Dexamethasone attenuates acute macromolecular efflux increase evoked by smokeless tobacco extract

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Gao, Xiao-Pei, Syed R. Akhter, Hiroyuki Ikezaki, Dennis Hong, and Israel Rubinstein. Dexamethasone attenuates acute macromolecular efflux increase evoked by smokeless tobacco extract. J. Appl. Physiol. 87(2): 619–625, 1999.—The purpose of this study was to determine whether dexamethasone attenuates the acute increase in macromolecular efflux from the oral mucosa elicited by an aqueous extract of smokeless tobacco (STE) in vivo, and, if so, whether this response is specific. Using intravital microscopy, we found that 20-min suffusion of STE elicited significant, concentration-related leaky site formation and an increase in clearance of fluorescein isothiocyanate-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 the bradykinin-induced leaky site formation and the increase in clearance of FITC-dextran from the cheek pouch. However, it had no significant effects on adenosine-induced responses. Dexamethasone had no significant effects on baseline arteriolar diameter and on bradykinin-induced vasodilation in the cheek pouch. Collectively, these data indicate that dexamethasone attenuates, in a specific fashion, the acute increase in macromolecular efflux from the cheek pouch that is elicited by STE. We suggest that corticosteroids mitigate acute oral mucosa inflammation elicited by smokeless tobacco.

oral mucosa; inflammation; microcirculation; plasma exudation; corticosteroids; bradykinin; hamster

IT IS ESTIMATED that each year ~304,000 individuals who are 11–19 yr of age become regular users of smokeless tobacco in the US (8, 32). Emerging clinical and epidemiological evidence suggests that regular use of smokeless tobacco is associated with chronic inflammation of the oral mucosa that might predispose to the development of oral cancer in susceptible individuals (14, 28). A characteristic feature of acute and chronic oral mucosa inflammation elicited by smokeless tobacco use in humans and laboratory animals is plasma exudation from postcapillary venules (12, 14, 27).

Previous work from our laboratory showed that short-term (20-min) suffusion of the in situ hamster oral mucosa with an aqueous extract of smokeless tobacco (STE) increases macromolecular efflux from postcapillary venules through local elaboration of bradykinin, a potent phlogistic peptide (4, 10–12). This response was abrogated by selective bradykinin B2 receptor antagonists (12). Whether other anti-inflammatory drugs that are commonly used in humans, such as glucocorticoids, have similar effects is uncertain.

To this end, dexamethasone, a potent albeit nonspecific anti-inflammatory drug, and other corticosteroids have been shown to attenuate plasma exudation elicited by short-term exposure to bradykinin and other phlogistic mediators in various microvascular beds of laboratory animals (1, 5–7, 16, 22, 25, 30). Akhter and colleagues (2) showed recently that pretreatment with high-dose dexamethasone (10 mg/kg iv) attenuates the acute increase in macromolecular efflux from the cheek pouch evoked by suffusion of grain sorghum dust extract and substance P. However, the effects of dexamethasone on the acute increase in macromolecular efflux from the cheek pouch that is elicited by STE are uncertain.

Hence, the purpose of this study was to begin to address this issue by determining whether dexamethasone attenuates the acute increase in macromolecular efflux from the oral mucosa evoked by an aqueous STE in vivo and, if so, whether this response is specific.

METHODS

Preparation of STE

The extract was prepared according to the method of Oh et al. (24), as previously described by our laboratory (10–12, 26, 29). Briefly, 10 g of smokeless tobacco (1S3 moist snuff; Tobacco and Health Research Institute, University of Kentucky, Lexington, KY) were mixed with 100 ml saline and incubated at 37°C for 2 h. The mixture was then centrifuged at 450 g for 10 min. The supernatant was collected and centrifuged at 13,000 g for 1 h. The supernatant was collected and centrifuged at 13,000 g for 1 h. After adjusting the pH to 7.4 by using 0.1 N HCl, the resulting supernatant, designated STE, was filtered through a Millipore filter (pore size: 0.45 µm), divided into 2-ml samples, snap frozen in liquid nitrogen, and stored at −70°C until used.

Preparation of Animals

Adult, male golden Syrian hamsters (weight, 129 ± 3 g; n = 36) were anesthetized with pentobarbital sodium (6 mg/100 g body weight ip). A tracheotomy was performed to facilitate spontaneous breathing. A femoral vein was cannulated to inject the intravascular tracer, fluorescein isothiocyanate-labeled dextran (FITC-dextran; mol mass 70 kDa), and supplemental pentobarbital sodium anesthesia (2–4 mg·100 g body weight−1·h−1). A femoral artery was cannulated to obtain arterial blood samples and to monitor arterial blood pressure, which did not change significantly during the
experiments. A heating pad was used to keep the body temperature constant (37-38°C) throughout the experiment. To visualize the microcirculation of the cheek pouch, we used a method previously described by our laboratory (10–12, 18, 26, 35). 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. The chamber contained the suffusion 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 bicarbonated buffer (37–38°C) that allowed continuous suffusion of the cheek pouch. The buffer was bubbled continuously with 95% N₂:5% CO₂ (pH 7.4). The chamber was also connected via a three-way valve to an infusion pump (model 341B; Sage Instruments, Boston, MA) that allowed constant administration of STE and drugs into the suffusate.

Determination of Clearance of Macromolecules

The cheek pouch microcirculation was visualized with an Olympus microscope coupled to a 100-W mercury light source at a magnification of ×40, as previously described by our laboratory (10–12, 18, 26, 35). Fluorescence microscopy was accomplished with the aid of filters that matched the spectral characteristics of FITC-dextran. 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.

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 (Cygnet; ISCO, Lincoln, NE). 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 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 previously 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 (13, 18, 19, 30, 35). 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 bicarbonated buffer used as background, a standard curve was generated 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 (in ng/ml) to plasma (in ng/ml) concentration of FITC-dextran and multiplying this ratio by the suffusate flow rate (2 ml/min). Previous work by our laboratory (18) has shown that there is a significant positive correlation between the number of leaky sites and clearance of FITC-dextran during suffusion of the cheek pouch with phlogistic compounds.

Determination of Arteriolar Diameter

The cheek pouch microcirculation was visualized with a Nikon microscope coupled to a 100-W mercury light source at a magnification of ×40. The microscope image was projected through a low-light TV camera (Panasonic TR-124 MA, Matsushita Communication Industrial, Yokohama, Japan) onto a video screen (Panasonic). The inner diameter of second-order arterioles (44–51 µm) was determined during the experiment from the video display of the microscope image by using a video micrometer (VIA 100; Boeckler Instruments, Tucson, AZ), as previously described by our laboratory (9, 29). In each animal, the same arteriolar segment was used to measure vessel diameter during the experiment.

Experimental Protocols

Effects of dexamethasone on STE-induced responses. The purpose of this study was to determine whether dexamethasone attenuates acute leaky site formation and the increase in clearance of FITC-dextran elicited by suffusion of STE on the 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, while no leaky sites were observed. Then two concentrations of STE (0.1 and 1%) were suffused in an arbitrary fashion for 20 min each, as previously described by our laboratory (10–12). The number of leaky sites was determined every minute for 7 min and at 5-min intervals for 60 min thereafter.

Clearance of FITC-dextran was determined before STE was suffused and every 5 min for 60 min during and after STE was suffused. The time interval between subsequent suffusions of STE was at least 60 min (10–12, 29). After suffusion of STE was stopped and the number of leaky sites returned to baseline, dexamethasone (10 mg/kg iv) was infused for 30 min by using an infusion pump (final volume, 1 ml), and suffusion of STE was repeated. The number of leaky sites and clearance of FITC-dextran were determined during each intervention as outlined above. In preliminary experiments, we found that repeated suffusions of STE (0.1 and 1%) for 20 min before and after suffusion of saline (vehicle) for 60 min were associated with reproducible leaky site formation (5 ± 2 and 4 ± 1/0.11 cm² and 10 ± 1 and 9 ± 2/0.11 cm², respectively; each group, n = 4; P > 0.5) and increase in clearance of FITC-dextran (25 ± 4 and 22 ± 3 ml/min × 10⁶, and 44 ± 6 and 40 ± 4 ml/min × 10⁶, respectively; each group, n = 4; P > 0.5). In addition, infusion of dexamethasone (10 mg/kg) alone had no significant effects on leaky site formation (nil) and clearance of FITC-dextran from the cheek pouch (12 ± 3 vs. 13 ± 4 ml/min × 10⁶ before and at the conclusion of the infusion, respectively; each group, n = 4; P > 0.5). Similarly, suffusion of saline (vehicle) for the entire duration of the experiment had no significant effects on leaky site formation (nil) and clearance of FITC-dextran (13 ± 4 vs. 12 ± 2 ml/min × 10⁶ at the start and the conclusion of saline suffusion, respectively; each group, n = 4; P > 0.5). The concentrations of STE and
Effects of dexamethasone on bradykinin-induced responses. 

Gao et al. (12) showed that STE-induced acute increase in clearance of macromolecules from the cheek pouch is mediated by local elaboration of bradykinin. Hence the purpose of this study was to determine whether dexamethasone attenuates bradykinin-induced leaky site formation and increase in clearance of FITC-dextran from the 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 bradykinin (0.5 and 1.0 µM) were suffused in an arbitrary fashion for 7 min (each 9, 35). The number of leaky sites was determined every minute for 7 min and at 5-min intervals for 60 min thereafter. Clearance of FITC-dextran was determined before bradykinin was suffused and every 5 min thereafter for 60 min. The time interval between subsequent suffusions of bradykinin was at least 45 min (9, 35). After suffusion of bradykinin was stopped and the number of leaky sites returned to baseline, dexamethasone (10 mg/kg iv) was infused, and suffusion of bradykinin was repeated. The number of leaky sites and clearance of FITC-dextran were determined during each intervention. In preliminary studies, we found that repeated suffusions of bradykinin (0.5 and 1.0 µM) for 7 min before and after suffusion of saline (vehicle) for 45 min were associated with reproducible leaky site formation (5 ± 1 and 6 ± 2/0.11 cm², and 15 ± 1 and 14 ± 2/0.11 cm², respectively; each group, n = 4; P > 0.5) and increase in clearance of FITC-dextran from the cheek pouch (27 ± 6 and 28 ± 4 ml/min ×10⁻⁶, and 45 ± 8 and 47 ± 6 ml/min ×10⁻⁶, respectively; each group, n = 4; P > 0.5). The concentrations of bradykinin used in these studies are based on previous studies by our laboratory and reports in the literature (4, 7, 9, 13, 21, 31, 35).

Effects of dexamethasone on adenosine-induced responses. The purpose of this study was to determine the specificity of dexamethasone attenuation of STE- and bradykinin-induced responses by determination of its effects on adenosine-induced leaky site formation and increase in clearance of FITC-dextran from the cheek pouch. Adenosine, like bradykinin, increases clearance of macromolecules from the hamster cheek pouch by a specific, receptor-mediated mechanism(s) (13, 21, 35). 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 on the cheek pouch for 7 min (13, 21, 35). The number of leaky sites was determined every minute for 7 min and at 5-min intervals for 45 min thereafter. Clearance of FITC-dextran was determined before adenosine was suffused and every 5 min thereafter for 45 min. After suffusion of adenosine was stopped and the number of leaky sites returned to baseline, dexamethasone (10 mg/kg iv) was infused, and suffusion of adenosine was repeated. 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 leaky site formation (11 ± 2 and 10 ± 1/0.11 cm²; each group, n = 4; P > 0.5) and increase in clearance of FITC-dextran from the cheek pouch (42 ± 11 and 40 ± 8 ml/min ×10⁻⁶; each group, n = 4; P > 0.5). The concentrations of adenosine used in these studies are based on previous studies by our laboratory and reports in the literature (2, 11, 13, 21, 35).

Effects of dexamethasone on arteriolar diameter. Suzuki et al. (29) showed that suffusion of STE at concentrations similar to those used in this study has no significant effects on baseline arteriolar diameter in the cheek pouch. However, dexamethasone may alter vasomotor tone and/or venular driving pressure in this organ, thereby accounting, in part, for its salutary effects during suffusion of STE and bradykinin. Hence, the purpose of this study was to determine whether dexamethasone-induced responses are related, in part, to changes in arteriolar diameter in the cheek pouch. In one series of experiments, after the equilibration period, dexamethasone (10 mg/kg) was infused intravenously for 30 min, and arteriolar diameter was determined before infusion, every minute during infusion of dexamethasone, and at 5-min intervals thereafter for 60 min. In another group of animals, after the equilibration period, bradykinin (1.0 µM) or saline (vehicle) was suffused on the cheek pouch for 7 min. Once arteriolar diameter returned to baseline, dexamethasone (10 mg/kg) was infused intravenously, and suffusion of bradykinin or saline was repeated. Arteriolar diameter was determined before, every minute during suffusion of bradykinin or saline, and at 5 min thereafter for 60 min. In preliminary studies, we found that repeated suffusions of bradykinin (1.0 µM) for 7 min before and after suffusion of saline (vehicle) for 45 min were associated with reproducible increases in arteriolar diameter from baseline (17 ± 3 and 20 ± 2 %, respectively; each group, n = 4; P > 0.5). Suffusion of saline (vehicle) for the entire duration of the experiment was not associated with a significant change in arteriolar diameter (data not shown). The concentration of bradykinin used in these studies is based on a previous study by our laboratory (9).

Drugs
FITC-dextran, dexamethasone, bradykinin, and adenosine were obtained from Sigma Chemical (St. Louis, MO). 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 on the cheek pouch, we determined the maximal change in the number of leaky sites and clearance of FITC-dextran, and we 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 and clearance of FITC-dextran returned to baseline between successive suffusions of test compounds, all vehicle (saline) control data are expressed as a single value for each experimental condition; n is given as the number of experiments, and each experiment represents a separate animal. Statistical analysis was performed by using two-way analysis of variance and the Newman-Kuels test for multiple comparisons. P < 0.05 was considered significant.

RESULTS
Effects of Dexamethasone on STE-Induced Responses

Suffusion of STE elicited significant, concentration-related leaky site formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 1; each group, n = 4; P < 0.05). These effects were observed within 7–10 min from the start of suffusion, were maximal within 20 min, and returned to baseline within 30 min after suffusion was stopped. STE-
induced responses were significantly attenuated by dexamethasone (10 mg/kg; Fig. 1; each group, n = 4; P < 0.05). The number of leaky sites decreased significantly from 10 ± 1/0.11 cm² during suffusion of STE (10%) alone to 2 ± 1/0.11 cm² during suffusion of STE (10%) after infusion of dexamethasone (10 mg/kg; Fig. 1A; each group, n = 4; P < 0.05). Similarly, clearance of FITC-dextran decreased significantly from 45 ± 7 ml/min × 10⁻⁶ during suffusion of bradykinin (1.0 µM) alone to 22 ± 4 ml/min × 10⁻⁶ during suffusion of bradykinin (1.0 µM) after infusion of dexamethasone (10 mg/kg; Fig. 1B; each group, n = 4; P < 0.05).

Effects of Dexamethasone on Bradykinin-Induced Responses

Suffusion of bradykinin elicited a significant, concentration-related leaky site formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 2; each group, n = 4; P < 0.05). The number of leaky sites increased significantly from baseline within 2–3 min of the start of bradykinin suffusion, was maximal within 5 min, and returned to baseline within 30 min after suffusion of bradykinin was stopped. Clearance of FITC-dextran was maximal within 5 min after the start of bradykinin suffusion and returned to baseline within 30 min after suffusion of bradykinin was stopped. Bradykinin-induced responses were significantly attenuated by dexamethasone (10 mg/kg; Fig. 2; each group, n = 4; P < 0.05). The number of leaky sites decreased significantly from 15 ± 1/0.11 cm² during suffusion of bradykinin (1.0 µM) alone to 1 ± 1/0.11 cm² during suffusion of bradykinin (1.0 µM) after infusion of dexamethasone (10 mg/kg; Fig. 2A; each group, n = 4; P < 0.05). Similarly, clearance of FITC-dextran decreased significantly from 42 ± 6 ml/min × 10⁻⁶ during suffusion of bradykinin (1.0 µM) alone to 20 ± 2 ml/min × 10⁻⁶ during suffusion of STE (10%) after infusion of dexamethasone (10 mg/kg; Fig. 2B; each group, n = 4; P < 0.05).

Effects of Dexamethasone on Bradykinin-Induced Responses

Suffusion of bradykinin elicited a significant, concentration-related leaky site formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 2; each group, n = 4; P < 0.05). The number of leaky sites increased significantly from baseline within 2–3 min of the start of bradykinin suffusion, was maximal within 5 min, and returned to baseline within 30 min after suffusion of bradykinin was stopped. Clearance of FITC-dextran was maximal within 5 min after the start of bradykinin suffusion and returned to baseline within 30 min after suffusion of bradykinin was stopped. Bradykinin-induced responses were significantly attenuated by dexamethasone (10 mg/kg; Fig. 2; each group, n = 4; P < 0.05). The number of leaky sites decreased significantly from 15 ± 1/0.11 cm² during suffusion of bradykinin (1.0 µM) alone to 1 ± 1/0.11 cm² during suffusion of bradykinin (1.0 µM) after infusion of dexamethasone (10 mg/kg; Fig. 2A; each group, n = 4; P < 0.05). Similarly, clearance of FITC-dextran decreased significantly from 42 ± 6 ml/min × 10⁻⁶ during suffusion of bradykinin (1.0 µM) alone to 22 ± 4 ml/min × 10⁻⁶ during suffusion of bradykinin (1.0 µM) after infusion of dexamethasone (10 mg/kg; Fig. 2B; each group, n = 4; P < 0.05).

Fig. 1. Effects of smokeless tobacco extract (STE) on leaky site formation (A) and clearance of FITC-dextran (B) in absence and presence of dexamethasone (10 mg/kg iv). Values are means ± SE; each group, n = 4 animals; *P < 0.05 vs. saline (vehicle); †P < 0.05 vs. STE alone.

Fig. 2. Effects of bradykinin on leaky site formation (A) and clearance of FITC-dextran (B) in absence and presence of dexamethasone (10 mg/kg iv). Values are means ± SE; each group, n = 4 animals. *P < 0.05 vs. saline (vehicle); †P < 0.05 vs. bradykinin alone.
ml/min × 10⁻⁶ during suffusion of bradykinin (1.0 µM) after infusion of dexamethasone (10 mg/kg; Fig. 2B; each group, n = 4; P < 0.05).

Effects of Dexamethasone on Adenosine-Induced Responses

Suffusion of adenosine (10 µM) elicited significant leaky site formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 3; n = 4; P < 0.05). The number of leaky sites increased significantly from baseline within 2–3 min of the start of adenosine suffusion, was maximal within 5 min, and returned to baseline within 30 min after suffusion of adenosine was stopped. Clearance of FITC-dextran was maximal with 5 min after the start of adenosine suffusion and returned to baseline within 30 min after suffusion of adenosine was stopped. Dexamethasone (10 mg/kg) had no significant effects on adenosine-induced responses (Fig. 3; 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) after infusion of dexamethasone (10 mg/kg; Fig. 3A; each group, n = 4; P > 0.5). 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) after infusion of dexamethasone (10 mg/kg; Fig. 3B; each group, n = 4; P > 0.5).

Effects of Dexamethasone on Arteriolar Diameter

Infusion of dexamethasone (10 mg/kg iv) had no significant effects on baseline arteriolar diameter in the cheek pouch. Arteriolar diameter increased by 1 ± 2% from baseline during infusion of dexamethasone and suffusion of saline (vehicle) for 30 min (n = 4; P > 0.5). In addition, dexamethasone had no significant effects on bradykinin (1.0 µM)-induced increase in arteriolar diameter from baseline (19 ± 1 and 19 ± 2% before and after infusion of dexamethasone, respectively; each group, n = 4; P > 0.5).

DISCUSSION

The new finding of this study is that dexamethasone, a potent, albeit nonspecific, anti-inflammatory drug, attenuates the acute increase in macromolecular efflux from the in situ hamster cheek pouch elicited by STE. This response is specific, because dexamethasone attenuates the acute increase in clearance of macromolecules elicited by bradykinin, a phlogistic mediator elaborated in the cheek pouch during short-term suffusion of STE (4, 12), but dexamethasone has no significant effects on adenosine-induced responses. Moreover, dexamethasone has no significant effects on baseline vasomotor tone and on bradykinin-induced vasodilation in the cheek pouch. Collectively, these data indicate that dexamethasone attenuates the acute increase in macromolecular efflux from the in situ oral mucosa elicited by STE in a specific fashion. We suggest that corticosteroids mitigate acute oral mucosa inflammation elicited by smokeless tobacco.

Consideration of Methods

The hamster cheek pouch is an established model to study the role of environmental toxicants and phlogistic mediators (such as smokeless tobacco, grain sorghum dust, bradykinin, and adenosine) and anti-inflammatory drugs, such as dexamethasone, in modulation of tissue inflammation in situ (2, 3, 6, 10–13, 17–19, 21, 23, 26, 27, 29–31, 35). We and other investigators have previously shown that successive suffusions of STE (at concentrations similar to those used in this study), bradykinin, and adenosine at appropriate time intervals on the cheek pouch are associated with reproducible formation of leaky sites and increases in clearance of FITC-dextran in the absence of tachyphylaxis (12, 13, 31, 35). Consequently, the acute effects of these compounds on macromolecular efflux can be tested repeatedly in the cheek so that each animal serves as its own control. This approach...
reduces the overall number of animals required to perform the study and facilitates data analysis.

High-dose dexamethasone has been recently shown to attenuate Escherichia coli lipopolysaccharide-grain sorghum dust extract-induced and substance P-induced acute increases in macromolecular efflux from the hamster cheek pouch (2, 6). The results of this study support and extend these observations by showing that high-dose dexamethasone attenuates the acute increase in clearance of FITC-dextran from the cheek pouch elicited by STE and bradykinin. Whether dexamethasone, at doses commonly used in humans, has similar effects on acute and chronic oral mucosa inflammation elicited by STE remains to be determined.

Vasoconstriction and/or a decrease in venular driving pressure may have mediated, in part, dexamethasone attenuation of STE-induced and bradykinin-induced responses. However, this possibility seems unlikely, because dexamethasone had no significant effects on baseline arteriolar diameter and on bradykinin-induced vasodilation in the cheek pouch. Moreover, if the salutary effects of dexamethasone are mediated, in part, by changes in vasomotor tone and/or venular driving pressure, the drug should have also attenuated adenosine-induced responses, because adenosine, like bradykinin, increases macromolecular efflux from this organ by a specific, receptor-mediated mechanism(s) (13, 21). Taken together, these data suggest that dexamethasone attenuation of STE-induced and bradykinin-induced acute increases in macromolecular efflux could not be attributed to nonspecific changes in vasomotor tone and/or venular driving pressure in the cheek pouch. This conclusion is consistent with previous studies in the hamster cheek pouch and other vascular beds and species that dissociate changes in vasomotor tone from macromolecular transport (20, 21, 33, 34).

For technical reasons, it is not feasible to suffice crude smokeless tobacco, which is dense and opaque, directly on the hamster cheek pouch while at the same time observing macromolecular efflux from postcapillary venules using intravital microscopy (3, 12, 24, 27). Hence, we had to prepare an aqueous STE and suffice it on the cheek pouch. This approach has been previously used by us and other investigators to study the biological effects of smokeless tobacco (10–12, 24, 26, 29). To this end, larger quantities of smokeless tobacco than those used in this study are placed daily on the human oral mucosa, where they are constantly being mixed with saliva and, in essence, producing an aqueous extract. Collectively, these data indicate that the use of an aqueous STE to test the hypotheses set forth in this study is justified.

Consideration of Previous Studies

The mechanisms and intracellular signal transduction pathway(s) underlying the salutary effects of dexamethasone on STE-induced and bradykinin-induced responses were not elucidated in this study. Gao et al. (12) showed that indomethacin has no significant effects on STE-induced acute increase in macromolecular efflux from the in situ hamster cheek pouch. This suggests that products of the arachidonic acid metabolic pathway are not involved in this response. Dexamethasone-induced responses are not related to nonspecific effects on the endothelium, because dexamethasone attenuates the acute increase in clearance of macromolecules elicited by bradykinin but has no significant effects on adenosine-induced responses. In addition, dexamethasone has no significant effects on baseline vasomotor tone and on bradykinin-induced vasodilation.

Gao et al. (12) showed that 20-min suffusion of STE on the cheek pouch is associated with a decrease in tissue neutral endopeptidase (NEP; EC 3.4.24.11) activity, a membrane-associated peptidase that is widely distributed in the microcirculation and cleaves and inactivates bradykinin very effectively (4, 9, 10, 35). NEP inhibitors have been shown to potentiate STE-induced and bradykinin-induced acute increases in macromolecular efflux from the cheek pouch by slowing bradykinin catabolism (10, 12, 35). Conceivably, dexamethasone could upregulate NEP activity in the hamster cheek pouch, thereby promoting local bradykinin catabolism during suffusion of STE and bradykinin and reducing macromolecular efflux (4, 15). This contention is supported, in part, by the study of Piedimonte et al. (25), who showed that dexamethasone upregulates NEP activity and attenuates neurogenic plasma exudation in the rat trachea in vivo.

Mancuso et al. (17) showed that dexamethasone increases the concentration of lipocortin-1, a protein that expresses a number of corticosteroid-like effects, in circulating neutrophils of hamsters. This, in turn, was associated with inhibition of agonist-induced leukocyte transmigration from postcapillary venules in the in situ cheek pouch. However, the role of lipocortin-1 in modulating macromolecular efflux from the cheek pouch was not elucidated in this study. Dexamethasone may also downregulate the number and/or affinity of bradykinin B2 receptors in postcapillary venules in the cheek pouch, thereby accounting, in part, for its salutary effects (4).

However, the extent of NEP and lipocortin-1 upregulation and/or bradykinin B2 receptor downregulation in the microcirculation during short-term infusion of high-dose dexamethasone may not be sufficient to completely circumvent the acute increase in macromolecular efflux elicited by the highest concentration of STE used in this study. This, in turn, may partly account for the observed residual response. Alternatively, high concentrations of STE may also activate other metabolic pathways in the microcirculation that are involved in regulation of macromolecular efflux and are dexamethasone resistant (6, 21–23). This contention is partly supported by the observation that high-dose dexamethasone had no significant effects of adenosine-induced responses. Clearly, additional studies that use molecular biology and biochemical and cell culture techniques are warranted to support or refute these hypotheses.

In summary, we found that dexamethasone attenuates the acute increase in macromolecular efflux from
the in situ hamster cheek pouch evoked by suffusion of STE in a specific fashion. We suggest that corticosteroids mitigate acute oral mucosa inflammation elicited by short-term exposure to smokeless tobacco.

This study was supported, in part, by grants from the National Institutes of Health (DE-10347), the American Heart Association of Metropolitan Chicago, and the Laerdal Foundation for Acute Medicine. I. Rubinstein is a recipient of a Research Career Development Award from the National Institutes of Health (DE-00386) and a University of Illinois Scholar Award.

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Received 24 September 1998; accepted in final form 14 April 1999.

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