Dexamethasone attenuates grain sorghum dust extract-induced increase in macromolecular efflux in vivo

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A GROWING BODY of clinical evidence indicates that exposure of farmers and grain handlers to grain sorghum dust is associated with incapacitating upper airway irritation (4, 5, 13, 21). A characteristic feature of this response is marked congestion of the upper airway mucosa that compromises upper airway patency (5, 13, 21). To this end, recent work from our laboratory showed that dexamethasone attenuates grain sorghum dust extract-induced increase in macromolecular efflux from the in situ hamster cheek pouch and, if so, whether this response is specific. By using intravitral microscopy, we found that an aqueous extract of grain sorghum dust elicited significant, concentration-dependent leaky site formation and increase in clearance of FITC-labeled dextran (FITC-dextran; mol mass, 70 kDa) from the in situ hamster cheek pouch (P < 0.05). This response was significantly attenuated by dexamethasone (10 mg/kg iv). Dexamethasone also attenuated substance P-induced leaky site formation and increase in clearance of FITC-dextran from the cheek pouch but had no significant effects on adenosine-induced responses. Dexamethasone had no significant effects on arteriolar diameter in the dorche pouch. On balance, these data indicate that dexamethasone attenuates grain sorghum dust extract- and substance P-induced increases in macromolecular efflux from the in situ hamster cheek pouch in a specific fashion.

METHODS

General Methods

Collection and preparation of grain sorghum dust extract. The extract was prepared by using methods previously described in our laboratory (6, 9). Briefly, settled dust from sorghum grains was collected from grain storage bins during the harvest season. One gram of grain sorghum dust was gently mixed with 10 ml of Hank's balanced salt solution for 60 min. The suspension was allowed to settle at room temperature for 90 min. Large-particulate debris were removed from the suspension by centrifugation at 5,000 g for 10 min. The supernatant was then decanted and filtered through a 0.22-μm pore filter (Millipore, Bedford, MA). Five-milliliter samples of the supernatant, designated arbitrarily as 100% grain sorghum dust extract, were snap frozen in liquid nitrogen and stored at −70°C until used. On the day of the experiment, some of these samples were thawed and diluted in saline to the desired concentrations.

Preparation of animals. Adult, male golden Syrian hamsters weighing 129 ± 4 g (n = 24) were anesthetized with pentobarbital sodium (6 mg/100 g body wt ip). A tracheotomy was performed to facilitate spontaneous breathing. A femoral ven was cannulated to inject the intravascular tracer, FITC-labeled dextran (FITC-dextran; mol mass, 70 kDa) and supplemental anesthetic (1 mg/mg 100 g body wt −1 h−1). A femoral artery was cannulated to inject arterial blood samples and monitor arterial blood pressure, which did not change significantly during the experiments. Body temperature was kept constant (37–38°C) throughout the experiment with the use of a heating pad.

To visualize the microcirculation of the cheek pouch, we used a method previously used by us and other investigators (2, 7–10, 12, 14, 17, 18, 26–29, 31). Briefly, the left cheek pouch was spread gently over a small plastic baseplate, and an incision was made in the outer skin to expose the cheek pouch membrane. The avascular connective tissue layer was removed, and a plastic chamber was positioned over the baseplate and secured in place by suturing the skin around the upper chamber. This chamber contains the suspension fluid. This arrangement forms a triple-layered complex: the baseplate, the upper chamber, and the cheek pouch membrane exposed between the two plates. After these initial procedures, the hamster was transferred to a heated microscope stage. The chamber was connected to a reservoir containing warmed bicarbonate buffer (37–38°C) that allowed continuous suffusion of the cheek pouch. The buffer was bubbled continuously with 95% O2-5% CO2 (pH 7.4). The

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chamber was also connected via a three-way valve to an infusion pump (model 341B, Sage Instruments, Boston, MA) that allowed constant administration of grain sorghum dust extract and drugs into the suffusate.

Determination of clearance of macromolecules. The cheek pouch microcirculation was visualized with an Olympus microscope (Jacobs Instruments, Shawnee Mission, KS) coupled to a 100-W mercury light source at a magnification of ×40. Fluorescence microscopy was accomplished with the aid of filters that matched the spectral characteristics of FITC-dextran (7–9, 14). Macromolecular leakage was determined by extravasation of FITC-dextran, which appeared as fluorescent “spots” or leaky sites around postcapillary venules. The number of leaky sites was determined by counting three random microscopic fields every minute for the first 7 min and then at 5-min intervals for 30–60 min after each intervention (see Experimental Protocols). The total number of leaky sites was averaged and expressed as the number of leaky sites per 0.11 cm² of cheek pouch, corresponding to the area of one microscopic field as previously described in our laboratory (7–9).

In experiments in which clearance of FITC-dextran was calculated, the suffusion fluid was collected at 5-min intervals throughout the experiment by a fraction collector (Microfractionator, Gilson Medical Electronics, Middleton, WI). Samples were collected in glass test tubes, and the concentration of FITC-dextran was determined. Arterial blood samples were collected in heparinized capillary tubes (70-μl volume; Scientific Products, McGaw Park, IL) beginning 5 min before and at 5, 30, 60, 120, 180, and 240 min after injection of FITC-dextran. The concentration of FITC-dextran was determined in all plasma samples. We and other investigators have shown that plasma concentration of FITC-dextran peaks within 10 min after intravenous injection and decreases slowly thereafter during the entire duration of the experiment (7–10, 14). To quantitate the concentration of FITC-dextran in the plasma and suffusate, a standard curve for FITC-dextran concentrations vs. percent emission was performed on a spectrophotofluorometer (Photon Technology International, Princeton, NJ). The standard was FITC-dextran prepared on a weight per volume basis. With the bicarbonate buffer used as background, a standard curve was established for each experiment, and each curve was subjected to linear regression analysis. The percent emission for unknown samples (plasma and suffusate) was measured on the spectrophotofluorometer, and the concentration of FITC-dextran was calculated from the standard curve. In preliminary experiments, minimal fluorescence signal (<2% above background) was detected when drugs were added to the buffer and when plasma and suffusate samples were examined before the addition of FITC-dextran. Clearance of FITC-dextran was determined by calculating the ratio of suffusate (ng/ml) to plasma (mg/ml) concentration of FITC-dextran and multiplying this ratio by the suffusate flow rate (2 ml/min) (7–9).

Determination of arteriolar diameter. The cheek pouch microcirculation was visualized with a microscope (Nikon, Tokyo, Japan) coupled to a 100-W mercury light source at a magnification of ×40. The microscope image was projected through a low-light television camera (Panasonic TR-124 MA, Matsushita Communication Industrial, Yokohama, Japan) onto a video screen (Panasonic). The inner diameter of second-order arterioles (41–53 μm) was measured during the experiment from the video display of the microscope image by using a videomicrot (model VIA 100, Boeckler Instruments, Tucson, AZ) as previously described in our laboratory (26). In each animal, the same arteriolar segment was used to measure vessel diameter during the experiment.

Experimental Protocols

Effects of dexamethasone on grain sorghum dust extract-induced responses. The purpose of these studies was to determine whether dexamethasone attenuates grain sorghum dust extract-induced leaky site formation and increase in clearance of FITC-dextran from the in situ cheek pouch. After buffer was suffused for 30 min (equilibration period), FITC-dextran was injected intravenously and the number of leaky sites and clearance of FITC-dextran were determined for 30 min. The concentration of FITC-dextran in the suffusate rose rapidly after the injection and stabilized within 30 min, whereas no leaky sites were observed (see below). Then, two concentrations of grain sorghum dust extract (1 and 10%) were suffused in a random order for 20 min each as previously described in our laboratory (9). The number of leaky sites was determined every 1 min for 7 min and at 5-min intervals for 60 min thereafter. Clearance of FITC-dextran was determined before and every 5 min for 60 min. In a previous and preliminary studies, we found that the number of leaky sites and clearance of FITC-dextran increased significantly from baseline within 30 min after the start of grain dust extract suffusion, reached maximal value within 30 min, and returned to baseline within 45 min (9). The time interval between subsequent suffusions of grain sorghum dust extract was 60 min (9). After suffusion of grain sorghum dust extract was stopped and the number of leaky sites returned to baseline, dexamethasone (10 mg/kg) was administered intravenously over a 30-min period by using an infusion pump (final volume, 1 ml) and suffusion of grain sorghum dust extract was repeated. The number of leaky sites and clearance of FITC-dextran were determined during each intervention. In preliminary studies, we determined that repeated suffusions of grain sorghum dust extract (1 and 10%) for 20 min before and after suffusion of saline (vehicle) for 60 min were associated with reproducible leaky site formation (9 ± 0.11 and 10 ± 0.11 cm² and 13 ± 1.01 and 15 ± 0.11 cm², respectively; each group, n = 4; P > 0.5) and increase in clearance of FITC-dextran (32 ± 8 and 36 ± 6 ml/min × 10⁻⁶ and 68 ± 8 and 72 ± 11 ml/min × 10⁻⁶, respectively; each group, n = 4; P > 0.5). In addition, intravenous administrations of dexamethasone (10 mg/kg) and suffusion of saline (vehicle) for the entire duration of the experiment had no significance effects on leaky site formation and clearance of FITC-dextran (data not shown). The concentrations of grain sorghum dust extract and dexamethasone used in these studies are based on previous studies in our laboratory (6, 9, 12, 18).

Effects of dexamethasone on substance P-induced responses. Gao et al. (9) showed that grain sorghum dust-induced increase in clearance of macromolecules from the cheek pouch is mediated, in part, by stimulation of sensory nerves to release substance P. Hence, the purpose of these studies was to determine whether dexamethasone attenuates substance P-induced leaky site formation and increase in clearance of FITC-dextran from the in situ cheek pouch. After the equilibration period, FITC-dextran was injected intravenously, and the number of leaky sites and clearance of FITC-dextran were determined for 30 min. Then, two concentrations of substance P (1.0 and 1.5 μM) were suffused in a random order for 7 min each (8). The number of leaky sites was determined every 1 min for 7 min and at 5-min intervals for 60 min thereafter. Clearance of FITC-dextran was determined before and every 5 min for 60 min. In a previous and preliminary studies, we found that the number of leaky sites increased significantly from baseline within 2–3 min of the start of substance P suffusion, reached maximal value within 5 min, and returned to baseline within 30 min (8). Clearance
of FITC-dextran was maximal 5 min after the start of substance P suffusion and returned to baseline within 30 min (8). The time interval between subsequent suffusions of substance P was 45 min (8). After suffusion of substance P was stopped and the number of leaky sites returned to baseline, dexamethasone (10 mg/kg) was administrated intravenously, and suffusion of substance P was repeated. The number of leaky sites and clearance of FITC-dextran were determined during each intervention. In preliminary studies, we determined that repeated suffusions of substance P (1.0 and 1.5 µM) for 7 min before and after suffusion of saline (vehicle) for 45 min were associated with reproducible leaky site formation (7 ± 1/0.11 and 8 ± 1/0.11 cm² and 13 ± 2/0.11 and 14 ± 1/0.11 cm², respectively; each group, n = 4; P > 0.5) and increase in clearance of FITC-dextran (26 ± 4 and 28 ± 2 ml/min × 10⁻⁶ and 42 ± 5 and 44 ± 3 ml/min × 10⁻⁶, respectively; each group, n = 4; P > 0.5). The concentrations of substance P used in these studies are based on a previous study in our laboratory and reports in the literature (8, 12, 18, 29).

Effects of dexamethasone on adenosine-induced responses.

The purpose of these studies was to determine the specificity of dexamethasone attenuation of grain sorghum dust extract- and substance P-induced responses. To accomplish this goal, we determined the effects of dexamethasone on adenosine-induced leaky site formation and increase in clearance of FITC-dextran from the cheek pouch. Adenosine, like substance P, increases clearance of macromolecules from the hamster cheek pouch by a specific, receptor-mediated mechanism(s) (8–10, 17). After the equilibration period, FITC-dextran was injected intravenously and the number of leaky sites and clearance of FITC-dextran were determined for 30 min. Then, adenosine (10 µM) was suffused for 7 min (8, 10). The number of leaky sites was determined every min for 7 min and at 5-min intervals for 45 min thereafter. Clearance of FITC-dextran was determined before and every 5 min for 45 min. After suffusion of adenosine was stopped and the number of leaky sites returned to baseline, dexamethasone (10 mg/kg) was administrated intravenously and suffusion of adenosine was repeated as outlined above. The number of leaky sites and clearance of FITC-dextran were determined during each intervention. In preliminary experiments, we determined that repeated suffusions of adenosine (10 µM) for 7 min before and after suffusion of saline (vehicle) for 45 min were associated with reproducible results. The concentration of adenosine used in these studies is based on previous studies in our laboratory and reports in the literature (8–10, 17).

Effects of dexamethasone on arteriolar diameter.

The purpose of these studies was to determine whether dexamethasone attenuation of grain sorghum dust extract- and substance P-induced responses is related, in part, to vasoconstriction in the cheek pouch. After the equilibration period, dexamethasone (10 mg/kg) was administrated intravenously over a 30-min period by using an infusion pump. Arteriolar diameter was determined before, every minute during infusion of dexamethasone, and at 5-min intervals thereafter for 60 min.

Drugs

FITC-dextran, dexamethasone, substance P, and adenosine were obtained from Sigma Chemical (St. Louis, MO). Hanks’ balanced salt solution was obtained from GIBCO (Grand Island, NY). All drugs were prepared fresh before each experiment and were diluted in saline to the desired concentrations.

Data and Statistical Analyses

When a test compound was suffused over the cheek pouch, we determined the maximal change in the number of leaky sites and clearance of FITC-dextran and used these values as the response to that compound. Data are expressed as means ± SE except for body weight, which is expressed as means ± SD. Because the number of leaky sites returned to baseline (nil) between successive applications of test compounds, all vehicle (saline) control data are expressed as a single value for each experimental condition. Statistical analysis was performed by using two-way analysis of variance and the Newman-Keuls test for multiple comparisons. A P < 0.05 was considered significant.

RESULTS

Effects of Dexamethasone on Grain Sorghum Dust Extract-Induced Responses

Suffusion of grain sorghum dust extract elicited significant, concentration-dependent leaky site formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 1; each group, n = 4; P < 0.05). Dexamethasone (10 mg/kg iv) significantly attenuated grain sorghum dust extract-induced responses (Fig. 1; each group, n = 4; P < 0.05). The number of leaky sites increased significantly from 13 ± 1/0.11 cm² during suffusion of grain sorghum dust extract (10%) alone to 2 ± 1/0.11 cm² during suffusion of grain sorghum dust extract (10%) in the presence of dexamethasone (Fig. 1, top; each group, n = 4; P < 0.05). Similarly, clearance of FITC-dextran decreased significantly from 69 ± 10 ml/min × 10⁻⁶ during suffusion of grain sorghum dust extract (10%) alone to 22 ± 1 ml/min × 10⁻⁶ during suffusion of grain sorghum dust extract (10%) in the presence of dexamethasone (Fig. 1, bottom; each group, n = 4; P < 0.05).

Effects of Dexamethasone on Substance P-Induced Responses

Suffusion of substance P on the cheek pouch elicited significant, concentration-dependent leaky site formation and increase in clearance of FITC-dextran that were significantly attenuated by dexamethasone (10 mg/kg iv; Fig. 2; each group, n = 4; P < 0.05). The number of leaky sites decreased significantly from 13 ± 1/0.11 cm² during suffusion of substance P (1.5 µM) alone to 2 ± 1/0.11 cm² during suffusion of substance P (1.5 µM) in the presence of dexamethasone (Fig. 2, top; each group, n = 4; P < 0.05). Similarly, clearance of FITC-dextran decreased significantly from 40 ± 5 ml/min × 10⁻⁶ during suffusion of substance P (1.5 µM) alone to 16 ± 3 ml/min × 10⁻⁶ during suffusion of substance P (1.5 µM) in the presence of dexamethasone (Fig. 2, bottom; each group, n = 4; P < 0.05).

Effects of Dexamethasone on Adenosine-Induced Responses

Suffusion of adenosine on the cheek pouch elicited significant leaky site formation and increase in clear-
ance of FITC-dextran that were not attenuated by dexamethasone (10 mg/kg iv; Fig. 3; each group, n = 4; P > 0.5). The number of leaky sites was 11 ± 2/0.11 cm² during suffusion of adenosine (10 µM) alone and 9 ± 3/0.11 cm² during suffusion of adenosine (10 µM) in the presence of dexamethasone (Fig. 3, top; each group, n = 4; P > 0.5). Similarly, clearance of FITC-dextran was 42 ± 11 ml/min × 10⁻⁶ during suffusion of adenosine (10 µM) alone and 38 ± 11 ml/min × 10⁻⁶ during suffusion of adenosine (10 µM) in the presence of dexamethasone (Fig. 3, bottom; each group, n = 4; P > 0.5).

Effects of Dexamethasone on Arteriolar Diameter

Dexamethasone (10 mg/kg iv) had no significant effects on arteriolar diameter in the cheek pouch. Arteriolar diameter decreased by 1 ± 2% from baseline in the presence of dexamethasone (n = 4, P > 0.5).

DISCUSSION

There are two new findings of this study. First, we found that dexamethasone, a potent anti-inflammatory drug, attenuates grain sorghum dust extract-induced increase in macromolecular efflux from the in situ hamster cheek pouch. This response is specific because...
Dexamethasone had no significant effects on adenosine-induced increase in clearance of macromolecules and arteriolar diameter in the cheek pouch. Second, dexamethasone attenuates substance P-induced increase in clearance of macromolecules from the cheek pouch. These effects could not be attributed to emerging tolerance or nonspecific microvascular dysfunction because repeated suffusions of grain sorghum dust extract and substance P were associated with reproducible increases in macromolecular efflux. On balance, these data indicate that dexamethasone attenuates grain sorghum dust extract- and substance P-induced increases in clearance of macromolecules from the in situ hamster cheek pouch in a specific fashion.

Consideration of Methods

We and others have used the hamster cheek pouch model extensively to investigate the effects of noxious stimuli and environmental toxicants, including grain sorghum dust extract and substance P, on macromolecular efflux in situ (2, 7–10, 14, 17, 27–29). Solute efflux is determined in this model by two reproducible parameters, leaky site formation and clearance of FITC-dextran, thereby providing quantitative appraisal of macromolecular transport across postcapillary venules in the cheek pouch during these interventions.

Vasoconstriction and/or a decrease in venular driving pressure may have mediated, in part, the salutary effects of dexamethasone on grain sorghum dust extract- and substance P-induced responses. However, this possibility seems unlikely because dexamethasone had no significant effects on adenosine-induced increase in macromolecular efflux. Adenosine, like substance P, increases clearance of macromolecules from the cheek pouch by a specific, receptor-mediated mechanism(s) (8–10, 17). In addition, dexamethasone had no significant effects on the diameter of resistance arterioles that regulate venular driving pressure in the cheek pouch (17, 26). If dexamethasone alters vasomotor tone and/or venular driving pressure in the cheek pouch microcirculation, it should also have attenuated adenosine-induced responses. The results of this study clearly show otherwise. Taken together, these data are consistent with previous studies in the cheek pouch and other vascular beds that dissociated changes in vascular tone from macromolecular transport (16, 17, 31, 33).

There are two inherent methodological limitations of this study. First, we used an aqueous extract of grain sorghum dust rather than dry dust. Conceivably, certain constituents of grain sorghum dust could have been removed and/or altered during preparation of the extract (4, 5, 24, 32). Although we cannot refute this hypothesis, Gao (7) showed that suffusion of tannic acid, a major constituent of organic dust (22), increases macromolecular efflux from the in situ hamster cheek pouch. Secondly, microvascular responses in the cheek pouch suffused with grain sorghum dust extract may not represent those in the upper airway mucosa of other laboratory animals and humans (1, 3, 4, 5, 11, 13, 24, 25, 32). Nonetheless, Gao (6) showed recently that grain sorghum dust extract similar to that used in this study increases macromolecular efflux from the in situ hamster cheek pouch and that this response is substance P dependent. Second, microvascular responses in the cheek pouch suffused with grain sorghum dust extract may not represent those in the upper airway mucosa of other laboratory animals and humans (1, 3, 4, 5, 11, 13, 24, 25, 32). Nonetheless, Gao (6) showed recently that grain sorghum dust extract similar to that used in this study increases macromolecular efflux from the in situ hamster nasal mucosa and that this response is mediated, in part, by substance P. Clearly, additional studies are warranted to determine the role of substance P in the pathophysiology of upper airway mucosa congestion observed in laboratory animals and humans exposed to dry grain sorghum dust.

Consideration of Previous Studies

Trapp et al. (32) showed that corticosteroids attenuate influx of inflammatory cells into the lower airway of healthy volunteers exposed to corn dust extract.
addition, dexamethasone inhibited transmigration of neutrophils from the in situ hamster cheek pouch elicited by substance P (12, 18). However, the effects of corticosteroids on macromolecular efflux in the airway mucosa were not determined in these studies. The results of this study show, for the first time, that dexamethasone attenuates grain sorghum dust extract- and substance P-induced increases in clearance of macromolecules from postcapillary venules in the cheek pouch. This response appears to be selective for the intracellular signal transduction pathway(s) activated by substance P in the cheek pouch microcirculation because dexamethasone had no significant effects on adenosine-induced responses.

The mechanisms underlying the salutary effects of dexamethasone were not elucidated in this study. Mancuso et al. (12) showed that dexamethasone increases the concentration of lipocortin-1, a protein that expresses a number of steroidlike effects, in circulating neutrophils of hamsters. This, in turn, was associated with inhibition of substance P-induced neutrophil transmigration from postcapillary venules in the cheek pouch. Piedimonte et al. (19, 20) showed that dexamethasone attenuates substance P-induced plasma exudation in the rat trachea and that this response was associated with upregulation of tissue neutral endopeptidase 24.11 and angiotensin I-converting enzyme activity, two membrane-bound peptidases widely distributed in the microcirculation that cleave and inactivate tachykinin-induced increase in microvascular clearance in hamster cheek pouch. Piedimonte et al. (19, 20) showed that dexamethasone inhibited neutrophil transmigration, but not rolling or adhesion, is selectively inhibited by dexamethasone in the hamster post-capillary venule. Involvement of endogenous lipocortin 1 in the microcirculation.

In summary, we found that dexamethasone attenuates grain sorghum dust extract- and substance P-induced increases in clearance of macromolecules from the in situ hamster cheek pouch in a specific fashion.

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