end to a holder, placed in water-jacketed 3 ml organ chambers containing modified Krebs-Henseleit solution (37° C) and attached at the other end to a force-displacement transducer for the measurement of isometric-tension responses. Resting tension was 0.4 g. The preparations were equilibrated, with washes at 15 min intervals with fresh bathing medium, for 1 hr prior to the beginning of the experiment.

Concentration-response relationships for ATP, adenosine (ADO), isoproterenol (ISO), and the various CBE preparations were obtained following the cumulative addition of these agents to the organ bath. When present, the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA; 10^{-6} M) and the adenosine transport inhibitor dipyridamole ($5X10^{-7}$ M) were added to the preparations 30 min prior to, and remained present during the addition of ATP and adenosine. To retard oxidation, the modified Krebs-Henseleit solution contained 0.1 mg/ml of ascorbic acid when ISO was used.

The results are expressed as means + S.E.M. n is the number of separate experiments. The data were evaluated for differences using Student's t-test for paired samples. The 0.05 level of probability was considered significant.

Results

The isolated GPT maintains intrinsic tone. In past experiments (Fedan et al., 1983) we observed that high concentrations of untreated CBE cause relaxation of the preparations below the level of resting tension. This effect was not observed in the present experiments in which untreated CBE ("Control") induced only contraction (Figure 1). Since similar but not identical botanical material was utilized in both cases to prepare the CBE, the reason for this discrepancy is not clear. Nevertheless, the responses of the tissues to CBE were very small with respect to those to methacholine, histamine,

5-hydroxytryptamine and KCl (Fedan et al., 1983). A comparison between the concentration-response curves for responses evoked by untreated, dialyzed and ashed CBE samples is also shown in Figure 1. Material which had dialyzed through the 12,000-14,000 cutoff molecular weight dialysis tubing ("Dialyzate") produced contractile responses which were not significantly different from those obtained with the untreated CBE ("Control") except for the response to 1 mg/ml which was significantly less than control. In contrast, the nondialyzable contents of the dialysis sack produced responses which were significantly smaller than those evoked with untreated CBE and with CBE dialyzate. In general, responses to ashed CBE were significantly less than untreated CBE (Figure 1). However, the maximum response to asked CBE was not different. As judged from the effective concentration producing 50% of the maximum response (EC50), ashed CBE was three-fold less potent than untreated CBE.

The effects of untreated CBE on relaxation responses induced by ADO and ATP are shown in Figure 2. In these experiments ADO and ATP produced relaxation of the tissues which had already developed spontaneous intrinsic tone. CBE (2.1 mg/ml) potentiated significantly the relaxation responses of the tissues to both ADO and ATP; the EC50 values of ADO and ATP were not affected. It seemed possible that this effect could have resulted artifactually from the small increase in tension which occurs when the EC50 concentration of CBE (see Figure 1) is applied to the tissues. This possibility was evaluated in two ways. In the first, a lesser concentration of CBE (0.3 mg/ml) which has a negligible effect on resting tension was employed. The results (Figure 2) indicated that this lesser concentration was able to potentiate significantly ATP-induced relaxation responses; the potentiation of responses to ADO was, however, lessened. The second approach which was used to evaluate the aforementioned possibility was to examine the possible effect of CBE on relaxation responses of the preparations to ISO, a beta-adrenergic receptor agonist. Were the potentiating effects of CBE on responses to ADO and ATP related solely to small increases in tension produced by CBE, then responses to ISO should be similarly enhanced. Shown in Figure 3 are results which demonstrate that responses of the preparations to ISO were not increased in the presence of CBE. In fact, the EC50 for ISO was reduced two-fold in the presence of CBE, indicative of a reduced sensitivity of the preparations to the agonist. The potentiating effect of CBE would appear to be relatively specific for responses mediated by purinergic receptors.

Discussion

Evidence has been obtained which indicates that the water-soluble agent(s) in our sample of Texas Southern Plains cotton which causes contraction and/or relaxation of the airway smooth muscle of guinea pigs and dogs (see Fedan et al., 1983) has a molecular weight less than 12,000-14,000, as assessed by dialysis. As such, these preliminary findings are compatible with those of Russell et al. (1982) who concluded that low molecular weight (less than 500) agents in a cotton bract extract which resemble 5-hydroxytryptamine could elicit contraction of isolated dog trachealis preparations. Our preparation of CBE differs from that of Russell et al., however, in that we do not utilize an initial acetone extraction step. While we have not been able to demonstrate 5-hydroxytryptamine-like activity in our CBE (unpublished observations), the work of Russell et al. and the present results would both seem to eliminate large proteins as candidate bronchoactive species in isolated muscle preparations.

A great deal of work has been done in recent years to isolate, characterize and test various organic and biological components of cotton plants for biological activity (Bell and Stipanovic, 1983). In view of this current emphasis in byssinosis research, we fully expected the ashing procedure to reduce substantially the airway smooth muscle contracting ability of our CBE, due to the destruction of organic compounds the procedure accomplishes. It was very surprising, therefore, that ashed CBE was nearly as effective as untreated CBE in evoking contraction of the GPT. It is widely known that many water-soluble divalent cations, such as barium, can initiate the contraction of smooth muscle or interfere with the mechanisms leading to contraction, and several minerals of the lanthanide series are active pharmacologically. For this reason it is tempting to speculate that the contraction induced by ashed CBE reflects the direct action of constituent minerals. This is an especially convenient hypothesis for two reasons. First, differences in bronchoactive activity in in vitro experiments on airway smooth muscle have been observed using bracts obtained from different varieties of cotton (Russell et al. 1983). The mineral content of the plants could be expected to have some relationship to cultivar type and soil conditions. Secondly, contractions evoked by CBE in our laboratory are not affected by muscarinic, H_1-histamine an 5-hydroxytryptamine receptor antagonists (atropine, pyrilamine and methysergide, respectively; unpublished observations). It is requisite to the hypothesis that the direct actions of minerals not be mediated by autonomic receptors. A strong conclusion regarding the importance of extractable minerals as important pharmacologically cannot be made at present, however, because the identity of the mineral salts formed when ashed CBE was dissolved in water is unknown. That is, the ashing procedure will have undoubtedly changed the salt form of the minerals to oxidized material, probably destroying many anionic species, and altered the oxidation state of the minerals as well. We suspect the formation of metal oxides may have occurred, as judged from the exothermic reaction which occurred when ashed CBE was dissolved. Insofar as anions as well as cations have pharmacological activity, the salts present in dissolved ashed CBE may well have been different from the endogenous ones. It will be necessary to obtain analytical information on dissolved ashed CBE to evaluate this possibility.

In previous experiments we observed that CBE caused a potentiation of NANC nerve-induced inhibitory responses (Fedan et al., 1983). The present results indicate that CBE potentiated relaxation responses induced by ADO and ATP. The identity of the NANC inhibitory neurotransmitter has not been defined unequivocally, but is regarded by many workers to be, possibly, ATP or ADO, its breakdown product. While a prejunctional action of CBE to facilitate the release of the NANC neurotransmitter cannot be evaluated from these or the previous experiments, the results of this study indicate that the inhibitory actions of ATP (or ADO) from a neural source, if they are in fact released neurogenically, would be potentiated in the presence of CBE. An earlier proposal (Fedan et al. 1983) that CBE does not inferfere with adrenergically-mediated inhibitory responses in dog or guinea-pig airway smooth muscles has received additional support in this study from the fact that relaxation responses to ISO were not potentiated by CBE. It should be mentioned that if ATP is demonstrated in the future not to be the NANC neurotransmitter, implying that the effect of CBE on responses to ATP and ADO observed here would be of little importance in byssinosis, the fact remains that NANC-induced neurogenic responses are nevertheless enhanced by CBE.

In virtually every experiment in which the effects of CBE on the GPT has been evaluated, contraction occurs at low concentrations of CBE while higher concentrations evoke relaxation (see Fedan et al., 1983). An exception to this pattern was seen, however, in the data of Figure 1. In preparations of dog trachealis the relaxation response to CBE has not ever been seen (see Fedan et al., 1983). The smooth muscle of the GPT contains a NANC inhibitory nervous innervation (Richardson and Bovehard, 1975; Souhrada et al., 1980); that of the dog does not (Richardson, 1979). The cumulative evidence suggests that CBE may contain an agent or agents which interact as agonists with postjunctional purinergic receptors, which mediate responses to ATP and ADO, or in some way facilitates responses mediated by these receptors. A hypothesis invoking the presence of purine-like compounds in CBE could explain the descending limb of the CBE concentration-response curves in GPT but not dog trachealis preparations: the effect could be manifest only when the NANC-inhibitory, possibly

purinergic, innervation exists in the muscle. It will be necessary in future experiments to evaluate the effects of purinergic receptor antagonists to test the validity of this hypothesis.

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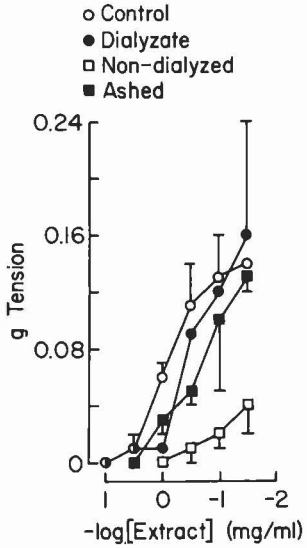

Figure 1. Comparison of concentration-response curves for contractile responses of isolated GPT to untreated CBE ("Control;" n=6), to the dialyzate of dialyzed CBE ("Dialyzate;" n=3), to the non-dialyzable contents of dialyzed CBE ("Non-dialyzed:" n=3) and to ashed CBE ("Ashed;" n=3).

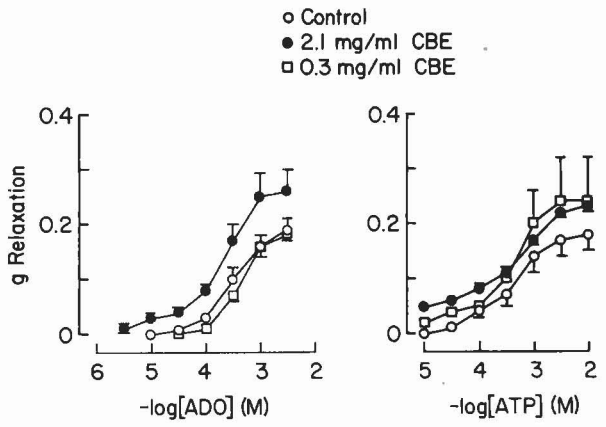

Figure 2. Effect of CBE on cumulative concentration-response curves for ADO- and ATP-induced relaxation of isolated GPT. The responses shown are for relaxation of the tissues which developed spontaneous resting tone. EHNA (10^{-6} M) and dipyridamole ($5X10^{-7}$ M) were present prior to and during the additions of ADO and ATP (see Materials and Methods). n=7 for ADO; n=4 for ATP.

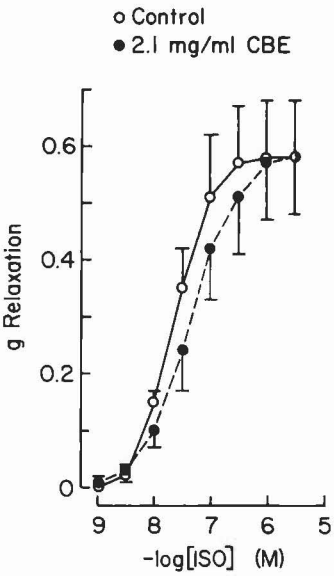

Figure 3. Effect of CBE on the cumulative concentration-response curve for ISO-induced relaxation of isolated GPT. The responses shown are for relaxation of the tissues which developed spontaneous resting time. n=6.

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