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#### Research Article

Inhalation of cigarette smoke is known to impair pulmonary mucociliary clearance. Active ion transport by airway epithelium plays an important role in maintaining effective mucociliary clearance by regulating the volume and composition of the airway secretions. To determine the effect of cigarette smoke on airway epithelial ion transport, the electrical properties and transepithelial Na and Cl fluxes were measured in canine tracheal epithelium. In vivo, the inhalation of the smoke from one cigarette acutely and reversibly decreased the electrical potential difference across the tracheal epithelium. In vitro, exposure of the mucosal surface of the epithelium to cigarette smoke decreased the short circuit current and transepithelial resistance. The decrease in short circuit current was due to an inhibition of the rate of Cl secretion with minimal effect on the rate of Na absorption. The effect of cigarette smoke was reversible, was not observed upon exposure of the submucosal surface to smoke, and was most pronounced when secretion was stimulated. The particulate phase of smoke was largely responsible for the inhibitory effect, since filtering the smoke minimized the effect. The effect of cigarette smoke was not prevented by addition of antioxidants to the bathing solutions, suggesting that the inhibition of Cl secretion cannot be entirely attributed to an oxidant mechanism. These results indicate that cigarette smoke acutely inhibits active ion transport by [...]



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## Cigarette Smoke Inhibition of Ion Transport in Canine Tracheal Epithelium

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ABSTRACT Inhalation of cigarette smoke is known to impair pulmonary mucociliary clearance. Active ion transport by airway epithelium plays an important role in maintaining effective mucociliary clearance by regulating the volume and composition of the airway secretions. To determine the effect of cigarette smoke on airway epithelial ion transport, the electrical properties and transepithelial Na and Cl fluxes were measured in canine tracheal epithelium. In vivo, the inhalation of the smoke from one cigarette acutely and reversibly decreased the electrical potential difference across the tracheal epithelium. In vitro, exposure of the mucosal surface of the epithelium to cigarette smoke decreased the short circuit current and transepithelial resistance. The decrease in short circuit current was due to an inhibition of the rate of Cl secretion with minimal effect on the rate of Na absorption. The effect of cigarette smoke was reversible, was not observed upon exposure of the submucosal surface to smoke, and was most pronounced when secretion was stimulated. The particulate phase of smoke was largely responsible for the inhibitory effect, since filtering the smoke minimized the effect. The effect of cigarette smoke was not prevented by addition of antioxidants to the bathing solutions, suggesting that the inhibition of Cl secretion cannot be entirely attributed to an oxidant mechanism. These results indicate that cigarette smoke acutely inhibits active ion transport by tracheal epithelium, both in vivo and in vitro. This effect may explain, in part, both the abnormal mucociliary clearance and the airway disease observed in cigarette smokers.

#### INTRODUCTION

Mucociliary clearance is an important pulmonary defense mechanism that serves to remove inhaled particulate material from the lung (1). The observation that inhalation of cigarette smoke impairs mucociliary clearance and that mucociliary clearance is abnormal in patients with chronic bronchitis, as well as young, asymptomatic cigarette smokers has suggested that an inhibition of effective pulmonary mucociliary clearance may be responsible for the airway disease observed in cigarette smokers (1). However, the mechanism by which cigarette smoke inhibits mucociliary clearance is unknown.

Effective function of the mucociliary clearance apparatus is dependent upon ciliary activity, the viscoelastic mucus, and ion transport by the airway epithelium (2). Since active ion transport by the airway epithelium plays an important role in regulating the volume and composition of the respiratory tract fluid, it is possible that smoke-induced alterations in mucociliary clearance might be due, at least in part, to an effect on the ion transport function of the airway epithelium. The purpose of this study was to determine the effect of cigarette smoke on ion transport by airway epithelium. We performed in vivo and in vitro studies on canine tracheal epithelium that has the capacity for both active secretion of Cl and absorption of Na (3).

#### METHODS

In vivo measurements. Mongrel dogs of either sex (20-35 kg) were lightly anesthetized with intravenous pentobarbital sodium (12-20 mg/kg) initially and small supplemental doses as needed to maintain anesthesia). The dogs were intubated, with the tip of the endotracheal tube positioned just distal to the larynx. Ventilation was spontaneous throughout.

The endotracheal tube was connected to a three-way adapter; one port was used for insertion of the exploring electrode, one port for attachment of a cigarette, and one port for ventilation. By closing the ventilation port and opening the port containing the lighted cigarette during the first half of a spontaneous inspiration, one puff of cigarette smoke was delivered to the dog. The cigarette port was closed and the ventilation port opened during the second half of inspiration and between puffs of smoke. During smoke delivery

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the dog inhaled 1 puff every 30 s for a total of 10–12 puffs (the smoke from one cigarette). For a sham-smoking period the same maneuver was performed without lighting the cigarette.

For measurement of the electrical potential difference across the tracheal epithelium, techniques similar to those reported by Boucher et al. (4) were used. A reference electrode (a Ringer bridge) was placed between the subcutaneous tissue of the neck and a saturated KCl solution. The exploring electrode consisted of PE-160 tubing filled with Ringer's solution flowing at a rate 0.1 ml·min<sup>-1</sup>. The flowing Ringer's solution was warmed (37°C) and oxygenated with 95%  $O_2$ -5% CO<sub>2</sub> in a reservoir and a constant flow rate was provided by a peristaltic pump (Buchler Instruments, Inc., Fort Lee, NJ). The flowing Ringer's bridge was connected to a saturated KCl reservoir with a Ringer agar bridge. The voltage difference between two calomel cells in the saturated KCl solutions was measured with a high input impedance electrometer (Keithley Instruments, Inc., Cleveland, OH) and recorded on a strip chart recorder (Watanabe Instruments Corp., Costa Mesa, CA). Identical solutions were used in both bridge circuits to avoid the problems of liquid junction potentials. The zero was checked before and after each series of measurements by placing both the exploring and reference bridges in either a Ringer's solution or the subcutaneous space (the same reading was obtained with either reference site). The transepithelial electrical potential difference  $(\Psi_t)^1$ was referenced to the submucosal tissue. The exploring electrode was positioned in the trachea so that a stable value of  $\Psi_t$  was obtained.  $\Psi_t$  was recorded continuously during the inhalation of smoke and during a subsequent recovery period.

In vitro measurements. The posterior membranous portion of the tracheal epithelium was removed and mounted in Ussing chambers (1.5 cm<sup>2</sup>) as previously described (5). The Ringer's solution contained (in millimolars): 118.9 NaCl, 2.4 K<sub>2</sub>HPO<sub>4</sub>, 0.6 KH<sub>2</sub>PO<sub>4</sub>, 20.4 NaHCO<sub>3</sub>, 1.2 CaCl<sub>2</sub>, 1.2 MgCl<sub>2</sub>, and 10 glucose. The solution was maintained at 37°C and bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH 7.4). The  $\Psi_t$  was automatically clamped to zero (the short circuit condition) by automatic voltage-current clamps (University of Iowa, Bioengineering). Transepithelial resistance (R<sub>1</sub>) was calculated from the change in current required to clamp  $\Psi_t$  to ±10 mV (pulses delivered by a pulse generator built into the voltage current clamp; duration 1 s; period 60 s).

Unidirectional and calculated net transepithelial fluxes of Na and Cl were determined from the fluxes of <sup>22</sup>Na and <sup>36</sup>Cl measured in paired tissues from the same dog.  $5 \,\mu$ Ci of <sup>22</sup>Na and 7  $\mu$ Ci of <sup>36</sup>Cl were added to the appropriate side of the tissue (10 ml of Ringer's solution bathed each surface of the epithelium). 30 min were allowed for isotope fluxes to reach a steady state and then three samples of both bathing solutions were taken at 20-min intervals during each control and experimental period. Isotope fluxes were performed during the steady state before and 15–20 min after the administration of cigarette smoke.

Tissues were exposed to smoke by bubbling whole cigarette smoke through either the mucosal or submucosal bathing solution. 60 ml of whole cigarette smoke was drawn into a syringe and then bubbled through the perfusion chamber at a rate of 0.7 ml  $\cdot$  s<sup>-1</sup> by a perfusion pump (Harvard Apparatus Co., Inc., S. Natick, MA). 300 ml of smoke (approximately equal to the smoke of one cigarette) was used for each intervention. During injection of cigarette smoke the tissue was continuously bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> at a rate at least three times the rate of smoke injection. Injection of air, rather than smoke, into the chamber did not alter the electrical properties of the epithelium. During injection of smoke the pH of the solution occasionally transiently decreased as low as 7.2. However, acidification of the mucosal bathing solution with HCl to pH 7.0 did not alter the electrical properties.

Indomethacin (10<sup>-6</sup> M) (Sigma Chemical Co., St. Louis, MO) was added to the mucosal bathing solution of some tissues to minimize the rate of Cl secretion. Indomethacin decreases the endogenous rate of prostaglandin production, decreases intracellular cyclic (c)AMP, and thus decreases the rate of Cl secretion (6, 7) without interfering with the subsequent response to secretagogues. Epinephrine  $(10^{-6} M)$ (Elkin-Sinn, Inc., Cherry Hill, NJ) was added to the submucosal bathing solution to stimulate Cl secretion. The use of indomethacin and epinephrine allowed the study of the epithelium under both Cl-secreting and -nonsecreting conditions. Other drugs and materials used were: superoxide dismutase, catalase, nicotine sulfate, D- $\alpha$ -tocopherol, prostaglandin E2 (all from Sigma Chemical Co.), acrolein (Eastman Kodak Co., Rochester, NY), the calcium ionophore A23187 (CalBiochem-Behring Corp., American Hoechst Corp., San Diego, CA), and activated coconut charcoal (Fisher Scientific Co., Pittsburgh, PA). Camel unfiltered cigarettes were used throughout.

All values are presented as means $\pm$ SEM. Statistical significance was evaluated using paired or unpaired t test as indicated; P < 0.05 was considered statistically significant.

#### RESULTS

In vivo measurements. Fig. 1 shows a representative recording of the  $\Psi_t$  in vivo in one dog.  $\Psi_t$  was stable and then decreased reversibly during the inhalation of 11 puffs of whole cigarette smoke. There was no change in  $\Psi_t$  during a sham period in which identical maneuvers were performed with an unlit cigarette.

Fig. 2 shows the values of  $\Psi_t$  obtained in seven dogs during a stable control period, following the inhalation of 10-12 puffs of cigarette smoke, and during a recovery period, 10-12 min following the cessation of cigarette smoke inhalation. Each set of points represents the mean of two-seven series of  $\Psi_t$  measurements obtained in one dog. The average value of  $\Psi_{t}$  decreased from  $-32\pm5$  mV to  $-28\pm4$  mV (P < 0.05) following inhalation of smoke. During the recovery period  $\Psi_{t}$ returned to the control value of 31±5 mV. Inspection of Fig. 2 also shows that at higher values of  $\Psi_t$ , the decrease produced by inhalation of cigarette smoke was greater than when the control value of  $\Psi_t$  was low. There is a direct correlation (r = 0.96) between the initial magnitude of  $\Psi_t$  and the decrease in  $\Psi_t$  produced by cigarette smoke. Furthermore, the effect of smoke appears to be minimal even though the value of  $\Psi_t$  remains at a negative value (i.e., the relation

<sup>&</sup>lt;sup>1</sup> Abbreviations used in this paper: cAMP, cyclic AMP; I<sub>sc</sub>, short circuit current; J<sup>Man</sup>, transepithelial flux of mannitol;  $\Psi_t$ , transepithelial electrical potential difference; R<sub>t</sub>, transepithelial resistance.



FIGURE 1 Time course of the effect of cigarette smoke on the  $\Psi_t$  in vivo. Tracing represents a representative recording from one tissue. Cigarette smoke was inhaled at the time indicated. An identical procedure was carried out with an unlit cigarette during the time marked Sham.

between the initial magnitude of  $\Psi_t$  and the smoke induced decrease in  $\Psi_t$  does not approach a  $\Psi_t$  of zero when there is no change in  $\Psi_t$ ). These observations suggest that the effect of cigarette smoke is not a nonspecific depression of the transport properties, but rather is dependent upon the magnitude of an epithelial electrogenic transport process.

In vitro measurements. To determine whether the decrease in  $\Psi_t$  observed in vivo was due to a decrease in the rate of active ion transport or to a decrease in epithelial resistance (i.e., an increase in ionic permeability) the tracheal epithelium was studied in vitro. Fig. 3 shows the time course of the effect of cigarette smoke on short circuit current (I<sub>sc</sub>) (which reflects the net rate of active ion transport). Cl secretion was stimulated by the addition of epinephrine (10<sup>-6</sup> M) to the submucosal bathing solution. Bubbling smoke through the submucosal bathing solution had no effect on I<sub>sc</sub> or R<sub>t</sub>. In contrast, bubbling smoke through the mucosal bathing solution, on two occasions, decreased I<sub>sc</sub> and



FIGURE 2 Effect of cigarette smoke on the  $\Psi_t$  in vivo. Each set of points was obtained from a different dog. Each set of values represents the mean of two to seven series of measurements made during a stable Control period, after the inhalation of 10-12 puffs of Smoke, and during a Recovery period, 10-12 min after the cessation of smoke inhalation.

increased  $R_t$ . These findings suggest that cigarette smoke inhibits the rate of Cl secretion.

To determine if the effect of cigarette smoke on the  $I_{sc}$  was specific for Cl, cigarette smoke was bubbled through the mucosal bathing solution in tissues in which the rate of Cl secretion was minimized by the addition of indomethacin ( $10^{-6}$  M, mucosal solution). In four tissues  $I_{sc}$  was  $21\pm3 \ \mu A \cdot cm^{-2}$  before exposure to cigarette smoke and was not significantly altered ( $19\pm3 \ \mu A \cdot cm^{-2}$ ) 10 min after exposure to cigarette smoke (data not shown). This indicates that when the rate of Cl secretion is minimized there is no substantial effect of smoke on ion transport by the epithelium.

The inhibition of  $I_{sc}$  by cigarette smoke was also reversible. In tissues in which Cl secretion had been stimulated by epinephrine, bubbling the mucosal solution with smoke decreased  $I_{sc}$ ; the subsequent replacement of the mucosal solution with fresh Ringer's partially reversed the decrease in  $I_{sc}$ . The effect of cigarette smoke on  $I_{sc}$  was  $75\pm3\%$  (n = 5) reversible when the solutions were exchanged 15 min after exposure to cigarette smoke and  $70\pm5\%$  (n = 2) reversible when the solutions were exchanged 30 min after exposure to smoke.

We also examined the effect of cigarette smoke on ion transport when Cl secretion was stimulated with prostaglandin  $E_2$  and the calcium ionophore, A23187; prostaglandin E2 increases intracellular levels of cAMP (6, 7) while A23187 stimulates secretion via a Ca-dependent mechanism without increasing cAMP (6, 8). When prostaglandin  $E_2$  (10<sup>-6</sup> M, submucosal solution) stimulated secretion, smoke decreased the Is from  $57\pm2$  to  $30\pm2$   $\mu$ A · cm<sup>-2</sup> and increased R<sub>t</sub> from  $388\pm70$ to  $509\pm123 \ \Omega \cdot \mathrm{cm}^2$  (n = 3). Likewise, when A23187 (10<sup>-6</sup> M, mucosal solution) stimulated secretion, smoke decreased I<sub>sc</sub> from 49±2 to 19±2  $\mu$ A·cm<sup>-2</sup> and increased R<sub>t</sub> from 341±112 to 563±273  $\Omega \cdot cm^2$  (n = 3). These changes are similar to those observed when epinephrine stimulates secretion. Thus, the inhibition of Isc produced by smoke does not appear to be dependent upon a specific secretagogue or intracellular mediator.

To directly examine the effect of cigarette smoke



FIGURE 3 Time course of the effect of cigarette smoke on  $I_{sc}$  and  $R_t$  in vitro. Epinephrine was added to stimulate Cl secretion at the time indicated. Whole cigarette smoke was bubbled through the submucosal (smoke s) or mucosal (smoke m) bathing solutions during the times indicated.

on the rate of Cl secretion and Na absorption, simultaneous fluxes of Na and Cl were measured; the results are shown in Table I. The decrease in  $I_{sc}$  was predominantly due to a decrease in the net rate of Cl secretion. While net Na absorption decreased in six of seven cases, the change was not statistically significant.

The results shown in Table I suggest that cigarette smoke has an effect on cellular transport processes rather than an effect on the paracellular pathway. To further determine whether cigarette smoke acutely influences the cellular or paracellular pathway, the effect of smoke on transepithelial mannitol fluxes was examined. Mannitol is a large nonelectrolyte that is not metabolized by the epithelium (7), is limited to the extracellular space (9), is not actively transported (i.e., the two unidirectional fluxes are equal) (5), and serves as a marker of paracellular pathway permeability in tracheal epithelium (5). The effect of cigarette smoke on the transepithelial flux of mannitol  $(J^{Man})$  in nonsecreting (indomethacin-treated) and secreting (epinephrine-treated) tissues is shown in Table II. For these studies, paired tissues from the same dog were used; one tissue served as a control while the other tissue was exposed to cigarette smoke. Smoke did

not alter J<sup>Man</sup> under either secreting or nonsecreting conditions, suggesting that there was no effect on the permeability of the paracellular pathway.

To determine if the effect of cigarette smoke is produced by a component in the particulate or vapor phase of whole cigarette smoke, smoke was passed through a 0.22- $\mu$ m filter (millex-GS, Millipore Corp., Bedford, MA) before being bubbled in the mucosal solution. As shown in Fig. 4, filtering cigarette smoke diminished the effect on I<sub>sc</sub>. The magnitude of the increase in R<sub>t</sub> produced by cigarette smoke (R<sub>t</sub> increased 75±14  $\Omega \cdot \text{cm}^2$ ) was also diminished by filtering the smoke (R<sub>t</sub> increased 34±5  $\Omega \cdot \text{cm}^2$ ) (n = 7 pairs, P < 0.01).

The effect of the particulate component of cigarette smoke was also examined by measuring the effect of the residue of cigarette smoke on the  $I_{sc}$ . 300 ml of whole cigarette smoke was passed through 0.22- $\mu$ m filters (Millipore Corp), and then the residue was washed off the filter with 10 ml of Ringer's solution. The solution containing the smoke residue was then warmed (37°C) and oxygenated and exchanged for the solution bathing the mucosal solution. The change in  $I_{sc}$  produced by the smoke residue was compared with

	I.	R,	J <sup>Na</sup>	Jsm	JNet	JCI Jms	Jsm	JNet
	$\left(\frac{\mu eq}{cm^2 \cdot h}\right)$	$(\Omega \cdot \mathrm{cm}^2)$			$\left(\frac{\mu \mathrm{eq}}{\mathrm{cm}^2 \cdot \mathrm{h}}\right)$		$\left(\frac{\mu eq}{cm^2 \cdot h}\right)$	
Base line	1.97±0.21	440±33	1.42±0.29	0.70±0.17	0.71±0.37	1.79±0.27	3.36±0.48	-1.58±0.37
Control	$1.76 \pm 0.21$	473±30	1.34±0.35	0.73±0.16	0.60±0.37	$1.71 \pm 0.30$	3.17±0.46	<b>−1.48±0.38</b>
Δ Control	-0.21±0.06•	+33±13°	-0.08±0.09	$+0.02\pm0.01$	-0.11±0.08	-0.08±0.03	-0.19±0.08	+0.10±0.11
Base line	2.03±0.24	381±34	$1.19\pm0.20$	0.76±0.11	0.43±0.23	2.06±0.23	<b>3.80±0.54</b>	-1.74±0.43
Smoke	0.89±0.12	522±51	$1.04\pm0.15$	$0.82 \pm 0.12$	$0.21 \pm 0.19$	1.49±0.24	2.04±0.27	-0.55±0.31
Δ Smoke	-1.14±0.15°‡	+141±26°‡	<b>−0.16±0.07</b>	+0.06±0.04	$-0.22\pm0.10$	-0.57±0.14°‡	<b>−1.76±0.37•</b> ‡	+1.18±0.36*‡

TABLE I Effect of Cigarette Smoke on Transepithelial Ion Fluxes Electrical properties and ion fluxes were measured during a base-line period and then during either a control period (no intervention) or following the bubbling of cigarette smoke through the mucosal bathing solution. Each group consists of seven pairs of tissues. Epinephrine (10<sup>-6</sup> M, submucosal solution) was present throughout to stimulate the rate of Cl secretion. J<sup>N</sup> and J<sup>Cl</sup> refer to the flux of Na and Cl, respectively, and the subscripts refer to the flux from mucosal solution to submucosal solution (ms), submucosa to mucosa (sm), and calculated net (Net). A positive net flux refers to the ms direction and a negative net flux is in the sm direction.  $\Delta$  refers to the difference between the two experimental periods.

• This indicates a  $\Delta$  significantly different from zero, P < 0.01.

) There is a significant difference between  $\Delta$  Control and  $\Delta$  Smoke, P < 0.02.

	Control			Smoke		
	Period 1	Period 2	Δ	Period 1	Period 2	Δ
Nonsecreting tissues						
$I_{sc} (\mu A \cdot cm^{-2})$	23±3	24±4	+1±1	$23\pm5$	17±1	-6±4
$R_t (\Omega \cdot cm^2)$	734±194	$888 \pm 294$	$+155 \pm 107$	870±308	711±152	$-159 \pm 180$
$\mathbf{J}^{Man}\left(n\boldsymbol{M}\boldsymbol{\cdot}\boldsymbol{c}\boldsymbol{m}^{-2}\boldsymbol{\cdot}\boldsymbol{h}^{-1}\right)$	3.3±1.0	3.0±0.9	$-0.3 \pm 0.4$	3.6±0.9	3.9±0.6	+0.3±0.6
Secreting tissues						
$I_{sc} (\mu A \cdot cm^{-2})$	83±9	75±8	-8±2°	81±6	33±3	-48±°‡
$R_t (\Omega \cdot cm^2)$	341±48	$383 \pm 58$	+42±12°	$347 \pm 47$	$532 \pm 98$	+185±59°‡
$\mathbf{J}^{Man}\left(n\boldsymbol{M}\boldsymbol{\cdot}\boldsymbol{cm}^{-2}\boldsymbol{\cdot}\boldsymbol{h}^{-1}\right)$	$3.8 \pm 1.7$	4.3±1.6	$+0.5\pm0.3$	$2.8 \pm 0.9$	3.6±0.9	+0.8±0.4

TABLE IIEffect of Cigarette Smoke on J

Paired tissues from the same dog were studied during two consecutive periods. In Control tissues no intervention was performed in period 2; in Smoke tissues the mucosal solution was bubbled with cigarette smoke between period 1 and period 2. Six pairs of tissues were studied under Nonsecreting conditions (indomethacin,  $10^{-6}$  M, mucosal solution) and six pairs were studied under Secreting conditions (epinephrine,  $10^{-6}$  M, submucosal solution).  $\Delta$  indicates the difference between period 1 and period 2. • There is a significant difference, P < 0.05, between period 1 and period 2.

t This indicates a significant difference, P < 0.05, between the Control  $\Delta$  and the Smoke  $\Delta$ .

the response observed following replacement of the mucosal solution with Ringer's solution used to wash nonsmoke-containing filters. As shown in Fig. 5, the residue from the filters containing cigarette smoke reversibly decreased  $I_{sc}$ , a response similar to the effect of whole cigarette smoke. Replacement of the mucosal bath with solution that had washed nonsmoke-containing filters produced no change in  $I_{sc}$ . The decrease in  $I_{sc}$  produced by the smoke residue was also accompanied by an increase in  $R_t$  from 330±35 to 409±44  $\Omega \cdot cm^2$  (n = 8) (P < 0.02).

In additional experiments we examined the effect of filtering smoke through charcoal before bubbling the mucosal solution. Fig. 6 shows the effect of filtering cigarette smoke through a 12 mm  $\times$  15-cm length of tubing filled with charcoal; the charcoal filtered smoke had less of an effect on I<sub>sc</sub> than nonfiltered smoke (P< 0.05).

Since cigarette smoke contains a number of potent oxidant substances (10) and since the pulmonary parenchymal injury associated with cigarette smoke may, in part, be related to an oxidant mechanism (11, 12)



FIGURE 4 Effect of filtering cigarette smoke on the I<sub>sc</sub>. Seven pairs of tissues were studied. Values represent the I<sub>sc</sub> during a Base-line period before addition of smoke and after the mucosal bathing solution was bubbled with cigarette smoke (Smoke) or cigarette smoke filtered through a 0.22- $\mu$ m filter (Filtered Smoke). Epinephrine (10<sup>-6</sup> M, submucosal solution) was present throughout to stimulate Cl secretion. The decrease in I<sub>sc</sub> produced by filtered smoke was less than that produced by whole cigarette smoke, P < 0.01.



FIGURE 5 Effect of smoke residue on  $I_{sc}$ . Values represent the  $I_{sc}$  in nine pairs of tissues taken during a Base-line period, an Experimental period, and a Recovery period after the tissues were washed with fresh Ringer's solution. The mucosal bathing solution was replaced with Ringer's that had washed the particulate phase of smoke from filters, Smoke Residue, or Ringer's that had washed nonsmoke-exposed filters, Control. Epinephrine ( $10^{-6}$  submucosal solution) was present during all periods to stimulate Cl secretion. The decrease in  $I_{sc}$  was greater in tissues exposed to smoke residue, P < 0.01.

it was important to determine if an oxidant mechanism might be involved in the acute effect of cigarette smoke on airway epithelial ion transport. Tissues were therefore incubated in a variety of antioxidants and oxygen radical scavengers before and during exposure to smoke. As shown in Fig. 7, addition of superoxide



FIGURE 6 Effect of charcoal filtered smoke on the  $I_{sc}$ . Values represent the  $I_{sc}$  obtained in eight pairs of tissues during a Base-line period before exposure to smoke, after bubbling the mucosal solution with whole cigarette smoke, Smoke, or smoke filtered through charcoal, Charcoal-Filtered Smoke. Epinephrine ( $10^{-6}$  M) was present in the submucosal solution throughout to stimulate Cl secretion. The decrease in  $I_{sc}$  produced by smoke was less when the smoke was filtered through charcoal, P < 0.05.



FIGURE 7 Effect of antioxidants on the percent change in  $I_{sc}$  produced by cigarette smoke. The mucosal bathing solution was bubbled with whole cigarette smoke in control tissues (open bars) or paired tissues in which both bathing solutions contained the substances indicated (crosshatched bars). Epinephrine  $(10^{-6} \text{ M})$  was present in the submucosal solution throughout.

dismutase (2,000 U·ml<sup>-1</sup>) and catalase 1,000 U·ml<sup>-1</sup>, vitamin E (D- $\alpha$ -tocopherol) (2.2 U·ml<sup>-1</sup>), or dimethyl, sulfoxide (10  $\mu$ l·ml<sup>-1</sup>) to both bathing solutions for 1 h before exposure to smoke did not prevent the decrease in I<sub>sc</sub>.

We also examined the effect of three other agents contained in whole cigarette smoke. The attainment of a 25% decrease in  $I_{sc}$  (n = 3) required the addition of 10 mM nicotine to the mucosal bathing solution, an amount certainly much greater than that obtained from bubbling smoke through the solution. Addition of acrolein ( $4 \times 10^{-4}$  M) (an irritant aldehyde contained in cigarette smoke) to the mucosal bathing solution decreased  $I_{sc}$  by 54% (n = 6). However, in contrast to the effect of cigarette smoke, the response obtained with acrolein was irreversible. Finally, bubbling carbon monoxide (0.3%) through the mucosal solution did not alter the  $I_{sc}$  (n = 3).

#### DISCUSSION

The results of this study indicate that cigarette smoke acutely inhibits active ion transport in canine tracheal epithelium both in vivo and in vitro. In vivo, the electrical potential difference across the tracheal epithelium was reversibly decreased during inhalation of cigarette smoke. The in vitro studies indicate that the decrease in voltage observed in vivo was primarily due to a decrease in the rate of Cl secretion. The inhibition of ion transport in vitro was only observed during exposure of the mucosal surface of the epithelium to smoke and was most pronounced in tissues in which Cl secretion had been stimulated. The observation that the effect of cigarette smoke was greatest in secreting tissues in vitro is consistent with the in vivo finding that the decrease in  $\Psi_t$  was dependent upon the initial value of transepithelial voltage; i.e., the largest decrease in  $\Psi_t$  observed in vivo may have occurred in tissues with a high rate of Cl secretion.

It has been well established that inhalation of cigarette smoke impairs overall pulmonary mucociliary clearance as measured by the rate of movement of particles along the airways (see reference 1 for a review). Abnormal mucociliary clearance has been observed following acute exposure to cigarette smoke in nonsmokers and in young, asymptomatic cigarette smokers. However, neither the mechanisms involved in the acute nor the long-term impairment of mucociliary clearance are known with certainty. Short-term exposure to cigarette smoke has been observed to decrease ciliary beat frequency (13, 14). While this observation suggests an effect of cigarette smoke on cilia. the results are also consistent with an alteration of the composition of the respiratory tract fluid, which might secondarily alter the observed ciliary activity. Cigarette smoke has also been observed to increase the permeability of the guinea pig airway epithelium to the electron-dense tracer horseradish peroxidase (15-17). However, these changes occurred at either much higher exposure levels, or during chronic exposure.

The exposure to high doses of cigarette smoke also produced an inflammatory reaction and altered the histology of the epithelium, changes that may have complicated the interpretation of the mechanisms involved.

The advantage of the methods used in this study is that they allow the examination of the effect of cigarette smoke on one component of the mucociliary clearance process. Effective mucociliary clearance requires ciliary activity, visoelastic mucus, and a pericilliary fluid layer (2). Active ion transport by the airway epithelium appears to play an important role in controlling the quantity and composition of the respiratory tract fluid, including the pericilliary fluid layer and the hydration of the mucus. The in vivo and in vitro results indicate that cigarette smoke has an acute reversible effect on the active ion transport function of the epithelium. An alternative explanation for the decrease in  $\Psi_{t}$  observed in vivo, an increase in ionic permeability of the epithelium, was not supported by the in vitro experiments (i.e., Rt increased and the permeability of the paracellular pathway, measured by J<sup>Man</sup>, did not decrease). A further advantage of the techniques used in this study is that they exclude the possibility that systemic factors or inflammation mediate the acute effects of smoke on ion transport. However, we can not eliminate the possibility of such factors mediating more long term, chronic alterations of ion transport. Furthermore, while the acute effect of cigarette smoke on ion transport was reversible, it is possible that chronic exposure would be less reversible.

The results also indicate that either the particulate phase of cigarette smoke, or some factor associated with the particulate material, is primarily responsible for the inhibition of Cl secretion, since filtering the smoke diminished its effect (Figs. 4 and 6). This conclusion is further supported by the finding that the effect of cigarette smoke could be reproduced by mucosal addition of smoke residue removed by the filter (Fig. 5). The observation that the particulate phase of smoke is primarily responsible for the effect on ion transport is in agreement with the observation that filtering cigarette smoke minimized its depressant effect on net mucociliary clearance (13, 18). The failure of filtering cigarette smoke to completely abolish its effect in the present study may have been due either to incomplete removal of all particulate material or to an effect on ion transport of some component of the vapor phase of smoke.

Cigarette smoke contains a number of potent oxidant substances that are thought to mediate, at least in part, the injury of lung parenchymal cells by cigarette smoke (10-12). The failure of antioxidants to completely prevent the effect of smoke in these studies suggests that the acute inhibition of Cl secretion cannot be entirely accounted for by an oxidant effect. However, it is possible that oxidants might mediate a more chronic injury to the epithelium. Finally, the results indicate that the effect of smoke is not produced by an effect of nicotine, acrolein, or carbon monoxide.

While the mechanism by which cigarette smoke inhibits Cl secretion cannot be determined with certainty, the data do provide some insight into the possible mechanisms involved. First, cigarette smoke only inhibited ion transport when bubbled through the mucosal bathing solution. This suggests that the effect may be localized to the apical cell membrane. Second, the decrease in Isc, increase in Rt, and decrease in the net transepithelial Cl flux indicate an effect on the electrogenic Cl transport process. Studies of the mechanism of Cl secretion in tracheal epithelium using intracellular microelectrode techniques (19) indicate that Cl exit from the cell across the apical cell membrane occurs via an electrically conductive transport process. Thus, the effect of cigarette smoke would be best explained by an inhibition of the apical membrane Cl permeability.

The results of this study indicate that cigarette smoke inhibits active ion transport both in vivo and in vitro in canine tracheal epithelium and thus suggest the possibility that smoke may alter ion transport in humans. The results, taken together with the important role of airway epithelial ion transport in maintaining the respiratory tract fluid, may explain, in part, the abnormal mucociliary clearance observed in cigarette smokers. The inhibition of ion transport might also be important in the pathogenesis of the airway disease observed in cigarette smokers.

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