Pharmacological characterisation of extracts of coffee dusts

EUGENIJA ZUSKIN, PAMELA G DUNCAN, AND J S DOUGLAS

From the John B Pierce Foundation Laboratory, New Haven, Connecticut 06519, USA, and the A Stampar School of Public Health, Zagreb, Yugoslavia

ABSTRACT The contractile or relaxant activities or both of aqueous extracts of green and roasted coffees were assayed on isolated guinea pig tracheal spirals. Contractile and relaxant activities were compared with histamine and theophylline, respectively. Green coffee extracts induced concentration dependent contraction, but the maximal tension never exceeded $76.3\% \pm 5.2$ of a maximal histamine contraction (0.69 ± 0.07 g/mm² v 0.52 ± 0.05 g/mm²; $p < 0.01$). One gram of green coffee dust had a biological activity equivalent to 1.23 ± 0.1 mg of histamine. The pD₂ value of histamine was -5.17 ± 0.05 . The potency of green coffee was unaffected by mepyramine maleate (1 µg/ml, final bath concentration) while that of histamine was reduced 500 fold. Tissues contracted with histamine were not significantly relaxed by green coffee extracts. By contrast, roasted coffee extracts induced concentration dependent relaxation of uncontracted and histamine contracted tissues. Tissues contracted with green coffee extracts were also completely relaxed by roasted coffee extracts. The pD₂ value of theophylline was -4.10 ± 0.03 . The relaxant activity of 1 g of roasted coffee was equivalent to 1.95 ± 0.16 mg of theophylline. The potency of these extracts was significantly reduced after propranolol (1 µg/ml; dose ratio 1.56). Our results show that coffee dust extracts have considerable biological activity which changes from a contractile to a relaxant action as a consequence of processing. The greater incidence of adverse reactions to green coffee dust(s) in coffee workers may be related to the contractile activity present in green coffee dust.

Symptoms such as asthma, chronic bronchitis, rhinitis, or conjunctivitis are often reported by workers exposed to green or roasted coffee dusts.¹⁻⁵ Positive immediate-type skin reactions to green coffee bean extracts and increased levels of immunoglobulins, notably IgE, have suggested that these adverse reactions are reagin mediated hypersensitivity reactions.⁶ The fact that sensitivity to green coffee beans could be passively transferred to Macaque monkeys and non-atopic man using allergic sera of coffee workers provided further evidence that these responses are allergic in nature.⁷

Provocative inhalation challenges with green coffee in subjects showing positive skin and RAST tests result in an immediate asthmatic attack with decreases in FEV₁ greater than 20%.⁸ We have

reported that acute reactions to coffee dust exposure were not necessarily correlated with the immunological status of the individual.⁹

A direct examination of the biological activities of coffee dusts has not been carried out. In this paper we describe the effects of green and roasted coffee extracts on isolated guinea pig tracheal tissues; airways whose responses are pharmacologically similar to isolated human bronchi.¹⁰

Methods

PREPARATION OF COFFEE DUST EXTRACTS

Green and roasted coffee beans were ground to a powder; 50 g of powder was extracted with 500 ml of buffer. The composition of the buffer was (nM): NaCl, 85.5; KH₂PO₄, 2.6; Na₂HPO₄, 49; C₆H₅OH, 42.6; pH, 8. The extract was clarified by filtration through filter paper and finally via a Seitz filter. Aliquots (7 ml) of the filtrate were freeze dried in sterile bottles and stored at 4°C until reconstituted (see below).

Requests for reprints to: Dr James S Douglas, John B Pierce Foundation Laboratory, 290 Congress Avenue, New Haven, Conn 06519.

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GUINEA PIG TRACHEAL TISSUES IN VITRO

Contractile activity

Tracheal spirals¹¹ from female albino guinea pigs (350–450 g, Hartley strain, Camm Research, Wayne, NJ) were equilibrated under an initial tension of 8 g in Tyrode's solution at 37°C (gassed with 5% CO₂ in O₂). The high initial tension ensured that, after the 1.5 h equilibration period, the resting tension was between 4 and 6 g. Under these conditions responses to agonists were reproducible since the muscle was near its maximal length.¹² Tension was measured isometrically with a Statham strain gauge (Model UC3) and was displayed on a Honeywell 2-channel chart recorder (Electronix 19). Cross sectional area was determined as a ratio of the length of the preparation (measured at the end of the equilibration period) to the wet weight of the preparation (determined after the experiment).¹³ The composition of the Tyrode's solution was (millimolar concentrations): NaCl, 139.2; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 0.49; NaHCO₃, 11.9; NaH₂PO₄, 0.4; glucose, 5.5; pH, 7.4. Freeze dried extracts were reconstituted in 6 ml of Tyrode's solution immediately before testing. Experiments were designed to determine the reproducibility of response and to determine the potency of the coffee dust extracts. In initial experiments we established that only extracts of green coffee dust caused contraction of the tracheal spirals. Active concentrations of this dust extract were added to the tissue bath, in random order several times to ensure that tachyphylaxis did not develop. The potency of the coffee dusts was determined by comparing their biological activity with that of histamine. Concentrations of histamine, in a volume less than 0.5 ml, beginning with the highest histamine dose (final bath concentration, 50 μ M) were added to the organ bath (10 ml). Subsequent concentrations were added in random order to determine a concentration effect curve. When the response to histamine reached a plateau, the bath fluid was exchanged for fresh Tyrode's solution. An interval of roughly 15 minutes was necessary to allow the preparation to return passively to its resting tension. A new concentration of histamine was then added to the bath. Concentration effect curves to extracts of green coffee dust were generated in a similar manner.

Relaxant activity

Preparations were equilibrated as above. The relaxant activity of the coffee dust extracts was assessed on the resting tone or after tone was induced with histamine. Experiments tested the reproducibility of the relaxant activity. Cumulative concentration effect curves to the relaxant theophylline and the coffee dust extracts were generated after tone was

induced in the preparation with a maximal concentration of histamine.¹⁴ When the induced tension reached a plateau, we added the relaxant to the bath. Relaxations produced were expressed as a percentage of the tension developed with histamine. A theophylline relaxation curve always preceded examination of the coffee extracts, thus enabling us to quantitate the relaxant activity relative to theophylline.

Drug treatment

A concentration effect curve was generated first to histamine and then to green coffee extract. Concentration effect curves were re-examined after the preparations were treated with mepyramine maleate (1 μ g/ml, final bath concentration). Tissues were incubated for 30 minutes after which the Tyrode's solution was exchanged with fresh medium containing mepyramine maleate (1 μ g/ml) — that is, preparations were treated with and then continuously exposed to the antihistamine.

We also determined the effects of dl-propranolol treatment on the relaxant potency of roasted coffee extracts. Concentration effect curves to roasted coffee were generated before (see above) or after preparations were treated with Tyrode's solution (as control) or with dl-propranolol (1 μ g/ml, final bath concentration). The treatment period was always 30 minutes, and the drug was washed out before a new concentration effect curve was generated.

Calculation of results

pD₂ values of histamine were derived from concentration effect curves; pD₂ values were defined as the negative logarithm of the molar EC₅₀ value (the concentration of histamine required to induce a tension that was 50% of maximum). Volumes of coffee extracts that induced 50% of their maximal responses were interpolated from concentration effect curves (EV₅₀). Biologically, the EV₅₀ has an effect equivalent to the EC₅₀ of histamine. Hence the activity of 1 g of coffee dust can be calculated as mg equivalents of histamine. Maximal tensions to histamine and coffee dust extracts were compared using Student's *t* test for paired variates.

pD₂ values of theophylline were derived from concentration effect curves in a similar manner. In this instance the concentration of relaxant which decreased the total tension by 50% was defined as the molar EC₅₀ value. The pD₂ value was the negative logarithm of the molar EC₅₀.

The activity of 1 g of coffee dust was calculated (using the EV₅₀ value) as mg equivalents of theophylline. For these experiments, experimental data were corrected for a small relaxation which was induced by the vehicle alone (see results). All values are the mean $\pm$ SEM.

Results

Extracts of green coffee increased the basal tone of tracheal spirals. Repeated additions of the same volume of green coffee extract gave reproducible responses (fig 1)—that is, tachyphylaxis did not develop. Responses to histamine were also reproducible and concentration dependent. The pD_2 value of histamine was -5.17 ± 0.05 for 13 experiments. This value is comparable to our previously published data.¹⁴ While all volumes of green coffee extract increased tracheal tone and there was generally a direct relationship between volume and response (fig 2), there was always a smaller response to 5 μ l of extract than to 3 μ l of extract ($p < 0.01$). Maximal responses to green coffee extracts were $76.3\% \pm 5.2$ of the maximal histamine contraction. Mean tensions induced in tracheal spirals by histamine and green coffee extracts were 0.69 ± 0.07 g/mm² and 0.52 ± 0.05 g/mm², respectively ($p < 0.01$). The potency of green coffee was determined by relating the volume of the extract which induced a 50% response with the pD_2 value of histamine (fig 2). To ensure that the biological activity was associated with green coffee per se, we also assayed freeze dried aliquots of buffer—that is, the extracting medium. These samples did not contract tracheal spirals. Green coffee extracts appeared to relax histamine contracted tissues but when compared with tissues treated with buffer alone, the effect was not significant (fig 3). The pharmacological activity of green coffee extract was equivalent to 1.23 ± 0.01 mg histamine/g green coffee dust. Treatment of preparations with the antihistamine, mepyramine maleate, reduced the sensitivity of the tissues to histamine approximately 500 fold (fig 4). The maximal response to histamine was also significantly depressed (see discussion). Volumes of green coffee extracts required to induce a 50% of maximal response (to green coffee) were not

Fig 1 Reproducibility of responses of guinea pig tracheal spirals to green coffee bean extracts. An original tracing shows repeated responses to green coffee extracts. One microlitre of green coffee extract is equivalent to 116.6 μ g of coffee dust. A maximal contraction with histamine (5×10^{-5} M) is shown for comparison. Note that there was an interval of at least 15 minutes (not shown) between additions of the extract.

Fig 2 Concentration (volume) effect curves to green coffee and histamine in guinea pig tracheal spirals. Histamine concentrations (abscissa) were added in random order to generate a concentration effect curve. Response (●) was expressed as a percentage of response to 5×10^{-5} M histamine. Aliquots of green coffee were added to tissues in random order. Response (□) was expressed as a percentage of maximal response to histamine. Values are mean $\pm$ SEM of 13 experiments for each agent. Arrows (abscissa) show concentrations inducing responses that were 50% of maximal for each agonist studied. *Signifies values where the mean response to 5 μ l extract was significantly less ($p < 0.01$) than mean response to 3 μ l extract. **Signifies values significantly less than maximal response to histamine ($p < 0.01$).

Fig 3 Concentration (volume) effect curves to theophylline and green and roasted coffee extracts. Tracheal spirals were contracted with 5×10^{-5} M histamine and relaxed by cumulative addition of theophylline (●; $n = 19$), roasted coffee extract (Δ ; $n = 5$), green coffee extract ($\square$; $n = 5$) or freeze dried buffer ($\circ$; $n = 4$). Values are means $\pm$ SEM. Concentrations which induced relaxations that were 50% of maximal were interpolated as shown in fig 2.

Fig 4 Antihistamine and airway contraction with green coffee dust extract. Concentration (volume) effect curves were generated (see methods) to histamine (●, $n = 2$) and then to green coffee extract (■, $n = 2$). Tissues were incubated for 30 minutes with mepyramine maleate (1 $\mu\text{g}/\text{ml}$, final bath concentration) and then continuously exposed to antihistamine during generation of another histamine (○) and green coffee extract (□) concentration effect curve. Values are mean of two experiments, whose values did not differ by more than 5%.

Fig 5 β -Adrenoceptor blockade and airway relaxation with roasted coffee extracts. Concentration (volume) effect curves to roasted coffee were determined in preparations contracted with histamine ($5 \times 10^{-5} \text{ M}$). Panel A shows mean data from two experiments whose values did not differ by more than 5% before (●) and after a 30 minute incubation in Tyrode's solution (○). Panel B shows similar relaxation curves before (●) and after (○) a 30 minute incubation in dl-propranolol (1 $\mu\text{g}/\text{ml}$ final bath concentration). Values are mean $\pm$ SEM of five experiments. *Signifies values significantly different from pretreatment values (●, in panel B).

significantly different after antihistamine treatment if the decreased responsiveness of the tissues to the extract was taken into account (fig 4).

Tissues were never contracted by roasted coffee extracts. When added to resting tracheal spirals, roasted coffee relaxed basal tone. This relaxant

activity was also demonstrable on histamine contracted tissues. Relaxation with roasted coffee was concentration dependent, and the efficacy of the relaxant(s) was sufficient to reverse completely a maximal histamine contraction (fig 3). Maximal contractions induced with extracts of green coffee beans were also completely reversed in a concentration dependent manner by roasted coffee extracts (data not shown). Relaxant activity was compared with the bronchodilator, theophylline. This drug completely relaxed preparations (pD_2 value— 4.10 ± 0.03 for 19 preparations) as we have previously reported.¹⁴ The potency of roasted coffee was calculated from concentration effect curves by relating the volume of the extract that induced a 50% of maximal relaxation to the pD_2 value of theophylline (fig 3). Relaxation curves were corrected for the small relaxant activity shown for freeze dried buffer before calculation. One gram of roasted coffee had a pharmacological potency equivalent to $1.95 \pm 0.16 \text{ mg}$ of theophylline. Concentration effect curves to roasted coffee were reproducible when repeated after incubation for 30 minutes in Tyrode's solution (fig 5A). The potency of roasted coffee was reduced 1.6 fold when preparations were treated with dl-propranolol ($p < 0.05$; fig 5B).

Discussion

We have shown that green coffee dust has components that can induce airway constriction by a direct action on airway smooth muscle. This contractile activity is not tachyphylactic (fig 1) and is concentration dependent (fig 2). In preliminary experiments we examined two sources of green coffee dust—namely, dust derived from sacks containing the raw green coffee beans, pericarps, and leaves and stems and dust derived from pure green coffee beans devoid of their brown covering, the pericarp. The pharmacological activities of these extracts were identical and fig 2 shows the combined dose response data. The amount of biologically active material(s) present in these coffee extracts is considerably greater than in other aqueous vegetable extracts when quantitated from assays on tracheal spirals. For example, green coffee dust extracts have 10 to 50 times more contractile activity per gram of dust than cotton dust extracts.^{15 16} When extracts of green coffee were added to tracheal preparations previously contracted with histamine (see above), the preparations either contracted further or relaxed. The nature of the response was dependent on the amount of extract added—that is, small volumes induced contraction while larger volumes induced relaxation (fig 3). When this pattern of response was compared with appropriate control

data—that is, freeze dried buffer—it was evident that green coffee has only contractile activity (fig 3). Our observations show the presence of a potent contractile agent present in large quantities in green coffee beans.

Contractile activity in other vegetable dust extracts has been principally associated with the presence of histamine or high concentrations of cations such as potassium or both. Although treatment with the antihistamine mepyramine appears to reduce the sensitivity of the tissues to green coffee extracts, interpretation of these data is complicated by the simultaneous decrease in the responsiveness of preparations after mepyramine maleate (ordinate, fig 4). If these results are analysed by determining the volumes of green coffee extract which induce responses that are 50% of maximal before and after the antihistamine then the antihistamine does not affect the potency of green coffee extracts. By contrast, even though the responsiveness of tissues to histamine was slightly reduced, the sensitivity of the preparations to histamine was reduced 500 fold after mepyramine treatment (abscissa, fig 4). We can therefore conclude that the contractile activity present in green coffee bean extracts is not due to the presence of histamine. Since the contractile activity is not demonstrable after green coffee beans have been roasted, the activity is unlikely to be due to the presence of cations in the extracts since these substances are not heat labile. Alternatively, the absence of contractile activity after roasting may be related to the production of potent bronchodilator substances that functionally antagonise the contractile agent (see below). We show that roasted coffee extracts can completely reverse airway constriction induced by green coffee extracts. The presence of a parasympathomimetic substance in regular and decaffeinated coffees has been reported by Kalsner.¹⁷ The substance we describe may be similar, since many plant products contain substances with cholinergic or anticholinesterase activity.^{18,19} Further experiments are in progress to characterise this potent contractile agent.

By contrast, roasted coffee bean extracts show strong relaxant activity that was demonstrable in tracheal tissues with or without induced tone (fig 3). Our experiments show that the relaxant activity is stable to high temperature and does not induce tachyphylaxis (fig 5). DL-propranolol reduces the potency of the extracts approximately 1.6 fold. It is unlikely, however, that the effects of this drug are related to its beta-adrenoceptor blocking activity, as this concentration of propranolol decreases the potency of catecholamines at the beta-adrenoceptor by at least tenfold. Additionally, most relaxant catecholamines are unstable in aqueous solution,

unstable to light, and rapidly lose biological activity, while the roasted coffee extracts have a persistent relaxant activity over many weeks without a loss in potency. Relaxation could be caused by a release of catecholamine stores from within tracheal tissues, but the small dose ratio and the reproducibility of relaxant curves do not support this hypothesis. Alternatively, dl-propranolol is known to affect the membrane transport of cations and to exhibit local anaesthetic properties. Reduction in the potency of roasted coffee may be related to either of these effects, though it is noteworthy that in the same situation the contractile response to histamine was unaffected. We therefore believe that our data with propranolol preclude a role for catecholamines as relaxant agent(s) in roasted coffee extracts.

Caffeine is a well known constituent of coffee²⁰⁻²³ and like theophylline (fig 3) is a potent smooth muscle relaxant.²⁴ We have determined that 1 g of roasted coffee has a biological activity equivalent to 1.95 ± 0.16 mg of theophylline. This would be equivalent to 1.48 mg of caffeine/g of roasted coffee if the relative potency of caffeine to theophylline were taken as 0.76.²⁴ Chemical extraction of roasted coffee beans has shown caffeine levels ranging from 8 mg/g²⁰ to 20 mg/g.²³ In one of the few papers that gave information about the weights of coffee powder used to prepare extracts the calculated caffeine content was 1.6 mg/g for a percolated extract and 1.8 mg/g for a drip extract.²¹ If our extraction procedure removed even these lower amounts of caffeine then the total activity could be due to the xanthine(s) present. Green coffee bean extracts show no relaxant activity, yet chemical assay indicates that the caffeine content of these beans is identical to that found in roasted coffee beans.²³ These results suggest that either the green coffee beans contain a potent heat labile contractile agent or the caffeine present in these beans is in a form that cannot be extracted into aqueous solution. Separation of the biologically distinct substances would resolve this question.

Adverse reactions of workers to coffee dusts have always been considered to be of an allergic nature, but no studies have examined the biological activities of the dusts. In this study we show a potent bronchoconstrictor activity in extracts of green coffee beans when assayed on isolated guinea pig airways that as previously noted are pharmacologically similar to human airway muscle.¹⁰ Our data suggest that green coffee dusts may induce acute bronchoconstriction in man without allergic hypersensitivity. Most respiratory symptoms occur in the handling of green coffee beans. In these areas the mean total dust concentration was 11 mg/m³ of which 0.3 mg/m³ is respirable.⁵ While small quantities of dust may

be deposited in the lungs, green coffee dust may possibly induce bronchospasm by direct or reflex mechanisms or both, especially in workers who do not show allergic hypersensitivity and in whom the airways are sensitive to inhaled agents.²⁵ Fewer respiratory symptoms are reported by workers handling roasted coffee. This might be related to the reduced mean total dust (4.3 mg/m^3) and mean respirable dust (0.08 mg/m^3) levels.⁵ In some healthy subjects roasted coffee extracts induce small but significant decrements in pulmonary function.²⁶ Only controlled experiments in man that examine the potencies of the constrictor and dilator components of coffee extracts will elucidate their effects in the workplace. Nevertheless, in future studies the presence and potential of potent airway muscle contractile and relaxant agents in coffee dusts in relationship to the incidence of respiratory symptoms should be considered. The dramatic change in the pharmacological profile of the coffee dusts that occurs during processing also bears investigation.

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